Pediatric Endocrinology

A Practical Clinical Guide

Third Edition

Sally Radovick
Madhusmita Misra
Editors

Springer

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This textbook is dedicated to Margaret MacGillivray (Aug. 30, 1930–Sept. 17, 2016), for her vision in editing the first edition and with thanks for asking me to join her. I knew Margaret as a colleague, a mentor, and a friend. For those of us that were lucky enough to know her, she will be remembered as an outstanding clinician, investigator, mentor, and leader. Her passion for pediatric endocrinology, her compassion for others, and her upbeat spirit made her not only a most respected colleague but also a deeply valued friend.

Margaret was a towering figure in pediatric endocrinology making groundbreaking contributions to it. A true professional and role model, Margaret contributed to almost every aspect of pediatric endocrinology: thyroid disease, disorders of growth and puberty, and diabetes. In the mid-1960s, she published a seminal study on growth hormone secretion, defining for the first time short stature in children commonly seen with delayed puberty. She was a true physician scientist, investigating the factors that regulate growth hormone in these affected children. Her legacy to our field will be the pioneering use of growth hormone treatment for children with dwarfism. With this impact, Margaret is alive today and for generations to come.

I met Dr. MacGillivray in 1995 when she was president of the Pediatric Endocrine Society. I believe she never sought to be a leader, but became one naturally through her brilliance, compassion, patience, and selflessness. Her presidential address to the society was inspirational as I was beginning my career. I got to know Margaret well as a member of a prestigious grant review panel and little did I know that she had recommended my membership. Her guidance was critical as I was beginning to develop my academic reputation. In her gentle well-meaning, but somewhat blunt, way, she asked me if I had considered the insecurity associated with my academic position and whether the benefits were sufficient (which I had only cursorily considered). My salary was being funded entirely by NIH grants, which were subject to the vagaries of federal funding; I had 2 children and was married to a physician scientist. It was this discussion that changed my career course. She asked me to consider "replacing her" (imagine that) in Buffalo as she was thinking about stepping down as division director. Unfortunately, this did not work out, but her "reality check" stayed with me as I made my future career decisions. Twenty years later, I followed in her footsteps and was elected president of the Pediatric Endocrine Society.

On several occasions, we discussed the need for a pediatric endocrinology textbook focused on the knowledge required by clinicians that was comprehensive, organized,

and relevant. Agreeing in principal, she gained the support of Humana Press and asked me to co-edit the book with her. This was again an example of her mentorship, allowing me to share her academic stature. Her main goal, reflected in the preface, was to encourage the senior author of each chapter to include "a junior coauthor" as an opportunity to learn, to be mentored, and to give the next generation recognition in the field. With this third edition, we continue her tradition of a junior colleague as coauthor.

My relationship with Margaret has taught me most about the importance of mentorship. She taught mentorship by example and never demanded of her mentees what she would not expect of herself. She brilliantly mentored a generation of doctors with her characteristic compassion, grace, wisdom, and clever sense of humor. She was and still is an inspiration to women who pursue a career in medicine – very seldom looking backward to difficulties she had to endure as a woman, rather looking always forward. Some women would be very angry and bitter, but she always looked back on that as a challenge, and she overcame it. There were no role models or mentors at the time. She broke the glass ceiling and became the role model. Although she was dedicated to her roles as professor, clinician, and researcher, she was passionate about her role as wife, mother, and grandmother.

She taught me that hard work, determination, refusal to give up when the going gets rough, and, above all, sticking to one's ideals make for a successful career and a contented life. Margaret was a star. She didn't just shine; she blazed.

In this spirit, I welcome Madhu Misra as a co-editor of the third edition. Dr. Misra is the Fritz Bradley Talbot and Nathan Bill Talbot professor of pediatrics, Harvard Medical School, and division chief of pediatric endocrinology at the Massachusetts General Hospital. Her clinical interests include disorders of the pituitary gland and bone. Her research interests include the neuroendocrine and bone consequence of conditions that span the nutritional spectrum from anorexia nervosa to exercise-induced amenorrhea to obesity and conditions such as autism spectrum disorder and major depressive disorders.

Additionally, Dr. Misra is known for her successful mentorship of the next generation of pediatric endocrinologists and her service to the field as exemplified by her distinguished service to the Pediatric Endocrine Society.

Sally Radovick, MD

Preface

We welcome you to the third edition of *Pediatric Endocrinology: A Practical Clinical Guide*. The aim of this edition remains similar to the previous: to provide practical detailed and concise guidelines for the clinical management of pediatric endocrine diseases and disorders. Thus, the audience includes pediatric endocrinologists, pediatricians, and primary care physicians who provide medical care for children and adolescents.

The scope of the text continues to include the most common and the most challenging diseases and disorders seen by both primary care physicians and pediatric endocrinologists. We have encouraged the involvement of a junior coauthor to give recognition to our young investigators in the field. We believe we have assembled a state-of-the-art, comprehensive text on the practice of pediatric endocrinology.

Although the main focus of this text is on diagnosis and treatment, each author has included a brief discussion on pathophysiology and molecular mechanisms. The chapters have been organized in such a way as to present the following elements in synchrony: (1) a table of contents and key points; (2) an introductory discussion with background information; (3) a brief overview of recent progress on the mechanism involved; (4) a discussion of the etiology and clinical features that characterize each condition; (5) a delineation of the criteria used to establish a diagnosis; (6) a therapy section which comprehensively reviews the options available and the risks and benefits of each approach corroborated by clinical trial and outcome data, includes information on the long-term safety and efficacy of the treatment modality, and cites guidelines when available; (7) where relevant, a discussion of psychosocial and quality-of-life issues; and (8) finally a new section in this edition which includes related case studies and relevant questions.

Due to the dynamic clinical practice of pediatric endocrinology, extensive revisions and significant changes have been made to reflect current knowledge and practice. We have added chapters and expanded chapter content on care of gender nonconforming/transgender youth, diagnosis and management of osteoporosis, mineralocorticoid disorders and hypertension, and delayed puberty and hypogonadism.

We are most thankful for the generous contributions of our author colleagues. We hope you find the textbook helpful, and we are, of course, open to your comments.

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Growth Disorders

Childhood Growth Hormone Deficiency and Hypopituitarism

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1

Key Points

- Severe GH deficiency in the newborn period may be characterized by hypoglycemia and conjugated hyperbilirubinemia, as well as a small phallus in boys, consistent with multiple anterior pituitary hormone deficiencies.
- Hypopituitarism due to mutations in genes involved in pituitary development may be associated with other developmental anomalies.
- The diagnosis of GH deficiency should be made on the basis of physical findings and the integration of auxologic, biochemical, and radiographic data.
- Potential adverse effects of GH therapy include benign intracranial hypertension, slipped capital femoral epiphysis, and progression of scoliosis.

1.1 Introduction and Background Information

The pituitary gland is formed of anterior (adenohypophysis) and posterior (neurohypophysis) sections, which are derived from two different sources [1]. The upward invagination of stomodeal ectoderm forms the primordium of the anterior pituitary, Rathke's pouch, while the posterior pituitary arises from the neural ectoderm of the forebrain [2]. Rathke's pouch can be identified by the third week of pregnancy [3].

The anterior pituitary, which comprises 80% of the pituitary, consists of three parts: the pars distalis (pars anterior or anterior lobe), the pars intermedia (intermediate lobe), and the pars tuberalis (pars infundibularis or pars proximalis). In humans, the pars distalis is the largest portion of the anterior pituitary and where most of the anterior pituitary hormones are produced [3]. The intermediate lobe is poorly developed in humans with only a tiny remnant in adults, although more obvious in the fetus and in pregnant women [4]. The upward extension of the pars distalis onto the pituitary stalk forms the pars tuberalis, which may contain a small number of gonadotropin-producing cells [3].

Peptide hormones produced in neurons of the hypothalamus are transported via a capillary plexus in the pituitary stalk to the anterior pituitary, where they regulate the release and synthesis of several hormones [5]. The anterior pituitary hormones are somatotropin or growth hormone (GH), prolactin (PRL), thyrotropin or thyroid-stimulating hormone (TSH), follicle-stimulating hormone (FSH), luteinizing hormone (LH), and adrenocorticotropin (ACTH). Posterior pituitary hormones are synthesized in cell bodies of neurons in the hypothalamus and transported along their axons through the neurohypophyseal tract of the pituitary stalk. These hormones, arginine vasopressin (also known as antidiuretic hormone [ADH]) and oxytocin, are stored in and secreted from the posterior pituitary [6].

Hypopituitarism is the deficiency in varying degrees of one or multiple pituitary hormones. In this chapter, GH deficiency (GHD) will be discussed, while other hormonal deficiencies are presented elsewhere in this book. To understand GHD, an understanding of GH physiology is important and follows below.

Growth hormone is a single-chain α-helical non-glycosylated polypeptide. The majority (90%) of circulating GH is a 22-kDA form consisting of 191 amino acids and two intramolecular disulfide bonds [3, 7]. There is also a 20-kDa variant form, which arises from alternative splicing during the processing of human GH pre-mRNA [8, 9]. The remainder of the GH produced by the pituitary is in the *N*-acetylated and desaminated forms and oligomers [3]. Secreted GH circulates both unbound and bound to binding proteins, which are portions of the extracellular domain of the GH receptor (GHR) [10].

The *GH1* gene encodes for GH and is part of a 50-kb cluster of five genes located on human chromosome 17q22–24: *GH1*, *chorionic somatomammotropin* (*CS*)*-like* (*L*), *CS-A*, *GH-2*, and *CS-B* [11]. The CS-L translated protein appears nonfunctional, while CS-A and CS-B encode human chorionic somatomammotropin (hCS), also known as human placental lactogen (hPL). The syncytiotrophoblastic cells produce hCS, which has 85% homology to GH. hCS also contains two disulfide bonds that occur at the same positions as in GH-N, but it only has 0.5% affinity for the GHR. Interestingly, hCS does not appear necessary for fetal or extrauterine growth, nor does it appear essential for maintenance of pregnancy or lactation [12]. The *GH-2* gene product, which is

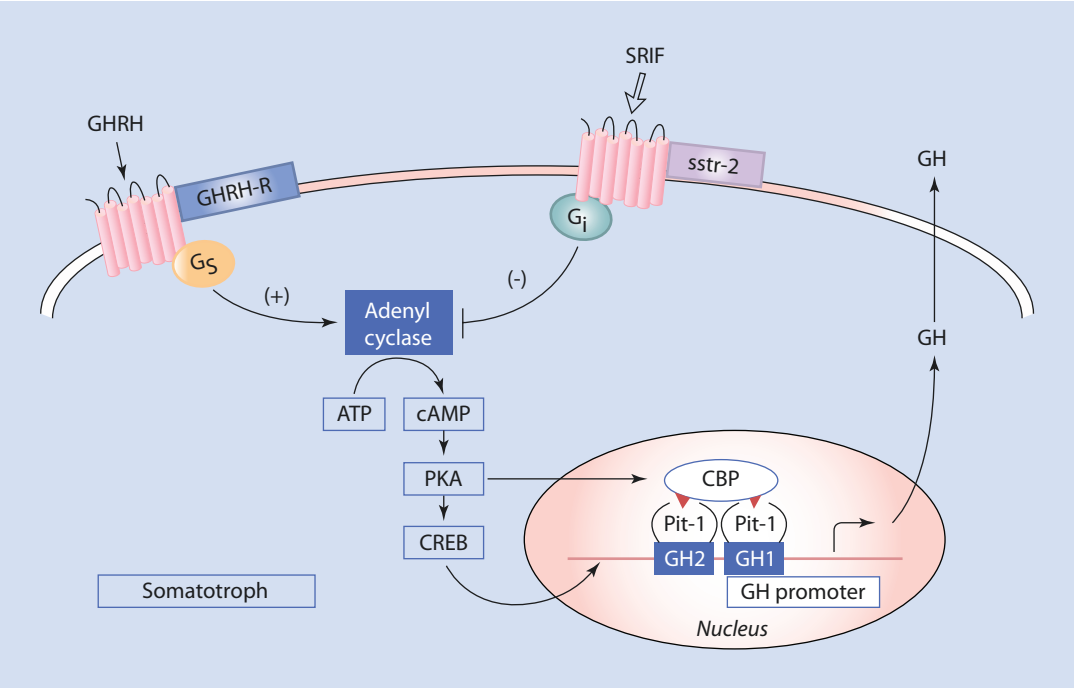

Fig. 1.1 GH secretion. Simplified model of growth hormone (GH) gene activation. GH synthesis and release from somatotrophs are regulated by growth hormone-releasing hormone (GHRH) stimulation and somatostatin (SRIF) inhibition. GHRH activation of its Gs-protein-coupled receptor leads to an increase in cyclic adenosine monophosphate (cAMP) and intracellular calcium, resulting in activation of protein kinase A (PKA). PKA phosphorylates and activates cAMP response element-binding protein (CREB), which binds to cAMP response elements in the GH promoter to enhance *GH1* gene transcription. There is also a PKA-dependent, CREB-independent mechanism of human GH gene activation by POU1F1 and CREB-binding protein (CBP). SRIF activation of its Gi-coupled protein leads to a decrease in cAMP and a reduction in calcium influx

known as GH variant (GH-V), differs from GH-N by 13 amino acids. It is expressed as at least four alternatively spliced mRNAs in the placenta and is continuously secreted during the second half of pregnancy, suppressing maternal pituitary *GH-1* gene function [13, 14].

GH is secreted in a pulsatile manner due to the opposing actions of growth hormone-releasing hormone (GHRH) and somatotropin release-inhibiting factor (SRIF), also known as somatostatin (SST). GHRH, a 44-amino-acid protein, binds to the GHRH receptor (GHRHR), which is a G-protein-coupled receptor with seven-transmembrane-spanning domains with three extracellular and three cytoplasmic loops [15]. Activation of the GHRHR results in an increase in cyclic adenosine monophosphate (cAMP) and intracellular calcium, leading to the activation of protein kinase A (PKA) [16, 17]. PKA phosphorylates and activates cAMP response element-binding protein (CREB), which binds to cAMP response elements in the *GH* promoter to enhance *GH-1* gene transcription [18, 19]. There is also a PKA-dependent, CREB-independent mechanism of *hGH* gene activation by POU1F1 (also known as Pit-1) and CREB-binding protein (CBP) [20] (Fig. 1.1).

SRIF, a 14-amino-acid neuropeptide, negatively regulates GH release primarily via the SRIF receptor subtype 2 (SSTR2) [20]. SRIF activates a G_i-coupled protein [21, 22], which decreases cAMP and reduces calcium influx, resulting in inhibition of GH secretion [23]. SRIF controls the pulse frequency of GH [24] (Fig. 1.1).

Infants have nonpulsatile GH secretion. There is a gradual increase in 24-h integrated GH secretion during childhood. The amplitudes of GH pulses are increased during puberty, which may be secondary to the effect of gonadal steroids on GHRH [25–27]. Although GH production continues throughout life, the levels decline in the elderly [28, 29].

There are multiple other factors that affect GH secretion. Thyroid hormone regulates GH secretion at the level of the hypothalamus and pituitary, and hypothyroidism is associated with a decrease in GH secretion [30]. Adiposity (in particular visceral fat) is associated with decreased GH secretion [31], while undernutrition leads to oversecretion of GH but low IGF-I levels indicating GH resistance [32].

Synthetic hexapeptides capable of stimulating GH secretion are termed GH secretagogues (GHS) or GH-releasing peptides (GHRP). These compounds can stimulate GH release but do not act through the GHRH or SRIF receptors [33, 34]. These peptides can initiate and amplify pulsatile GH release; however, this is accomplished via the GHS receptor (GHS-R), which is distinct from the GHRHR [34]. The GHS-R is a seven-transmembrane G-protein-coupled receptor that acts via protein kinase C activation and is expressed in the hypothalamus and in pituitary somatotrophs [35].

An endogenous ligand for the GHS-R, ghrelin, stimulates GH release in a dose-related manner, as well as potentiates GHRH-dependent secretion of GH [36, 37]. It is produced mainly by the oxyntic cells of the stomach but is also found throughout the gastrointestinal tract, as well as in the hypothalamus, heart, lung, and adipose tissue [38]. Several studies have demonstrated that ghrelin has a wide range of effects, including acting as a physiological mediator of feeding [39, 40]. Thus, it is difficult to separate the direct effects of ghrelin from those related to GH secretion.

Approximately 50% of circulating GH is bound to GH-binding protein (GHBP). GHBP is produced in multiple tissues, with the liver being the predominant source. GHBP acts as a circulating buffer or reservoir for GH, prolonging the half-life of plasma GH and competing with the GHR for GH, probably forming an unproductive heterodimer. In general, GHBP levels reflect GHR levels and activity. In rodents, GHBP appears to be synthesized de novo from alternative splicing of *GHR* mRNA. In humans, rabbits, and others, it is shed from membrane-bound GHR by proteolytic cleavage [10, 41].

The GHR is a 620-amino-acid protein that belongs to the cytokine family of receptors [42]. It consists of a large extracellular domain, a single transmembrane helix, and an intracellular domain [43]. The highest level of *GHR* expression is in the liver, followed by the muscle, fat, kidney, and heart. GH binds to a homodimer complex of the GHR in order to activate its intracellular signaling pathways. The subunits of the GHR are constitutively dimerized in an unbound or inactive state [44, 45]. The GH-binding sites on the extracellular domains of the two subunits are placed asymmetrically; GH binding to the constitutive dimer induces rotation of the two subunits, which allows downstream kinase activation by phosphorylation of Janus kinase 2 (Jak2) [45]. Subsequently, the Jak2 molecule induces tyrosine phosphorylation on the intracellular portion of the GHR, which then provides docking sites for intermediary signal transducers and activators of transcription (STAT) proteins [46–48]. After phosphorylation, STATs dimerize and move to the nucleus, where they activate gene transcription [49, 50] (Fig. 1.2).

Many of the actions of GH, both metabolic and mitogenic, are mediated by insulin-like growth factors (IGFs) or somatomedins, initially identified by their ability to incorporate sulfate into rat cartilage [51]. IGF-I, which is a basic 70-amino-acid peptide, is produced under the direction of GH predominantly in the liver [52]. It plays an important role in both embryonic and postnatal growth. Both systemic and local IGF-I have been shown to stimulate longitudinal bone growth [53–57].

Human fetal serum IGF-I levels, which are approximately 30–50% of adult levels, have been positively correlated with gestational age [58, 59]. The levels of IGF-I gradually increase during childhood and peak during pubertal development, achieving two to three times the normal adult values [60, 61]. IGF-I production is also augmented by the rise in gonadal steroids, which contribute to the pubertal growth spurt. After adolescence, serum IGF-I concentrations decline gradually with age [59, 62]. IGFs circulate within the plasma complexed to

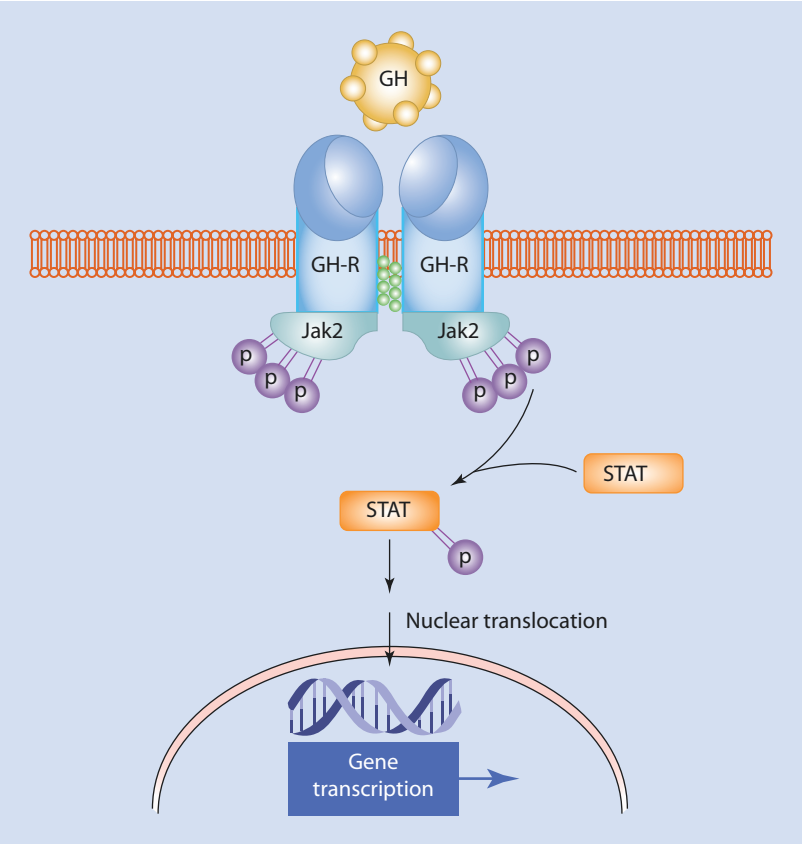

Fig. 1.2 GH action. Schematic model of growth hormone receptor (GHR) binding and signaling. A single GH molecule binds asymmetrically to the extracellular domain of two receptor molecules, causing a conformational change. This leads to interaction of the GHR with Janus kinase (Jak2) and tyrosine phosphorylation of both Jak2 and GHR, followed by phosphorylation of cytoplasmic transcription factors known as signal transducers and activators of transcription (STATs). After phosphorylation, STATs dimerize and move to the nucleus, where they activate gene transcription

high-affinity binding proteins or IGF-binding proteins (IGFBPs). IGFBPs extend the serum half-life of IGFs, transport IGFs into target cells, and modulate the interaction of IGFs with their receptors [59, 63]. Six distinct IGFBPs have been cloned and sequenced [64, 65]. IGFBP-3, which is GH dependent, is the major IGFBP in human serum and transports over 90% of the circulating IGF-I [3].

The IGF-I receptor (IGF-IR), which is structurally related to the insulin receptor, is a heterotetramer comprised of two-membrane-spanning α-subunits and two intracellular β-subunits [66, 67]. The subunits contain binding sites for IGF-I, are linked by disulfide bonds, and are composed of a transmembrane domain, an adenosine triphosphate (ATP)-binding site, and a tyrosine kinase domain that mediates the presumed signal transduction mechanism for the receptor [3, 68].

1.2 Etiology of Growth Hormone Deficiency

1.2.1 Congenital Forms of GH Deficiency (▸ Box 1.1)

The incidence of congenital isolated GHD (IGHD) has been reported as between 1:4000 and 1:10,000 live births [69, 70]. Congenital cranial malformations, including holoprosencephaly, septo-optic dysplasia (SOD) spectrum, and midline craniocerebral or midfacial abnormalities, can be associated with anomalies of the pituitary gland, including pituitary hypoplasia or aplasia [6]. Clinically, they may be associated with pituitary hormone deficiencies at birth or with the risk for developing future hormone deficiencies. Although these conditions often have no identifiable etiology, ongoing advances in understanding

Box 1.1 Congenital Forms of Hypopituitarism. Congenital Causes of or Associations with Growth Hormone Deficiency

- Cranial and central nervous system abnormalities
  - Septo-optic dysplasia
  - Cleft lip ± palate
  - Empty sella syndrome
  - Holoprosencephaly, anencephaly
  - Pituitary aplasia or hypoplasia
  - Thin or absent pituitary stalk
  - Hydrocephalus
- Genetic (mutations, deletions)
  - GHRH receptor
  - Ventral diencephalon factors
    - FGF8
    - GLI2
  - Pituitary developmental factors
    - Pituitary primordium factors
      - HESX1
      - OTX2
      - PITX2
      - LHX3
      - LHX4
      - SOX3
      - SOX2
    - Pituitary transcription factors
      - PROP1
      - POU1F1
  - GH-1
  - Types Ia, Ib, II, and III
  - Multiple GH family gene deletions
  - Bioinactive GH
  - GH receptor
  - IGF-I
  - IGF-I receptor
  - Stat5b

pituitary development have provided a genetic basis to account for pituitary pathology. Mutations have been found in genes necessary for pituitary development and function. The following presents a summary of reported genetic defects associated with pituitary pathology.

1.2.1.1 GHRH Receptor Mutations

Inactivating mutations reported in the GHRHR are often classified as a type of IGHD. The little mouse (*lit*/*lit*), which demonstrates dwarfism and decreased number of somatotrophs, has a recessively inherited missense mutation in the extracellular domain of the gene for *Ghrhr* [71–73]. In addition to GHD, these mice exhibit postnatal growth failure and delayed pubertal maturation [73]. The first human mutation identified was a nonsense mutation that introduced a stop codon at position 72 (E72X) in two cousins who presented clinically with the typical phenotype of severe GHD [74]. Subsequently, a nonsense mutation was found in codon 50 in "Dwarfism of Sindh" in Pakistan [75]. Since then, more than 30 nonsense, missense, and splice site mutations in the *GHRH* gene and deletions and regulatory mutations of the POU1F1-binding sites in the GHRHR promotor have been identified [76].

1.2.1.2 Pituitary Developmental Factor Mutations

The normal development of the pituitary is a complex cascade of events that has been shown to be dependent on several pituitary-specific transcription factors, which are expressed in a specific spatial and temporal pattern. The coordination of expression of these factors ultimately leads to the development of the pituitary-specific cell types (Fig. 1.3). Although mutations in these factors are often rare, it is important for the clinician to recognize the genetic basis for the pathology of idiopathic hypopituitarism. Mutations in genes involved in pituitary development may be associated with other developmental anomalies (Table 1.1).

Developmental Factors

Gli2 Gli transcription factors mediate Sonic hedgehog (Shh) signaling, which controls cell fate specification and proliferation in multiple tissues. Gli2/Shh signaling controls the expression of genes in the ventral diencephalon that are necessary for the early patterning of Rathke's pouch, as well as proliferation of pituitary progenitors [80]. Mice deficient in Gli2 have early forebrain, spinal cord, skeleton, and ventral diencephalon defects with variable pituitary loss. Pituitary cell types develop, but corticotrophs, somatotrophs, and lactotrophs do not proliferate [81]. Mutations in GLI2 have been found in patients with GH deficiency alone or with one or more other anterior pituitary deficiencies with and without holoprosencephaly and in patients with holoprosencephaly with and without pituitary hormone deficiencies. When pituitary hormone deficiencies are present, the GLI2 protein is usually truncated; when pituitary hormone deficiencies are absent, there is usually a missense mutation. The anterior pituitary may be absent or hypoplastic, and there may or may not be an ectopic posterior pituitary (EPP). Polydactyly is often an associated finding [82].

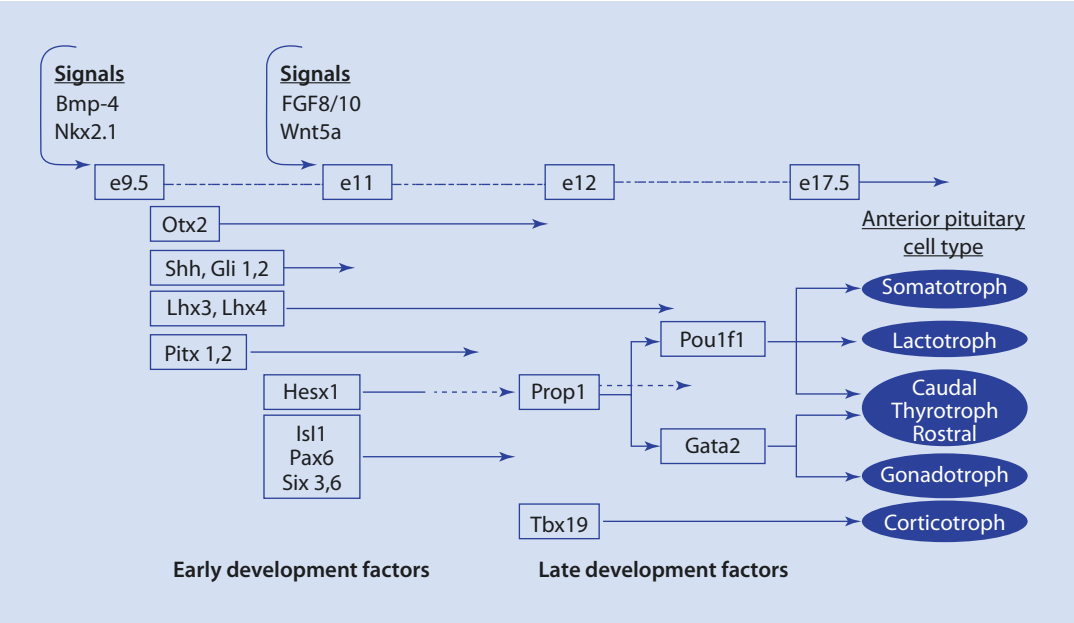

Fig. 1.3 Anterior pituitary development. The development of the mature pituitary gland initiates with the contact of the oral ectoderm with the neural ectoderm followed by a cascade of events consisting of both signaling molecules and transcription factors expressed in a specific temporal and spatial fashion. This figure presents a modified overview of pituitary development adapted from previous embryological studies performed in murine species by illustrating the temporal expression of various developmental factors. Early on, bone morphogenetic protein 4 (Bmp-4) and NK2 Homeobox 1 (Nkx2.1) are expressed along with Sonic hedgehog (Shh) in order to form the primordial Rathke's pouch, which will become the mature pituitary. Also expressed are Gli1 and 2, Lhx3, and Pitx1 and 2, which all play a role in the development of progenitor pituitary cell types. Subsequently, the expression of Hesx1, Isl1, paired box gene 6 (Pax6), and Six3 assists in appropriate cellular development, proliferation, and migration. The *hashed arrows* denote the attenuation of an expressed factor, such as seen with Hesx1, and are often required for the expression of another factor. The attenuation of Hesx1, for example, is required for the expression of Prop1. Similarly, Pou1F1 (Pit-1), which is required for somatotroph, lactotroph, and thyrotroph development, is expressed upon the attenuation of Prop1 expression. Ultimately, the mature pituitary gland is marked by the differentiated cell types: somatotrophs, lactotrophs, thyrotrophs, gonadotrophs, and corticotrophs [77–79]

Hesx1 (Rpx) Hesx1, a member of the paired-like class of homeobox genes originally described in *Drosophila melanogaster*, is one of the earliest known specific markers for the pituitary primordium, although no target genes for Hesx1 have been identified [83, 84]. Hesx1 null mutant mice demonstrate abnormalities in the corpus callosum, anterior and hippocampal commissures, and septum pellucidum, a phenotype similar to the defects seen in humans with SOD [83]. The initial report of a human HESX1 mutation was in two siblings with agenesis of the corpus callosum, optic nerve hypoplasia, and panhypopituitarism who were found to have a homozygous mutation at codon 53 (arginine to cysteine) in the homeodomain (DNA-binding domain) of *HESX1*, resulting in a drastic reduction in DNA binding [83]. Subsequently, autosomal recessive and dominant HESX1 mutations have been found in association with SOD (although a rare cause of SOD) or with combined pituitary hormone deficiency (CPHD) [85].

Several investigators have screened patients with a wide spectrum of congenital hypopituitarism for mutations in *HESX1*. Thomas et al., for example, evaluated 228 patients: 85 with isolated pituitary hypoplasia (including isolated GH deficiency and combined pituitary hormone deficiency [CPHD]), 105 with SOD, and 38 with holoprosencephaly or related phenotypes. In this cohort, three missense mutations were identified [86]. In another study, approximately 850 patients were studied for mutations in *HESX1* (300 with SOD; 410 with isolated pituitary dysfunction, optic nerve hypoplasia, or midline brain anomalies; and 126 patients with familial inheritance). Only 1% of the group was found to have coding region mutations, suggesting that mutations in *HESX1* are a rare cause of hypopituitarism and SOD [87]. As the described mutations in *HESX1*

Table 1.1 Developmental abnormalities seen in association with hypopituitarism due to mutations in genes involved in pituitary development

Gene	Associated findings
GLI2	Holoprosencephaly Midline defects Polydactyly
FGF8	Holoprosencephaly
HESX1	Optic nerve hypoplasia Absent septum pellucidum and corpus callosum
OTX2	Micro- or anophthalmia
LHX3	Cervical spine/vertebral anomalies Deafness Hyperextensible joints
LHX4	Hypoplastic corpus callosum Chiari syndrome
SOX2	Micro- or anophthalmia Esophageal atresia Sensorineural hearing loss
SOX3	Absent corpus callosum Craniofacial abnormalities

present with variable phenotypes, it has been suggested the hormone abnormalities may be affected by modifier genes or environmental factors [88].

Otx2 *Otx* genes are expressed in the rostral brain during development and are homologous to the *Drosophila orthodenticle* (*otd*) gene, which is essential for the development of the head in *Drosophila melanogaster* [89]. Otx2 is expressed in the ventral diencephalon, where it interacts with Hesx1, and in Rathke's pouch. Homozygous inactivation of Otx2 in mice leads to extreme brain defects, while heterozygous inactivation results in eye abnormalities, commonly pituitary hypoplasia, and sometimes holoprosencephaly. Heterozygous mutations of the *OTX2* gene, which have been implicated in severe ocular malformations such as anophthalmia, have also been reported in patients with hypopituitarism ranging from GH deficiency to multiple pituitary hormone deficiencies [90–93]. There are variable findings of hypoplastic pituitary, EPP, and Chiari syndrome [94–98].

Pitx2 (Ptx2) Pitx2 is a paired-like homeodomain transcription factor closely related to the mammalian *Otx* genes [89]. Pitx2 null mice showed embryonic lethality; however, a hypomorphic allele model of Pitx2 demonstrated pituitary hypoplasia and cellular differentiation defects in proportion to the reduced dosage of Ptx2. The gonadotrophs were most severely affected, followed by somatotrophs and thyrotrophs [99–101].

RIEG is the human homologue of Pitx2, and clinical mutations of PTX2 have been described in patients with Axenfeld-Rieger syndrome. This syndrome is an autosomal dominant condition with variable manifestations including anomalies of the anterior chamber of the eye, dental hypoplasia, a protuberant umbilicus, mental retardation, and pituitary alterations [102]. One group of investigators described mutations in six out of ten families with autosomal dominant Rieger syndrome [103, 104]. Five of the six mutations reported were in the homeobox region, and several showed loss of DNA-binding capacity.

Lhx3 (Lim-3, P-Lim) and Lhx4 Lhx3 is a LIM-type homeodomain protein expressed in the anterior and intermediate lobes of the pituitary gland, the ventral hindbrain, and the spinal cord. Lhx3 expression persists in the adult pituitary, suggesting a maintenance function in one or more of the anterior pituitary cell types [105]. In addition, its expression is associated with cells that secrete GH and PRL, as well as the expression of the α-glycoprotein subunit (α-GSU), suggesting a common cell precursor for gonadotrophs, thyrotrophs, somatotrophs, and lactotrophs [105, 106].

In humans, homozygous loss-of-function mutations in LHX3 have been identified in patients with hypopituitarism including GH, TSH, PRL, LH, and FSH deficiencies, anterior pituitary defects, and cervical abnormalities with or without restricted neck rotation [107–109]. Among 366 studied patients with idiopathic GHD or CPHD, only 7 patients from 4 families were found to have LHX3 mutations, suggesting *LHX3* mutations are a rare cause of CPHD [109]. A compound heterozygous mutation of LHX3 was described that leads to a short protein inducing a dominant negative effect (from a paternally derived change) and a protein with impaired transactivational ability (from a maternally derived change) [110]. As with other described *LHX3* mutations, the patient presented with pituitary hormone deficiencies, in addition to deafness and limited neck rotation.

Lhx4 is a closely related transcription factor to Lhx3. Heterozygous sporadic and familial LHX4 mutations have been reported. Pituitary hormone

deficiencies range from IGHD to panhypopituitarism, and the pituitary may be hypoplastic with or without an EPP. Some patients also have corpus callosum hypoplasia or Chiari syndrome with pointed cerebellar tonsils [111].

Other transcription factors In addition to the more commonly cited factors, several other mutated developmental factors have been reported to cause CPHD [111]. Sox2, for example, has roles both in pituitary development and in the stem cell compartment [112]. Patients with reported Sox2 mutations presented with phenotypes including hormone deficiencies (primarily isolated gonadotroph deficiency), pituitary hypoplasia, and eye abnormalities [113, 114]. Another interesting development has been the association of pituitary hormone deficiencies with mutations in the gonadotroph genes prokineticin receptor 2 (*PROKR2*), fibroblast growth factor 8 *(FGF8)*, and FGF receptor 1 (*FGFR1*), which have been traditionally reported in patients with isolated hypogonadotropic hypogonadism [115].

Pituitary-Specific Transcription Factors

Prop1 Prop1 is a paired-like homeodomain transcription factor with expression restricted to the anterior pituitary during development [2, 116]. During pituitary development, Prop1 acts as a repressor in downregulating Hesx1 and as an activator of Pou1f1 [77]. Recent evidence suggests that Prop1 may play a more central role in pituitary stem cell differentiation than previously recognized [117].

A considerable variation in clinical phenotypes of patients with PROP1 mutations has been demonstrated, even in patients bearing identical genotypes [116, 118, 119]. Several reports have shown that the hormone deficiencies may be variable and dynamic; some patients may develop hypogonadotropic hypogonadism despite the progression into spontaneous puberty or cortisol deficiency over time [116, 120–122]. Interestingly, some patients present with pituitary hyperplasia prior to developing hypoplasia, which is speculated to be due to pituitary progenitors accumulating in the intermediate lobe rather than differentiating into more mature cell types [123].

At least 25 heterozygous or compound heterozygous human mutations have been described [111]. The most common is a recurring homozygous autosomal recessive mutation of PROP1, delA301, and G302 (also known as 296delGA) in exon 2, which changes a serine to a stop codon at codon 109 in the homeodomain, resulting in a truncated gene product. It has been found in nonconsanguineous patients from at least eight different countries [124–126].

Pou1f1 Pou1f1 (Pit-1, GHF-1) is a member of a family of transcription factors, POU, which are responsible for mammalian development, and its expression is restricted to the anterior pituitary lobe [127, 128]. Pit-1 has been shown to be essential for the development of somatotrophs, lactotrophs, and thyrotrophs, as well as for their cell-specific gene expression and regulation [128].

Mutations in *POU1F1* in humans were described in 1992 by four different groups in patients with CPHD consisting of GHD, TSH, and PRL deficiencies and variable hypoplastic anterior pituitaries on MRI [129–132]. At least 28 different mutations have been described, with 23 demonstrating autosomal recessive inheritance and 5 demonstrating dominant inheritance [78]. The most common mutation is an R271W substitution affecting the POU homeodomain; this leads to a mutant protein that binds normally to DNA but acts as a dominant inhibitor of transcription and may act by impairing dimerization [130, 132–140]. In another single allele mutation, K216E, the mutant Pit-1 is able to bind DNA but unable to support retinoic acid induction of the *Pit-1* gene distal enhancer either alone or in combination with wild-type Pit-1. This ability to selectively impair the interaction with the superfamily of nuclear hormone receptors is thus another mechanism responsible for CPHD [141]. Several other point mutations in the Pit-1 gene resulting in CPHD have been described. Some alter residues important for DNA binding and/or alter the predicted α-helical nature of the Pit-1, while others have been shown to or postulated to impair transactivation of target genes [78, 142].

1.2.1.3 Isolated GHD

Four forms of IGHD have been described, and its classification is based upon the clinical presentation, inheritance pattern, and GH secretion.

IGHD Type IA results primarily from large deletions, along with microdeletions and single base-pair substitutions of the *GH1* gene, which ultimately prevents synthesis or secretion of the hormone. This condition is associated with growth

retardation in infancy and subsequent severe dwarfism. Heterogeneous deletions of both alleles ranging from 6.7 to 45 kb have been described [143–146]. In addition to *GH1* gene abnormalities, a recent report, in siblings with IGHD, described two homozygous variants in the proximal GH1 promoter within a highly conserved region and predicted binding sites [147]. Patients with IGHD type 1A frequently develop antibodies to exogenous GH therapy, which is attributed to the lack of immune tolerance because of prenatal GHD [148, 149]. Some patients may eventually become insensitive to GH replacement therapy demonstrating a decreased clinical response; subsequently, recombinant IGF-I therapy may be an alternative option.

IGHD Type IB is a less severe autosomal recessive form of GHD resulting from mutations or rearrangements of the *GH1* gene, such as splice site mutations that lead to partial GH deficiency [144, 150, 151]. In one study, a homozygous splice site G to C transversion in intron 4 of the *GH-1* gene was identified, causing a splice deletion of half of exon 4 as well as a frameshift within exon 5. These changes ultimately affected the stability and biological activity of the mutant GH protein [152]. Several other deletions or frameshift mutations have been described by others [153–155].

IGHD Type II is an autosomal dominant condition considered the most common genetic form of IGHD. Several patients have been found to have intronic transitions in intron 3, inactivating the donor splice site of intron 3 and deleting exon 3 [151, 152, 156–160].

IGHD Type III is a partial GH deficiency with X-linked inheritance due to interstitial Xq13.3-Xq21.1 deletions or microduplications of certain X regions. Patients may also have hypogammaglobulinemia, suggesting a contiguous Xq21.2-Xq22 deletion [161, 162].

Bioinactive GH has been reported in patients with short stature demonstrating normal GH immunoreactivity but reduced biopotency. A child, with an autosomal arginine to cysteine mutation at codon 77, was described with severe growth retardation, high serum GH levels, elevated GHBP, low IGF-I levels, and increased GH levels after provocative testing. The child expressed both mutant and wild-type GH; however, the mutant GH had a higher affinity for GHBP, a lower phosphorylating activity, and an inhibitory or dominant negative effect on wild-type GH activity [163]. In another patient, an aspartic acid to lysine mutation at codon 112 was identified and suggested to prevent appropriate GHR dimerization [164].

There are also patients with the phenotype of growth hormone insensitivity who do not demonstrate mutations of the GHR gene, but have identified mutations in downstream GHR signaling molecules. Homozygous mutations in STAT5B, a major GH-dependent mediator of IGF-I gene transcription, have been identified as a cause of GH insensitivity [165, 166]. The first mutation characterized was a point mutation resulting in a marked decrease in phosphorylation of tyrosine [166], a critical step in the pathway to STAT activation of *IGF-I* gene transcription; while the second characterized mutation was an insertion in exon 10, leading to early protein termination [165, 167, 168]. In addition to growth retardation, both patients had evidence of immune dysfunction presumably because STAT5B is involved in downstream signaling for multiple cytokines.

1.2.1.4 GHR Mutations

Laron dwarfism is an autosomal recessive disorder characterized by clinical features of severe GH deficiency along with low IGF-I levels but with normal to high levels of GH after provocative testing [169]. Several deletions and point mutations of several GHR exons have been described [170–179]. Many of these mutations affect the extracellular domain and, therefore, lead to absent or decreased levels of GHBP [180]. Recombinant IGF-I therapy has been demonstrated to effectively treat these patients [181, 182]. It has also been hypothesized that some patients with idiopathic short stature, normal GH secretion, and low serum concentrations of GHBP may have partial insensitivity to GH due to mutations in the *GHR* gene [178].

1.2.1.5 IGF-I and IGF-IR Mutations

A patient noted to have a homozygous partial IGF-I gene deletion with undetectable levels of IGF-I presented with severe prenatal and postnatal growth failure, bilateral sensorineural deafness, mental retardation, moderately delayed motor development, and behavioral difficulties. His evaluation did not demonstrate a significant delay in his bone age, and an IGFBP-3 level was normal [183]. Subsequently, a few other cases of IGF-I mutations have been described.

Studies with African Pygmies demonstrate normal levels of GH but decreased IGF-I levels and unresponsiveness to exogenous GH. Although

IGF-I deficiency has been hypothesized, Bowcock et al. found no differences in restriction fragment length polymorphisms in the IGF-I gene in Pygmy versus non-Pygmy black Africans [184]. Furthermore, Pygmy T cell lines show IGF-I resistance at the receptor level with secondary GH resistance [185, 186]. In subsequent studies, it was demonstrated that adult Pygmies demonstrate a reduction in both GH gene expression (1.8-fold) and GHR gene expression (8-fold). This decrease of the GHR expression in Pygmies was associated with reduced serum levels of IGF-I and GHBP [187].

Abnormalities in the IGF-IR gene have also been reported and are often associated with intrauterine growth retardation (IUGR). Several heterozygous mutations of the IGF-IR gene, as well as an association with deletions in chromosome 15q, have been reported in patients with growth retardation [188–193]. The majority of these reported patients carried the diagnosis of IUGR along with progressive postnatal growth retardation; however, other phenotypic characteristics not universal in these patients included findings of developmental delay, microcephaly, or skeletal abnormalities. In addition, IGF-I levels were found to be either normal or high, whether at baseline or after provocative testing.

Other patients are suspected to have IGF-I resistance, as they have elevated GH levels and elevated IGF-I levels [194–196]. In one patient, cultured fibroblasts had a 50% reduction in IGF-I binding capacity [194]. Another patient had a markedly diminished ability of IGF-I to stimulate fibroblast α-aminoisobutyric acid uptake compared to control subjects [195]. Their birth lengths, which were less than the fifth percentile, suggest the importance of IGF-I in fetal growth.

Other post-signal transduction defects and mutations in IGF-binding proteins may occur but have not been demonstrated as of yet.

1.2.2 Acquired Forms of GH Deficiency (► Box 1.2)

Hypopituitarism can be caused by anything that damages the hypothalamus, pituitary stalk, or pituitary gland. Head trauma can injure the pituitary stalk and infundibulum and lead to the development of transient and permanent diabetes insipidus, as well as other hormonal deficiencies [197, 198]. There are a number of reports suggesting an association between hypopituitarism and a complicated perinatal course, especially breech delivery [199, 200]. It is not clear if a complicated perinatal course causes hypopituitarism or if a brain anomaly leads to both a complicated delivery and hypopituitarism. The finding that some of these patients have a microphallus at birth suggests that pituitary dysfunction may precede the birth trauma [6].

Infiltrative conditions can also disrupt the pituitary stalk. Diabetes insipidus can be the first manifestation of germ cell tumors, Langerhans cell histiocytosis [201–203] or sarcoidosis [204]. Lymphocytic hypophysitis, usually in adult women in late pregnancy or in the postpartum period, can result in hypopituitarism [205].

Metabolic disorders can cause hypopituitarism through destruction of the hypothalamus, pituitary stalk, or pituitary. Hemochromatosis is characterized by iron deposition in various tissues, including the pituitary. It may be idiopathic or secondary to multiple transfusions (e.g., for thalassemia major); gonadotropin deficiency is the most common hormonal deficiency, but GHD has also been described [206, 207].

Hypothalamic or pituitary tissue can also be destroyed by the mass effect of suprasellar tumors or by their surgical resection. These tumors include craniopharyngiomas, low-grade gliomas/hypothalamic astrocytomas, germ cell tumors, and pituitary adenomas [208]. Treatment of brain tumors or acute lymphoblastic leukemia (ALL) with cranial

Box 1.2 Acquired Forms of Hypopituitarism. Etiologies of Acquired Growth Hormone Deficiency

- Trauma
- Head injury
- Perinatal events
- Infiltrative and autoimmune diseases
- Langerhans histiocytosis
- Sarcoidosis
- Lymphocytic hypophysitis
- Infections
- Meningitis
- Granulomatous diseases
- Metabolic
- Hemochromatosis
- Cerebral edema
- Neoplasms
- Craniopharyngioma
- Germinoma
- Hypothalamic astrocytoma/optic glioma
- Cranial irradiation

irradiation may also result in GHD. Lower radiation doses preserve pharmacologic response of GH to stimulation, but spontaneous GH secretion may be lost [209]. Discordancy between failure to provoke an adequate GH response to insulin-induced hypoglycemia and normal response to exogenous GHRH stimulation suggest that the hypothalamus is more vulnerable than the anterior pituitary [210]. Data, however, from Darzy et al. show that spontaneous GH secretion is maintained in adults after low-dose cranial RT, suggesting there is not GHRH deficiency. There is a normal but decreased peak GH response to stimulation testing indicating decreased somatotroph reserve. They postulated that there is compensatory increase in hypothalamic stimulatory input (GHRH) and suggested that "neurosecretory dysfunction" after low-dose cranial RT may only be seen in puberty during the time of increased GH demand [211].

The higher the radiation dose, the more likely and the earlier GHD will occur after treatment [212, 213]. Clayton et al. reported that 84% of children who received greater than 30 Gy to the hypothalamic-pituitary area had evidence of GH deficiency more than 5 years after irradiation [212]. Higher doses also increase the likelihood of the development of other anterior pituitary hormone deficiencies [213]. Cranial radiation can also be associated with precocious puberty, leading to premature epiphyseal fusion [198], and spinal irradiation can lead to skeletal impaired spinal growth [214], both of which will further compromise adult height.

1.3 Clinical Presentation of Growth Hormone Deficiency

Growth failure presenting in infancy and childhood is the most common sign of GH deficiency. Children with mild GH deficiency usually present after 6 months of age when the influences of prenatal environment wane [215]. They generally have normal birth weights and lengths slightly below average [216]. The height percentile of a child with GH deficiency will progressively decline, and typically the bone age will be delayed. They develop increased peri-abdominal fat [217] and decreased muscle mass and may also have delayed dentition, thin hair, poor nail growth, and a high-pitched voice [215]. Severe GH deficiency in the newborn period may be characterized by hypoglycemia and conjugated hyperbilirubinemia, as well as a small phallus in boys, consistent with multiple anterior pituitary hormone deficiencies [215].

1.4 Diagnostic Evaluation of Growth Hormone Deficiency

There is much debate as to the proper methods to diagnose GHD in childhood. It is clear that there is a spectrum of GHD, and the clinical presentation varies with the degree of hormonal deficiency. The diagnosis of GHD should be based on the integration of auxological, biochemical, and radiographic criteria.

GHD should be considered in children with short stature, defined as a height more than 3 SD below the population mean or height more than 2 SD below the population mean with a growth velocity more than 1 SD below the mean, and in children with a very low growth velocity (more than 2 SD below mean) irrespective of current height. In considering who should undergo evaluation for GHD, it is important to first exclude other causes of growth failure and then assess the patients for clinical features that can coexist with GHD. These features include hypoglycemia, prolonged jaundice, microphallus, traumatic delivery in the neonate, and craniofacial midline abnormalities. Additionally, history of other pituitary hormone deficiencies, cranial radiation, and central nervous system infection, as well as family history of GHD, should be ascertained [218]. When present, the majority of these features are seen in patients on the severe end of the spectrum of GHD. These patients are typically easy to diagnose and have low growth velocity and biochemical markers of GHD, including low IGF-I levels [219] and low peak GH levels after stimulation tests [220]. Nonetheless, the majority of patients with GHD will present with short stature without any of these other features.

1.4.1 IGF-I and IGFBP-3

GH induces the expression of IGF-I in the liver and cartilage. The use of age and puberty-corrected IGF-I levels has become a major tool in the diagnosis of GHD [221]. Because of little diurnal variation, their quantification in random

samples is useful. However, sensitivity is still limited due to a significant overlap with normal values. Low levels of IGF-I may be found in normal children, especially in those less than 5 years of age. Similarly, low levels are reported in children with malnutrition, hypothyroidism, renal failure, hepatic disease, and diabetes mellitus. Serum levels of IGF-I do not correlate perfectly with GH status as determined by provocative GH testing [222, 223].

IGFBP-3 is the major carrier of IGF-I [224]. It is GH dependent but has less age variation and is less affected by the nutritional status compared to IGF-I [225]. Although low levels of IGFBP-3 are suggestive of GH deficiency, up to 43% of normal short children have been reported to have low concentrations [226]. Similarly, normal values have been reported in children with partial GHD [222, 227].

The combination of a low growth velocity and a low IGF-I level for pubertal status may remove the need for provocative testing in patients [228] where other causes for growth failure, especially malnutrition and gastrointestinal illness, have been excluded. This is particularly true in patients with CPHD.

1.4.2 Growth Hormone Stimulation Tests

GH is secreted episodically, mostly during slow-wave sleep. Between the pulses of pituitary GH secretion, serum concentrations are typically low, even in GH-sufficient children, and thus a single sample is not useful. Thus, a variety of pharmacological tests have been implemented to assess the GH secretory capacity of the pituitary gland [229]. These tests are expensive, are not free of side effects, and require fasting conditions, as high glucose levels inhibit GH secretion [230, 231].

GH provocative tests have been divided into two groups: screening tests including levodopa and clonidine and definitive tests including arginine, insulin, and glucagon. Due to their low specificity and sensitivity and to exclude normal children who might fail a single stimulation test, the performance of two different provocative tests has been implemented [230, 231]. Conventionally, the gold standard for the diagnosis of GHD has been a peak serum GH <10 μg/L after two different GH stimulation tests. This cutoff is arbitrary and has increased from <3 to <10 μg/L as the supply of GH has increased with the production of recombinant human GH for therapy. An inappropriate low peak GH response in the second test supposedly is confirmatory of GH deficiency. While very low GH peak levels on provocative testing are consistent with severe GHD and generally correlate with a favorable response to GH replacement, there is considerable overlap of GH peak levels in patients with partial GHD and healthy subjects. Multiple studies have shown that many children diagnosed with isolated GHD based on peak GH levels <10 μg/L will have normal GH secretion on retesting both in childhood [232] and as adults [233, 234]. The requirement for two stimulation tests to be performed per patient highlights the large prevalence of inadequate responses in healthy individuals and the poor reproducibility of the same and different tests in the same subject [235].

The sensitivity and specificity of these tests are limited by the use of different laboratory techniques for the measurement of GH. Radioimmunoassays (RIAs) used in early studies employ polyclonal antibodies, which render low specificity and higher GH levels when compared with the more specific immunoradiometric assays used today that employ two highly specific monoclonal antibodies. Discrepancies up to two- to fourfold have been reported among different assays [236]. The discrepancies between GH assays are also addressed in current guidelines, which recommend that institutions require laboratories to provide harmonized GH assays using the somatropin standard 98/574 [237].

Furthermore, in normal children, serum levels of GH are age and sex dependent and show a sharp pubertal increase. Immediately before puberty, GH secretion may normally be very low, making the discrimination between GHD and constitutional delay of growth and puberty difficult. Sex steroid priming with estrogen [238] or androgen [239] to prepubertal boys older than 11 and prepubertal girls older than 10 is suggested by experts [237] and can reduce unnecessary treatment of children with slow growth due to constitutional delay of growth and puberty. This can be achieved with β-estradiol orally two evenings before the test is performed for both boys and girls or with one dose of intramuscular testosterone 1 week prior to testing in boys [240, 241]. While children with GHD might have an attenuated response, those with constitutional growth delay

will have a normal pattern. In a study by Marin et al. [242], 61% of normal stature prepubertal children who were not primed with sex steroids failed to raise their peak serum GH concentration above 7 μg/L following a provocative test.

Obesity also affects the results of GH stimulation testing. There is a negative impact of adipose tissue on GH secretion [235, 243]. These lower stimulated GH values in obese children normalize after weight loss [244].

Recent guidelines from the Pediatric Endocrine Society suggest that in patients who meet auxological criteria and have a hypothalamic-pituitary insult (congenital malformation, tumor, history of radiation, etc.) and deficiency of one additional pituitary hormone, diagnosis of GHD can be established without provocative testing of GH secretion [240]. Similarly, in newborns with congenital hypopituitarism and deficiency of at least one additional pituitary hormone (or imaging findings of EPP with pituitary hypoplasia and an abnormal stalk), GHD can be diagnosed without formal provocative testing if GH fails to rise above 5 μg/L during hypoglycemia. In patients who do undergo GH provocative testing, their results should not be used as the sole diagnostic criterion for GHD [237].

In summary, the threshold to define GH deficiency to various provocative stimuli is arbitrary and based on no physiological data. Pharmacological tests involve the use of potent GH secretagogues, which may not reflect GH secretion under physiological circumstances, masking the child with partial GH deficiency. Age, gender, and body weight all affect responses. GH stimulation tests are reliable only in the diagnosis of severe or complete GH deficiency. In addition to their low reproducibility [245], a "normal" secretory response does not exclude the possibility of various forms of GH insensitivity or partial GH deficiency. The usefulness and reliability of GH provocative tests for the diagnosis of GHD remain a matter of ongoing debate.

1.4.3 Physiologic Assessment of Growth Hormone Secretion

In addition to pharmacological tests of growth hormone secretion, frequent blood sampling can be performed overnight to test for spontaneous GH secretion. The term GH neurosecretory dysfunction refers to patients with an abnormally slow growth rate and low integrated GH concentration (mean serum 24-h GH concentration) but appropriate GH response to provocative tests [246, 247]. The pathophysiology and the incidence of this condition remain unknown. Although the integrated GH concentration has better reproducibility compared to the standard provocative tests, there are still significant intraindividual variation and overlapping with the values found in normal short children [248]. Lanes et al. reported decreased overnight GH concentrations in 25% of normally growing children [249]. As sampling is required every 20 min for a minimum of 12–24 h, this test is not practical for routine clinical care. Current guidelines advise against the use of overnight GH sampling as there is very limited data on its usefulness to distinguish between GH-deficient and normal subjects, and thus, it does not warrant the burden to the patient [240].

1.4.4 Bone Age Evaluation

The evaluation of skeletal maturation is crucial in the assessment of growth disorders, as osseous growth and maturation are influenced by nutritional, genetic, environmental, and endocrine factors. Skeletal maturation is significantly delayed in patients with GHD, hypothyroidism, hypercortisolism, and chronic diseases. Children with constitutional growth delay will show a delayed bone age (BA), which corresponds with the height age.

In children over 1 year of age, the radiograph of the left hand is commonly used to evaluate the skeletal maturation. The skeletal age or BA is determined by comparing the epiphyseal ossification centers with chronological standards from normal children. Comparison of the distal phalanges renders better accuracy. Several methods to determine the BA are available, with the Greulich and Pyle [250] and Tanner-Whitehouse 2 (TW2) [251] methods most widely used. For the Greulich and Pyle method, a radiograph of the left hand and wrist is compared with the standards of the Brush Foundation Study of skeletal maturation in normal boys and girls [250]. The standards correspond to a cohort of white children, so its applicability to other racial groups may be less accurate. The TW2 method assigns a score to each one of the epiphyses. It is more accurate but also more time-consuming. Bone age estimation

has technical difficulties due to inter- and intra-observer variations, as well as ethnic and gender differences among children.

1.4.5 Prediction of Adult Height

The growth potential of an individual must be evaluated according to the parents' and siblings' heights, as genetic influences play a crucial role in determining the adult height. An approximation of the ultimate adult height is obtained by calculating the midparental height. For girls, midparental height is (mother's height + father's height − 13 cm)/2 and for boys (mother's height + father's height + 13 cm)/2. The child's target height is the midparental height ± 2 SD (approximately 10 cm or 4 in) [252]. When the growth pattern deviates from the parental target height, an underlying pathology must be ruled out.

Four methods to predict adult height are available: (1) Bayley-Pinneau is based on current stature, chronologic age, and BA obtained by the Greulich and Pyle method [253]. This method probably underpredicts growth potential [254]. (2) The TW2 method considers current height, chronologic age, TW2 assessment of BA, midparental stature, and pubertal status [251]. (3) The Roche-Wainer-Thissen method requires recumbent length, weight, chronologic age, midparental stature, and Greulich and Pyle BA assessment [255]. (4) The Khamis-Roche (KR) algorithm directly calculates predicted adult height from a linear combination of child's height and weight, together with midparental height. Sex- and age-specific coefficients for both sexes are provided [256]. However, there is wide variation in predicted adult heights using height prediction algorithms, and different methods are useful under certain circumstances, with accuracy varying according to subjects' age, gender, and BA [257]. In addition, predictions of adult height may be of limited value in patients with underlying pathology.

1.4.6 Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) of the brain is a sensitive and specific indicator of hypopituitarism: A high proportion of children with IGHD with normal or small pituitary glands showed normalization of GH secretion at the completion of GH treatment, whereas GHD was permanent in all patients with congenital anatomical abnormalities, such as pituitary hypoplasia with pituitary stalk agenesis and an EPP [233]. Structural abnormalities are more common in patients with CPHD or panhypopituitarism (93%) and in those with severe GH deficiency (80%) compared to those with isolated GH deficiency [258]. Mass lesions such as suprasellar tumors or thickening of the pituitary stalk due to infiltrative disorders such as histiocytosis may be found in patients with acquired GHD.

1.5 Treatment of Growth Hormone Deficiency

There is wide variability in the dose of recombinant human GH used to treat GHD. Traditionally, GH dosage has been based on weight, and consensus guidelines recommend doses of 22–35 μg/kg/day given 6–7 days per week (0.16–0.24 mg/kg/week) with possible, but not routine, increase in GH dose during puberty [240]. There is great variability in response to GH therapy. IGF-I levels should be monitored and maintained within laboratory-defined normal range for the age or pubertal stage of the patient. Treatment should be discontinued once growth velocity falls below 2–2.5 cm/year [240].

In order to decrease variability and improve adult height outcomes, two strategies have evolved to help refine GH dosing. The first strategy employs prediction models which calculate expected growth velocity based on baseline parameters [259]. These prediction models are derived from large pharmaceutical company GH registries and provide important insights into GH responsiveness. Peak GH levels during stimulation testing, age at onset of GH therapy, and height deficit from midparental target height have been found to be the most significant predictors of first-year growth velocity [260]. This indicates that individuals with more severe GHD, younger age at therapy start, and greater genetic potential will have the greatest response to therapy. Growth velocity in subsequent years is highly dependent on growth response in the prior year. One study has shown that using individualized GH doses based on a prediction model decreased variability in response without compromising efficacy [261].

The second strategy for refining GH dosing involves using IGF-I levels to target therapy. One study showed that targeting higher IGF-I levels leads to an increase in height gain without apparent adverse effects [262]. With this strategy, there is still a range of responses, which depend on the individual patient's GH sensitivity. To date, there is no consensus on the optimal IGF-I level that results in favorable growth while minimizing long-term risk. For this reason, current guidelines propose aiming to keep IGF-I within normal range for age and pubertal status [240].

Regardless of the strategy used to select a dose for initiation of GH therapy, one must assess response to therapy and make further decisions about dose adjustments or discontinuation of therapy. Typically, response is assessed after a year of therapy, and the most important parameters are height velocity and change in height standard deviation score (SDS). Height velocity varies by age and gender, while change in height SDS intrinsically corrects for these factors [263, 264]. In patients with severe GHD defined as a peak GH level < 5 μg/L on stimulation testing, a change in height SDS less than 0.4 in the first year of therapy is a poor response, while in those with less severe GHD, the corresponding value is 0.3 [264]. A suboptimal response may be indicative of an incorrect diagnosis of GH deficiency, lack of adherence to therapy, improper preparation and/or administration of the GH, associated hypothyroidism, concurrent chronic disease, complete osseous maturation, and, rarely, anti-GH antibodies. Development of antibodies to exogenous GH has been reported in 10–30% of recipients of GH. This finding is more common in children lacking the *GH* gene. However, the presence of GH antibodies does not usually attenuate the hormonal effect, as growth failure has been reported in less than 0.1% [265]. Additionally, one can compare actual growth response to predicted growth response based on the aforementioned growth prediction models. As GH dose is included in the models, a poor actual versus predicted response indicates either decreased GH sensitivity or nonadherence [264]. Finally, there is some evidence that underlying genetic variants in the GHR influence response to therapy [266], but this area requires further research.

To evaluate the response to GH, the most important parameter is the determination of the height velocity (expressed as the change in height SDS score). Monitoring of IGF-I levels has gained wide acceptance to assess safety and adherence [240]; however, the level does not always correlate with the obtained increment in growth velocity. Although recommended by some [267], regular monitoring of the BA in children receiving GH therapy is questionable. Interobserver differences in BA interpretation and erratic changes over time in osseous maturation make the estimation of adult height inaccurate. Similarly, predictions of adult height may be overestimated, as GH may accelerate the bone maturation in advance to any radiographic evidence [268].

1.6 Outcomes and Possible Complications

1.6.1 Short-Term Follow-Up

Potential adverse effects of GH treatment include benign intracranial hypertension, slipped capital femoral epiphysis, and progression of scoliosis, all of which should be monitored during physical exams on follow-up visits and require counseling of patients prior to start of treatment.

1.6.1.1 Benign Intracranial Hypertension

This neurological complication has been described in patients receiving GH, but with a low incidence. A prospective study collecting data on 3332 children in Australia and New Zealand found a low incidence of 1.2 cases per 1000 patients [269]. Data from the Kabi International Growth Study further demonstrated the incidence is lower than previously reported [270]. Nevertheless, an ophthalmologic evaluation is mandatory in GH recipients in the event of persistent headaches, nausea, visual symptoms, and dizziness. Symptoms usually resolve with discontinuation of treatment.

1.6.1.2 Slipped Capital Femoral Epiphysis (SCFE)

SCFE occurs with an incidence of 73 per 100,000 treatment years in GH-treated patients. The incidence is significantly lower in patients with IGHD (12.2) than in those with Turner syndrome (56.4), congenital GHD (54.5), Prader-Willi syndrome (PWS) (68.3), and chronic renal insufficiency (147.8) and not increased from the general population in children with ISS [270]. It occurs from 0.01 to 1.3 years after onset of therapy in various diag-

nostic groups, but up to 2.5 years into treatment in other series. Typically, these children were older and heavier and grew more slowly during the first year of GH treatment than those who did not [265]. SCFE, however, can be associated with not only obesity, but also untreated endocrine conditions (e.g., hypothyroidism), trauma, and radiation exposure. Although the incidence of SCFE in all databases appears to remain greater than for the general population, it is difficult to assess the risk of SCFE in the general population because of several variables (age, sex, race, geography) [270, 271].

1.6.1.3 Scoliosis Progression

Rapid growth during GH treatment can worsen existing scoliosis. Scoliosis is more frequent during GH treatment in groups with a higher baseline incidence, such as Turner syndrome and PWS [240].

1.6.1.4 Diabetes and Insulin Resistance

The incidence of type I diabetes mellitus in children and adults is not increased by GH treatment. GH decreases insulin sensitivity, which can result in higher insulin requirements for patients with coexisting diabetes or result in the development of type II diabetes mellitus in those at risk. The increase in insulin resistance appears to happen early and returns to baseline with time or after discontinuation of treatment. Monitoring for impaired glucose metabolism and potential diabetes mellitus should be focused on patients at risk [272].

1.6.1.5 Other Side Effects

Other recognized side effects include musculoskeletal symptoms such as edema, carpal tunnel syndrome, and muscular pain related to fluid retention which might correlate with overdosage. They are less likely to occur if the starting dose is low and titrated up as needed. GH can cause tonsillar hypertrophy and can exacerbate obstructive sleep apnea, which is a particularly important risk in patients with PWS. Polysomnography prior to initiating treatment and monitoring during treatment is recommended for those patients. Pancreatitis, although rare, is another listed side effect of GH, but whether there is a causal relationship from GH treatment is still unknown. Lastly, increases in metabolism of cortisol and thyroid hormone may help unmask adrenal insufficiency or hypothyroidism after starting GH treatment [240].

1.6.2 Long-Term Risks

In 2016, the European Society of Paediatric Endocrinology (ESPE), the GH Research Society (GRS), and the Pediatric Endocrine Society (PES) released a position statement to address GH safety. GH exposure and treatment data throughout studies are incomplete and lack harmony in how patients are characterized, diagnosed, and treated, *how and which risk factors are quantified*, and *risk metrics reported. The available* evidence to date does not support an association between GH therapy and all-cause mortality, and there is inadequate data to reach a conclusion regarding the association to cause specific mortality [272].

1.6.2.1 Cancer Recurrence

GH and IGF-I, which both have anabolic and mitogenic effects, have been suggested to cause proliferation of normal and malignant cells. Therefore, several possible mechanisms regarding GH's potential role in tumor growth have been investigated [273]. Initial data from the Kabi International Growth Study (KIGS) [274] and the National Cooperative Growth Study (NCGS) [275] did not support an increased risk of brain tumor recurrence. Follow-up data by the NCGS in 2010, which comprises approximately 20 years of GH therapy and 192,345 patient-years, continued to report no increase in new malignancies or recurrences of CNS tumors in GH-treated patients without risk factors [271].

1.6.2.2 Primary and Secondary Malignancies

Although there are only a small number of subjects with long-term follow-up, the available data in children do not substantiate an increased risk of new primary malignancies, including leukemia in GH-treated patients without associated risk factors, in agreement with adult GH evidence. Therefore, cancer surveillance is not recommended beyond local standard practice, including in children and adults with prior malignancy [240, 272]. Ergun-Longmire et al. reported that cancer survivors treated with GH appeared to have an increased risk of developing SN compared to survivors not treated (relative risk 2.15), although the elevation of risk appeared to diminish with increasing length of follow-up [276]. The latter should be discussed with patients and families before starting treatment [272].

1

1.6.2.3 Skin Cancer

The statistics of the NCGS have not shown a higher incidence of melanocyte nevi or skin cancer in individuals treated with rhGH [277].

1.6.2.4 Stroke

An association between GH treatment and subarachnoid hemorrhage, intracerebral hemorrhage, and ischemic stroke was found in a French Registry [278, 279]. An increased incidence in cardiovascular events has not been seen in other European countries [280]. Given the lack of information on potential confounders such as family history and coexisting conditions in the French study and the lack of association in other studies, the evidence is felt to be insufficient at this time.

Case Study

A 12-month-old boy presents with growth failure. He was a 3 kg, 48 cm product of a full-term uncomplicated gestation and delivery. At birth, he was noted to have bilateral polydactyly. He has been an otherwise healthy child who has only had an occasional upper respiratory infection. His growth chart is seen in Fig. 1.4. Laboratory evaluation includes a normal chemistry panel, urinalysis, CBC, and thyroid function tests. IGF-I level is 42 μg/L (reference range 55–327).

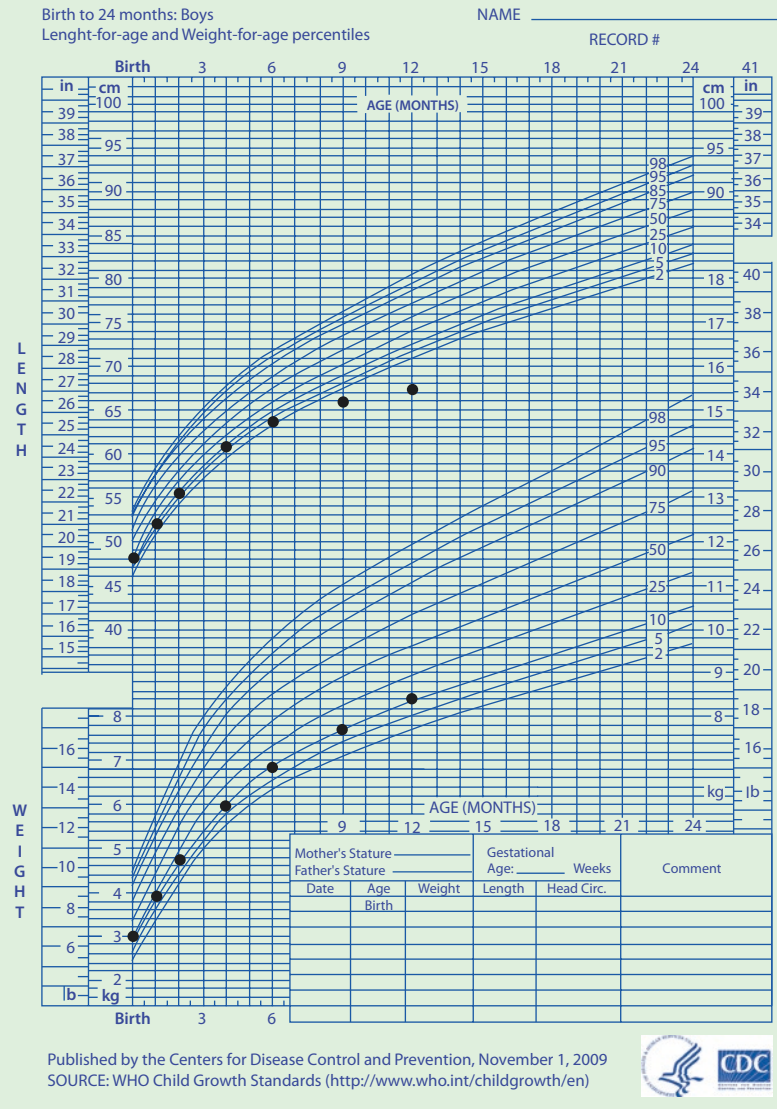

Fig. 1.4 Case study—growth chart

1.7 Summary

Congenital anomalies or anything that damages the hypothalamus, pituitary stalk, or pituitary gland can result in GHD. It is now recognized that there are molecular defects at multiple levels of the GH axis that cause GHD. Diagnosis of GHD, however, remains problematic. Once it is diagnosed, GH therapy is an effective treatment.

Review Questions

1. The statement that is MOST correct about his low IGF-I level is that it:
   A. Confirms GH deficiency
   B. May indicate GH deficiency
   C. Rules out GH deficiency
   D. Suggests liver disease
2. The patient undergoes cranial magnetic resonance imaging and is discovered to have a hypoplastic anterior pituitary and an ectopic posterior pituitary. Of the following choices, the diagnosis MOST likely to be the etiology of his growth failure is a:
   A. GHRH mutation
   B. GLI2 mutation
   C. IGF-I mutation
   D. STAT5B mutation
3. You initiate therapy with recombinant human growth hormone. He is subsequently MOST likely to develop:
   A. Adrenal insufficiency
   B. Diabetes mellitus
   C. Leukemia
   D. Pancreatitis

Answers

1. B
2. B
3. A

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Growth Hormone Insensitivity

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Key Points

- Growth hormone (GH) exerts its growth effects via IGF-I activation of transcription and transduction at the cellular level.
- Mutation of cell surface GH binding protein, IGF-I, IGF-I binding protein, and other intracellular growth factors is rare, whereas secondary GH insensitivity due to undernutrition, renal failure, and disease states is common.
- rhIGF-I replacement therapy is only partially effective in restoring growth in IGF-I deficiency states.

2.1 Introduction and Background

2.1.1 The Growth Hormone (GH)-Insulin-Like Growth Factor (IGF)-I Axis

GH synthesis and secretion by the anterior pituitary somatotrophs are under the control of stimulatory GH-releasing hormone (GHRH) and inhibitory somatostatin (SS) from the hypothalamus; however, other GH secretagogues (e.g., ghrelin and various synthetic hexapeptides) may also play a role (Fig. 2.1). The stimulation and suppression of GHRH and SS result from a variety of neurologic, metabolic, and hormonal influences; of particular importance to discussions of GHI is the feedback stimulation of SS by IGF-I, with resultant inhibition of GH release [1].

After its release from hypophyseal somatotrophs, GH binds to its receptor in the liver to initiate signaling of the growth cascade; however, after binding occurs, GH remains attached to the external domain of its receptor – known as GH binding protein (GHBP) – that is the proteolytic cleavage product of the full-length membrane-bound receptor molecule [2]. This binary complex circulates in equilibrium with approximately equal amounts of free GH. Because the binding sites for the radioimmunoassay of GH are not affected by the GHBP, both bound and unbound GH are measured [3]. This characteristic permits assaying circulating GHBP as a measure of cellular-bound GH receptor (GHR), which usually correlates [1].

The GH molecule is enveloped by a cell surface dimerized GHR molecule [4]. The intact receptor lacks tyrosine kinase activity but is closely associated with JAK2, a member of the Janus kinase family. JAK2 is activated by binding of GH with the GHR dimer, which results in self-phosphorylation of the JAK2 and a cascade of phosphorylation and activation of cellular proteins, including signal transducers and activators of transcription (STATs), which couple ligand binding to the activation of gene expression and mitogen-activated protein kinases (MAPK). STAT5b is the most important of these activator proteins. This is a mechanism typical of the GH/prolactin/cytokine receptor family that includes receptors for erythropoietin, interleukins, and other growth factors [3].

The effect of GH on growth is indirect, via stimulation of IGF-I production in the liver and growing tissues, particularly bone and muscle [1]. Hepatic (endocrine) IGF-I circulates almost exclusively bound to IGFBPs, less than 1% being unbound. The IGFBPs are a family of seven structurally related proteins with a high affinity for binding IGFs [5, 6]. IGFBP-3 is the most abundant IGFBP, binding 75–90% of circulating IGF-I in a large (150–200 kilodalton) complex which includes IGFBP-3 and an acid labile subunit (ALS). Both ALS and IGFBP-3 are produced in the liver as a direct effect of the GH cell surface binding. The ALS stabilizes the IGF-IGFBP-3 complex, reduces the passage of IGF-I to the extravascular compartment, and extends its half-life.

IGFBP-1 production is highly variable, with the highest concentrations in the fasting, lower insulinemic state. Since IGFBP-1 can inhibit invasion and metastasis of hepatocellular carcinoma, it has been suggested that its levels could be used as a prognostic marker in this malignancy [7]. In addition, low levels of IGFBP-1 reflect hepatic and widespread insulin resistance and deterioration of glucose balance, including T2DM [8]. IGFBP-1 levels are elevated in subjects with growth hormone insensitivity, probably reflecting the decreased insulin resistance found in this condition [9].

The circulating concentration of IGFBP-2 is less fluctuant and is partly under the control of IGF-I; levels are increased in GHRD states but increase further with IGF-I therapy of such patients [6, 10]. It has been proposed that IGFBP-2 influences extracellular and nuclear activities of IGF1, thereby contributing to its widespread effects in physiological growth and metabolism. It

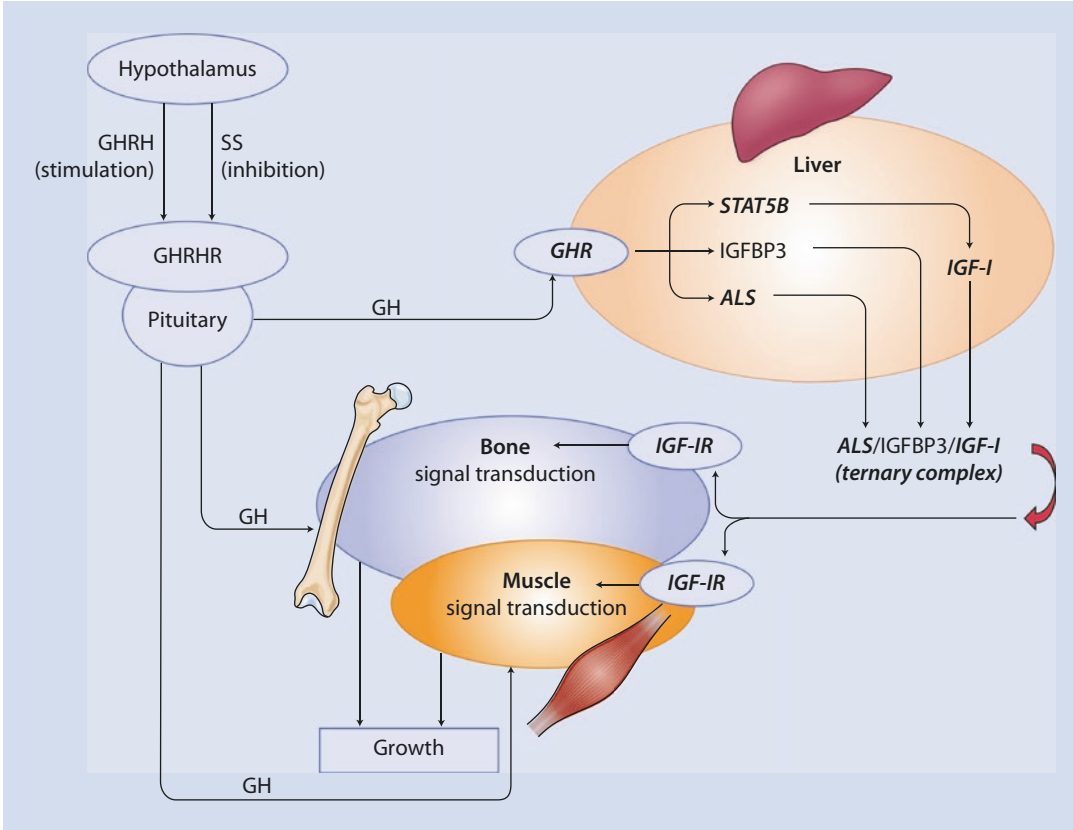

Fig. 2.1 Simplified diagram of the hypothalamic-pituitary-GH/IGF-I axis, showing mutational targets resulting in GH insensitivity indicated in italics and bold

also promotes transcriptional activation of genes that could influence malignant growth [11]. The IGFBPs modulate IGF action by protean actions such as controlling storage and release of IGF-I in the circulation, by influencing the binding of IGF-I to its receptor, by facilitating storage of IGFs in extracellular matrices, and by independent and miscellaneous actions of IGF-I [5].

Autocrine and paracrine production of IGF-I occurs in tissues other than the liver; in growing bone, GH stimulates differentiation of prechondrocytes into chondrocytes able to secrete IGF-I, which stimulates clonal expansion and maturation of these chondrocytes, thus ensuring subsequent growth. It is estimated that at least 20% of GH-stimulated growth results from this autocrine-paracrine IGF-I mechanism [12].

IGF binding involves three types of receptors: the structurally homologous insulin receptor and type 1 IGF receptor and the distinctive type 2 IGF-II/mannose-6-phosphate receptor. Considering that IGF-I is present in the circulation at molar concentrations that are 1000 times those of insulin, and despite the fact that the insulin receptor has a low affinity for IGF-I, even a small insulin-like effect of IGF-I could be more important than that of insulin itself were it not for the IGFBPs that control the availability and activity of IGF-I. In fact, intravenous infusion of recombinant human IGF-I (rhIGF-I) can induce hypoglycemia [13].

Deranged activation of the IGF1 receptor by usual circumstances such as hyperinsulinemia might be present in the etiology or associated with a variety of pathologic conditions, including polycystic ovarian disease, diabetes mellitus, and malignancy [14].

2.2 Etiology

GH insensitivity (GHI) or resistance is defined as the absence of an appropriate growth and metabolic response to endogenous GH or to GH administered at physiologic replacement dosage [1].

Table 2.1 Conditions characterized by unresponsiveness to endogenous or exogenous growth hormone: clinical and biochemical characteristics

Condition	Growth failure	GH	GH binding protein	IGF-I	IGFBP3
Genetic					
GHR def recessive forms	Severe	Elevated	Absent–low[a]	Very low	Very low
GHR def dom neg forms	Mild–moderate	Elevated	Increased	Very low	Low/normal
STAT5b mutation	Severe	Elevated	Normal	Very low	Very low
ALS mutation	None–moderate	Normal	Normal	Very low	Very low
IGF-I gene mutation	Severe	Elevated	Normal	Absent/high[b]	Low/normal
IGF-I receptor mutation	Mild–moderate	Normal–elevated	Normal	Normal–elevated	Normal–elevated
Acquired					
GH inhib antibodies	Severe	Absent	Normal	Very low	Low
Malnutrition	None–mild	Elevated	Decreased	Variable	Variable
Diabetes mellitus	None–mild	Elevated	Decreased	Decreased	Increased
Renal disease	Mild–severe	Normal	Decreased	Normal	Increased
Hepatic disease	Mild–severe	Elevated	Normal–increased	Decreased	Normal

[a]Increased in mutations of or near the transmembrane domain of the GH receptor
[b]Absent with partial IGF-I gene deletion; very high with have abnormal IGF-I

Table 2.1 lists the known conditions associated with GH resistance and their clinical and biochemical features. The genetic disorders that interfere with the response to GH include mutations affecting the GH receptor (GHRD), STAT5b, ALS, IGF-I, and the IGF-I receptor. In addition to these well-documented genetic causes of GHI, several other congenital and acquired conditions may be associated with GHI [15].

The conditions that have been associated with acquired GHI may not demonstrate low levels of IGF-I or even consistent growth failure (Table 2.1). Acquired GH resistance occurs in some patients with GH gene deletion for whom injections of recombinant human GH (rhGH) stimulate the production of GH-inhibiting antibodies [16]. Growth failure associated with chronic renal disease is thought to be related to increased concentrations of IGFBPs with normal or elevated GH and usually normal total IGF-I levels [17].

The complex nature of the influences of the GH-IGF1 axis in humans is highlighted by the apparently contradictory concomitant findings found in subjects with IUGR and insulin resistance who also have a normal GH/IGF-I/IGFBP3 axis and severe short stature [18].

2.2.1 Molecular Etiology

2.2.1.1 GHR Gene Mutations

The GHR gene (Fig. 2.2) is on the proximal short arm of chromosome 5, spanning 86 kilobase pairs. The 5′ untranslated region (UTR) is followed by nine coding exons. Exon 2 encodes the

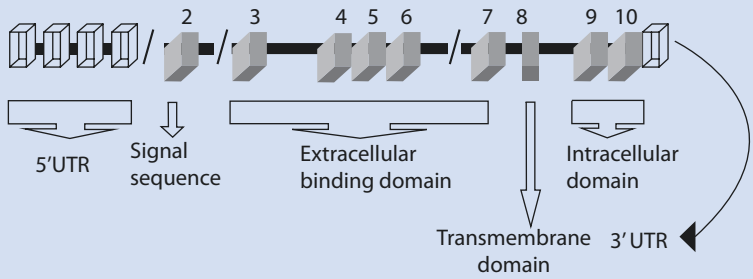

Fig. 2.2 Representation of the GHR gene. The black horizontal line represents intron sequence; breaks in lines indicate uncloned portions of the intron and the boxes represent exons, which are enlarged for clarity. UTR refers to untranslated regions of the transcripts. Translated regions are exons 2–10

last 11 base pairs of the 5'-UTR sequence, an 18-amino acid signal sequence, and the initial 5 amino acids of the extracellular hormone binding domain. Exons 3 to 7 encode the extracellular hormone binding domain, except for the terminal three amino acids of this domain, which are encoded by exon 8. Exon 8 further encodes the 24-amino acid hydrophobic transmembrane domain and the initial 4 amino acids of the intracellular domain. Exons 9 and 10 encode the large intracellular domain. Exon 10 also encodes the 2 kb 3'-UTR [3]. More than 50 mutations in the GHR have been described in the approximately 300 known patients with GHRD (Laron syndrome), which result in a clinical picture identical to that of severe GHD, but with elevated serum GH concentrations. The report of the characterization of the complete GHR gene included the first description of a genetic defect of the GHR, a deletion of exons 3, 5, and 6 [2]. Recognition that the exon 3 deletion represented an alternatively spliced variant without functional significance resolved the dilemma of explaining deletion of nonconsecutive exons. In addition to the original exon 5 and 6 deletion, another deletion of exon 5 has been described, along with numerous nonsense, missense, frame shift, and splice mutations, as well as a unique intronic mutation resulting in insertion of a pseudo-exon. A number of other mutations have been described that are either polymorphisms or have not occurred in the homozygous or compound heterozygous state [1, 19, 20].

All but a few of the defects result in absent or extremely low levels of GH binding protein (GHBP). Noteworthy is the D152H missense mutation that affects the dimerization site, thus permitting the production of the extracellular domain in normal quantities but failure of dimerization at the cell surface, which is necessary for signal transduction and IGF-I production. Two defects that are close to (G223G) or within (R274T) the transmembrane domain result in extremely high levels of GHBP. These defects interfere with the normal splicing of exon 8, which encodes the transmembrane domain, with the mature GHR transcript being translated into a truncated protein that retains GH-binding activity but cannot be anchored to the cell surface.

All these homozygous and compound heterozygous defects, whether involving the extracellular domain or the transmembrane domain, whether associated with very low or unmeasurable GHBP, or with the less common transmembrane defects that can be associated with elevated GHBP levels, result in a typical phenotype of severe GH deficiency. In contrast, the intronic mutation present in the heterozygous state in a mother and daughter with relatively mild growth failure (both with standard deviation score [SDS] for height of −3.6) [3] and resulting in a dominant-negative effect on GHR formation, is not associated with other phenotypic features of GHD [19]. This splice mutation preceding exon 9 results in an extensively attenuated, virtually absent intracellular domain. Japanese siblings and their mother have a similar heterozygous point mutation of the donor splice site in intron 9, also resulting in mild growth failure compared to GHR deficiency but with definite, although mild, phenotypic features of GHD [21].

GHBP levels in the Caucasian patients with the dominant-negative mutation were at the upper limit of normal with a radiolabeled GH binding

assay and in Japanese patients twice the upper limit of normal, using a ligand immunofunction assay. These heterozygous GHR mutants transfected into permanent cell lines have demonstrated increased affinity for GH compared to the wild-type full-length GHR, with markedly increased production of GHBP. When co-transfected with full-length GHR, a dominant-negative effect results from overexpression of the mutant GHR and inhibition of GH-induced tyrosine phosphorylation and transcription activation [22]; naturally occurring truncated isoforms have also shown this dominant-negative effect in vitro [23].

A novel intronic point mutation was discovered in a highly consanguineous family with two pairs of affected cousins with GHBP-positive GHI and severe short stature, but without the facial features of severe GH deficiency or insensitivity. This mutation resulted in a 108-bp insertion of a pseudoexon between exons 6 and 7, predicting an in-frame, 36-residue amino acid sequence, in a region critically involved in receptor dimerization [20].

2.3 Genetic Disorders Affecting GH-GHR Signal Transduction and Transcription

2.3.1 STAT5 Gene Mutations

There are seven members of the STAT family of proteins activated by multiple growth factors and cytokines, participating in a wide range of biological activities, particularly relating to growth and immunocompetence. While GH activates four members of this family, STAT5b is the one relevant for growth [24]. The GH-activated GHR recruits the STAT5b which docks to specific tyrosine residues on the receptor, undergoing tyrosine phosphorylation by the receptor-associated JAK2. The phosphorylated STAT dissociates rapidly from the receptor, forms a dimer, and translocates to the nucleus, where it binds to DNA, interacts with other nuclear factors, and initiates transcription.

Ten patients have been described with seven autosomal recessively transmitted mutations of STAT5b [25]. Similar to children with severe GHD and GHRD, but unlike those with IGF-I gene or IGF-I receptor mutations (below), birth size is normal, indicating GH-independent IGF-I production in utero. Also, as in GHD and GHRD, postnatal growth shows rapid decline in SDS ranging from −9.9 to −5.6 at the time of diagnosis. Serum concentrations of IGF-I, IGFBP-3, and ALS were markedly low; basal and stimulated GH concentrations were either normal or elevated. Except for GHBP, their phenotypic features, growth patterns, facial disproportion, and biochemistry have been identical to those of subjects with severe GHRD.

As might be expected because STAT5b is involved in intracellular signaling for other cytokine receptors besides the GHR, immunodeficiency with serious complications has been described in all but one of the reported patients with STAT5b mutations. Reported abnormalities include T-cell functional defects, low numbers of NK and γδT-cells, and IL-2 signaling defects.

2.3.2 PTPN11 Gene Mutations

Fifty percent of children with Noonan syndrome, characterized by short stature, cardiac defects, skeletal abnormalities, and facial dysmorphia, have been found to carry a gain-of-function mutation of *PTPN11*. This gene encodes a non-receptor-type tyrosine phosphatase (SHP-2) involved in intracellular signaling for a variety of growth factors and cytokines. Activated SHP-2 is thought to serve as a negative regulator of GH signaling. Children with this syndrome and bearing the PTPN11 mutation have more severe statural deficit than those without this genetic abnormality. They also have lower serum concentrations of IGF-I and IGFBP-3 with higher GH concentrations and less robust growth response to rhGH treatment, all suggesting mild GH insensitivity [24].

2.3.3 Mutations of the IGF-I Gene

Failure of IGF-I synthesis due to a gene deletion was described in a male subject with a homozygous partial deletion of the IGF-I gene [26]. His profound intrauterine growth retardation (IUGR) persisted into adolescence; he also had sensorineural deafness with severe mental retardation and micrognathia. A second individual with the same clinical phenotype but a different mutation also resulting in near absence of circulating IGF-I was subsequently described [27]. A third similarly affected subject had a defect in IGF-I synthesis

resulting in production of a nonfunctioning IGF-I molecule circulating in high concentration [28].

The absence of the craniofacial phenotype of severe GHD or GHRD and the presence of normal IGFBP-3 in these individuals, despite absent IGF-I function, indicate that the craniofacial features and low IGFBP-3 of GHD and GHRD are related to an absence of the direct effects of GH that do not act through the medium of IGF1 synthesis. It is also noteworthy that profound IUGR and mental retardation are not characteristic of GHD or GHRD, but IGF-I knockout mice have defective neurological development as well as growth failure. Thus, IGF-I production in utero does not appear to be GH-GHR dependent.

A milder molecular defect in IGF-I synthesis due to a homozygous missense mutation of the IGF-I gene has also been described. It results in IUGR and postnatal growth failure, microcephaly and mild intellectual impairment but with normal hearing, and undetectable IGF-I by highly specific monoclonal assay but elevated levels with a polyclonal assay [29]. In summary, findings in these individuals highlight the importance of IGF-1 during intrauterine life [30].

2.3.4 Mutations of the Acid Labile Subunit (ALS) Gene

ALS is an 85 kDa glycoprotein that modulates IGF-I bioavailability by stabilizing the binary complex of IGF-I and IGFBP-3. It is a member of a family of leucine-rich repeat (LRR) proteins that can participate in protein-protein interactions; ~75% of the mature protein corresponds to the consensus motif for the LRR superfamily. ALS is a donut-shaped molecule that binds readily to the binary complex of IGF-I and IGFBP-3 but does not interact directly with free IGF-I and has low affinity for IGFBP-3 that is not bound to IGF-I. Functional mutation of the ALS gene was first reported in 2004 and, by 2010, included 21 individuals from 16 families. They were proven to have 16 discrete mutations that induced homozygous or compound heterozygous states [31–37]. ALS is undetectable and serum IGF-I and IGFBP-3 concentrations are extremely low. Nonetheless, statural impairment is generally modest, with near-adult or adult height, available for 11 of the subjects, being better than target height in 1 individual, less than 1 SD below mean in an adopted child with unknown target height, and within 1.5 SD of target height in 6 others. The modest, at worst, effect on growth despite circulating concentrations of IGF-I that are similar to those of severe GHI or GHD emphasizes the compensatory capability of local IGF-I production [12, 38].

2.3.5 Mutations of the IGF-I Receptor Gene

Mouse studies demonstrated that deletion of the IGF-I receptor resulted in intrauterine growth retardation and perinatal death. Thus, it is not surprising that only heterozygous mutations in the IGF-I receptor gene (*IGF-IR*) have been described in humans. The initial report of *IGF-IR* mutation followed systematic examination in two groups of children with intrauterine growth retardation (IUGR) who remained greater than 2 SD below normal for length after 18 months of age. This population was selected because IGF-I receptor knockout mice have more severe IUGR than do IGF-I knockout mice. Among 42 US subjects who did not have low IGF-I and IGFBP-3 concentrations, a single subject with compound heterozygosity for mutations of the IGF-I receptor resulting in amino acid substitutions was identified. She had severe IUGR (birth weight 1420 grams at 38 weeks), poor postnatal growth, and elevated concentrations of IGF-I. Integrated GH concentration when prepubertal was consistent with IGF-I resistance. In general, the location of the mutations was within a putative ligand binding domain, and the heterozygous parents were subnormal in stature and had also had low birth weight. In the same study, a European group of 50 IUGR subjects was selected who had elevated circulating IGF-I concentrations and a second subject identified. He had a heterozygous nonsense mutation reducing the number of IGF-I receptors on fibroblasts; two other affected first-degree relatives were also found [39]. In all, 17 cases in 7 families, each family with a unique mutation, were described from 2003 to 2009 with in vitro confirmation of failure of IGF-I binding and function [39–44].

There is wide phenotypic variability among these individuals, with height SDS ranging from −1.6 to −5.7, substantial delay in osseous maturation to normal bone ages for chronologic age,

normal timing of puberty, and normal to markedly increased serum concentrations of IGF-I and IGFBP-3. The effect of IGF-I gene mutations on intrauterine growth, however, is uniformly replicated in this less severe circumstance.

2.3.6 Mutations of the PAPP-A2 Gene

Pregnancy-associated plasma protein A2 (PAPP-A2) mutation is associated with short stature and elevated levels of IGF-I, IGFBP-3, and ALS. PAPP-A2 is a metalloproteinase that cleaves IGFBP-3 and IGFBP-5 thereby releasing IGF-I. The underlying mechanism in this syndrome appears to be diminished bioactivity of IGF-I due to low availability generated by the lack of the proteolytic activities of the PAPP-A2 enzyme, resulting in impaired release of IGF-I [45].

2.4 Epidemiology

2.4.1 Race/Nationality

Among the approximately 350 affected individuals identified worldwide with growth failure due to GHR mutations, about two-thirds are Semitic and half of the rest are of Mediterranean or South Asian origin. The Semitic group includes Arab, Oriental, or Middle Eastern Jews and, the largest group, the genetically homogeneous 100+ Conversos in Ecuador (Jews who converted to Christianity during the Inquisition). The identification of an Israeli patient of Moroccan origin with the E180 splice mutation found in the Ecuadorian patients indicated the Iberian provenance of this mutation, which readily recombined in the isolated communities of these sixteenth-century immigrants established in the southern Ecuadorian Andes [46]. Recently, additional individuals with the E180 splice mutation on the same genetic background have been identified in Chile, Mexico, and Brazil, likely of the same origin. Among those who are not of Semitic, South Asian, or Mediterranean origin, there is wide ethnic representation, including Northern European, Eurasian, East Asian, African, and Anglo-Saxon (Bahamas) [15].

The individuals with STAT5b mutation include Kuwaiti siblings, two unrelated Argentinians, siblings from Brazil, one patient from Turkey, and one patient from the Caribbean [24].

ALS mutations were reported in three Kurdish brothers, three unrelated and two sibling Spanish subjects, three Norwegian/German siblings, two unrelated Swedish patients, and individual patients of Turkish, Argentinian, Ashkenazi Jewish, Pakistani, mixed European, and Mayan origin. Families with heterozygous mutations of the IGF-I receptor were of Dagestani, European, and Japanese origin [31–37].

Subjects with IGF-I receptor mutations have been reported from the USA, Germany, Russia, Korea, Japan, and the Netherlands [39–44].

2.4.2 Gender

Among individuals observed from the original description of the GHR deficiency syndrome by Laron, Pertzelan, and Mannheimer in 1966 [47], and until 1990, a normal sex ratio was noted. The initial report of 20 cases from a single province in Ecuador included only 1 male [48], but subsequent observations from an adjacent province indicated a normal sex ratio, and a few more males were subsequently identified in the initial province [49–51].

The abnormal sex ratio for that locus remains unexplained. All but 3 of the 21 individuals with *IGFALS* mutations are male, but this may reflect ascertainment bias because of the relatively modest effects on stature being of less concern in girls than in boys.

2.4.3 Morbidity and Mortality

The only available report of the effect of GHR deficiency on mortality comes from the Ecuadorian population [49, 50, 52–54]. Because families in the relatively small area from which the Ecuadorian patients originate had intensive experience with this condition, lay diagnosis was considered reliable. Of 79 affected individuals for whom information could be obtained, 15 (19%) died under 7 years of age, as opposed to 21 out of 216 of their unaffected siblings (9.7%, $p < 0.05$). The kinds of illnesses resulting in death, such as pneumonia, diarrhea, and meningitis, were no different for affected than for unaffected siblings [55].

The complete life span in the Ecuadorian cohort provided an opportunity to look at adult mortality risk factors. Twenty-three adults with GHRD had elevated cholesterol levels, normal HDL-cholesterol levels, elevated LDL-cholesterol levels, and normal triglycerides compared to relatives and non-related community controls. It was postulated that the effect of IGF-I deficiency due to GHRD was to decrease hepatic clearance of LDL-C, since the triglyceride and HDL-C levels were unaffected. This effect was independent of obesity or of IGFBP-1 levels, which were used as a surrogate for insulinemia. The key pathogenic factor was thought to be the absence of GH induction of LDL receptors in the liver [56].

Interestingly, despite obesity, Ecuadorian subjects with GHRD have enhanced insulin sensitivity and absence of diabetes despite a background of frequent diabetes in relatives; this protection was attributed to the absence of GH counter-regulation [10, 53].

2.5 Clinical Presentation

2.5.1 Clinical Features of Severe IGF-I Deficiency Due to GH Deficiency or GH Receptor Deficiency

- **Growth**
  - Birth weight, normal; birth length, usually normal
  - Growth failure, from birth, with velocity one-half normal
  - Height deviation correlates with (low) serum levels of IGF-I, IGF-II, and IGFBP-3
  - Delayed bone age but advanced for height age
  - Small hands or feet
- **Craniofacial Characteristics**
  - Sparse hair before age 7; frontotemporal hairline recession all ages
  - Prominent forehead
  - Head size more normal than stature with impression of large head
  - "Setting-sun sign" (sclera visible above iris at rest) 25% < 10 years of age
  - Hypoplastic nasal bridge, shallow orbits
  - Decreased vertical dimension of face
  - Blue scleras
  - Prolonged retention of primary dentition with decay; normal permanent teeth may be crowded; absent third molars
  - Sculpted chin
  - Unilateral ptosis, facial asymmetry (15%)
- **Musculoskeletal/Body Composition**
  - Hypomuscularity with delay in walking
  - Avascular necrosis of femoral head (25%)
  - High-pitched voices in all children, most adults
  - Thin, prematurely aged skin
  - Limited elbow extensibility after 5 years of age
  - Children underweight to normal for height, most adults overweight for height; markedly decreased ratio of lean mass to fat mass, compared to normal, at all ages
  - Osteopenia indicated by DEXA
- **Metabolic**
  - Hypoglycemia (fasting)
  - Increased cholesterol and LDL-C
  - Decreased sweating
- **Sexual Development**
  - Small penis in childhood; normal growth with adolescence
  - Delayed puberty
  - Normal reproduction

2.5.2 Growth

Individuals with GHI due to GHRD usually have normal intrauterine growth [57]. Nonetheless, IGF-I is required for normal intrauterine growth as demonstrated by patients with IUGR with a proven IGF-I gene defect or IGF receptor mutation [58]. It is thereby suggested that intrauterine IGF-I synthesis, which appears to not be GH-dependent, is necessary during intrauterine life for normal somatic and brain development [30].

SDS for length declines rapidly after birth in GHRD indicating the GH dependency of extrauterine growth (Fig. 2.3). Growth velocity with severe GHD or GHRD is approximately half

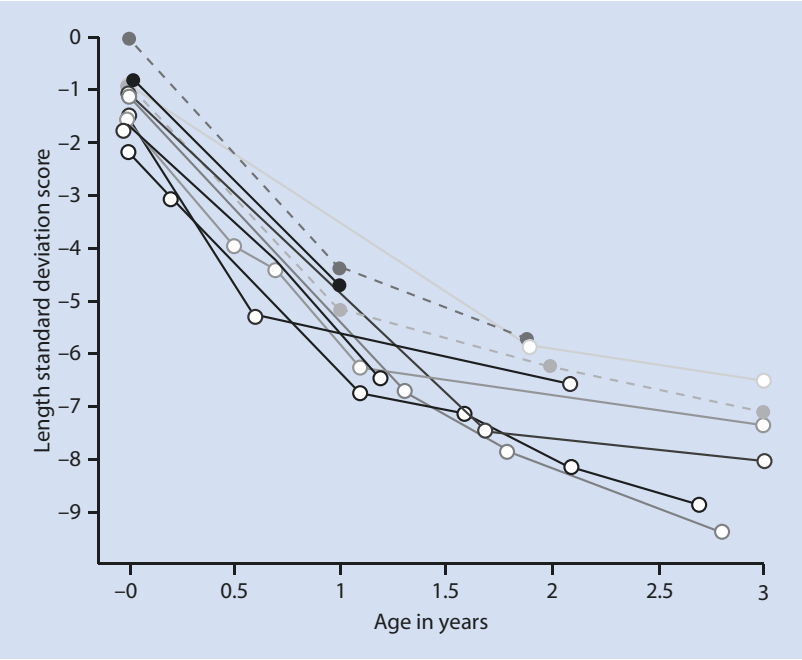

Fig. 2.3 Length standard deviation scores of nine girls from Ecuador (open circles, solid lines) and two brothers from southern Russia (solid circles, dashed lines) with known birth lengths, followed over the first 2–3 years of life (From Rosenbloom et al. [57]. Reprinted with permission from Elsevier)

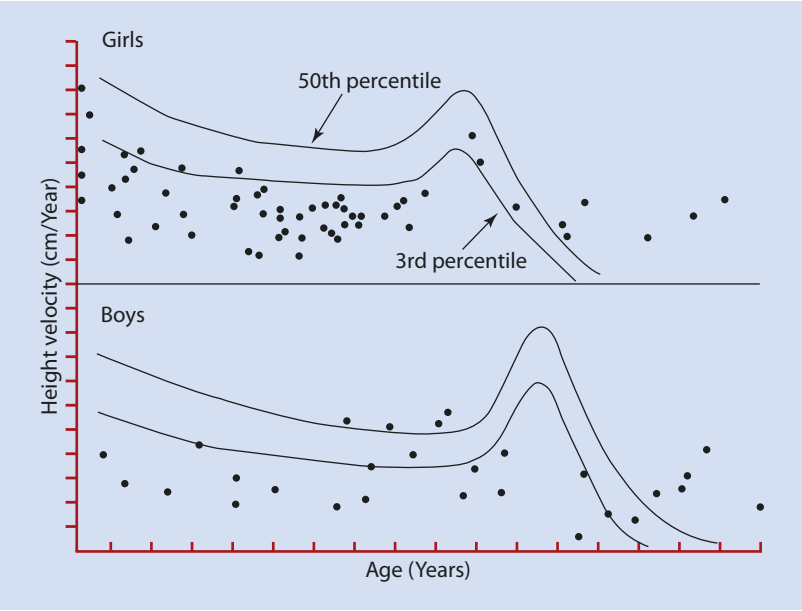

Fig. 2.4 Growth velocities of 30 Ecuadorian patients (10 males) with GH receptor deficiency; repeated measures were *at* least 6 months apart. Third and 50th percentiles are from Tanner and Davies [59] (From Rosenbloom et al. [57]. Reprinted with permission from Elsevier)

normal (Fig. 2.4). Occasional periods of normal growth velocity may be related to improved nutrition [60, 61].

Despite normal sexual maturation, the pubertal growth spurt is minimal or absent in GHRD, as documented in the most extensive available data from Israel and Ecuador [57, 62]. The adolescent growth spurt is GH dependent, reflected in significantly elevated circulating levels of GH and IGF-I compared to preadolescence and adulthood [63].

Adult stature in GHRD varies from −12 to −5.3 SDS in Ecuadorian subjects and −9 to −3.8 SDS in others in the literature, using the US standards (Fig. 2.5) [57]. This is a height range of 95–124 cm for women and 106–141 cm for men in the Ecuadorian population. This wide variation in the effect of GHRD on stature was not only

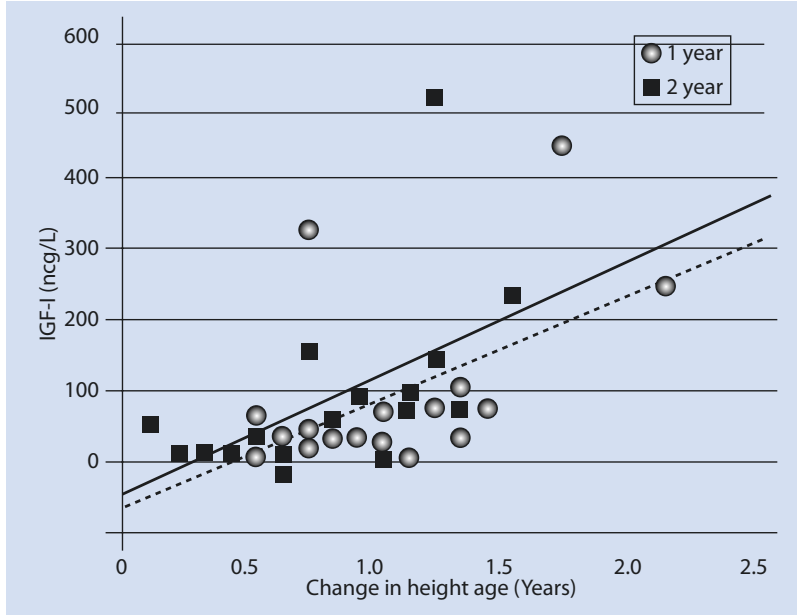

Fig. 2.5 Correlation of annual changes in height age and differences between baseline and trough levels of serum IGF-I with rhIGF-I treatment in 22 children with GHRD. The dashed line represents the 1-year correlation ($r = 0.54$, $p = 0.009$), and the solid line represents the 2-year correlation ($r = 0.58$, $p = 0.005$) (From Guevara-Aguirre et al. [64]. Reprinted with permission from Oxford University Press)

seen within the population but also within affected families; such intrafamilial variability has also been described with severe GHD due to GH gene deletion [16].

Some patients with GHRD may have an appetite problem in addition to their IGF-I deficiency, as highlighted by Crosnier et al. [61], in their observations of a child aged 3 1/2 years with GHRD who had severe anorexia. With his usual intake of approximately 500 kcal/day, he grew at a rate of 2 cm/year. With moderate hyperalimentation to approximately 1300 kcal/day, growth rate increased to 9 cm/year without significant change in plasma IGF-I level. The hyperalimentation period was associated with an increase in the IGFBP-3 bands on Western ligand blots, from total absence in the anorexic period to levels comparable to those seen in GHD. The catch-up growth noted could not be explained by hyperinsulinism, which has provided the explanation for accelerated or normal growth in children with GHD and obesity following removal of a craniopharyngioma. There was no appreciable increase in circulating basal or stimulated insulin during the hyperalimentation. In this patient, there was speculation that a nutrition-dependent autocrine/paracrine increase in IGF-I concentration at the cartilage growth plate might have occurred, independent of the GHR. The importance of adequate nutrition for catch-up growth was emphasized by this study, which also reinforced the notion that normal periods of growth in subjects with GHRD without IGF-I replacement therapy, as noted in Fig. 2.4, might be explained by periods of improved nutrition alone [60, 61, 65].

2.5.3 Craniofacial Characteristics

Children with GHR deficiency are recognized by knowledgeable family members at birth because of craniofacial characteristics of frontal prominence, depressed nasal bridge, and sparse hair, as well as small hands or feet, increased lines of facial expression, and hypoplastic fingernails. Decreased vertical dimension of the face is demonstrable by computer analysis of the relationships between facial landmarks and is present in all patients when compared with their relatives including those without obviously abnormal facies [66]. Blue scleras, the result of decreased thickness of the scleral connective tissue, permitting visualization of the underlying choroid, were originally described in the Ecuadorian population and subsequently recognized in other populations with GHRD, as well as in severe GHD [48, 49, 67].

Unilateral ptosis and facial asymmetry may reflect positional deformity due to decreased muscular activity in utero, although mothers do not recognize decreased fetal movement in pregnancies with affected infants [68].

As noted above, individuals with STAT5b mutations have growth impairment and facial dysmorphism indistinguishable from those with homozygous or compound heterozygous GHR gene abnormalities. IGF-I mutations result in micrognathia and microcephaly but no craniofacial abnormalities similar to those with GHR or STAT5b mutations.

2.5.4 Musculoskeletal and Body Composition

Hypomuscularity is apparent in radiographs of infants with GHRD and is thought to be responsible for delayed walking, despite normal intelligence and timing of speech onset [55, 68]. Radiographs of the children also suggest osteopenia; dual photon absorptiometry and dual energy x-ray absorptiometry in children and adults confirm this. A study of dynamic bone histomorphometry in adults with GHRD, however, demonstrated normal bone volume and formation rate, with the only abnormality being reduction in trabecular connectivity. This study suggested that some of the densitometry findings were artifactual, due to the small bone size [69].

Limited elbow extensibility seen in most patients over 5 years of age in the Ecuadorian population is an acquired characteristic, absent in younger children and increasing in severity with age [48, 49, 68]. That this feature is not peculiar to the Ecuadorian population or to IGF-I deficiency due to GHRD has been confirmed by finding a Brazilian patient having a different GHR mutation with limited elbow extension [70] and observing this finding in all but the youngest patient in a family with eight individuals affected by multiple pituitary deficiencies [67]. The cause of this elbow contracture is unknown, but poorly developed musculature of the fetus might contribute to it.

Although children appear overweight, they are actually underweight to normal-weight-for-height, while most adults, especially females, are overweight with markedly decreased lean to fat ratios [10, 55, 68].

2.5.5 Reproduction

GHRD is associated with small penis size with normal penile growth at adolescence or with testosterone treatment in childhood. Although puberty may be delayed 3–7 years in some 50% of individuals, there is normal adult sexual function with documented reproduction by males and females [50, 55]. Females require delivery by cesarean section.

2.5.6 Intellectual and Social Development

2.5.6.1 GHRD

Intellectual impairment was originally considered a feature typical of Laron syndrome based upon uncontrolled observations [71]. Among 18 affected children and adolescents administered the Wechsler Intelligence Scale for Children, only 3 had IQs within the average range [90 to 110]; of the remaining 15 subjects, 3 were in the low average range (80 to 89), 3 in the borderline range (70 to 79), and 9 in the intellectually disabled range (<70). These studies were done without family controls, so that the possibility of other factors related to consanguinity that might affect intellectual development and the appropriateness of the testing materials in this Middle Eastern population could not be addressed. In a follow-up study 25 years later, the investigators reexamined 8 of the original 18 patients and 4 new patients with GHRD, excluding 5 patients with mental disabilities who were in the original study [72]. This group had mean verbal and performance IQs of 86 and 92 on the Wechsler scale without evidence of visual motor integration difficulties that had been noted in the earlier group, but there was a suggestion of deficient short-term memory and attention. The investigators hypothesized that early and prolonged IGF deficiency might impair normal development of the central nervous system or that hypoglycemia common in younger patients may have had a deleterious effect.

Sporadic anecdotal reports of patients with GHRD suggested a normal range of intelligence. The collective data from the European IGF-I treatment study group, which includes a wider range of clinical abnormality than either the Ecuadorian or Israeli population, notes a mental retardation rate of 13.5% among 82 patients, but formal testing was not carried out [73]. Here again, the high rate of consanguinity was proposed as a possible explanation; hypoglycemia could not be correlated with these findings.

In the Ecuadorian population, exceptional school performance was reported among 51 affected individuals of school age or older who had attended school, with 44 typically in the top 3 places in their classes and most thought to be as bright or brighter than the smartest of their unaffected siblings [74]. The first controlled documentation of intellectual function in a population with GHRD was in the Ecuadorian children, a study of school-age individuals compared to their close relatives and to community controls. No significant differences in intellectual ability could be detected among these groups, using nonverbal tests with minimal cultural limitations. It was hypothesized that the outstanding school performance in this population might have been related to the lack of social opportunities due to extreme short stature, permitting greater devotion to studies and superior achievement in school for IQ level [75]. In the above context, it has been shown that IGF1, IGFBP-1, and IGFBP-3 circulate in higher concentrations in the cerebrospinal fluid of young infants than later in life and that these peptides might be important for myelination and synapse formation in the developing brain [76].

However, in an apparent paradox, GHRD children who have lower endocrine IGF1 and IGFBP-3 also display normal to above normal intelligence than their age-matched relatives, despite common episodes of grand mal-type seizures [74, 75]. These apparently contradictory events might partially be explained and reconciled by the finding that very young GHRD children also have very high IGFBP-1 levels [9] and improved insulin efficiency (as further indicated by their concomitant hypoglycemia), thereby indicating that insulin and IGFBP-1 action might be relevant determinants for the developing brain [10, 77].

2.5.6.2 STAT5b Gene Mutation

Consistent with other evidence that the IGF-I importance for intrauterine brain development is independent of the need for an intact GH activation pathway, individuals with STAT5b mutations do not have impaired brain development [24].

2.5.6.3 IGF-I Gene Mutation

There was marked intellectual impairment in the patients with severe IGF-I deficiency due to mutation of the IGF-I gene indicative of the dependence of intrauterine brain development, as well as intrauterine growth, on IGF-I. That this intrauterine IGF-I production is not dependent on GH is suggested by the intellectual normality with severe GHD and GHRD, consistent with gene disruption studies in mice. The IGF-I deleted mouse is neurologically impaired, while the GHRD mouse is behaviorally normal [78, 79]. Thus, GH-dependent IGF-I production is not necessary for normal brain development and function.

2.5.6.4 IGF-I Receptor Gene Mutation

The effect of heterozygous IGF-I receptor mutation on head growth and brain development is less impressive than with IGF-I gene defects. Small head size was recorded frequently, including in a proband with above-average IQ and her daughter with slight motor delay at 1 1/2 years of age. Except for one individual with a reported IQ of 60, intellectual development of the other probands ranged from normal to mild retardation [39–44].

2.5.6.5 IGFALS Gene Mutation

Normal neurological development with *IGFALS* mutation would be expected, consistent with normal brain development in children with severe GHD or GHRD who would not be stimulating intrauterine synthesis of ALS. This would imply that the GH-independent IGF-I production necessary for normal intrauterine somatic and brain growth is local (paracrine or autocrine) rather than endocrine.

2.6 Diagnostic Evaluation

2.6.1 Growth Hormone (GH)

Affected children with GHRD have random GH levels that are greater than 10 ng/ml, and may be as high as 200 ng/ml, with enhanced responsiveness to stimulation and paradoxical elevations following oral and intravenous glucose, as is seen in acromegaly [55]. The GH levels show normal diurnal fluctuation [13]. Twenty-four-hour profiles demonstrate marked GH variability among adult patients with suppression by exogenous recombinant human IGF-I [13]. Thus, the normal sensitivity of the GH secretion is preserved, despite lifelong elevated GH levels and lack of feedback suppression from IGF-I.

Postpubertal subjects with GHRD may have normal or elevated basal levels of GH but invariably demonstrate hyperresponsiveness to stimulation, which is even more impressive considering their obesity, which suppresses GH responses in normal individuals. This hyperresponse might also be due to the enhanced insulin sensitivity seen in these subjects that could theoretically induce deeper hypoglycemia or, alternatively, that the higher insulin efficiency sensitizes GH release via other hypothalamic mechanisms. ALS mutations are associated with normal GH levels, despite very low circulating IGF-I concentrations. IGF-I receptor deficiency, which is an IGF-I, rather than GH resistant state, can also be associated with normal GH levels.

2.6.2 Growth Hormone Binding Protein (GHBP)

The absence of GHBP in the circulation was initially considered a requirement for the diagnosis of GHRD, along with the clinical phenotype, very low concentrations of IGF-I and IGFBP-3, and elevated (in children) or normal to elevated (in adults) GH levels. Chromatographic analysis for serum GHBP, however, showed measurable though reduced levels in several subjects. The ligand-mediated immunofunction assay (LIFA) used to measure GHBP serum levels since 1990 uses an anti-GH monoclonal antibody to measure the amount of GH bound to GHBP. As a largely functional assay, this should not detect structurally abnormal though expressed GHBP.

As noted above, certain genetic defects in the GHR, those affecting dimerization or anchoring of the GHBP to the cell membrane, and dominant-negative mutations of the cytoplasmic domain can result in normal or markedly elevated GHBP levels. In the Ecuadorian population, despite in vitro evidence for failure of production of normally spliced receptor, 4 children and 4 adults out of 49 subjects had serum GHBP levels higher than 40% of the sex-specific lower limit for controls, and one adult male had a level in the lower portion of the normal adult male range.

There were no age-dependent changes, indicating that the difference in IGF values between children and adults was not related to the GHBP levels which did not correlate with stature or with serum IGF-I levels [68]. Although finding of extremely low or undetectable levels of GHBP serves as an important diagnostic feature, it is not a sine qua non, for the diagnosis of GHRD [80].

2.6.3 Insulin-Like Growth Factors (IGFs)

The lowest serum levels of IGF-I are seen in severe congenital defects in GH synthesis (GH gene deletion, GHRH-R deficiency), with deletion of the IGF-I gene, and with GHRD. IGF-II is not as severely suppressed, its reduction, likely related to diminution of IGFBP-3 rather than to decreased synthesis. In chronic disease states associated with acquired GHI, IGF-I levels are more likely to be reduced than are concentrations of IGF-II and IGFBP-3.

Among 50 Ecuadorian patients homozygous for the E180 splice site mutation, IGF-I levels were significantly greater in adults 16–67 years of age (n = 31, 25 ± 19 mcg/L) than in the 19 subjects under 16 years of age (3 ± 2 mcg/L, $p < 0.0001$), although still markedly below the normal range of 96–270 mcg/L. The children's levels were too low to correlate with stature but in the adults' IGF-I levels correlated inversely with statural SDS with a coefficient of 0.64 ($p < 0.001$). IGF-II levels in adults were also significantly greater than in children (151 ± 75 mcg/L versus 70 ± 42 mcg/L, normal 388 to 792 mcg/L, $p < 0.0001$). The correlation between serum IGF-I and IGF-II levels was highly significant, $r = 0.53$, $p < 0.001$. With no indication of age difference in GHBP levels, the increased levels of IGF-I and IGF-II with adulthood suggest effects on synthesis of these growth factors which are not mediated through the GHR and initially thought to be under the influence of sex steroids. This hypothesis was challenged by findings in patients with GHRH resistance due to mutation of the GHRH receptor. Sexually mature individuals with GHRH receptor mutation and affected children have comparably very low IGF-I (and IGFBP-3) serum concentrations [81]. The correlation of IGF-I levels with stature in adults with GHRD indicates that, despite the markedly low levels, the influence of IGF-I on stature remains important in these subjects.

2.6.4 IGF Binding Proteins (IGFBPs)

In IGF deficiency states that are the result of GHD or GHRD, IGFBP-3 is reduced, and, as noted above, in children and adults with GHRD, this reduction correlates with statural impairment [57]. In renal disease, elevated IGFBP-3, as well as IGFBP-1 and IGFBP-2, is thought to impair the delivery of normal levels of IGF-I [17].

Short-term and extended treatment of GHI with rhIGF-I has failed to result in increases in IGFBP-3 [13, 60, 64, 82–84], whereas treatment of GHD with rhGH restores levels to normal. This indicates that IGFBP-3 production is under the direct influence of GH.

IGFBPI is elevated in GHD and GHRD; in GHRD it is the most abundant IGFBP and is strongly inversely related to insulinemia. IGFBP-2 is present at a mean 300% of control concentrations in children with GHRD and 175% of control in affected adults, a significant difference. The IGFBP-3 levels in adults with GHRD are significantly greater than those in affected children [85]. In both children and adults, IGFBP-3 concentration correlated significantly with height SDS ($p = 0.06$).

2.7 Diagnostic Issues

GHI due to deficiency is readily diagnosed in its typical and complete form because of severe growth failure, the somatic phenotype of severe GHD, elevated serum GH levels, and marked reduction in IGF-I, IGF-II, and IGFBP-3 concentrations, with increased concentrations of IGFBP-1 and IGFBP-2. Most such individuals will also have absent to very low concentrations of GHBP, although the less common GHBP-positive forms make absence of GHBP an important but not essential criterion. As noted in Table 2.1, some of the biochemical features of GHRD may be shared by conditions associated with acquired GH insensitivity, such as malnutrition and liver disease.

The demonstration of a homozygous mutation or a compound heterozygous mutation affecting the GHR usually provides definitive diagnosis. Thirty-one of the 82 patients reported by Woods et al. [73] had a genetic study of the GHR, of whom 27 had abnormalities affecting both alleles of the GHR gene, in association with clinically and biochemically unequivocal GHRD. Identification of heterozygous mutations, however, is not necessarily helpful because, as noted earlier, polymorphisms have been described which appear to have no phenotypic consequences.

2.7.1 Partial GH Resistance

GH resistance might be expected to occur in an incomplete form, analogous to insulin resistance, androgen insensitivity, or thyroid hormone resistance. Affected children might have growth failure with normal or slightly increased GH secretion, variable but usually decreased GHBP levels, and decreased IGF-I concentrations, but not as severely reduced as in GHD or GHRD, and might respond to supraphysiologic doses of GH. It might also be expected, given the need for dimerization of the GHR for signal transduction, that certain mutations could have a dominant-negative effect in the heterozygous state.

Credibility for a heterozygous defect as a cause of short stature requires the demonstration of functional significance, not only by transfection of the mutant allele but by co-transfection with wild-type GHR gene; to approximate the circumstance in vivo, Goddard et al. [86] identified six mutations in eight children with short stature (SDS for height −5.1 to −2.0) and normal or increased stimulated GH levels. One patient had compound heterozygosity involving a novel mutation in exon 4 (E44K) and a mutation in exon 6 previously associated with GHRD in the homozygous state (R161C). Two other subjects were heterozygous for this mutation. The other five individuals included two who were heterozygous for the same novel mutation in exon 7 (R211H) and one each with novel mutations of exon 5 (C122X), exon 7 (E224D), and exon 10 (A478T). Expression in vitro of these four novel mutations involving the extracellular domain has shown functional effects, although co-transfection studies have not been reported. The defect involving exon 10 has not been expressed in vitro. Other defects without demonstrable significance have been described involving exon 10. None of these putative partial GHI patients had the clinical phenotype of GHD. Five of the eight subjects were treated with GH with variable improvement in growth velocity, from slight to dramatic, in the first year. This variable response is typical of that

seen with rhGH treatment of idiopathic short stature (ISS).

The subjects studied by Goddard et al. [86] were selected from the large Genentech National Cooperative Growth Study database in pursuit of the question raised by the observation that GHBP concentrations are low in children with ISS, i.e., short children without a recognizable syndrome or GHD. Using a ligand-mediated immunofunction assay, Carlsson et al. [87] studied a large number of short children with known causes of growth failure such as GHD and Turner syndrome, or ISS, and compared their GHBP concentrations in serum to those of normal controls. Ninety percent of the children with ISS had GHBP concentrations below the control mean, and nearly 20% had concentrations that were two standard deviations or more below the normal mean for age and sex. In a further analysis of the ISS group in this database, Attie et al. [88] identified over 500 patients who had been treated with rhGH and had normal GH stimulation tests, of whom, as noted above, 20% had low GHBP concentrations. While those with the low GHBP levels had significantly lower IGF-I concentrations and higher mean 12-h serum GH levels, the GH differences were numerically unimpressive (2.8 μg/L ± 1.1 versus 2.3 ± 1.1). Particularly relevant to the supposed GH resistance, there was no correlation of GHBP levels with the growth response to exogenous GH in these individuals. The search for defects in the GHR to explain ISS in the 100 subjects with low GHBP yielded 7 heterozygous mutations, but in studies of the families of these children, short stature did not segregate with the heterozygous state.

More recently, the Genentech database was analyzed for evidence of GH insensitivity among ~5000 patients entered between 1993 and 1996, with short stature (height standard deviation score <−2) being treated with GH. Over 40% were deemed IGF-I deficient, and half of these were to have the novel diagnosis of "primary IGF-I deficiency," i.e., normal GH responses with low IGF-I. Considered as a whole, the ISS group had a similar growth response to GH as did GH-deficient patients during the first year of treatment, with growth response correlating inversely with IGF-I baseline levels, exactly the opposite of the correlation that would be expected if they had GH insensitivity [89]. This evidence of GH sensitivity in the presence of low IGF-I concentrations is consistent with the observation that the growth response to rhGH in children with ISS who have low levels of IGF-I is greater than in those with more normal levels [90] and with the lack of correlation of growth response to GH in ISS relative to peak stimulated GH levels.

What cannot be appreciated from such a cross-sectional analysis of data from hundreds of pediatric endocrinologists is the clinical context in which the biochemical measures were obtained. Decreased circulating IGF-I with normal or elevated GH levels occurs with chronic illness and undernutrition. Many of the children seen with what is termed ISS are poor eaters with decreased body mass index and may be receiving treatment for hyperactivity which can suppress appetite and growth.

IGF-I levels decline substantially with fasting, which is considered a means of protecting against potential insulin-like effects on circulating glucose levels. Clinical investigations of children with ISS and varying responses to GH stimulation tests or IGF-I generation tests (in which GH is given for several days to stimulate IGF-I synthesis) have indicated that GH insensitivity is, at most, an uncommon finding [91].

Nonetheless, efforts and clinical investigations have been based on the hypotheses that much, if not most, ISS was due to IGF-I deficiency as the result of GH insensitivity and that exogenous IGF-I was appropriate growth-promoting therapy [91]. These hypotheses were not databased and disproven by the manufacturers' clinical trial data in which subjects had dubious IGF-I deficiency, normal GH sensitivity, and responses to rhIGF-I in relation to bone age advance which were no different than in control untreated subjects [92, 93].

The possibility of an effect of heterozygosity for a mutation which causes GHRD in the homozygous state was explored in the unique Ecuadorian cohort with a single mutation, permitting genotyping of numerous first-degree relatives. There was a minor difference in stature between carrier and homozygous normal relatives, and no difference in IGF-I or IGFBP-3 concentrations, indicating minimal, if any, influence of heterozygosity for the E180 splice mutation of the GHR [94]. A more general indication of the lack of influence of heterozygosity for GHR mutations involving the extracellular domain on growth comes from studies of the large multicenter European-based GHI study [73]. In both

the European and Ecuadorian populations, the stature of parents and of unaffected siblings does not correlate with statural deviation of affected individuals, while expected high correlation exists between parents and unaffected offspring. If the mutations that cause growth failure in the homozygous state also affected growth in heterozygotes, heterozygous parents and predominantly heterozygous siblings would have height SDS values which correlated with those of affected family members. In the Ecuadorian families, there was no difference in height correlations with parents between carriers and homozygous normal offspring.

2.8 Treatment

Soon after the cloning of the human IGF-I cDNA, human IGF-I was synthesized by recombinant DNA engineering (rhIGF-I), and physiologic studies were undertaken with intravenously administered rhIGF-I. Side effects of the intravenous administration terminated its further investigation which awaited the development of a subcutaneous form [95].

2.8.1 rhIGF Treatment of GHRD

During administration of rhIGF-I at a dose of 40 mcg/kg subcutaneous (sc) every 12 h over 7 days to six Ecuadorian adults with GHRD, hypoglycemia was avoided by having the subjects eat meals after the injections [13].

Elevated 24-h GH levels typical of the condition were rapidly suppressed, as was clonidine-stimulated GH release. Mean peak serum IGF-I levels were 253 ± 11 ng/ml reached between 2 and 6 h after injection, and mean trough levels were 137 ± 8 ng/ml before the next injection, values not different from those of normal control Ecuadorian adults. Although IGFBP-3 levels did not increase, elevated baseline IGFBP-2 levels (153% of control) increased 45% ($p < 0.01$). The short-term studies demonstrated that there was an insignificant risk of hypoglycemia despite low levels of IGFBP-3. There remained, however, concern whether the low IGFBP-3 levels would result in more rapid clearance of IGF-I, with blunting of the therapeutic effect.

The initial report of treatment for longer than 10 months was in two children with GHRD who had height velocities of 4.3 and 3.8 cm/year at 8.4 and 6.8 years of age. Their elevated serum GH levels were suppressed, and serum procollagen-I levels increased shortly after starting treatment; 6-month height velocities increased to 7.8 and 8.4 cm/year, but in the second 6 months of treatment, the velocities decreased to 6.6 and 6.3 cm/year and in the subsequent 5 months returned to pretreatment values. These patients were treated with a dose of 40 mcg/kg subcutaneously twice daily (bid), and the waning of their growth response after a year suggested that this dosage was not adequate for sustained effect [96].

The first IGF-I treatment report from the large Ecuadorian cohort was of growth and body composition changes in two adolescent patients treated with a combination of rhIGF-I (120 mcg/kg bid) and long-acting gonadotropin-releasing hormone analog to forestall puberty. A girl aged 18 and boy aged 17 years, with bone ages of 13 1/2 and 13 years, experienced an approximate tripling of growth velocity, increased bone mineral density, and maturation of facial features with rhIGF-I treatment for 1 year. There was initial hair loss followed by the recovery of denser and curly hair with filling of the frontotemporal baldness, appearance of axillary sweating, loss of deciduous teeth, and appearance of permanent dentition. They had coarsening of their facial features. Submaxillary gland enlargement was noted in one patient and fading of premature facial wrinkles in the other patient. Serum IGF-I levels increased into the normal range for age during the 2–8 h following IGF-I sc injection [97].

rhIGF-1 pharmacokinetic/pharmacodynamic profiles were done at doses of 40, 80, and 120 mcg/kg, and results suggested a plateau effect between 80 and 120 mcg/kg per dose. It was considered that the carrying capacity of the IGFBPs was saturated at this level. Mean serum IGF-II levels decreased concurrently with the increase in IGF-I, and serum IGFBP-3 levels did not respond to prolonged rhIGF-I treatment. There was no apparent change in the half-life of IGF-I during the treatment period, indicating no alteration of IGF-I pharmacokinetics induced by prolonged treatment.

Seventeen prepubertal Ecuadorian patients entered a randomized double-blind, placebo-controlled trial of rhIGF-I at 120 mcg/kg sc

Table 2.2 Treatment with rhIGF-I for 1–2 years of children with GH insensitivity

	Europe (64)	Ecuador (63)		Israel (78)	International (79)
Number	26[a]	7	15	9	56[b]
Age (years)	3.7–19.6	3.1–15.2	4.7–17.1	0.5–14.6	1.7–17.5
Dose (/kg)	40–120 μg bid	80 μg bid	120 μg bid	150–200 μg/d	60–125 μg bid
Ht velocity-cm/year (SD) Pre-Rx	N/A	3.0 (1.8)	3.4 (1.4)	4.7 (1.3)	2.8 (1.8)
Year 1	8.8 (1.9)[c]	9.1 (2.2)	8.8 (1.1)	8.2 (0.8)	8.0 (2.2)
Year 2	7 (1.4)[c]	5.6 (2.1)	6.4 (1.1)	6.0 (1.3)[d]	5.8 (1.5)[e]
Height SDS (SD) Pre-Rx	−6.5 (13)[c]	−8.0 (1.8)	−8.5 (1.3)	−5.6 (1.5)	−6.7 (1.8)
Year 1	−5.8 (1.5)[c]	−7.2 (1.8)	−7.5 (1.1)	−5.2 (1.7)	−5.9 (1.8)
Year 2	−5.4 (1.8)[c]	−6.7 (1.4)	−7.0 (1.2)	−5.8 (1.2)[d]	−5.6 (1.8)[e]

[a]Includes two patients with GH-neutralizing antibodies
[b]Includes eight patients with GH-neutralizing antibodies
[c]For 15 subjects treated for 4 years
[d]For six of the nine subjects
[e]48 subjects

bid for 6 months, following which all subjects received rhIGF-I. Such a study was considered necessary because of the observation of spontaneous periods of normal growth in these youngsters, the suggestion that nutritional changes that might accompany intervention would be an independent variable, and the need to control for side effects, particularly hypoglycemia, which occurs in the untreated state. The nine placebo-treated patients had a modest but not significant increase in height velocity from 2.8 + 0.3 to 4.4 + 0.7 cm/year, entirely attributable to three individuals with 6-month velocities of 6.6–8 cm/year [60]. Although this response was attributed to improved nutritional status, there was no accompanying increase in IGFBP-3 as noted with nutrition-induced catch-up growth in the French GHRD patient with anorexia [61].

For those receiving rhIGF-I, the height velocity increased from 2.9 + 0.6 to 8.8 + 0.6 cm/year, and all 16 patients had accelerated velocities during the second 6-month period when all were receiving rhIGF-I. No changes or differences in circulating IGFBP-3 concentrations were noted. There was no difference in the rate of hypoglycemia events, nausea or vomiting, headaches, or pain at the injection site between the placebo and rhIGF-I-treated groups. Initial hair loss occurred in 90% of subjects, similar to what is seen with treatment of hypothyroidism, reflecting more rapid turnover [60]. In the 2-year treatment study comparing 120 mcg/kg bid dosage to 80 mcg/kg bid treatment of GHRD in Ecuadorian subjects, no differences in growth velocity or changes in height SDS, height age, or bone age between the two dosage groups (Table 2.2) were observed. A group of six individuals receiving the higher dose followed for a third year continued to maintain second year growth velocities. The annual changes in height and age in both the first and the second year of treatment correlated with IGF-I trough levels which tended to be in the low normal range despite a failure of serum IGFBP-3 levels to increase (Fig. 2.6). The comparable growth responses to the two dosage levels and the similar IGF-I trough levels confirmed the plateau effect at or below 80 mcg/kg body weight twice daily observed in the first two patients [64, 97].

The Israeli report of 3 years' treatment of nine patients is the only one in which patients were given rhIGF-I as a single daily dosage (150–200 mcg/kg) [98], and the Ecuadorian patients had an improvement of 1 SDS over 1 year and 1.5 over 2 years at the higher dose and 0.8 SDS over 1 year

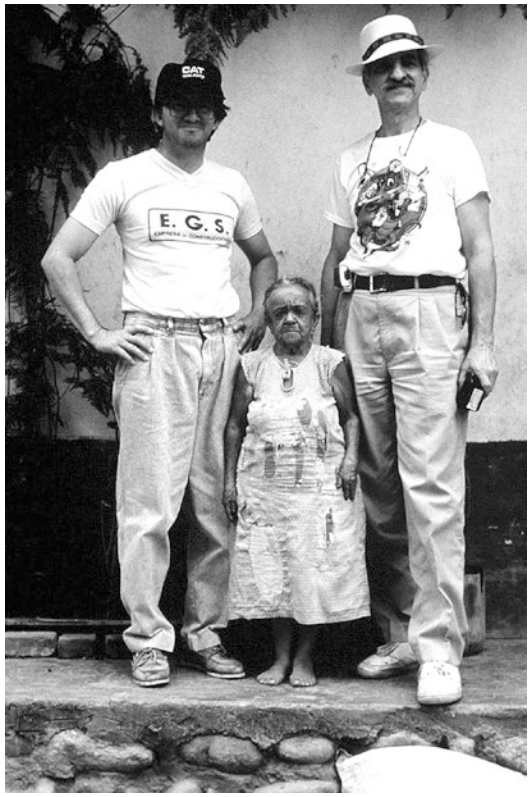

Fig. 2.6 The authors with the oldest subject of the Ecuadorian cohort with GH insensitivity. Height SDS from left to right: JGA −1.5 SDS; PRG −9.3 SDS (66 years old); ALR 0.3 SDS

and 1.3 SDS over 2 years at the dosage of 80 mcg/kg twice daily. The Israeli patients had an improvement in height SDS of only 0.4 over 1 year and for the six patients with 2-year data, 0.2 over 1 year and 0.4 over 2 years. The kinetic studies that originally formed the rationale for twice-daily administration were supported by these observations.

The collective experience of treating the rare conditions in which responsiveness to GH is severely impaired includes approximately 150 individuals, mostly with GHRD, and fewer than 10% with GH inactivating antibodies (Table 2.2). The growth velocity increment in the first year was 4.3 cm in the European and mecasermin (Genentech/Tercica/Ipsen) study populations [99] and 5.6 cm in the Ecuadorian population, all groups receiving comparable doses of rhIGF-I administered twice daily.

In the Israeli population given a single injection of a comparable total daily dose, the increment was only 3.6 cm/year. Height SDS improvement in the first year of treatment paralleled these increments at 0.7, 0.8, and 0.6 for the twice-daily rhIGF-I in the European, Ecuadorian, and international-mecasermin groups, respectively, and 0.2 for the Israeli population. The stimulatory effect on growth wanes rapidly after the first year, with only modest continued improvement. Among 76 patients treated for a mean 4.4 years, overall height SDS improvement was 1.4, almost all of which was achieved in the first 2 years of treatment [99].

Comparison of the growth response of 22 rhIGF-I treated GHRD patients and 11 GH-treated GHD patients in the same setting demonstrated mean growth velocity increment in those with GHRD to be 63% of that achieved with rhGH treatment of GHD in the first year and less than 50% in the second and third years (Table 2.2). The inadequate growth response compared to GH treatment of GHD persisted over this longer-term treatment period, with a mean improvement in height SDS of only 1.4, from −5.6 to −4.2, thus only sustaining the improvement of the first 2 years of treatment, as noted in Table 2.2. The importance of GH effects beyond hepatic IGF-I, IGFBP-3, and ALS synthesis is confirmed by this experience with attempted IGF-I replacement therapy in which only endocrine IGF-I can be replaced [12]. Near total deletion of the GHR in the liver only in the mouse model had no effect on total body or bone linear growth [100].

2.8.2 rhIGF-I Therapy of PAPP-A2 Deficiency

Short-term treatment with rhIGF-I has been effective in other human autosomal recessive conditions such as PAPP-A2 deficiency, a condition in which serum levels of IGF-I, IGFBP-3, and ALS are elevated but the proteolytic activity of PAPP-A2 is missing. rhGH administration was not considered since it would have induced a further elevation of the IGFBPs. rhIGF-I administration was initiated attempting to bypass the diminished bioactivity of the endogenous IGF-I [101].

2.8.3 Limitations of Endocrine rhIGF-I Replacement

The observation that growth failure due to GHRD cannot be adequately corrected with endocrine IGF-I replacement is not explained by

concomitant IGFBP-3 deficiency. Substantial tissue delivery is reflected in profound effects on adipose tissue, facies, and lymphoid tissue in treated patients (see below). This indicates that twice-daily injection provides more than adequate replacement of endocrine IGF-I, despite both IGFBP-3 and ALS deficiency which are not corrected by IGF-I treatment. The maintenance of circulating levels of IGF-I despite severe IGFBP-3 and ALS deficiency may be the result of binding to other IGFBPs; IGFBP-2 is elevated in GHRD and increases further with rhIGF-I therapy [13].

If the tissue dose of IGF-I in patients with GHI treated with IGF-I is supraphysiologic, as indicated by increases in body fat and acromegaloid facial changes, why then do we not see sustained growth acceleration as in GH-treated GHD? At this time, the dual effector hypothesis remains as the most appropriate explanation for the inadequate growth response [12]. With diminished ability to stimulate prechondrocyte differentiation and local IGF-I production, children with GHI can expect only partial recovery of normal growth with IGF-I replacement. Thus, rhIGF-I replacement therapy of GHI may need to continue longer than GH treatment of GHD to achieve more normal height. This goal will likely require suppressing adolescence in most children with GHI, using GnRH analogs [97].

In addition to statural attainment, goals of replacement therapy with rhIGF-I in GHI include improvement in body composition, normalization of facial appearance, and possible reduction of risk factors for childhood and adult mortality. All studies that have monitored body composition have verified lean mass increases, including increased bone density. Unlike GH replacement therapy, however, which restores normal lipolysis, IGF-I therapy is lipotropic, increasing or sustaining the high percentage of body fat. Normalization of craniofacial features has also been apparent [102]. Voice change has not been remarked on but can be expected.

The reduction of risk factors for the higher mortality in infancy and childhood with GHRD is to be expected with rhIGF-I therapy, but the reason for this increased risk is unknown. Leukocytes share in the general upregulation of IGF-I receptors in GHRD and appear to function normally in this condition [103]. In a study of one affected infant (who died at 7 months with bronchitis) and five adults with GHRD from Ecuador, Diamond et al. [104] demonstrated a variety of immune disturbances in the infant and three of the adults. The pathologic significance of these findings remains uncertain [105].

2.8.4 Purported Partial GHI

The definition adopted by the FDA for "severe primary IGFD" was height SDS <−3, basal IGF-I SDS <−3, and normal or elevated GH concentration. Growth velocity, osseous maturation, and projected height relative to mean parental stature, factors that are important in clinical evaluation of short children, were not considered. Younger children may have quite low values for IGF-I that are not diagnostically useful, and at any age a single measurement may vary considerably from a subsequent determination. There is also inconsistency between laboratories, and normal ranges vary widely. In one analysis, three of four laboratories failed to identify 15–20% of Ecuadorian patients with molecularly proven GHRD using the FDA criterion for IGF-I concentration <−3 SD. Values may be spuriously low because of the high susceptibility of IGF-I to post sampling proteolysis [91]. Children, especially boys, with constitutional delay in growth and maturation (CDGM), who do not have biochemical markers of undernutrition, may be hypermetabolic and have mean IGF-I concentrations that are only 40% of those of normal age-mates, which could lead to an inappropriate diagnosis of IGFD [106]. This interpretation may be artifactual because of comparison to norms for chronologic age rather than biologic (bone) age.

Further insight into the wide variability in IGF-I concentrations in the absence of endocrine deficiency comes from a study comparing African and Italian children of comparable height but with the African children having significantly lower weight and BMI and mean IGF-I and IGFBP-3 levels <1/3 those of the Italian children [107]. Wide fluctuations in IGF-I concentrations can be seen in normal prepubertal children, including levels <2 SDS [108].

Finally, IGF-I generation tests have poor reproducibility [51]. The off-label promotion of rhIGF-I has been based on two considerations which are not evidence-based, that many children with ISS have partial GH insensitivity and that

appropriate therapy for these individuals is recombinant human IGF-I. The absence of convincing evidence for this hypothesis, the limited ability of endocrine IGF-I to restore normal growth in those with unequivocal GH unresponsiveness, the suppression of local GH effects on growth with IGF-I administration, the risk profile, and the absence of data on efficacy in other than proven severe GH insensitivity led the Drug and Therapeutics Committee of the Pediatric Endocrine Society to conclude that rhIGF-I use is only justified in conditions approved by the US Food and Drug Administration (FDA) and that use for growth promotion in other children should only be investigational [54]. A manufacturer of rhIGF-I "estimates that approximately 30,000 children in the US are affected by primary IGFD, which is also similar to the estimated [European Union] EU market size" [91]. Considering that fewer than 200 children with GHI due to GHRD, transduction defects, or GH-inhibiting antibodies had been identified worldwide in the previous 20 years, it is apparent that there were exuberant anticipation and extensive promotion of off-label use. Indeed, current clinical trials demonstrate the loosening of criteria from those in the approval by FDA [► www.clinicaltrials.gov].

In January 2008, a pharmaceutical manufacturer of rhIGF-I announced that they had begun dosing the first patient in a phase II clinical trial evaluating the combination of GH and IGF-I in a study scheduled for completion at the end of 2011 to involve 100 subjects over 5 years of age. This is a four-arm study involving rhGH alone in a dose of 45 mcg/kg daily and the same dose of GH with once-daily injections of either 50, 100, or 150 mcg/kg IGF-I. Inclusion requires height SDS ≤ -2 (which is less stringent than the criterion of SDS ≤ -2.25 for GH treatment of ISS) and IGF-I SDS ≤ 1, along with normal response to GH stimulation testing and bone age $\leq$ 11 years for boys and ≤ 9 years for girls. There are no growth velocity criteria. This study is likely to include a substantial number of children, especially males, with constitutional delay of growth and maturation (CDGM), the most common explanation for short stature in the growth clinic, and other normal short children. It is noteworthy that there is not an IGF-I monotherapy arm and that the IGF-I is given as a single daily injection in contrast to the pharmacokinetic studies and clinical experience with this drug.

There is no reason to expect better growth response with IGF-I in patients who do not have proven insensitivity to GH than with recombinant GH, based on the absence of evidence of GH resistance as a cause of their short stature. In fact, monotherapy with rhIGF-I in individuals who have normal or even somewhat reduced GH production and action should result in suppression of endogenous GH, which occurs rapidly with rhIGF-I administration in both normal and GHRD subjects [13]. This GH suppression will reduce IGFBP-3 and ALS production, and most importantly, decrease GH delivery to growing bone, with reduction of chondrocyte proliferation and autocrine/paracrine IGF-I production, potentially decreasing growth velocity.

Data presented by the manufacturer of mecasermin in 2009 provided evidence that countered the notion that "primary IGFD" due to partial GHI was a valid diagnosis. In their clinical trial of individuals carrying this supposed diagnosis, supraphysiologic doses of IGF-I were required, resulting in circulating IGF-I levels of +2 SDS, to obtain a growth effect; thus, a pharmacologic rather than physiologic replacement therapy was required, which would be inconsistent with replacement therapy for primary IGFD. Also, inconsistent with the diagnosis of primary IGFD were the normal baseline growth velocities in these subjects. When these children underwent IGF-I generation tests (administration of GH for 5 days), there was a 76.5% increase in mean IGF-I level and 34% increase in mean IGFBP-3 concentration [58]. Both are approximately 50% greater responses than in normals and indicative of better than normal GH sensitivity. After a year, controls were growing at the same rate as IGF-I-treated subjects. Furthermore, half of them now had IGF-I levels that would have disqualified them from the study.

2.8.5 Safety Concerns with rhIGF-I Treatment

Episodes of hypoglycemia, which may be severe, are common in infants and children with GHRD. In contrast to the far less common

hypoglycemia of GHD which is corrected by GH replacement therapy, IGF-I treatment increases the risk of hypoglycemia in children with GHRD. Hypoglycemia has been the most common early adverse event, reported in 49% of subjects in the largest series, including 5% with seizures [99]. In the 6-month, placebo-controlled Ecuadorian study, hypoglycemia was reported in 67% of individuals receiving placebo and 86% of those treated with rhIGF-I, an insignificant difference [60]. Finger stick blood glucose measurements in 23 subjects residing at a research unit indicated frequent hypoglycemia before breakfast and lunch, which did not increase in frequency with rhIGF-I administration [99]. Five of the subjects participated in a crossover, placebo-controlled study for 6 months with a 3-month washout period with fasting glucose determinations performed three times daily by caregivers for the entire 15-month study. The percentage of glucose values <50 mg/dL was 2.6% with placebo and 5.5% with rhIGF-I, not a significant difference. In practice, hypoglycemia associated with IGF-I treatment appears reasonably controllable by adequate food intake.

Pain at the injection site is common. Injection site lipohypertrophy is frequent, affecting at least one-third of subjects; this is the result of failure to rotate injections and injection into the lumps, which can attenuate growth response. The inotropic effect of IGF-I results in asymptomatic tachycardia in all treated patients, which clears after several months of continued use [109]. Benign intracranial hypertension or papilledema has been noted in approximately 5% of IGF-treated subjects. While headache is frequent, the placebo-controlled study found no difference in incidence between those receiving placebo injections and those receiving rhIGF-I. Parotid swelling and facial nerve palsy have been described. Lymphoid tissue hypertrophy occurs in over 25% of patients, with hypoacusis, snoring, and tonsillar/adenoidal hypertrophy that required surgical intervention in over 10% of patients. Thymic hypertrophy was noted in 35% of subjects having regular chest radiographs. It is worth noting that some of these side effects may be more frequent than reported because they take time to develop; for example, snoring incidence in the first year for the 25 longest treated subjects in the mecasermin study was only 4% but increased to 65% for the entire study period [99].

Anti-IGF-I antibodies have developed in approximately half of the rhIGF-I-treated patients during the first year of treatment, but these have had no effect on response [98, 99]. Urticaria has been noted in subjects participating in the trial of IGF-I in combination with GH. Transient elevation of liver enzymes has also been noted [98]. Coarsening of facial features with disproportionate growth of the jaw reminiscent of acromegaly has been common, particularly among those of pubertal age [97]. In contrast to the increase in lean body mass and decreasing percentage of body fat that occurs with GH treatment of GHD, both lean and fat mass increase with rhIGF-I therapy [69, 95].

Mean body mass index (BMI) increased from +0.6 SDS to +1.8 SDS during 4–7 years of treatment with rhIGF-I in the European multicenter trial, and severe obesity has occasionally occurred [65]. BMI measurement may not accurately reflect the degree of obesity, which can be a doubling or tripling of body fat as demonstrated by dual energy x-ray absorptiometry [77].

Hyperandrogenism with oligomenorrhea or amenorrhea, acne, and elevated serum androgens has been described in prepubertal and young adult patients given single daily injections of rhIGF-I [110]. There have been two instances of anaphylaxis from rhIGF-I treatment [93].

It is not known whether there might be long-term mitogenic effects of extended therapy with rhIGF-I in growing children. The role of IGF-I in carcinogenesis, as an antiapoptotic agent favoring the survival of precancerous cells, together with the increased cancer risk in hypersomatotropic states, and the evidence for aberrant tissue effects in rhIGF-I-treated patients dictate a need for long-term follow-up of rhIGF-I-treated patients [111]. The recommended IGF-I dosage (120 mcg per kg twice daily) is efficacious and induces salutary effects on height; however, it may be too high, as indicated by excessive fat accumulation, accelerated bone maturation, and acromegaloid facial changes (Fig. 2.7). Concordant with this assertion, if a lower dose is given (80 mcg per kg twice daily), improved adult height and leaner body composition were observed (Fig. 2.8) [112].

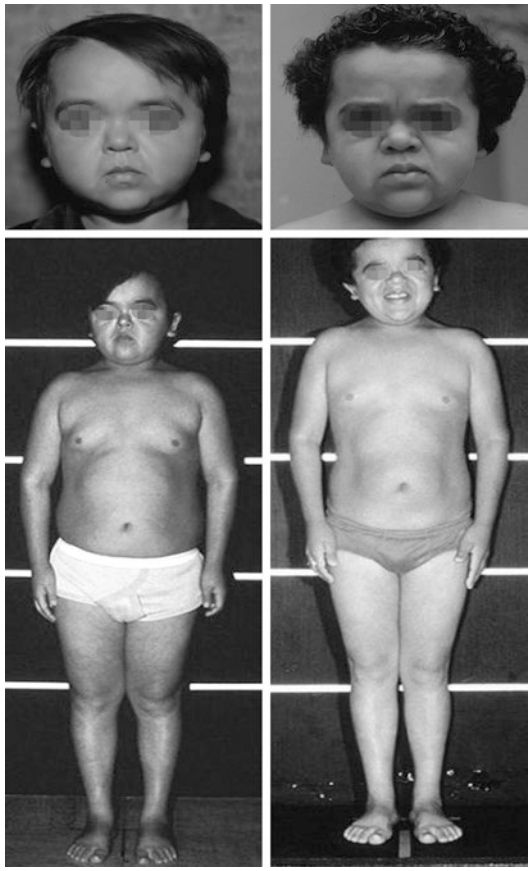

Fig. 2.7 Adolescent subject with GHRD due to the E180 splice mutation on the GH receptor gene. Left panel: 3 years before treatment. Right panel: the subject after 1-year treatment with 120 μg/kg bid of rhIGF-I and leuprolide acetate 7. 5 mg sc monthly

Fig. 2.8 Four subjects with GH receptor deficiency due to the E180 splice mutation on the GH receptor gene. From left to right, the first woman, age 22, was treated from age 4 to age 14 with rhIGF-I at a dose of 80 μg /body weight bid. The other three women were treated for 3 years with 120 μg /kg bid and are 30, 23, and 27 years of age. Their heights are comparable to those treated by Chernausek et al. [99] for upward of 10 years [112, 121.2, and 120.8 cm] (From Guevara-Aguirre et al. [112]. Reprinted with permission from the Endocrine Society)

2.8.6 rhGH Therapy of GHI

rhGH therapy has been used with limited success in GHI; indeed, a moderate response to rhGH administration in children with GHI due to IGF-I receptor mutations (chromosome 15q26 deletion) has been reported; however, the catch-up growth seen with GH treatment of GHD was not observed, and despite improvement in height SDS during therapy, adult height remained reduced [113].

Moreover, a less robust growth response to rhGH administration has been observed in certain subjects with Noonan syndrome, a condition in which mild GHI has been suggested. The decreased response to therapy occurs in those bearing PTNP11 mutations; however, in those without these genetic changes, an improvement in clinical and biochemical growth parameters, including serum IGF-I levels, was seen when rhGH was administered. This might be explained by the hyperactivity of the RAS/MAPK system that has been described in this syndrome [114].

2.9 Conclusions

Genetic causes of GHI from the GHR to IGF-I action remain rare, but their identification has greatly enhanced understanding of growth processes and introduced challenging questions, for example, about phenotypic variability between genetic defects at various sites with comparable biochemical effects and among individuals with the same or similar mutations of a particular gene. Acquired GHI is relatively common as a complication of a variety of chronic problems associated with growth failure. Treatment of the genetic causes of GHI remains inadequate because of the inability of exogenous rhIGF-I to replicate GH effects at the growth plate; however, the efficacy and effect of the rhIGF-1 dose schedule on final height need to be further explored [112].

Review Questions

1. Mutations of which of the following genes are associated with GHI in fewer than 50 children?
   A. GH receptor
   B. IGF-I receptor
   C. STAT5b
   D. A and B
   E. B and C
2. rhIGF-I has limited effects on growth because it does not replace the direct effect of GH on?
   A. Chondrocyte proliferation
   B. Autocrine/paracrine IGF-I generation at the growth plate
   C. ALS and IGFBP-3 production by the liver
   D. Because it suppresses endogenous GH production
   E. All of the above
   F. None of the above
3. Partial GHI has been established in a large number of short children by?
   A. Measurement of serum GH concentrations
   B. Measurement of serum IGF-1 concentrations
   C. Growth response to high-dose rhGH administration
   D. Response to rhIGF-1 administration
   E. All of the above
   F. None of the above

Answers

1. E
2. E
3. F

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Normal Variant and Idiopathic Short Stature

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Key Points

- Familial short stature and constitutional delay of growth and puberty are normal physiologic variants of growth.
- Familial short stature, constitutional delay of growth and puberty, and idiopathic short stature do not have an organic etiology. Idiopathic short stature is distinguished from normal variant short stature in that predicted adult height is below the genetic target height range.
- In 2003, the FDA approved growth hormone for the treatment of idiopathic short stature which generated controversy within the medical community.
- Height outcome data for growth hormone treatment of children with idiopathic short stature is variable with a mean gain in height of 4–7 cm.

3.1 Introduction

Short stature is defined as a height ≤−2 standard deviations (SD) below the mean for age, sex, and population. Height in a given population follows a normal Gaussian distribution; therefore, it is expected that the height of 2.3% of a population will fall 2 SD below the mean for age and sex [1]. Among this 2.3% of the population, the majority of these individuals will have a normal variant of short stature or idiopathic short stature, while some may have pathological causes of short stature.

Normal variant short stature is comprised of familial short stature (FSS) and constitutional delay of growth and puberty (CDGP).These individuals attain a final adult height consistent with their target genetic height, while individuals with idiopathic short stature have predicted adult heights below their target height range. Frequently, FSS and CDGP are considered as subtypes of ISS. Since the growth patterns of individuals with FSS and/or CDGP are usually consistent with that of a first degree relative and their final adult height conforms with their target height, these diagnoses most suitably represent "normal variants" of growth [2].

Normal variant and idiopathic short stature constitute forms of nonpathologic short stature, meaning that the short stature is not caused by intrauterine growth restriction, chronic disease, an endocrine disorder, skeletal dysplasia, or genetic disorder.

3.2 Auxologic Methods to Assess Growth

3.2.1 Length and Height Measurement

Accurate length and height measurements are critical for assessing a child's growth pattern. Until a child can stand, an infant or adult horizontal measuring board is used to measure length. The infant or child is placed supine on the board with one person holding the head in the Frankfort plane, flush with the headboard (Fig. 3.1). The Frankfort plane is defined as the linear plane created by the intersection of the outer epicanthus of the eye with the upper 1/3 of the ear. The second person should maintain the infant's knees fully extended and feet upright and parallel to the footboard. Height is measured with a wall-mounted stadiometer, and the child is positioned with their head, shoulders, back, buttocks, and heels flush against the back of the stadiometer. The child's knees are extended with their feet together and head positioned in the Frankfort plane (Fig. 3.1) [3]. To improve the accuracy of length and height measurements, a minimum of three measurements should be obtained and the mean of the three measurements used. Weight, length, weight for length, and head circumference should be plotted on the Centers for Disease Control and Prevention (CDC) growth charts from birth through age 3. When the child is able to stand for a height measurement, weight, height, and body mass index should be plotted on the CDC growth charts for children age 2–20 [4].

Within the first 2 years of life, it is common for an infant's weight and length to cross growth percentiles, gravitating toward percentiles consistent with their genetic potential. Thereafter, crossing length or height percentiles should not occur, with the exception of children with CDGP, who may continue to exhibit a decline in length or height percentile until ages 3–4. Thereafter, they maintain a normal linear growth velocity and will grow at a consistent percentile. Height measurements are 1 cm less than length measurements which frequently accounts for the apparent

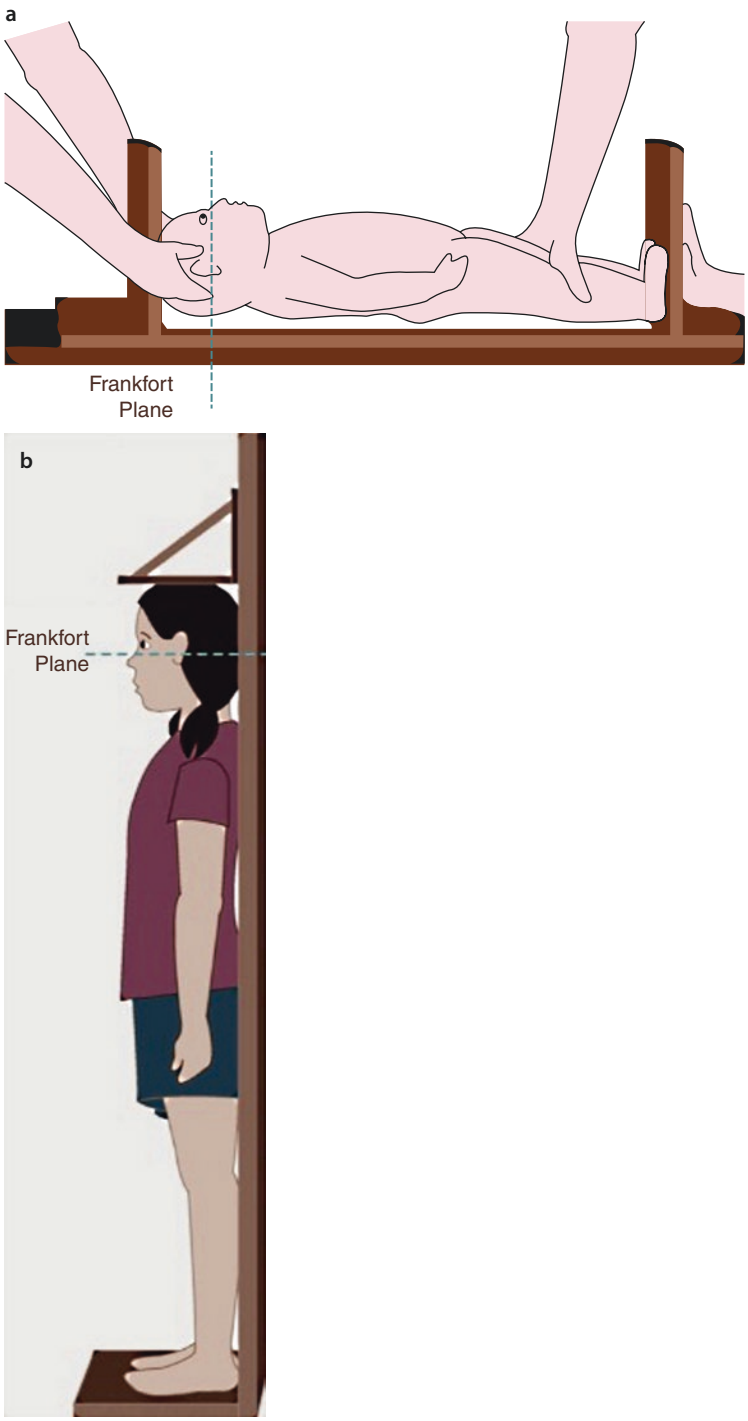

Fig. 3.1 Length and height measurements with an infant board and wall-mounted stadiometer. Note the position of both the infant's **a** and child's **b** heads in the Frankfort plane (From Foote et al. [3]. Reprinted with permission from Elsevier)

decline in growth rate when a child is transitioned from a length measurement to a standing height.

Assessing a child's pattern of weight gain, linear growth, and gain in head circumference from their growth chart is an important part of an evaluation for short stature. Pathological causes of poor growth secondary to nutritional causes or chronic disease initially affect weight gain and then linear growth followed by head circumference. In contrast, children with endocrine disorders typically present with normal or increased weight gain and poor linear growth. Sometimes,

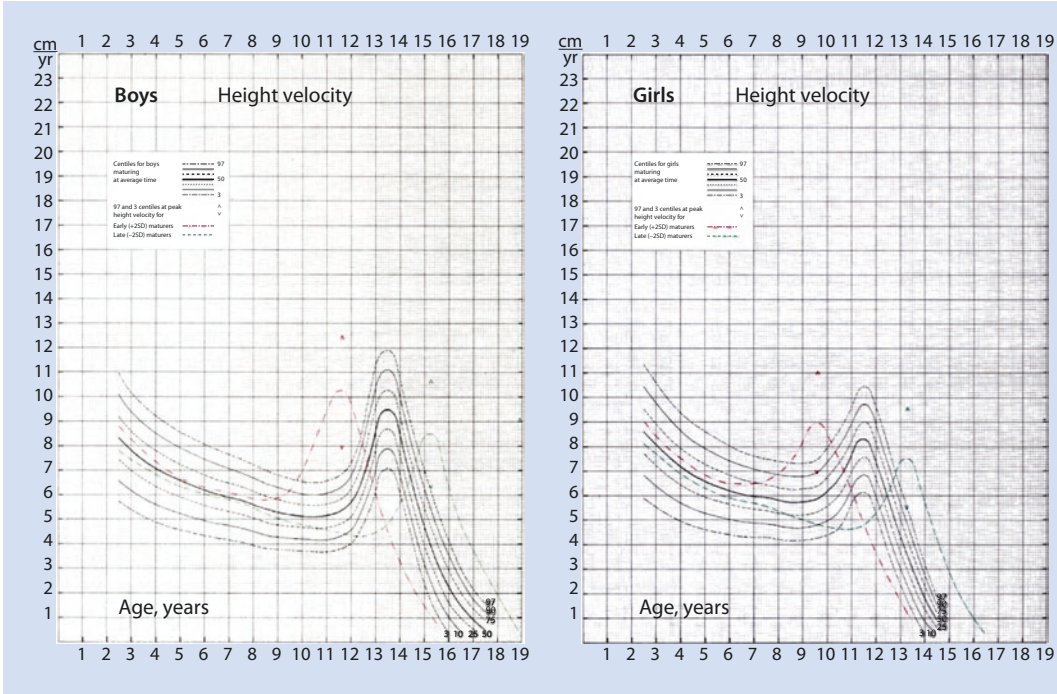

Fig. 3.2 Height velocity charts for boys and girls (From Tanner and Davies [5]. Reprinted with permission from Elsevier)

disorders of malabsorption, such as celiac disease, may initially present with normal weight gain, but poor growth.

3.2.2 Growth Velocity

Determination of growth velocity, expressed as growth rate per year, is essential in the evaluation of a child's growth. In the first year of life, infants grow at a rate of 23–27 cm/year, and by the second year of life, their growth rate declines to 10–14 cm/year. Between 2 and 4 years of age, the growth rate further declines to 7 cm/year and by age 5 to 5 cm/year until the onset of puberty. The pubertal growth spurt in girls peaks at Tanner stage 3 with a growth rate of 6–10 cm/year, while in boys the growth rate peaks at Tanner stage 4 with a growth rate of 7–12 cm/year. Figure 3.2 illustrates the growth velocity charts devised by Tanner et al. for North American boys and girls to assess growth rates throughout childhood and adolescence. These growth velocity charts also contain separate growth rate curves for early and late maturers who achieve their peak growth velocity 2 SD earlier or later than the general population [5].

3.2.3 Anthropometric Measurements

The evaluation of a child with a height 2 SD below the mean for age and sex should include anthropometric measurements of lower segment or sitting height, arm span, and occipitofrontal head circumference to assess for a skeletal dysplasia. Arm span is measured with the child standing in an erect position against a wall with outstretched arms. The distance between the tips of the middle phalanges is measured. In prepubertal children, the arm span is shorter than height, and after midpuberty the arm span exceeds height. In an adult male, arm span exceeds height by 5.2 cm and in an adult female by 1.2 cm. Ethnic differences exist as well, with longer arm spans noted in the African American population. A sitting height (SH) is measured with the child seated on a chair flush against the stadiometer. The sitting height provides the height of the trunk and when subtracted from the child's standing height (HT) provides the value of their lower segment (LS). The SH/HT ratio or SH/LS ratio can be plotted on their appropriate charts to determine whether these body proportions are

suggestive of a skeletal dysplasia. An alternative approach is to measure the child's lower segment from the symphysis pubis to the floor and subtract this value from the child's standing height for their truncal height. The upper to lower segment ratio decreases throughout childhood and adolescence. Due to an increased truncal length relative to limbs, infants have an upper to lower segment ratio of 1.7. By age 3 the upper to lower segment ratio decreases to 1.3, by age 10 this ratio decreases to 1, and in pubertal children the ratio is <1. Children with CDGP have increased leg length and shortened truncal height which accounts for their decreased upper to lower segment ratios of 0.8–0.9.

3.2.4 Bone Age

Skeletal maturation is evaluated by assessing epiphyseal maturation on bone age films, which can be determined by the methods of Greulich and Pyle or Tanner-Whitehouse. The former method compares a child's left hand radiograph to standard bone age radiographs of the left hands of boys and girls from birth through age 18 [6]. The Tanner-Whitehouse method assigns a value to each of the 27 epiphyses in the hand and wrist and sums these values for the skeletal maturity score, which correlates with specific bone ages as determined for select populations [7]. Bayley-Pinneau also provides a table for the percent of adult height achieved at given bone ages which can be used to calculate a child's predicted adult height [8]. Variations in bone age readings among providers and inaccurate height measurements can limit the accuracy of a child's height prediction.

3.2.5 Dental Age

Loss of the primary teeth and eruption of permanent teeth correlate with specific ages and bone ages but vary due to environmental influences. Tooth calcification as determined on an orthopantomogram is less variable and corresponds better with dental age [9, 10]. GHD, hypothyroidism, and CDGP are associated with delayed dental age and bone age.

3.2.6 Target Height

In the 1970s, Tanner et al. defined target height as either the addition or subtraction of 6.5 cm from the mean parental height for a boy or girl, respectively, with 2 SD representing 10 cm above or below the calculated target height [11]. The target height is used to determine a child's expected adult height based on their genetic potential. Measuring the parents' heights at the child's visit improves the accuracy of the target height. Starting in the 1990s researchers devised alternative formulas to calculate the target height because the method of Tanner was determined to either underestimate or overestimate the height of a child with either short or tall parents, respectively. These formulas also tried to account for population mating trends and secular height trends. The 2008 consensus statement from the Growth Hormone Research Society, the Lawson Wilkins Pediatric Endocrine Society and the European Society for Paediatric Endocrinology Workshop recommended using a corrected target height SD in lieu of the Tanner method. The corrected target height is calculated as 0.72 X average of father's and mother's height SD with the lower limit of the target height as the corrected target height minus 1.6 SD [12].

The evaluation of a child with short stature requires a review of their previous growth data, accurate length and/or height measurements, a calculated target height, bone age, predicted adult height and possibly arm span, and upper to lower segment ratio. Analyzing auxologic data in conjunction with a detailed history and physical examination is essential for determining whether a child's short stature is a *normal variant* or due to an underlying disorder.

3.3 Etiology and Clinical Presentation

3.3.1 Normal Variant Short Stature

3.3.1.1 Constitutional Delay of Growth and Puberty

In CDGP, within the first 2–3 years of life growth rate declines, crossing percentiles on the growth chart. Usually, by age 3–4 growth proceeds at a normal growth rate. Other features include a

delayed bone age (often a 2–3 SD delay), normal growth velocity, delayed pubertal onset, and eventual attainment of an adult height consistent with genetic heights. Most children with CDGP exhibit a pattern of weight gain that mirrors their linear growth at less than 2 SD below the mean for age, sex, and population. Individuals with CDGP are frequently referred to as "late bloomers" and often have either a first- or second-degree relative(s) with a history of delayed onset of puberty (defined as the onset of puberty for males as >13 years of age and for females as >12 years of age) and a similar growth pattern.

Prior to the onset of puberty there is a normal decline in growth velocity as seen on the growth velocity charts devised by Tanner et al. [5] (◘ Fig. 3.2). Individuals with CDGP commonly have a more pronounced or longer decline in their growth rate, which is usually more pronounced in boys than girls, causing their height to deviate further away from their prepubertal height percentile. The growth velocity of children with CDGP should be plotted on the growth velocity curves for late maturers and interpreted with respect to a child's bone age as opposed to chronologic age.

Forty percent of children with constitutional delay of growth and puberty also have familial short stature [13]. Even with a strong family history of CDGP, as with FSS, other identifiable causes of short stature and delayed puberty should be excluded which is discussed in the ▶ Sect. 3.4.

Several studies report a 2:1–5:1 male to female predominance of CDGP [14, 15]. Wehkalampi et al.'s retrospective study evaluated the prevalence of a positive family history in children with CDGP. The findings in this study challenge this male predominance because a nearly equal number of mothers as fathers had CDGP. The authors suggest that referral bias may account for the reported male prevalence in CDGP. The data from this study and others support an autosomal dominant pattern of inheritance for CDGP [14–16]. Heterozygous mutations in the growth hormone secretagogue receptor gene (GSHR), a ghrelin receptor gene, were identified in two females with CDGP, suggesting an autosomal dominant mode of inheritance with incomplete penetrance. Further studies are needed to better define the association of mutations in this gene with CDGP [17]. Researchers have not found mutations in the acid labile subunit, leptin, or the leptin receptor in individuals with CDGP [18, 19].

3.3.2 Familial Short Stature

Children with FSS have normal linear growth at ≤−2 SD below the mean for age, sex, and population with a predicted adult height consistent with their target height. They also have a bone age consistent with their chronologic age, a normal growth velocity, and onset of puberty, and either one or both of their parents have short stature.

Extreme cases of FSS in which a parent's height is near or below 3 SD below the mean for age and sex should elicit an evaluation for a genetic disorder other than normal variant short stature. To establish a diagnosis of FSS, one should discern that the patient and/or parent's short stature is not due to familial isolated growth hormone deficiency, a skeletal dysplasia, or some other identifiable etiology (refer to the ▶ Sect. 3.4). Factors that may have affected a parent's height should be considered, such as a history of precocious puberty, acquired hypothyroidism, chronic disease, or malnutrition. ◘ Figure 3.3 represents a normal growth chart of a girl with FSS.

3.3.3 Idiopathic Short Stature

As with normal variant short stature, ISS is defined as a height ≤−2 SD below the mean for age, sex, and population. ISS is discerned from normal variant short stature by a predicted adult height more than 2 SD below the child's target height range. The term "idiopathic" implies that the etiology of the short stature is unknown and should be established in the absence of FSS, CDGP, or other identifiable cause as discussed in detail in the ▶ Sect. 3.4).

Molecular defects in the growth hormone receptor gene, STAT 5, acid labile subunit, and IGF-1 gene have been described in patients with a prior diagnosis of ISS [20]. Whole exome sequencing led to the identification of genetic causes of short stature in 5 of 14 children with prior diagnoses of ISS with a height at or ≤−3 SD below mean for age and sex. Prior to the whole exome testing, these children had extensive testing, including standard genetic testing, that did not identify a cause for their short stature. Two of the five patients had the progeroid form of Ehlers-Danlos syndrome, and the other diagnoses included 3-M syndrome (overlapping features with Russell-Silver syndrome), Floating-Harbor

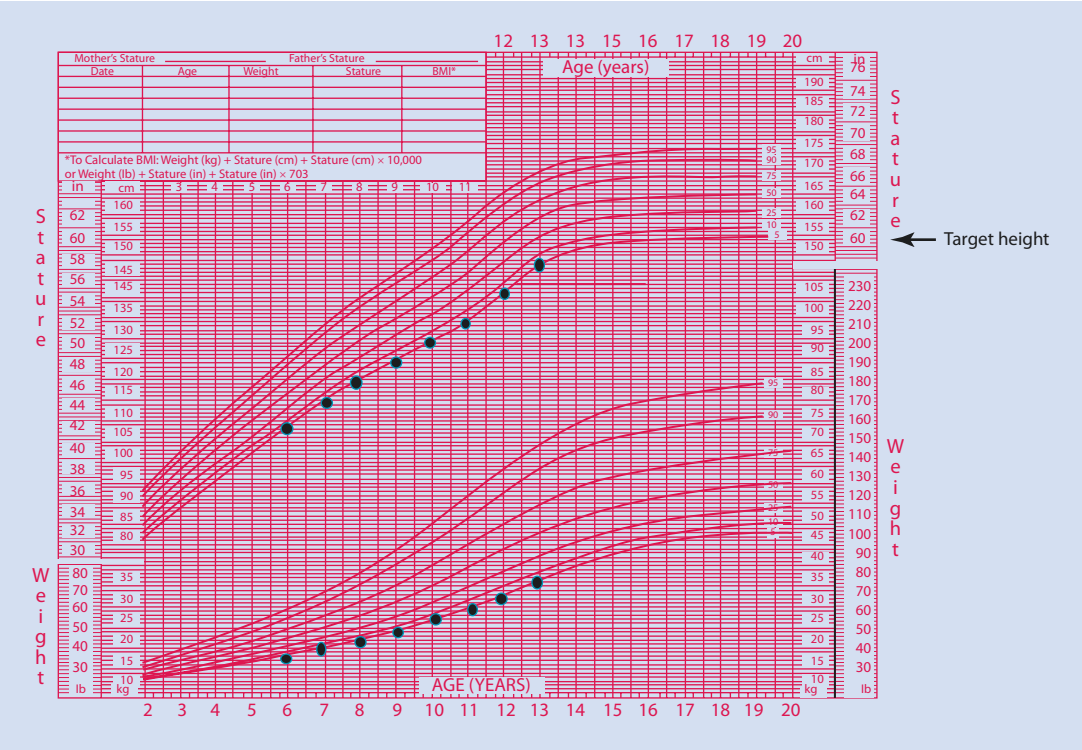

Fig. 3.3 Weight and height data for a girl with familial short stature plotted on the CDC growth chart [4]. Note the normal growth velocity along the 5th percentile

syndrome, and a variant of the Kenny-Caffey syndrome with normocalcemia. In addition to short stature these patients had dysmorphic features and/or other medical conditions [21].

In summary, ISS is also a diagnosis of exclusion, determined after a comprehensive evaluation fails to find an identifiable etiology for the short stature. Rare genetic disorders should be considered as a cause for the short stature in children with a height at or ≤−3 SD below the mean for age and sex, especially in the presence of dysmorphic features and/or other medical diagnoses.

3.4 Diagnostic Evaluation

The evaluation of a child with a height ≤−2 SD below the mean for age, sex, and population should include a comprehensive history and physical examination with anthropometric measurements. A detailed history should assess for risk factors for hypopituitarism, such as a traumatic birth, postnatal hypoglycemia, jaundice, and/or a microphallus in male infants. A low birth weight may suggest short stature secondary to small for gestational age or Russell-Silver syndrome. A history of lymphedema, pedal edema, and/or cardiac anomalies may suggest Turner syndrome or Noonan syndrome. In girls with short stature, it is well established that Turner syndrome should be excluded even in the absence of overt clinical findings. A karyotype is not routinely done in boys but should be considered when predicted adult height is below target height, because 45X/46 XY may present with a normal phenotype and short stature. Growth hormone treatment is indicated for this condition [22]. Determining whether to test for a SHOX gene mutation is best determined by the established clinical scoring system for this condition. Rappold et al. found that an increased body mass index, SH/HT ratio, decreased arm span/height ratio, Madelung's deformity, short bowed forearms, dislocation of the ulna at the elbow, and muscular hypertrophy were most predictive of this gene mutation [23]. Children with a history of developmental delay and findings suggestive of a chromosomal disorder warrant further evaluation with a geneticist. Furthermore,

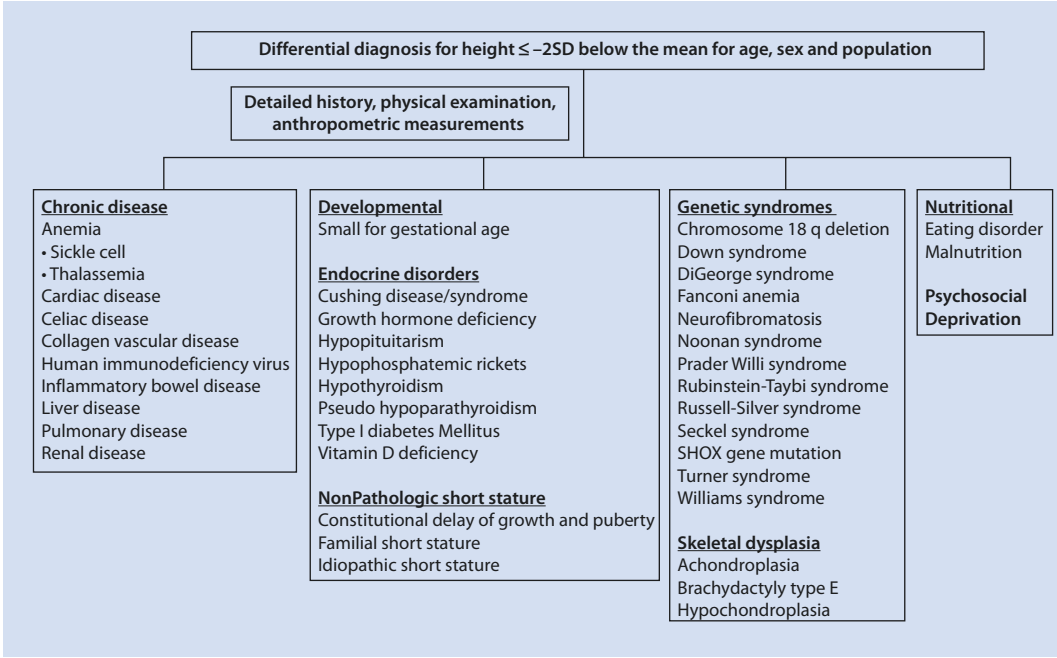

Fig. 3.4 Diagnoses to consider when evaluating a child with short stature

the evaluation should include an assessment for endocrine disorders such as Cushing disease/syndrome, hypothyroidism, and isolated growth hormone deficiency. Other causes of short stature to consider are chronic diseases, eating disorders, and psychosocial deprivation.

Physical examination findings of shortened metacarpals may indicate Turner syndrome, pseudohypoparathyroidism, brachydactyly type E, or other associated disorder. Some of the brachydactyly type E skeletal dysplasias are of autosomal dominant inheritance or may be caused by a spontaneous new mutation. Determining a diagnosis may be facilitated by examination of the parents for the presence of similar findings, especially if parental height is near or ≤−3 SD below the mean for age and sex [24]. A skeletal survey to evaluate for a skeletal dysplasia should be considered in the presence of abnormal body proportions and/or height SD significantly below target height. Figure 3.4 shows the different diagnostic categories to consider when evaluating a child for short stature.

If the comprehensive history, physical examination, and auxologic data are not suggestive of a specific disorder, then screening studies (electrolytes, alkaline phosphatase, calcium, phosphorous, albumin, CBC, TSH, free T4, IGF-1, IGF BP-3, ESR, and celiac panel) to exclude an identifiable etiology are indicated [12]. The diagnostic studies to consider when evaluating a child with short stature are listed below. A retrospective chart review of 235 asymptomatic patients (defined as normal history, review of systems, and physical examination) referred to a large academic pediatric endocrinology center for short stature (height at ≤−2 SD below the mean for age, sex, and population) determined that performing these screening studies had a low yield for organic disease and was not cost-effective. Only 37% of the patients had prior growth records available as part of their evaluation. The authors suggest reconsidering the recommendations from the 2008 Consensus Statement on the Diagnosis and Treatment of Children with Idiopathic Short Stature (ISS) in that in asymptomatic children a height velocity should be monitored over a 6 month period, and if abnormal for age and sex then proceed with screening studies [25].

Assessing these children for growth hormone (GH) deficiency is an important part of the evaluation. Since GH is secreted in a pulsatile fashion throughout the day with more frequent pulses overnight, random GH levels are usually low. GH mediates the production of IGF-1 and IGF BP-3 from the liver, and these levels are stable throughout the day, serving as a better screening test for GH deficiency. Normal ranges for IGF-1 and IGF BP-3 are based on age and Tanner stage in prepubertal and pubertal children, respectively. Low or low/normal IGF-1

Diagnostic Screening Studies for Evaluation of Short Stature and/or Delayed Puberty

Screening studies for height ≤−2 SD mean for age, sex, and population

- CBC
- Electrolytes
- Calcium, phosphorous, alkaline phosphatase
- ESR
- Celiac panel (total IgA and tissue transglutaminase IgA)
- TSH, Free T4
- IGF-1, IGF BP-3
- Karyotype for girls (consider for boys)
- Bone age
- Consider skeletal survey based on anthropometric measurements and/or growth rate

Screening studies for delayed puberty

- Gonadotropins (*pedi-LH and pedi-FSH, recommend 3rd-generation assay)
- Testosterone or estradiol.
- Prolactin.
- Depending on the above results, a karyotype or head MRI may be indicated.

and/or IGF BP-3 levels should prompt further evaluation with provocative GH stimulation testing. GH is measured at several time points following the administration of a GH secretagogue, such as clonidine, arginine, L-Dopa, propranolol, glucagon, or insulin. A single GH value of ≥10 ng/ml on two provocative GH stimulation tests indicates GH sufficiency. In peripubertal and early pubertal children, priming with sex steroids, testosterone, or estrogen, prior to the testing, is indicated to prevent misdiagnosis of GH deficiency. Priming with sex steroids is controversial, because of concern for a missed opportunity to treat partial GH deficiency or underdiagnose GH deficiency [26–28]. An increase in GH amplitude and mean 24 h GH levels occur between Tanner stages 2–4 and 3–5 in girls and boys, respectively. Girls exhibit increased mean nocturnal GH levels and GH amplitude prior to the onset of breast development which coincides with the start of their pubertal growth spurt. In contrast, boys in the early stages of puberty have lower mean nocturnal GH levels and GH amplitude that are comparable to that of a prepubertal boy [29]. This data supports the role of sex steroids on GH secretion and explains the decline in growth rate exhibited in boys prior to the onset of puberty. Saggese et al. showed that lower growth hormone releasing hormone (GHRH) levels in children with delayed puberty may explain their subnormal GH response to stimulation tests. When retested in mid- to late puberty, these adolescents had a normal GH response, and their GHRH levels were comparable to the control group [30]. A prospective study in Turkey evaluated the final height of boys with a history of delayed growth and normal testosterone primed GH stimulation studies. These boys had normal final adult heights, consistent with their mid-parental heights [31]. Müeller et al. and others showed that children with true GH deficiency have an abnormal GH response to sex steroid primed provocative GH stimulation tests [32, 33].

Adolescents with delayed puberty should have additional analyses (gonadotropin levels, serum prolactin, estradiol, or testosterone) to assess for causes of pubertal delay. A girl with hypergonadotropic hypogonadism requires further evaluation with a karyotype for Turner syndrome, a common cause of primary ovarian failure and short stature with an incidence of 1 in 2500 live births. Often boys and girls with Noonan syndrome manifest delayed puberty, but boys more frequently have primary gonadal failure [34]. 46 XX SRY-positive males present with normal male external genitalia, short stature, and primary gonadal failure [35]. Other causes of primary gonadal failure include autoimmune primary ovarian failure, triple X syndrome, and Klinefelter's syndrome, but these disorders are associated with normal or tall stature. Primary gonadal failure may also be a sequela of radiation therapy and/or chemotherapy for childhood cancer.

Hypogonadotropic hypogonadism (HH) may be isolated or associated with other pituitary hormone deficiencies or genetic syndromes. Head trauma, CNS tumors, neurosurgical resection of a cranial tumor, cranial irradiation, or transcription factor mutations (PROP-1, HESX-1, LHX3 and SOX 3) can cause HH, frequently in association with other pituitary hormone deficiencies. A prolactinoma causes delayed puberty secondary to suppression of gonadotropin release and may or may not be associated with galactorrhea. Kallmann syndrome and isolated hypogonadotropic hypogonadism (IHH) due to mutations in the GnRH receptor cause IHH, although Kallmann syndrome is often associated with tall stature. Functional causes of HH include chronic illness, eating disorder, or other endocrine disorder (hypothyroidism, Cushing disease/syndrome). Genetic syndromes associated with HH and short stature include Prader-Willi syndrome and Werner syndrome. Table 3.1 shows the differential diagnosis and evaluation for delayed puberty.

Table 3.1 Diagnoses to consider in the evaluation of a child for delayed puberty

Differential diagnosis for delayed puberty	
Hypergonadotropic hypogonadism	**Hypogonadotropic hypogonadism**
Short stature Turner syndrome Noonan syndrome Normal male phenotype of 45 X/46XY 46XX SRY-positive male Gonadal radiation/chemotherapy for cancer (e.g., cyclophosphamide, ifosfamide, procarbazine)	*Short stature* Brain malformations (e.g., hydrocephalus) Brain tumor (craniopharyngioma, prolactinoma) Craniospinal irradiation Head trauma Hypothyroidism Neurosurgical treatment Pituitary transcription factor mutations (Prop-1, HESX-1, LHX-3 SOX-3)
	Short stature (GH deficiency excluded) Constitutional delay of growth and puberty Isolated hypogonadotropic hypogonadism
Normal/tall stature Primary ovarian failure Triple X syndrome Klinefelter's syndrome	*Normal/tall stature* Kallmann syndrome

Distinguishing idiopathic IHH from CDGP is often a challenge, because basal and stimulated levels of gonadotropins are low in both conditions until activation of the hypothalamic-pituitary-gonadal axis. Many studies, limited by a small number of study subjects, were conducted to determine whether basal and GnRH-stimulated FSH and LH levels, basal inhibin B levels, HCG-stimulated testosterone, and/or combinations of these tests can most effectively diagnose IHH. Binder et al.'s retrospective study showed that a combination of basal LH <0.3 IU/L and inhibin B < 111 pg/ml had a specificity of 100% and sensitivity of 98% in establishing a diagnosis of IHH [36]. Another retrospective study determined that a peak stimulated LH of 2.8 U/L and 4-day and 19-day HCG-stimulated testosterone cutoff peaks of 1.04 mcg/L and 2.75 mcg/L, respectively, had 100% sensitivity and specificity for diagnosing IHH [37]. Two prospective studies of basal inhibin B as a diagnostic test for IHH reported discrepant cutoff values. Further studies of basal inhibin B are indicated before establishing it as a routine test to discern IHH from CDGP [38–40].

3.5 Management of Normal Variant and Idiopathic Short Stature

Children with NVSS and ISS are healthy and do not have an identifiable cause for their short stature. Management should include reassurance and follow-up to monitor growth, pubertal development, and other auxologic growth parameters to confirm that the child follows the anticipated pattern of growth. Ongoing surveillance of the child's growth pattern is important because sometimes, despite, an extensive evaluation, a diagnosis such as a mild hypochondroplasia or pseudohypoparathyroidism may not be clinically and diagnostically evident until the child is older [41–43].

Treatment of children with normal variant short stature and ISS should be individualized and expectations of treatment clearly discussed with the family.

As peers progress through puberty and differences in pubertal development and stature become more apparent, despite reassurance, many boys with CDGP have a difficult time coping with this discrepancy. The short stature and/or delay in puberty from CDGP can significantly impact the psychological and emotional well-being of an adolescent, causing poor school performance and self-esteem, withdrawal from social activities, and even depression and anxiety [44].

Treatment with low-dose testosterone is an option for boys with difficulty coping with their short stature and delayed puberty. Boys treated with low-dose testosterone should have a bone age of at least ≥12 to prevent significant bone age advancement [45]. Depot testosterone enanthate

or cypionate 50 mg SQ or IM every 4 weeks for 3–6 months will frequently invoke an increase in growth rate and pubertal progression without bone age advancement. Testosterone enanthate contains a sesame seed oil and testosterone cypionate, a cotton seed oil, and should be avoided in individuals with allergies to these oils. The response to treatment should be evaluated within 4–6 months following the last testosterone dose. If the response is inadequate, then an additional 3- to 6-month course of testosterone 50–75 mg monthly may be administered [46, 47].

In contrast to boys, girls have a growth spurt in early puberty. Therefore, girls infrequently seek treatment for CDGP because their onset of puberty is accompanied by a pubertal growth spurt. In extreme cases of CDGP, a girl can be treated with either a low-dose oral estrogen, ethinyl estradiol 5 mcg daily, or low-dose estrogen transdermal patch, 3.1–6.2 mcg per 24 h (1/8 to ¼ of a 25 mcg patch), to initiate puberty [46].

Oxandrolone, a weak synthetic, non-aromatizing anabolic steroid derivative of testosterone, is an oral treatment option for CDGP. Oxandrolone 0.1 mg/kg/day for 3–4 months is recommended for pubertal initiation. Crowne and colleagues' double-blind, placebo-controlled trial showed comparable increases in growth velocity and testicular development in boys treated with either a 3-month course of oxandrolone 2.5 mg daily or testosterone 50 mg every 4 weeks [47].

Increased interest in aromatase inhibitors as a potential treatment option for boys with CDGP and ISS stems from its inhibition of the conversion of androgens to estrogen. Cessation of growth depends on estrogen-mediated fusion of the epiphyses; therefore, lower estrogen levels may preserve epiphyseal patency, improving final height prognosis. Many of the studies conducted to date included a small number of male study subjects with CDGP and/or ISS. Hero et al.'s prospective randomized, placebo-controlled study compared predicted adult height in 8 boys with CDGP treated with low-dose testosterone and placebo and 9 boys treated with testosterone and letrozole. Both groups were treated with a 6-month course of testosterone 50 mg every 4 weeks and either placebo or letrozole for 2 years. Initial study data, following 1 year of treatment, showed a gain of 5.1 cm in predicted adult height in the letrozole-treated group compared to the control group. After 2 years of treatment the cohorts were followed until near final height, defined as a bone age ≥15.75 years. The study group achieved a final adult height (175.8 cm) consistent with their target height (177.1 cm), while the control group's final adult height (169.1 cm) was less than their target height (173.9 cm) [48]. In a separate randomized controlled study in boys with ISS, Hero et al. compared the effect of 24 months of placebo vs. letrozole on predicted adult height and bone mineralization. In the letrozole-treated group, predicted adult height increased 5.9 cm, whereas the control group's predicted adult height was unchanged. Bone mineral density of the lumbar spine and femoral neck was comparable in the two groups, but the apparent bone mineral density was higher in the letrozole-treated cohort [49].

Another randomized, double-blind, placebo-controlled trial compared the effects of letrozole and oxandrolone on predicted adult height, pubertal development, bone mineral density, serum IGF-1, and blood lipoproteins in boys with CDGP and ISS. Following 2 years of treatment, predicted adult height in the letrozole-treated group improved by 6.1 cm, while the oxandrolone group's predicted adult height was unchanged. A greater increase in testicular volume and bone mineral density occurred in the oxandrolone-treated group compared to the letrozole- and placebo-treated groups. HDL was lower in the letrozole-treated group and unchanged in the oxandrolone group. The increases in IGF-1 levels were comparable in the oxandrolone and letrozole groups, significantly increased from that of the placebo group [50]. Currently, Dr. Nelly Mauras is conducting a 2–3-year randomized controlled trial in pubertal boys with ISS and a bone age <14.5 years, comparing three different treatment arms, an aromatase inhibitor (letrozole or anastrozole) vs. growth hormone alone (0.3 mg/kg/week) vs. combination therapy (aromatase inhibitor and growth hormone). Outcome measures include change in predicted adult height, bone density, bone markers, IGF-1, lean body mass, and the degree of estradiol suppression by the individual aromatase inhibitors [51]. In terms of adverse effects of aromatase inhibitors, there is only one published report of an increased incidence of vertebral deformities in 5 of 11 prepubertal/early pubertal boys with ISS treated with letrozole [52]. Karmazin et al. reported that 25% of study subjects treated with letrozole developed adrenal suppression determined by a low-dose ACTH stimulation test [53].

The aromatase inhibitor, anastrozole, increases sperm count in males with infertility, yet concern exists as to whether it can affect sperm production in adolescent males. Mauras et al. analyzed sperm counts in 11 young adults, age 18, with GH deficiency treated with anastrozole for 29 months. No difference was found in the sperm counts between the treated and untreated cohorts, but low sperm counts were found in both groups [54]. Currently, due to the paucity of data on side effects and final adult height, aromatase inhibitors are not recommended for the treatment of ISS and CDGP.

In 2003, the Food and Drug Administration (FDA) approved GH treatment (0.3–0.37 mg/kg/week) for children with ISS defined as a height of ≤−2.25 SDS (1.2 percentile) below the mean for age and sex "and associated with growth rates unlikely to permit attainment of adult height in the normal range, in pediatric patients whose epiphyses are not closed and for whom diagnostic evaluation excludes other causes associated with short stature that should be observed or treated by other means" [55]. The lower cutoff for the normal range adult height is defined as 160 cm (63 inches) for a male and 150 cm (59 inches) for a female. This statement issued by the FDA does not clearly exclude GH treatment for children with FSS and CDGP.

Prior to the FDA approval of GH treatment for ISS, several nonrandomized, longitudinal studies were published that used variable dosing regimens of GH. Hintz et al. reported final adult height outcomes in children with ISS treated for 2–10 years with a 0.3 mg/kg/week of thrice weekly GH. The data demonstrated a gain in height of 5 ± 5.1 cm for boys and 5.9 ± 5.2 cm for girls above the initial predicted adult height. The researchers did not find any predictive factors that correlated with an improved response to GH [56]. MacGillivray et al.'s 4-year open-label randomized study compared a daily regimen and thrice weekly regimen of 0.3 mg/kg/week of growth hormone in prepubertal children with ISS and found superior growth rates in the cohort treated with daily GH. No difference in bone age advancement or onset of puberty occurred between these groups. Skeletal maturation and pubertal onset did not differ between the placebo- and GH-treated cohorts [57]. The Cochrane Metabolic and Endocrine Disorders Group analyzed the data on 10 randomized controlled studies published between 1989 and 2004. Two of the studies reported on final adult height outcomes, and the remainder of the studies had short-term height outcome data. This analysis included the National Institutes of Health (NIH) randomized, double-blind placebo-controlled study that treated peripubertal children with GH 0.22 mg/kg/week divided into three doses per week. Adult height outcome data was available in nearly 50% of the cohort and showed a gain of 3.7 cm in adult height compared to the placebo-treated group. Intent-to-treat analysis for final adult height showed a similar gain in final adult height compared to the control group. The other randomized controlled study that reported final adult height outcomes was a small study with only 10 girls in the treatment group (GH 30 IU /m2/week of daily injections), 8 in the randomized control group, and 22 in the control group that refused to consent to randomization. A gain of 7.5 cm and 6 cm in final adult height was reported in the treated group compared to the randomized control and the nonrandomized control groups, respectively. The remainder of the randomized controlled studies evaluated short-term gains in height which ranged from no improvement to 0.7 SD improvement over 1 year [58, 59]. Other meta-analysis of randomized and nonrandomized controlled studies indicates a variable response with gains in height ranging from 2.3 to 8.7 cm in children [60, 61]. The GH adverse effect profile reported in these studies did not differ from that of children treated with GH for other conditions. Some studies suggest that adult height outcomes are optimized with earlier initiation of GH treatment and with higher doses [12, 62, 63]. Kamp et al.'s randomized controlled study analyzed the effects of 2 years of high-dose GH treatment in prepubertal children with ISS. Five years after initiation of treatment, when compared to the control group, for the GH-treated cohort, height improved (−1.4 SD vs. −2.2 SD), bone age advanced significantly (3.6 year/2-year chronologic age vs. 2 year/2-year chronologic age), and pubertal onset was earlier (11 of 13 GH-treated study subjects vs. 7 of 13 control subjects) [64].

GH treatment requires monitoring of growth velocity and adjustment of GH dose to achieve an adequate response. The 2007 international consensus meeting on ISS recommended monitoring IGF-1 levels to assess dosing, adherence and to prevent overtreatment, with a goal of a level above 0 SDS and within the normal limits for age [65].

Lee et al. calculated a cost analysis of treating a 10-year-old child with GH 0.37 mg/kg/week with an endpoint of a 1.9 inch gain in height over

5 years. The gain in height and GH dose used for this analysis was derived from the two studies that the FDA based its decision on to approve GH for ISS [59, 66]. The analysis revealed a cost of approximately $52,000 per inch of height gain [67]. An estimated 400,000 children would qualify for GH treatment based on the FDA's criteria for GH treatment of children with a height of <1.2nd percentile. Although the FDA statement that children with a "form of short stature that can be observed or otherwise managed by other means" is suggestive of children with FSS and CDGP, these diagnoses are not clearly excluded from the FDA indications for GH treatment for short stature. The exorbitant cost of GH weighed against treating an otherwise healthy child has generated controversy over the use of GH as a "lifestyle drug," a term used to refer to a "pharmaceutical product characterized as improving quality of life rather than alleviating disease" [68].

Conflicting study outcomes have been reported on the efficacy of combination treatment with GH and a GnRH agonist for pubertal children with ISS and SGA. Many of the studies are small and nonrandomized and do not include control groups. Kamp and colleague's randomized controlled study of combination treatment in pubertal children with ISS and SGA showed that predicted adult height in the treated groups improved by 8 cm in girls and 10.4 cm in boys [69]. These researchers also analyzed the final height and bone mineralization of 32 of the 40 participants who participated in the original 3-year study and found that the treated group had a net gain of 4.9 cm above their predicted adult and target heights compared to the control group. The bone mineralization was similar between the treated and control groups except for a lower lumbar spine bone mineralization SD score in the treated boys ($n = 6$) compared to the control group ($n = 2$) [70].

3.6 Outcomes and Possible Complications

Adult height outcome data in children with CDGP vary, with some studies showing impaired adult height and in others an adult height consistent with target height. These studies found that the Bayley-Pinneau method as opposed to the Tanner-Whitehouse method of height prediction correlated best with the child's adult height. Factors negatively impacting the adult height prognosis in children with CDGP included a shorter period of time between the onset of puberty and peak height velocity, a lower peak height velocity, a shorter sitting height, and a decreased duration of puberty [71].

Proponents of treatment advocate that short stature negatively impacts a child's psychosocial development [72, 73]. In contrast, others have shown that GH does not affect psychosocial function. The Wessex study, a longitudinal controlled study, evaluated whether short stature impacts a child's academic, social, and behavioral development. The study and control groups consisted of 5-year-olds recruited from two school districts in the South of England. The study group had a height at or below the 3rd percentile and was matched with a control group of peers of the same sex, age, and grade with normal stature (10–90th percentile). These cohorts had testing at ages 7–9, 11–13, and 18–20 years to determine whether their short stature effected their academic and psychosocial development. Analysis of the data from these studies showed that after adjusting for socioeconomic status, the study and control groups did not differ with respect to intelligence quotient, self-esteem, and behavior [74–76]. Other studies that examined participants referred to pediatric endocrinology clinics for evaluation of short stature indicated no effect on psychosocial function [77, 78].

The Sante Adulte GH Enfant (SAGhE) study led by French investigators reported on the long-term mortality of approximately 7000 GH-treated patients for diagnoses of isolated GH deficiency, ISS, and SGA. The mortality was evaluated 16.9 years following the cessation of GH treatment and compared to that in the general French population. This study found an increase in mortality due to cerebrovascular events and bone tumors among individuals treated with GH, in particular in those treated with higher doses of GH, exceeding 50 mcg/kg/day [79]. The SAGhE study design had several shortcomings that may have influenced these findings. In particular, mortality in the treated group was compared to the mortality rate in the general French population as opposed to an identical matched, untreated control group. Also, the reported association between the higher dose of GH and increased mortality was among a small number of the cohort, many of whom had a diagnosis of SGA [80]. A preliminary report from the EU SAGhE

Study conducted in Belgium, the Netherlands, and Sweden did not show an association between adult mortality in 2543 patients treated with GH during childhood for isolated GH deficiency, ISS, or SGA [81]. Due to the limitations of the SAGhe study design the FDA, Endocrine Society, Pediatric Endocrine Society, and Hormone Research Society concluded that it is safe to continue to treat children with ISS, isolated GH deficiency, and SGA with GH [80].

Case Study

LA was referred at age 16 to a pediatric endocrinologist for evaluation of short stature and delayed puberty. His growth chart below shows normal linear growth below and parallel to the 3rd percentile before the age of 12. Between ages 12 and 16, his growth velocity declined to 3 cm/year. A comprehensive history revealed no significant past medical history, and a complete review of systems was unremarkable. Family history was significant for CDGP; both the patient's father and brother completed their growth in their early to mid-20s. LA was doing well in school, but was upset by the lack of secondary sexual development. His physical examination revealed Tanner 2 pubic hair and genitalia with testicles measuring 4 ml. LA had a delayed bone age consistent with a 12-year-old boy. He had an extensive workup that revealed a normal CBC, complete metabolic panel, ESR, IGA 110 mg/dl, negative tissue transglutaminase antibody titer, TSH 1uIU/ml, free T4 1.2 ng/dl, IGF-1 201 ng/ml (normal Tanner 2: 56–432 ng/ml), IGF BP-3 4.4 mg/L (normal Tanner 2: 2.3–6.3 mg/L0), normal prolactin 9 ng/ml, and gonadotropins consistent with early puberty (FSH 3.9 uIU/ml, LH 0.94 uIU/ml, and testosterone 22 ng/ml).

How Should LA Be Managed?

LA was treated with low-dose testosterone 50 mg IM every 4 weeks for 6 months, and his growth velocity started to increase. At age 17 he had Tanner 3 genitalia and a growth rate of 5–6 cm/year and a bone age consistent with a 12.5-year-old boy. He was followed every 6 months to monitor his growth velocity (Fig. 3.5).

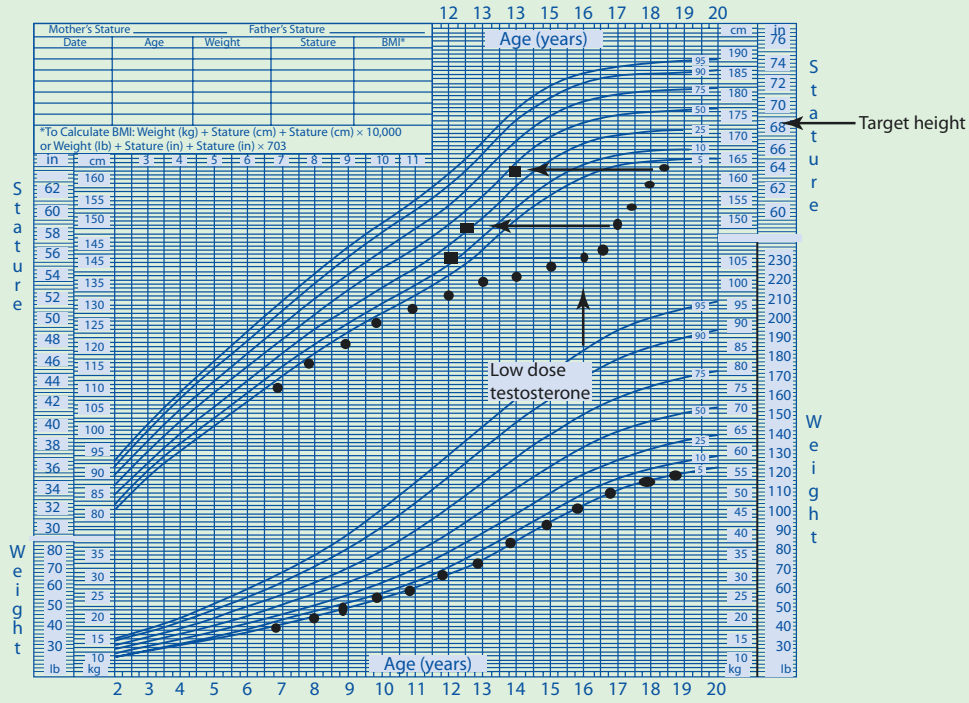

Fig. 3.5 Weight and height data for a boy with constitutional delay of growth and puberty treated with a 6-month course of low-dose testosterone. Data points are plotted on the CDC growth chart [4]. • represents height and ■ represents bone age

3.7 Summary

A comprehensive history, physical examination, current and past auxologic data, and diagnostic studies are essential for evaluating children with short stature to exclude an organic etiology. The growth patterns of children with normal variant short stature typically reflect a familial pattern of growth in that a child with FSS has at least one parent with short stature. Children with CDGP frequently have a family history of a first or second degree relative with a similar pattern of growth. Often children with CDGP also have FSS as the cause of their short stature. Children with normal variant short stature require reassurance and follow-up to ascertain that they follow the expected course of growth and pubertal development for these normal physiologic variants of growth. Low-dose testosterone treatment effectively initiates puberty in boys with CDGP without advancing their bone age and compromising their final adult height.

ISS is differentiated from normal short stature in that the predicted adult height falls below the target height range for that child. These children are GH sufficient and do not have an identifiable cause for their short stature. Treatment of ISS with GH remains controversial in the medical community because of the costs and variable response to treatment. GH treatment for ISS should be individualized and the growth expectations explained to families. Children who are treated with GH should be closely monitored for side effects and assessed for an adequate response by monitoring growth velocity.

Although the SAGhE study initially raised concern regarding a potential increase in long-term mortality from GH treatment of isolated GH deficiency, SGA, and ISS, the limitations of this study created an awareness that well designed controlled studies are needed to effectively determine the long-term safety of GH.

Review Questions

1. Which of the following findings distinguishes ISS from CDGP and FSS?
   A. Bone age
   B. Predicted adult height is less than target height range
   C. Family medical history
   D. Height ≤−2 SD below the mean for age, sex, and population
2. Based on the FDA 2003 approval of GH for the treatment of children with short stature, which diagnoses could potentially qualify for treatment with GH growth hormone?
   A. ISS
   B. FSS
   C. CDGP
   D. All of the above
3. Which of the following provisions were included in the FDA approval statement for GH treatment for children with short stature?
   A. Height at or below 1.2nd percentile for age and sex.
   B. Predicted adult height is below the 150 cm (4′11″) for a woman and 160 cm (5′3″) for a man.
   C. The short stature is not due to an identifiable cause.
   D. All of the above.
4. Which of the following is not an auxologic measure of growth?
   A. Growth velocity
   B. Head circumference
   C. Arm span
   D. Bone density
5. You are asked to evaluate a 5-year-old boy with both height and weight at the 1st percentile. Your evaluation includes a comprehensive history and physical examination. Which of the following data are *essential* for evaluating the child's short stature?
   A. Prior growth data, target height, and bone age
   B. Bone age, target height, and head circumference
   C. Target Height, bone density, and growth velocity
   D. Sitting height, arm span, and upper to lower segment ratio

Answers

1. (B) Individuals with ISS have a predicted adult height more than 2 SD below their target height. Individuals with CDGP and FSS have a predicted adult height within 2 SD of their target height.
2. (D) In 2003 the FDA approved GH for treatment of children with a height less than 2.25 SD below the mean for age,

sex, and population "and associated with growth rates unlikely to permit attainment of adult height in the normal range, in pediatric patients whose epiphyses are not closed and for whom diagnostic evaluation excludes other causes associated with short stature that should be observed or treated by other means." Based on this statement, children with ISS, FSS, and CDGP may qualify for treatment with GH based on these criteria.

3. (D) All of the above statements are included in the FDA 2003 criteria for growth hormone treatment of children with short stature.
4. (D) Bone density is not an auxologic measure of growth. Choices A, B, and C are all auxologic measures of growth.
5. (A) All three of these parameters provide essential information for the initial assessment of this child's short stature. Prior growth data provides information about growth velocity. Target height is an auxologic growth measure utilized to determine whether a child's growth potential falls within the expected range for their genetic potential and should be utilized cautiously as certain growth disorders are genetic. Lastly, bone age provides information about skeletal maturity and potential adult height. The auxologic growth parameters in the other choices would not be essential for the initial assessment.

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Growth Hormone Treatment of the Short Child Born Small for Gestational Age

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4

Key Points

- Intrauterine growth retardation is caused by a variety of conditions that affect the offspring in multiple ways.
- The majority of individuals born small for gestational age (SGA) show catch-up growth by age 2 years and reach normal adult height.
- For those who do not show catch-up growth, growth hormone treatment accelerates growth and increases stature, but the responses are variable.
- Treatment at relatively young ages and with doses of growth hormone above those used for growth hormone deficiency is associated with improved response.

4.1 Introduction

Intrauterine growth retardation (IUGR) is a pathologic condition where fetal growth is restrained by either extrinsic (maternal) factors or a disorder intrinsic to the fetus itself. Each year nearly 14 million infants are born following IUGR worldwide [1]; rates are especially high in developing countries because of poor nutrition and limited prenatal care. This is a significant problem because of the morbidity that accompanies IUGR. Complications in the immediate postpartum period include hypoglycemia, necrotizing enterocolitis, and persistence of the fetal circulation, to name a few [2]. Moreover, the first year survival rate is substantially reduced in infants who have experienced IUGR [3], and stunting of growth is prevalent [4].

There are long-term sequelae of IUGR as well [5]. Affected children may have poor school performance and attenuated intellectual development [6]. There is evidence that intrauterine nutrient deprivation leads to obesity, insulin resistance, and hyperlipidemia later in life, an effect thought to be due to in utero "programming" of metabolic status [7, 8]. Postnatal growth is also affected adversely [4]. Somewhere between 10% and 40% of children who are born following IUGR remain growth retarded in childhood [9, 10]. Many never reach normal adult size. The effect of IUGR on subsequent growth and its amelioration by growth hormone (GH) therapy are the focus of this chapter.

The percentage of newborns said to have had IUGR depends on the definition applied but generally is around 3% in the United States and 10% in developing countries. It is important to consider definitions used to define IUGR as they have some bearing on interpretation of published reports. Intrauterine growth retardation (or intrauterine growth restriction) is a failure to grow at a normal rate in the in utero environment. Sequential measurements of fetal size in utero are becoming increasingly common and begin to allow IUGR to be documented with greater precision, and the phase of pregnancy during which the growth aberration occurred is defined [11]. The more commonly used definition is small for gestational age (SGA), which is simply a statistical definition for low birth weight at the calculated gestational age. Typical lines of demarcation are −2 SDs or <3rd percentile. Though newborns who fall below this are assumed to have had a period of IUGR, in some cases they simply represent the end of the normal spectrum of birth size. Similarly, infants may have experienced IUGR, especially in the last trimester, but have a birth weight that surpasses the minimal standards. These newborns may have other features of IUGR such as decreased subcutaneous tissue, hypoglycemia, etc.

4.2 Etiology

The etiologies of IUGR are as varied as those for postnatal short stature but typically fall into one of three classes. Maternal factors that lead to IUGR include deprivation of oxygen or nutrients (severe maternal malnutrition, multiple gestation, cigarette smoking, high-altitude living), infection (CMV, toxoplasmosis, AIDS), and toxins (alcohol). Placental deficiency due to suboptimal implantation site, placental undergrowth, infarction, or vascular anomalies such as velamentous cord insertion and placental hemangiomas can also lead to IUGR. Factors intrinsic to the fetus constitute the final classification and are exemplified by chromosome aneuploidy (Turner syndrome, trisomy 13 and 18) and specific genetic defects.

A deficiency of insulin secretion (such as that which occurs in pancreatic agenesis) or action (e.g., insulin receptor deficiency/leprechaunism) severely impairs fetal growth and, though specific, is a rare cause of IUGR [12]. More common fetal insults that produce IUGR are hypoxia and nutrient deprivation. Restriction of oxygen or nutrients result in adaptive responses on the part of the fetus which tend to preserve organ differentiation and maturation at the expense of physical growth and energy stores (fat and glycogen). It is clear that the insulin-like growth factor (IGF) axis is intimately involved in these adaptive responses. Because this chapter deals predominantly with practical aspects of diagnosis and treatment of short stature in patients born SGA, a detailed review of the components of the IGF system and their roles in control of fetal growth is beyond its scope (for reviews see works by Gicquel [13] and Netchine [14]). However, it is worthwhile to consider the in vivo experiments using mouse mutant models that have defined major hormonal influences on prenatal and postnatal growth. The physiology is summarized as follows: IGF-1 and IGF-2 are the major hormonal regulators of fetal growth and can compensate, at least partially, for deficiency of each other [15–18]. The growth-promoting effects of the IGFs are principally mediated by the type I or IGF-1 receptor, a homologue of the insulin receptor [15]. The IGF-2 "receptor," in contrast, serves as a clearance mechanism of IGF-2 and thereby modulates tissue IGF-2 abundance [19, 20]. During fetal life, the IGF system operates largely independently of GH, which has little influence on body size before birth in humans and before 2 weeks in rodents [21]. Thereafter the influence of IGF-2 declines, and IGF-1, under the control of GH, becomes the dominant growth regulator of postnatal life.

The extent to which the GH/IGF axis influences growth in the rodent has been detailed by genetic manipulations [21]. In mature animals, approximately 70% of body size is due to the actions of IGF, of which about half relate to GH-mediated changes in IGF concentration, while the remainder reflects IGF direct effects (i.e., not related to GH stimulation of IGF production). GH also appears to have direct effects, independent of IGFs, on body size, but the magnitude of these effects are relatively modest. When these elements are accounted, only about 17% of body size in the adult mouse relates to factors other than GH or IGF.

Though much of our insight into mechanisms come from studies of rodents, many of the experimental findings have been confirmed in humans (Table 4.1). Children with GH insensitivity due to receptor deficiency are near normal size at birth, indicating a modest role for GH in prenatal growth [44]. In contrast, children with significant disruptions in the IGF-1 gene [22, 23, 45] and IGF-1 receptor gene [28, 46, 47] show severe intrauterine growth retardation and subsequent postnatal growth deficiency, just as predicted from murine deletion mutants. Such data, when considered in the context of the reports describing positive correlation between cord blood IGF-1 concentration and birth size [48–50], illustrate the pivotal role of the IGF axis in controlling prenatal and postnatal growth.

4.3 Clinical Presentation

After birth, most SGA newborns increase their growth velocity substantially and eventually catch up [9, 10]. However, it should be noted that there is a relationship between birth weight and stature that is maintained for several years during childhood, with patients being born especially small remaining short on the average [51].

Patients generally present to the endocrinologist in one of two ways. The first is immediately following birth when categorized as SGA. The questions that arise at this time relate to potential causes of IUGR and whether patient will have normal growth thereafter. The extent of the evaluation will depend on the severity of growth retardation, the existence of concurrent medical conditions or dysmorphic features, and whether the cause of IUGR is evident.

A more common presentation is that of the short child between the ages of 3 and 8 years. The child was born with a low birth weight and was expected to "catch up." However, catch-up never occurred, and the child has had the same relative degree of short stature for many years. That is, when plotted on the growth chart, the trajectory seems to parallel the norm, just three to five SDS

Table 4.1 Important genetic anomalies causing intrauterine growth retardation in humans

Gene(s)	Disorder	Major clinical features	Comments	References
IGF1	Short stature	Severe pre- and postnatal growth failure, microcephaly, deafness, carbohydrate intolerance	Very rare	[22–24]
IGF2	Silver-Russell syndrome	Severe pre- and postnatal growth failure	Epigenetic variation at imprinted locus	[25–27]
IGF1R (IGF1 receptor)	Short stature	Severe pre- and postnatal growth failure, variable CNS abnormalities	Variable increases in circulating levels of IGF-1	[22, 28–30]
INS	Congenital diabetes	Fetal growth retardation, diabetes mellitus	10% of permanent neonatal DM	[31]
KCNJ11, *ABCC8* (KATP channel)	Congenital diabetes	Fetal growth retardation, diabetes mellitus, transient or permanent	40% of permanent neonatal DM CNS disease in some *KCNJ11* mutations Activating mutations	[32–34]
Chromosome 6 ICR abnormality	Congenital diabetes	Fetal growth retardation, transient diabetes mellitus	70% of transient neonatal DM Paternal isodisomy or DNA methylation defect	[35]
INSR (Insulin receptor)	Donohue (leprechaun) syndrome, Rabson-Mendenhall syndrome	Fetal growth retardation, diabetes mellitus, moderate to severe insulin resistance	Treatment with rhIGF-1 may be beneficial	[36, 37]
PTF1A, IPF1	Pancreatic agenesis	Fetal growth retardation, diabetes mellitus	Cerebellar involvement with *PTF1A*	[38]
FANC A-M	Fanconi syndrome	Severe pre- and postnatal growth failure, absent thumb/radius	Associated with GH deficiency, hypothyroidism, hypogonadism, and malignancy	[39, 40]
BLM	Bloom syndrome	Severe pre- and postnatal growth failure	Increased risk for neoplasms	[41]
ACAN	Short stature	Variable growth phenotype with advanced bone age and midface hypoplasia	Early-onset osteoarthritis	[42, 43]

below average. The child has otherwise been healthy, and parents are concerned that the short stature will become increasingly problematic as the patient ages and wonder whether anything can be done to improve stature. It is not always evident that the persistent small size is related to a growth disorder that began prior to birth. Only by reviewing the birth weight and history of pregnancy and delivery does this information come to light.

4.4 Diagnostic Evaluation

The causes of growth failure are many, as are the tests that can be applied to such patients. One should consider the likely possibilities and apply the diagnostic tests that are reasonably expected to be helpful. There are important reasons for establishing a diagnosis; however, it should be emphasized that in many cases, it is impossible to ascertain the precise cause of prenatal growth fail-

ure. Since this chapter deals primarily with the use of GH in augmenting growth in such children, the diagnostic discussion is directed toward determining whether GH therapy is warranted. The diagnostic approach is framed under several relevant questions.

? 1. Does the patient have a disorder that limits both pre- and postnatal growth?

Certain common endocrine disorders, such as hypothyroidism and GH deficiency, only affect postnatal growth substantially even when the condition is congenital. Thus, these are simply eliminated as diagnostic possibilities when prenatal growth restriction is evident. The same applies for common, acquired causes of growth failure such as celiac disease, etc. Patients who are born SGA and show catch-up growth during the first year do not require further evaluation. Patients being evaluated for IUGR who are still in the first few months of life should simply be tracked in terms of growth if they have no dysmorphic features, malformations, or suspicious symptoms. It is clear that most patients destined to catch up will demonstrate increased growth velocity during the first 6 months of life and have caught up by the end of the first year [9, 52, 53]. Patients who have shown no evidence for improved growth following birth need further evaluation.

? 2. Is the cause of IUGR obvious?

A careful history and physical examination can be very helpful in explaining the IUGR. Maternal hypertension and poor weight gain during pregnancy suggest a maternal factor. Dysmorphic features in the baby imply that a syndrome associated with IUGR is present. Useful diagnostic tests for evaluating patients with IUGR are listed in ▶ Box 4.1. A karyotype is particularly important for females because Turner syndrome is typically accompanied by mild prenatal growth restriction. Patients with dysmorphic features should have karyotyping as well or be considered for other specialized genetic tests and further evaluated by a geneticist/dysmorphologist. It is particularly important to recognize Bloom and Fanconi syndromes, recessive disorders associated with severe IUGR, and poor postnatal growth. These patients have increased chromosomal breakage and usually develop malignancies later in childhood. For these reasons, GH therapy may be contraindicated. The diagnosis of Bloom syndrome is often suspected with routine chromosome studies in which there is increased chromosome breakage and formation of triradial chromosomes. Confirmation requires specialized chromosomal studies which examine rates of sister chromatid exchange or direct gene analysis.

Assessment of renal function is required because mild forms of renal dysplasia can produce IUGR and moderate postnatal growth failure that is otherwise not evident. These patients may manifest oligohydramnios as a clue to the diagnosis.

Box 4.1 Useful Tests for IUGR-Associated Short Stature

General
- Complete blood count
- Erythrocyte sedimentation rate
- BUN/creatinine
- Serum electrolytes
- IGF-1/IGFBP-3
- T4, TSH
- Radiological skeletal survey

Specialized
- Karyotype (Turner syndrome)
- Cytogenetic studies to assess chromosome stability (Bloom syndrome, Fanconi syndrome)

? 3. Why has the patient not shown catch-up growth?

If more were known of the mechanisms involved in catch-up growth, it would be easier to explain why, in certain cases, it does not occur. In some situations the fetal growth retardation may have been so severe and the cellular mass at birth is so low that overall somatic size is ultimately restricted. Even with normalization of nutritional and hormonal factors, simply restoring normal growth (body size doubling at normal intervals) still leaves a person small relative to the peers. In other cases, patients do not reach normal size following birth because of persistence of a defect in growth regulation or cellular growth and replication. From a practical point of view, it is important to consider that catch-up growth may be impaired in patients whose nutritional status is compromised. A careful dietary history and review by a

nutritionist can be helpful and is especially indicated in a patient with low weight for height.

There is evidence that short SGA children may have relative resistance to IGF-1 [54]. Cutfield et al. [55] showed that circulating concentrations of IGF-1, while lower than normal in short SGA children, were higher than those with idiopathic short stature of the same age. Chatelain et al. [56] demonstrated that short SGA children required higher circulating concentrations of IGF-1 during GH treatment to achieve growth rates equal to children with GH deficiency or familial short stature treated with GH.

There is also evidence that GH secretion is reduced, limiting catch-up growth in some patients. Though absence of GH clearly cannot explain intrauterine growth retardation, studies by Boguszewski et al. [57] and de Waal et al. [58] have suggested that there is an increased incidence of low GH secretion in patients with short stature following IUGR. The data imply that reduced pituitary GH secretion contributes to the relatively poor postnatal growth in some cases. However, the ability of indices of GH secretion to predict response to GH therapy for this group of patients is not clear. Earlier reports suggested that low overnight GH concentrations or low IGF-1 concentrations were associated with an improved response [59, 60], whereas later reports, examining greater numbers, found no predictive value in these measures [61–63]. However, such studies frequently differ in the patient selection (e.g., severity of IUGR and short stature), the dosing of GH, and the tests of GH release. Studies that examine larger numbers of patients only slightly SGA likely include a significant proportion that does not necessarily have disorders of prenatal growth and/or have differing etiologies from those patients who are −4 to −5 SD below average for birth size. In addition, large doses of GH administered could obscure underlying differences in sensitivity to GH.

Most patients do not meet biochemical criteria for classic GH deficiency or other known endocrine disorders, and thus the most likely explanation for their poor postnatal growth is the persistence of a problem intrinsic to the fetus. There may be a specific genetic defect that continues to limit growth, or the early growth restriction has, in some way, reprogrammed the growth regulating system so that the child remains small. The evidence above suggests that the reprogramming involves alterations in the GH/IGF axis that results in a situation analogous to that seen with type 2 diabetes mellitus. Type 2 diabetes is characterized by insulin resistance coupled with reduced insulin secretion. Thus, the short child born SGA could be thought of as having "type 2 growth deficiency," where there are inadequate circulating levels of IGF-1 in the face of relative IGF resistance.

The number of patients for whom a specific etiology cannot be established continues to decline as methods to establish molecular genetic diagnoses in IUGR patients improve and become more commonplace. One study found a 14% prevalence of *ACAN* mutations in short children born SGA with advanced skeletal maturation [42]. Another study found that 16% of dysmorphic individuals with pre- and postnatal growth retardation had presumptive pathologic genomic copy number variations [64]. Application of whole exome sequencing identified pathologic mutations in over a third of highly selected cases, most of whom were SGA [65]. Although these reports survey small numbers of select individuals, for patients without features that suggest a specific diagnosis, microarray for CNV and whole exome sequencing are reasonable and recommended as diagnostic procedures [66]. With over 700 genes identified that determine adult height [67], it is certain that many are involved in determining pre- and postnatal growth patterns. With this new knowledge will come the ability to further categorize growth anomalies and to use the information to optimize therapy.

4.5 Treatment

Growth hormone was approved by the US Food and Drug Administration in 2001 and the European Agency for the Evaluation of Medicinal Products (EMEA) in 2003 for treatment of non-GH-deficient short stature in children born SGA, with some differences in specific recommendations (Table 4.2). Though changes in the IGF axis appear to mediate the alterations in growth, primary disturbances of GH/IGF are unlikely to be the root cause for most cases of IUGR with poor postnatal growth. Certainly classical GH deficiency is uncommon as an explanation for poor growth following IUGR. Why then would one expect that GH treatment to benefit patients with short stature is

Table 4.2 Indications for GH use in short children born small for gestational age

Parameter	FDA approval (2001)	EMEA approval (2003)	Consensus statement (2007)
SGA defined	Not defined	Birth weight or length < −2 SD	Birth weight or length <−2 SD
Youngest age to start Rx	2 years	4 years	2–4 years
Height at start	Not defined	<−2.5 SDS & < −1 SD parents	<−2 to <−2.5 SDS
Growth rate at start	No catch-up	< 0 SDS	No catch-up
Dose	70 μg/kg/day	35 μg/kg/day (1 mg/M^2/day)	35–70 μg/kg/day

Data are from somatropin package insert for the United States, from report 3478/03 by the Committee for Proprietary Medicinal Products of the EMEA and from Clayton et al. [42] for consensus statement
SGA small for gestational age, *SDS* standard deviation score

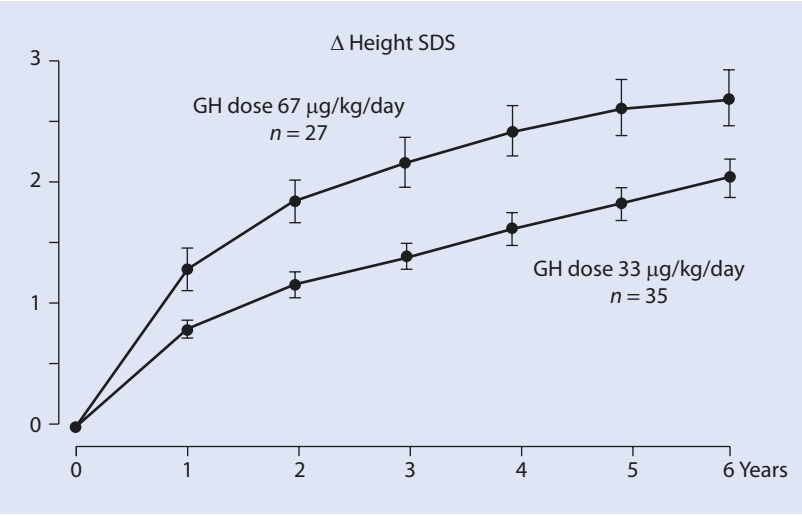

Fig. 4.1 Height SD score from baseline in patients with IUGR-associated short stature randomized to receive GH daily at two distinct doses. Patients were approximately 5 years of age on average at the start of treatment. Note the clear dose-response relationship most apparent in the first years of treatment (From de Zegher et al. [75]. Reprinted with permission from Oxford University Press)

associated with IUGR? The most straightforward answer is that GH administered at pharmacological dosages stimulates the system sufficiently to overcome whatever cellular condition has not allowed the expected "catch-up growth" to restore age-appropriate size.

There is now a general agreement that one should consider administration of GH to significantly short patients who experienced IUGR. A consensus statement published in 2007 [68] provides additional guidance, though leaves many practical considerations unaddressed. In the following sections, newer evidence from the literature and recommendations of the consensus statement are melded to yield a practical approach to the short child born SGA and to deal with the complex and controversial issues that surround the topic.

4.5.1 Effects on Somatic Growth

The earliest reports of GH administration to patients with IUGR-associated short stature indicated that short-term linear growth was stimulated by GH [69–71]. However, enthusiasm was diminished by the suggestion that the growth stimulation was not sustained [72] and that undesirable bone age advancement was negating the effect [73]. Such data implied that patients were unlikely to have meaningful increases in final height with long-term GH therapy. However, the doses employed were modest by today's standards, being similar to those given to patients with GH deficiency at the time. Subsequent studies showed clearly that exogenous GH stimulates growth in short children born SGA and that such growth can be sustained for several years [74] (Fig. 4.1). Table 4.3 displays

Table 4.3 Selected trials of GH given continuously to short children born SGA

Study format	*N*	Age at start (years)	Treatment duration (years)	GH dose (μg/kg/day)	SDS start	SDS end	SDS gain	Comments	Ref
Controlled clinical trial to final height	91	12.6	2.7	66	−3.2	−2.1	1.1	Older subjects and short duration. RCT design provides proof of principal that GH therapy increases adult height	[76]
	33	12.9	NA	0	−3.2	−2.7	0.5		
Clinical trial to final height	36	8.9	8.5	33	−3.1	−1.2	1.9	Showed that duration of GH treatment prior to pubertal onset had positive impact on final height	[77]
	34	8.3	NA	0	−2.2	−2.0	0.2		
Clinical trial to final height	28	7.9	7.9	33	−2.9	−1.1	1.8	Shows what can be achieved with 7 + years of treatment. Modest or nonexistent dose effect in terms of final height	[78]
	26	8.2	7.5	67	−3.0	−0.9	2.1		
	15	7.8	NA	0	−2.6	−2.3	0.3		
Clinical trial to final height	70	10.3	4.6±2.5	20	−2.9	−2.0	0.9	Untreated "controls" had normal GH stimulation test; treated patients had GH peak <10 ng/ml	[79]
	40	10.0	NA	0	−2.8	−2.2	0.6		

Dosage of GH is approximate because in some cases doses were given on the basis of body surface area or described in international units rather than mass. Conversion employed was 1 mg = 3 IU

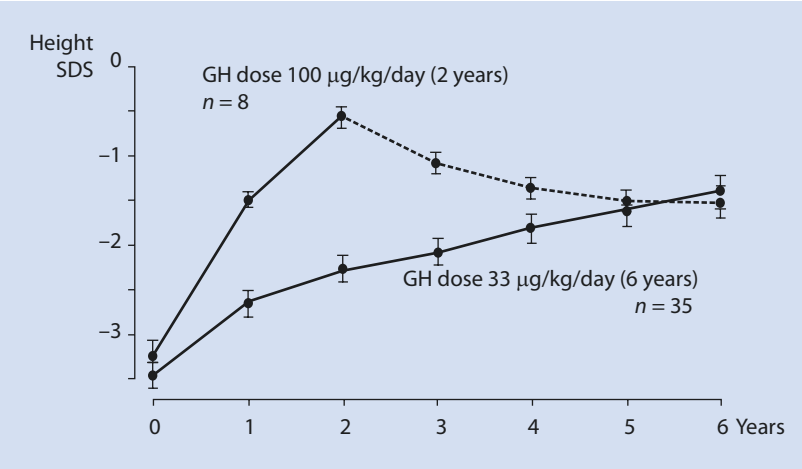

Fig. 4.2 Height SD score in patients treated with a 2 year course of high dose (100 μg/kg) daily GH and followed for 4 additional years untreated (dotted line). They are compared to patients treated with a lower dose of GH continuously for 6 years. Note that patients on high dose grew very well during GH therapy but that height SD score was not maintained when GH supplementation was withdrawn (From de Zegher et al. [75]. Reprinted with permission from Oxford University Press)

results from several studies from which the following conclusions can be drawn: (1) GH treatment increases adult height in the short child born SGA. (2) Meaningful gains require a dose of at least 33 μg/kg/day on average and several years of treatment. (3) An increase of about 2 SDS in height over the SDS at treatment initiation can be expected when GH is given for 5 years or more. (4) A modest gain in SDS will occur in the absence of treatment.

Though the growth-promoting effects of GH on such patients is clear, many questions remain in terms of patient selection criteria, dosage and dosing schedules, and monitoring for side effects. Continuous versus intermittent schedules have been evaluated [75]. Short-term treatment makes some sense since the underlying growth rate of patients may be near normal. In theory, therapy that could boost a patient to a higher percentile growth channel might be all that is needed for long-term benefit. Data thus far supports this concept but suggest that the effect is not complete. Figure 4.2 shows the result from a study that compared a short high-dose period of treatment with a more moderate sustained therapy. The high-dose group lost some ground in the years following GH withdrawal such that after 5 years, heights were equivalent. Data such as these has led some to propose intermittent dosing schedules for treatment, though continuous treatment is the more common approach.

4.5.2 Criteria for GH Therapy

Treatment should be limited to those patients in whom short stature is at least moderately severe (<2.5 SDS) and where there is little expectation of meaningful catch-up growth over the next several years. If significant catch-up is going to occur, it is usually evident during the first year of life. Since patterns can be variable, careful measures over at least 6 months (preferably 12) should be performed to assess underlying growth velocity in all patients prior to treatment. Patients that present after age 2 with persistent short stature typically have a growth rate in the low-normal range and are unlikely to show substantial improvement in height SDS over the next several years, with the possible exception of babies born very prematurely. Assessing final height prognosis with a bone age measure is not helpful because the patients are generally quite young and may have a pathological condition, both of which render the prediction inaccurate. Since younger patients appear to respond better, treatment can be initiated once it is clear that the current growth velocity will be insufficient to normalize height. Patients in mid-childhood would likely benefit as well, but those well into puberty are at a disadvantage because of limited time before epiphyseal fusion; however, meaningful height gains have been reported with

higher GH doses especially when combined with a GnRH analog [80].

Few studies evaluate the growth response of patients with specific genetic syndromes in numbers sufficient to assess efficacy, with the exception is Russell-Silver syndrome. These patients, although typically shorter than the average SGA patient, appear to show an equivalent growth response to GH [81]. For other syndromic patients, in the absence of data to the contrary, it is reasonable to consider that the responses to GH may be equal to that of non-syndromic patients.

Measures of GH secretion, though frequently performed, do not appear to predict growth response and were not recommended in the 2007 consensus unless "growth velocity is persistently reduced and signs of GH deficiency or hypopituitarism present." The author's approach is to measure IGF-1 and IGFBP-3 and only perform standard GH stimulation testing if these parameters are subnormal or there are other reasons to suspect pituitary involvement. If the IGFs and measures of GH release are all low, this suggests that a lack of GH is limiting the current skeletal growth and the need for supplementation seems clear. Above-average concentrations of IGF-1 may signify IGF resistance in a child with both pre- and postnatal growth failure [28, 46, 47]. Baseline IGF-1 also may be useful to help interpret serum concentrations measured during therapy. However, since many patients with apparently normal GH secretion respond to therapy, the testing does not necessarily alter the decision to treat.

4.5.3 GH Dosing and Monitoring

Though relatively high doses may be ultimately required for the best growth response (the USFDA approved dose is 70 μg/kg/day), beginning therapy at a dose around 40–50 μg/kg/day offers certain advantages. Since the response of patients is highly variable, acceptable improvement may be observed on such a regimen. After 6–12 months of therapy, the dose may be increased if the growth rate is insufficient to produce catch-up and the medication is being tolerated without safety concern. Alternatively, one could begin therapy at the relatively higher dose in order to achieve more rapid growth initially, keeping the absolute dose constant and allowing the patient to "grow into" a more modest weight-based dose once a satisfactory height percentile is reached. The higher variability of response in this population (likely reflecting the heterogeneous etiologies) is a challenge and supports adopting treatment regimens that are individualized for the patient. Prediction models have been developed and may be useful in some cases [82, 83].

Patients should have reevaluation at a minimum of 6 months intervals with careful history and physical examination, seeking signs and symptoms of scoliosis, other orthopedic abnormalities, pseudotumor cerebri, and assessment of growth response to therapy. Early on there was concern that the relatively high GH dosing would lead to glucose intolerance or diabetes in this population because being born SGA already imparted increased risk for these conditions. Consequently prior recommendations included periodic measurement of glucose tolerance and/or insulin sensitivity along with assessment of lipids. Such measures now appear unwarranted [68]. As noted previously GH therapy in short children born SGA reduces insulin sensitivity but rarely causes glucose intolerance, and metabolic parameters return to baseline or even improve following GH withdrawal [84].

Periodic assessment of circulating IGF-1 during GH therapy has been recommended for patients receiving GH [85]. It is perhaps most valuable for determining dose replacement of GH for adults with GH deficiency [86] but has been advocated as a safety measure for other conditions where GH is used [87, 88]. The notion is that doses of GH that produce supranormal levels of IGF-1 may be hazardous to the patient, perhaps increasing the risk of future malignancy or portending other GH-related side effects. The theory is reasonable, but its value is unproven, and for the short child born SGA, it may be particularly problematic since it is possible that a degree of IGF-1 resistance plays a role in growth limitation. In that circumstance, raising IGF-1 concentrations above normal might be needed, especially if the growth response is less than that desired [89]. Furthermore the range of IGF-1 blood levels is very wide among normal children suggesting varied sensitivity of individuals to IGF or that circulating IGF-1 is a poor reflection of signal strength at the cellular level. In fact, titrating GH dose by circulating IGF-1 in hopes of optimizing treatment appears less effective

than standard dosing strategy [90]. Clearly a lot more work needs to be done to define how measures of GH secretion and action can be used in the selection of therapeutic regimens for patients. In the meantime a reasonable approach is to use GH in the dose ranges as outlined above, with adjustments to achieve growth rates that resulting in catch-up when the patient is below the 5th percentile for height and prescribe GH at doses that will maintain normal growth when the patient is solidly in the normal range for height. With this approach IGF-1 levels are often within the upper normal range during the growth maintenance phase.

4.6 Outcomes and Complications

The goal of GH therapy is a height in the normal range without complication. Although this is achieved in the majority [74], a significant number still fall short of the goal (Fig. 4.3) because treatment was initiated too late, the short stature too severe, or the response to GH therapy suboptimal. Younger age and greater height at start of treatment are associated with improved outcome. Nearly all studies show a definite dose-response relationship when short-term growth is measured; however, the benefit of higher doses over the long term is less clear [78]. Lem et al. have shown that increasing GH dose to 2 mg/m^2/day (approx. 67 μg/kg/day) during puberty increases adult height which can be augmented further by adding a gonadotropin-releasing hormone analog [80]. Other therapies that might augment the response include adding recombinant human IGF-1 to the treatment and slowing epiphyseal maturation with aromatase inhibition. These treatments have been evaluated in limited clinical trials and demonstrate efficacy but are considered experimental at this time [91, 92].

An important issue facing the treating physician is the possibility of adverse effects. Even though GH, used for decades in large numbers of children, rarely causes serious morbidity [93], use in the short child born SGA presents new issues. First, the dose of GH prescribed is higher than that used for most patients in the past. Clearly, GH is being used as a pharmacological agent to stimulate the reluctant biologic pathways involved in somatic growth. Hence, the side-effect profile of GH may be altered now that the dose is increased. Second, this patient population may have unique susceptibilities to certain pharmacological properties of GH. The

Fig. 4.3 Final height of children born SGA treated with GH. Open triangles are subject at treatment initiation. Closed triangles are the same individuals at final height (From Dahlgren and Wikland [77]. Reprinted with permission from Nature Publishing Group)

issue of insulin resistance is pertinent. Epidemiological and experimental data indicate that humans born SGA have an increased incidence of obesity and type II diabetes as adults, with the implication that the period of fetal undernutrition results in a resetting of intrinsic insulin sensitivity [94, 95]. Patients with IUGR already show evidence of decreased insulin sensitivity as children [96]. Could GH, which diminishes insulin sensitivity, add to the risk of developing obesity, hyperlipidemia, and insulin resistance (syndrome X) later in life? Data from patients treated thus far show only modest effects on basal insulin levels [97] and no clinically significant impact on glucose or lipid metabolism [98]. Moreover, another report found that 6.5 years after completion of GH treatment, measures of carbohydrate metabolism were no different in those treated compared to untreated adults born SGA and that if anything, lipid profile and blood pressure indices were better in those treated [99]. Nonetheless the number of patients treated with the highest doses remains too few to detect uncommon, but significant, long-term sequelae.

Additional important theoretic or potential complications of pharmacological GH therapy include orthopedic problems such as carpal tunnel syndrome in adults and scoliosis and slipped capital femoral epiphysis in children. Another consideration is the possible increased risk of malignancy [86, 100], which has been difficult to quantify. Analyses of risk of GH treatment in patients who developed GH deficiency as a consequence of tumor treatment do not indicate much of a role for GH in the development of relapse [101, 102]. However, large epidemiological studies find that risks for prostate cancer [103] and breast cancer [104] are increased for people with serum IGF-1 concentrations in the upper ranges of normal. The studies do not prove cause and effect, but tumor cells in culture frequently express IGF-1 receptors and replicate in response to IGF-1 [105]. This raises the question as to whether the high IGF-1 levels that generally accompany high-dose GH therapy might increase risk over the long term. Despite these concerns, after careful review the Pediatric Endocrine Society Drug and Therapeutics Committee recently concluded that GH could be safely administered to children without known risk factors for malignancy [106].

The majority of published studies of GH safety in children have focused on short−/intermediate-term events. Only recently has it been possible to begin to assess late effects in adults who were treated with GH during childhood for nongrowth hormone-deficient conditions like that found in the short child born SGA. SAGhE (Safety and Appropriateness of Growth hormone treatments in Europe) is an ongoing large epidemiologic study (n = approx. 24,000) that attempts to examine such long-term outcomes of treatment [107]. Initial reports have suggested that all-cause mortality and stroke may be increased in adults treated with GH in childhood and that the risk may increases with higher doses, at least for mortality [108, 109]. However, the numbers of adverse events detected were very low, the appropriateness of reference populations challenged, and results not yet confirmed. Thus, a recent, society-sponsored, position paper concluded "the aggregate evidence from available data sets does not support an association between ongoing or previous GH therapy and all-cause mortality" [89].

Determining the true benefit of GH treatment in this population remains a significant challenge. This is important in light of the expense of the treatment and the potential for long-term adverse effects. While there is little doubt of the efficacy (i.e., growth stimulation) of GH therapy, exactly how much the treatment benefits the individual, in terms of quality of life, and society as a whole is unclear. There are data supporting a lower quality of life in short children and indications that GH therapy improves this [110, 111]. However, much more work needs to be done in this area [112], and we, as healthcare providers, must continue to ask whether GH treatment will be of meaningful benefit for an individual patient and continue to press for clarity on the risks and benefits of GH therapy for the short child born SGA.

Despite these justifiable concerns about GH safety, most data published to date are reassuring. Bell et al. [113] reported on the safety of GH in over 55,000 children with nearly 200,000 patient years of therapy. Even though individuals born SGA were not analyzed separately, it is clear that the risk of serious adverse effects is very low in individuals without predisposing risk factors, and there was no indication that GH therapy caused new malignancies or diabetes.

Case Study

A 30-year-old professional woman becomes pregnant with triplets. She is healthy, and the early phase of pregnancy is relatively uneventful. However, ultrasonography at 25 weeks gestation shows discordance in size with one of the triplets being below the 10th percentile for estimated fetal weight (Fig. 4.4). Subsequent ultrasonography shows growth attenuation, and intrauterine growth retardation is diagnosed. The babies are born at 27 weeks gestation due to premature rupture of membranes. The SGA child requires ventilation for 21 days and 8 weeks of nasogastric feeding to promote weight gain prior to discharge. Laboratory studies to determine the etiology of the intrauterine growth retardation are negative. During the subsequent 3 years, despite efforts to optimize nutrition and encouraged higher caloric intake, the child's height remains several standard deviations below the mean for age and sex (Fig. 4.4).

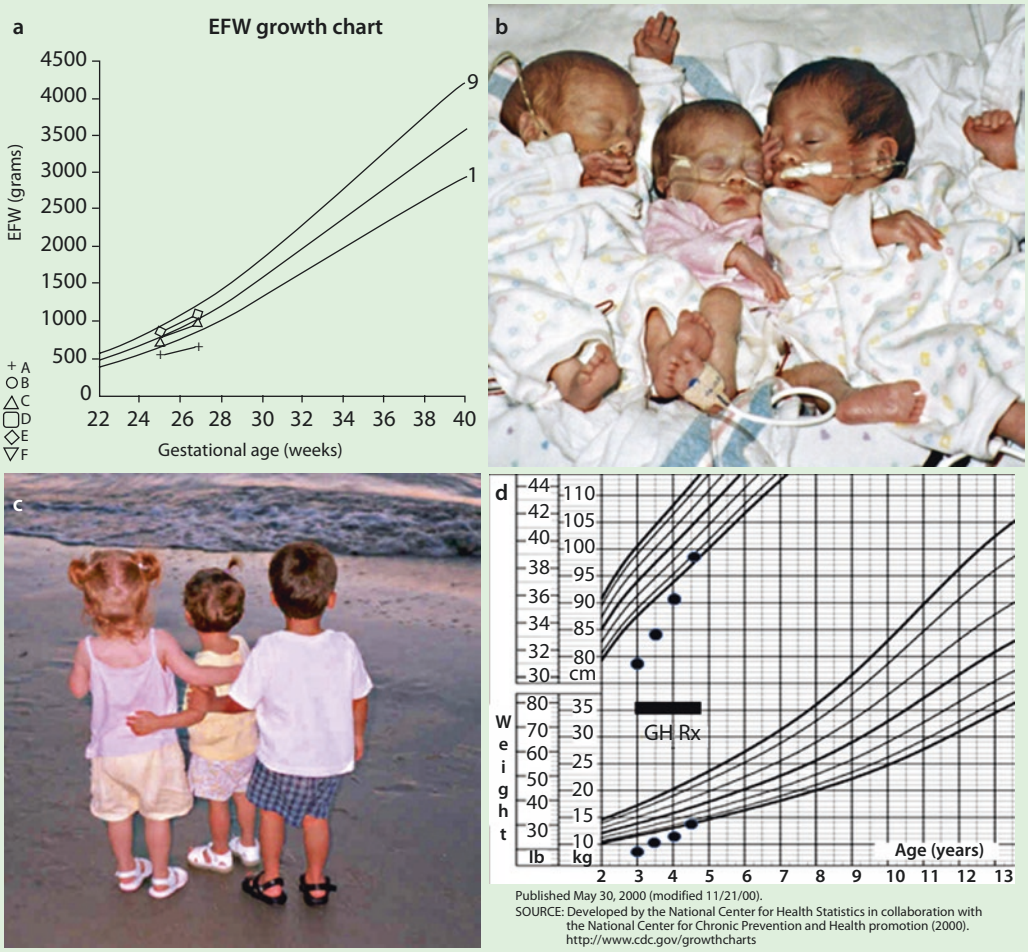

Fig. 4.4 Case study. **a** Prenatal growth of triplets. **b** Triplets at birth. **c** Triplets at age 3 years. **d** Growth response of IUGR triplets to GH treatment

4.7 Summary

There are many reasons why a child is born SGA; at least half the time, the cause is not evident. Nevertheless, 80–90% of the children will show improved growth after birth and reach heights in the normal range by age 2 years. For those that remain short, stature below the 3rd percentile is likely to persist. Growth hormone treatment is effective at stimulating growth and improv-

ing adult height and is generally safe. Tests of growth hormone secretion and action are typically not very helpful in making specific diagnoses or predicting response in this population. Careful monitoring during treatment and attention to appropriate dosing is important because responses are variable. Some patients will not respond sufficiently, and treatment is unlikely to benefit them. Advances in our ability to make specific diagnoses using molecular genetic techniques will likely improve our ability to establish causes of IUGR and determine appropriate treatment regimens.

Review Questions

Consider the case study for the following questions.

1. Immediately following the birth of her child, the mother asks what are the chances that her small baby will grow and be "on the growth chart" by age 2 years. Which of the following best approximates the percentage of children born SGA who show such catch-up growth?
   A. 80%
   B. 40%
   C. 20%
   D. 10%
2. At age 3 years, growth hormone therapy is being considered. Which of the following tests should be performed at this time if not done previously?
   A. Sweat chloride
   B. Test of growth hormone release
   C. Karyotype
   D. Oral glucose tolerance test
3. GH treatment is initiated at age 3 years at a weight-based dose of 50 μg/kg/day. Substantial catch-up growth occurred and at age 4 and 1/2 she is now at the 10th percentile for height. Which of the following represents the best approach to continued management?
   A. Discontinue GH as she has now reached a height in the normal range.
   B. Double the dose of GH to account for the declining response expected after 2 years of therapy.
   C. Adjust GH dose to achieve a circulating IGF-1 concentration that is within 1 standard deviation of the average for age and sex.
   D. Maintain current regimen of weight-based dosing with continuing monitoring of growth response.

Answers

1. (A) Although results vary by study, all find that the majority of straightforward cases of IUGR show catch-up growth in the first 2 years of life. The largest study [9] indicated that 84% catch-up by age 2.
2. (C) According to the guidelines [68], Turner syndrome needs to be excluded in short SGA girls. Patients with growth failure due to cystic fibrosis (tested by sweat chloride) do not show prenatal growth retardation. Tests of growth hormone release are only indicated when there is suspicion. In this case it would appear that the fetal growth retardation was a result of placental compromise/competition. Although one might be concerned about changes in insulin sensitivity that occur in conjunction with being born SGA and which might be aggravated by growth hormone treatment, specific diagnostic measures such as an OGTT are not recommended.
3. (D) The idea that the short child or an SGA would maintain a relative stature once in the normal range was disproven scientifically [75]. The patient has responded well so there is no reason to increase the dose which would exceed the label dose. IGF-1-based dosing has not proven advantageous in this condition.

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Growth Hormone Therapy in Children with Prader-Willi Syndrome

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Key Points

In infants and children with PWS, GH therapy:

- Increases linear growth, lean body mass, and bone density, decreases fat mass, and favorably affects markers of metabolic health in children with PWS
- Enhances early-life motor skill and possibly cognitive skill development
- Requires concomitant screening for and correction of sleep-related breathing disorders
- Has efficacy that is not significantly influenced by PWS genotype

5.1 Introduction

Prader-Willi syndrome (PWS) is a neurogenetic developmental disorder caused by the absence of expression of genes on the paternally inherited chromosome 15. This was initially described by Prader, Labhart, and Willi in 1956 and is characterized by obesity, hypotonia, hyperphagia, delayed motor skill acquisition, short stature, mental retardation, hypothalamic dysfunction, and hypogonadism [1]. The genetic abnormality positioned on chromosome 15 (q11–13) is a critical region active only in the paternally inherited chromosome. PWS occurs due to deletion of the paternal allele (approximately 70–75% of cases) or presence of maternal uniparental disomy (UPD, approximately 25% of cases). Thus, PWS was the first human disorder associated with imprinting [2, 3]. Rare cases of PWS involve translocations, molecular defects, or errors of the imprinting center. Although several genes and gene products of the PWS region of chromosome 15q11–13 have been identified, the specific genes involved in the pathogenesis are not completely understood [4, 5]. With an incidence of 1 in every 12,000 births, PWS is the most common syndrome causing marked obesity.

Many features of PWS suggest hypothalamic dysfunction, some with endocrine implications including, but not limited to, short stature, hyperphagia, sleep disorders, deficient growth hormone (GH) secretion, central hypothyroidism, and hypogonadism [6]. Other fundamental defining features in children with PWS include abnormal body composition with increased fat mass and decreased lean body mass [7]. Infants with PWS typically demonstrate poor weight gain and hypotonia that precede hyperphagia and obesity. However, even at this young age, percent body fat measurements are increased [8]. Early abnormalities in body composition in PWS, therefore, are present prior to the onset of characteristic hyperphagia and progressive obesity and are qualitatively similar to that observed in patients with GH deficiency (increased percent body fat and decreased muscle mass). Diminished GH secretion in children with PWS is well documented [9] and distinguished from reduced GH secretion observed in nutritional obesity by relatively low IGF-1 levels in addition to the aforementioned distinct abnormalities in body composition.

To alleviate abnormalities in linear growth and body composition, and because most children with PWS show evidence for subnormal GH secretion, recombinant human GH (hGH) therapy for children with PWS has been actively investigated. Several studies show that hGH treatment increases growth and improves (but does not normalize) body composition, energy expenditure, and strength and agility in children with PWS [10–14] and increases growth and enhances gain of motor skills in *infants and toddlers* with PWS [15, 16].

This chapter reviews current knowledge regarding causes of and potential treatments for impaired growth, body composition, and physical function observed in children with PWS. Growth failure due to PWS has become an approved indication for hGH therapy in the USA (with disordered body composition being an additional hGH indication in Europe). However, treatment of these children has raised awareness of other potential benefits of hGH therapy (improvements in body composition and physical strength and function and increased energy expenditure) – which may exceed linear growth promotion in importance to children with PWS.

5.2 Growth and Growth Hormone in PWS

Linear growth of children with PWS is characterized by mild to moderate intrauterine (average − 1 SDS) as well as postnatal growth delay. Historically between ages 2 and 4, when caloric intake increases and obesity begins to develop, growth rates normalize, but catch-up in

length/height relationship is unusual. The hands and feet tend to be particularly small. Childhood growth rates are close to normal, but lack of normal pubertal growth frequently results in reduced adult stature (mean 152 cm for adult PWS male, 146 cm for adult PWS female) [17, 18]. Growth impairment in PWS cannot be attributed to any known intrinsic bone or cartilage abnormality. Consequently, attention has focused on possible defective hypothalamic regulation of the growth process. Growth hormone responses to insulin, arginine, clonidine, L-dopa, or GH-releasing hormone (GHRH) are low-normal or blunted in PWS, as are sleep-induced GH secretion and 24-h integrated GH concentrations. One study of 54 consecutive children with PWS revealed GH-deficient levels (<10 ng/mL following clonidine provocation) in all patients [10].

Analysis of these results is complicated by the fact that GH secretion is often suppressed in non-GHD obese individuals and is partially returned toward normal with weight loss [19]. The reason for this effect of obesity on GH secretion remains unclear, although negative feedback by IGF-1 levels sustained by a state of overnutrition has been proposed [20]. Nevertheless, substantial evidence supports the existence of a true GH-deficient state in PWS. Children with PWS display borderline normal or diminished growth rates and delayed skeletal maturation, in contrast to normal or accelerated growth and bone age advancement typically seen in healthy non-PWS obese children. It is possible that overnutrition and its effects on insulin and IGF-1 production in children with PWS ameliorate growth retardation and skeletal maturation delay normally associated with severe GH deficiency, as it does in some children following craniopharyngioma surgery. Insulin levels are lower in children with PWS than in "healthy obese" children, suggesting relatively heightened insulin sensitivity compatible with reduced GH secretion and action [21].

Levels of IGF-1 are relatively low in children with PWS (mean − 1.5 SDS) compared to normal-weight age-matched children but not as low as in those with severe GHD. This moderation in IGF-1 reduction likely reflects responsiveness of IGF-1 levels to food intake as well as GH secretion; thus, moderately reduced IGF-1 levels in obese PWS children support underlying GHD [22]. That nutrition-stimulated IGF-1 production is helping to sustain near-normal growth in children with PWS is supported by the observation that strict caloric restriction curtails growth more severely in PWS patients than in obese children. Additionally, IGF-1 levels tend to be slightly higher in PWS children treated with hGH compared to non-PWS children treated with hGH [22].

Finally, even children with PWS with normal weight/height ratios show low GH responses to provocation [23]. While a normal weight/height does not indicate normal body composition in PWS (which could theoretically affect GH secretion), these important differences in body composition between PWS patients and individuals with "simple" obesity constitute a strong indication of abnormal GH secretion in PWS. The role of GH insufficiency during early development is discussed below as this relates to accretion of lean body mass and fat mass.

Early studies of the effect of exogenous hGH treatment of children with PWS focused on growth rate acceleration and improvement in stature as primary therapeutic goals. Multiple groups have consistently demonstrated increase in average growth in children with PWS; average growth rate increased from −1.9 to +6.0 SDS during the first year of hGH administration (0.1 IU/kg/day) compared to a decrease from −0.1 to −1.4 SDS in non-treated PWS children. However, now longer-term studies (5 years) have provided additional evidence supporting a significant and sustained growth response to daily hGH administration [14, 24, 25].

While studies have consistently shown that hGH increases short-term growth velocity in children with PWS, questions regarding long-term change in height had remained. In a comparison of the linear growth in 21 children with PWS treated with hGH for 6 years to that of 27 age- and gender-matched children with PWS naïve to hGH, the hGH-treated children exhibited significantly greater height (131 ± 2 vs. 114 ± 2 cm; $p < 0.001$) [26]. Similarly, 36 children with PWS demonstrated a significant increase in mean height of 1.2 standard deviations during 2–3 years of hGH therapy, and the increment of improvement did not differ significantly by subtype (deletion versus UPD of 15q11-13 region). In addition, response to GH in 56 other children with PWS also did not differ by genetic subtype during the first year of therapy [27]. With regard to eventual height attainment, PWS children with PWS receiving long-term hGH therapy reached a mean adult height standard deviation score (SDS)

of −0.3 (compared to −3.1 SDS in untreated controls, $p < 0.0001$). Together, these findings indicate that hGH is effective at increasing growth rate and adult height attainment in children with PWS regardless of genetic subtype [17, 28].

5.3 Body Composition in PWS

Children with PWS display markedly abnormal body composition characterized by very high (predominantly subcutaneous) fat mass and very low lean body mass. Combined with short stature, relatively low IGF-1 (insulin-like growth factor-1) compared to BMI-matched peers, and low GH responses to provocation, this phenotype strongly resembles other GH deficiency (GHD) states. Recognition of GHD as a possible component of PWS led to enthusiasm for trials of hGH therapy in children with PWS in an effort to improve body composition and height.

Infants with PWS demonstrate hypotonia and often have failure to thrive due to poor sucking and swallowing reflexes. However, elevated body fat determined by skinfold measurements in underweight infants with PWS suggests early alterations in their body composition prior to the development of obesity [29], and this has been confirmed by determination of reduced lean body mass and energy expenditure [8]. Between the second and fourth year of life, progressive obesity usually occurs primarily as a consequence of excessive caloric intake but also due to decreased energy expenditure and reduced physical movement. The inherent body composition abnormality of childhood PWS dominates responses to caloric restriction, so that while weight gain can be minimized, the ratio between lean body mass and fat remains abnormal. Since resting energy expenditure (REE) is largely determined by the metabolic activity of lean body mass, REE is significantly reduced in individuals with PWS (~60% of predicted caloric utilization for non-PWS individuals with similar body surface area) [8]. This extremely low "caloric tolerance" accounts for progressive weight gain in PWS children in whom caloric restriction has been successfully maintained.

The body composition seen in PWS [8, 30] is clearly distinguishable from the parallel increase in lean body and fat mass observed in overnourished obese but otherwise healthy (non-PWS) individuals. The distinctive replacement of lean body mass by fat mass in PWS suggests that diminished GH secretion is secondary to hypothalamic dysfunction rather than obesity and that abnormal body composition and reduced energy expenditure, linear growth, muscle strength, and pulmonary function might be improved in PWS by hGH therapy [12, 31–33].

5.4 Effects of hGH Treatment on Body Composition

Growth hormone is an anabolic hormone that promotes development of lean muscle mass and stimulates lipolysis of fat for energy usage. Administration of hGH to GH-deficient children not only restores linear growth but also promotes growth of lean body mass, decreases fat mass by increasing fat oxidation and total body energy expenditure, increases bone mineral density following an initial period of increased bone resorption, and improves cardiovascular risk factors [34]. Consistent evidence shows that hGH therapy for 12–48 months in children with PWS decreases fat mass, increases lean body mass, increases linear growth [12, 22, 35], and, in one study, increases fat utilization [10]. Similarly, children with PWS respond to hGH therapy with increased height and improvements in body composition. In this population of children, these "non-height" clinical effects, which impact physical function (see later section), are arguably more valuable than change in growth velocity [3, 36–39] (Fig. 5.1).

Like effects on growth rate, prolonged effects of hGH upon body composition are also dose-dependent. Further changes in body composition (lack of increase in fat mass and increase in lean body mass), growth velocity, and REE occurred with administration of either 1 mg/m^2/day or 1.5 mg/m^2/day of hGH, but not with 0.3 mg/m^2/day [11]. Prior improvements in BMD and strength and agility, which occurred during an initial 24 months, were sustained during these additional 24 months (48 months total) regardless of dose. The rate of change in body composition slowed but did not regress during more prolonged hGH therapy at doses ≥1.0 mg/m^2/day. It is important to note that changes in BMD and body composition occur with normal growth and advancing age. Nevertheless, changes in these PWS children exceed those reported over a 24-month period in healthy non-PWS late-childhood subjects based on reference data for BMD and fat-free mass.

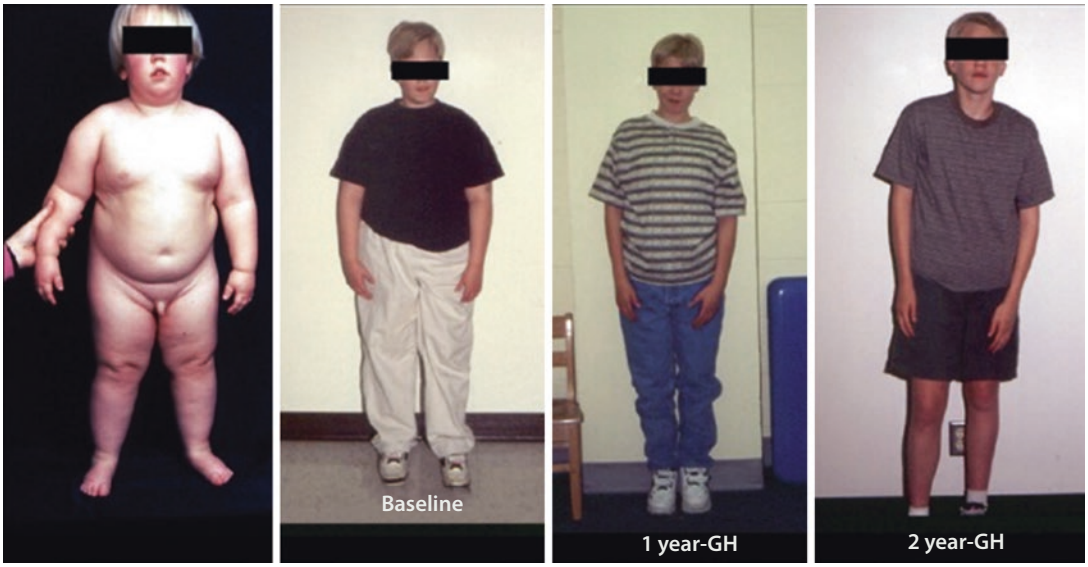

Fig. 5.1 Photographs of a child with PWS as a toddler and over a few years of GH therapy demonstrating changes in body composition

Response of children with PWS to hGH is greatest during the first 12 months. Thus, a diminution in response to hGH during prolonged hGH therapy, observed in virtually all growth studies of hGH therapy, applies to other GH metabolic effects in children with PWS.

5.5 Effect of hGH Treatment on Energy Expenditure

Deficiency of GH is associated with lipogenesis and fat storage predominating over the accretion of lean mass, even in the absence of overt obesity. Preference for fat utilization as an energy source is reflected in a reduction of respiratory quotient (RQ). RQ normally ranges from 0.7 (strong predominance of fatty acid oxidation) to 1.0 (exclusive oxidation of carbohydrate) to <1.0 (indicating lipogenesis from carbohydrate). Two years of hGH treatment in PWS children was associated with a decrease in RQ values (0.81 + 0.07 at baseline to 0.75 + 0.06 at 24 months, $p < 0.05$), indicating increased utilization of fat for energy. Thus, compared with non-hGH-treated PWS controls, hGH-treated PWS patients demonstrated a shift in energy derived from oxidation of fat, coinciding with reductions in fat mass. Clinically, reduced body fat can be seen, consistent with an increase in fat utilization.

5.6 Effects of hGH on Strength and Agility

Specific changes in increased muscle mass and decreased body fat are seen with hGH therapy compared to non-treated PWS children. Documented changes in physical function (strength and agility testing) in PWS children treated with GH, which translate to acquisition of new gross motor skills, appear to be important "real-life" benefits for these children. One of the most debilitating aspects of PWS is hypotonia. Increases in motor strength and function therefore are an extremely important area of assessment for children with PWS. In one study, a modified Bruininks-Oseretsky test was used to test strength and agility, with four subtests for different muscle groups of the body. Children with PWS who received early hGH treatment demonstrated improved functional motor strength of increased standing broad jump with an adjusted least squares mean of 22.9 ± 2.1 in. vs. 14.6 ± 1.9 in. ($p = 0.01$) and sit-ups 12.4 ± 0.9 vs. 7.1 ± 0.7 ($p < 0.001$). Clear trends were seen in the two other areas of the Bruininks-Oseretsky testing, including improved agility run (8.9 ± 1.3 s vs. 11.6 ± 1.1 s; $p = 0.1$) and weight lifting (63.9 ± 6.6 vs. 49.6 ± 5.7; $p = 0.09$), although these did not reach statistical significance. While these findings suggest that hGH therapy may potentially lessen some disabilities associated with PWS,

determining the long-term value of this intervention requires demonstration of sustained benefits during more prolonged therapy.

Substantial documentation supports beneficial effects of hGH therapy on improving body composition and linear growth in children with PWS. However, perhaps of greatest importance to patients and their families is the hope that hGH therapy would improve the child's physical strength, activity, and ability. Early reports included anecdotal reports of dramatic gains in physical activity abilities, and many parents of subjects describe striking improvements in physical stamina, strength, and agility. Specifically, these include new gross motor skills (e.g., independently climbing up the school bus steps, carrying a gallon carton of milk at the grocery store, participating in a normal gym class without restrictions, being able to join a karate class). More recently, these claims have been supported in controlled studies [10, 11, 14, 24, 32, 35].

The authors' research includes objective measures of changes in physical function during hGH treatment, including a timed run, sit-ups, and weight lifting [40]. Improvements in running speed, broad jump, sit-ups, and arm curls after 12 months of hGH treatment compared to controls were documented. Following 48 months of hGH treatment, improvements in broad jumping and sit-ups were maintained, while further improvement was found in running speed and arm curls. Increases in both respiratory muscle forces were seen after 1 year of therapy and maintained at 24 months. Nevertheless, in spite of these gains in physical function, PWS children still scored well below 2 SDS compared to non-PWS children for all parameters studied.

5.7 Does hGH Significantly Change the "Natural History" of Prader-Willi Syndrome During Childhood?

While the findings described above suggest that measured improvements in strength and agility are associated with "real-life" functional benefit to the children and their families, lack of a blinded, placebo-controlled study design admittedly weaken the scientific validity of these findings. We addressed this problem by comparing body composition, physical function, linear growth, and lipid metabolism in children with PWS who received early-life (i.e., started prior to 2 years of age) hGH treatment of to an age-matched group of hGH-naïve subjects recruited for a previous randomized, controlled study of hGH treatment for school-age children with PWS (Table 5.1). These data demonstrated that PWS children treated with hGH from early in life demonstrated lower body fat (mean, 36.1 ± 2.1% vs. 44.6 ± 1.8%, $p < 0.01$), greater height (131 ± 2 cm vs. 114 ± 2 cm; $p < 0.001$), greater motor strength [increased standing broad jump 22.9 ± 2.1 in. vs. 14.6 ± 1.9 in. ($p < 0.001$) and sit-ups 12.4 ± 0.9 vs. 7.1 ± 0.7 in 30 s ($p < 0.001$)], increased HDL cholesterol (58.9 ± 2.6 mg/dL vs. 44.9 ± 2.3 mg/dL, $p < 0.001$), decreased LDL cholesterol (100 ± 8 mg/dL vs. 131 ± 7 mg/dL, $p < 0.01$), and no differences in fasting glucose or insulin. Thus, this comparison of school-age children with PWS treated with hGH since infancy showed significant gains in muscle mass, energy expenditure, and motor milestones [35].

Figures 5.2, 5.3, and 5.4 illustrate the differences between children with PWS who received hGH from an early age to hGH-naïve PWS children.

5.7.1 Body Composition and Growth (Fig. 5.2)

Percent body fat and muscle mass (lean body mass) were assessed by DXA. Lower percent body fat was evident in early hGH treatment children with PWS when compared to the hGH-naïve PWS subjects (adjusted least squares means of 36.1 ± 2.1% vs. 44.6 ± 1.8%; $p = 0.006$). Fat-free mass (muscle mass) was greater in the children with PWS who received early hGH treatment-treatment group compared to the hGH-naïve PWS subjects (24.1 ± 1.1 kg vs.16.7 ± 0.9 kg; $p = <0.001$).

5.7.2 Carbohydrate and Lipid Metabolism (Fig. 5.3)

Effects on metabolic health have typically focused upon carbohydrate and lipid metabolism. Carbohydrate and lipid metabolism were evaluated using fasting AM blood samples.

Table 5.1 The least squares means (± SE) adjusted (for age and gender) of body composition, motor function, and lipid profile parameters for the two cohorts

	GH-naïve cohort ($N = 27$)	Early treatment cohort ($N = 21$)	p-value[a]
	Mean[b] ± SE	Mean[b] ± SE	
% Body fat	44.6 ± 1.8	36.1 ± 2.1	0.006
Fat-free mass (kg)	16.7 ± 0.9	24.1 ± 1.1	<0.0001
Height (cm)	114.5 ± 1.8	131.4 ± 2.1	<0.0001
Height z-score	−1.6 ± 0.3	1.2 ± 0.2	<0.0001
Weight (kg)	32 ± 2.3	38 ± 2.6	0.062
Standing broad jump (inches)	14.6 ± 2.0	22.9 ± 2.1	0.012
Sit-ups	7.1 ± 0.7	12.4 ± 1.0	0.0003
20-yard agility run (s)	11.6 ± 1.1	8.9 ± 1.3	0.17
Weight lift repetitions	63.9 ± 6.6	49.6 ± 5.7	0.09
IGF-1 (ng/mL)	112 ± 18	346 ± 20	0.001
IGF-1 SDS	−1.45 ± 0.30	1.39 ± 0.34	0.0001
HDL cholesterol (mg/dL)	44.9 ± 2.3	58.9 ± 2.6	0.0005
LDL cholesterol (mg/dL)	131.3 ± 7.1	100.2 ± 8.0	0.0099
Total cholesterol (mg/dL)	189.9 ± 7.3	177.3 ± 8.2	0.29
Triglycerides (mg/dL)	68.4 ± 10.6	94.2 ± 11.9	0.14
Fasting insulin	7.1 ± 1.3	10.2 ± 1.5	0.14
HOMA-IR	1.4 ± 0.3	2.1 ± 0.3	0.1

[a]Based on two-sided t-test
[b]Least squares mean, adjusted for age and gender

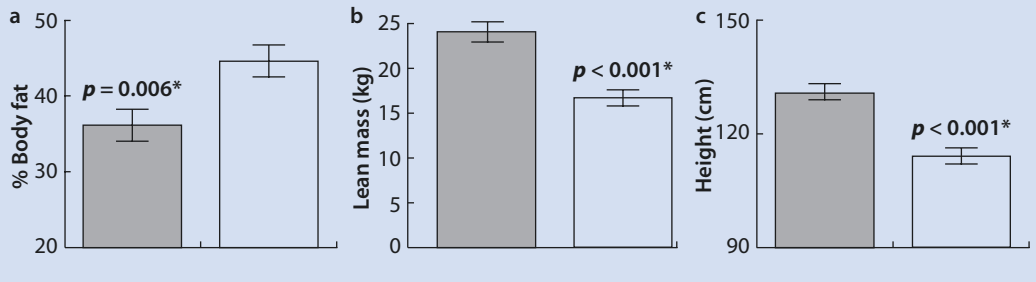

Fig. 5.2 Effect of growth hormone treatment on anthropometrics in PWS (hGH-treated (shaded) vs. untreated). **a** Demonstrates % body fat, **b** demonstrates lean mass (muscle mass), and **c** demonstrates height. * Age- and gender-matched analysis using ANCOVA

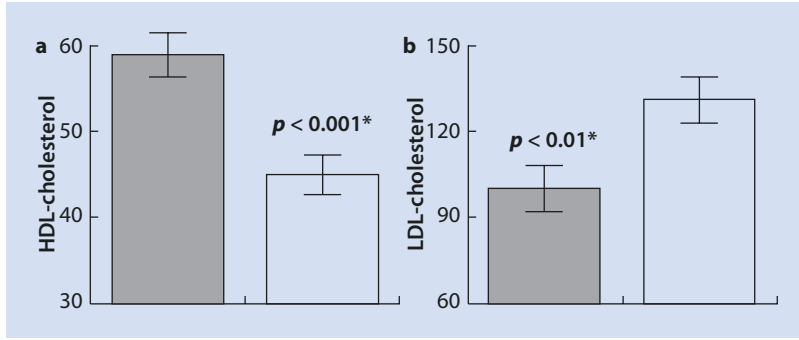

Fig. 5.3 Effect of growth hormone treatment on lipids in PWS (hGH-treated (shaded) vs. untreated). **a** Demonstrates HDL and **b** demonstrates LDL. * Age- and gender-matched analysis using ANCOVA

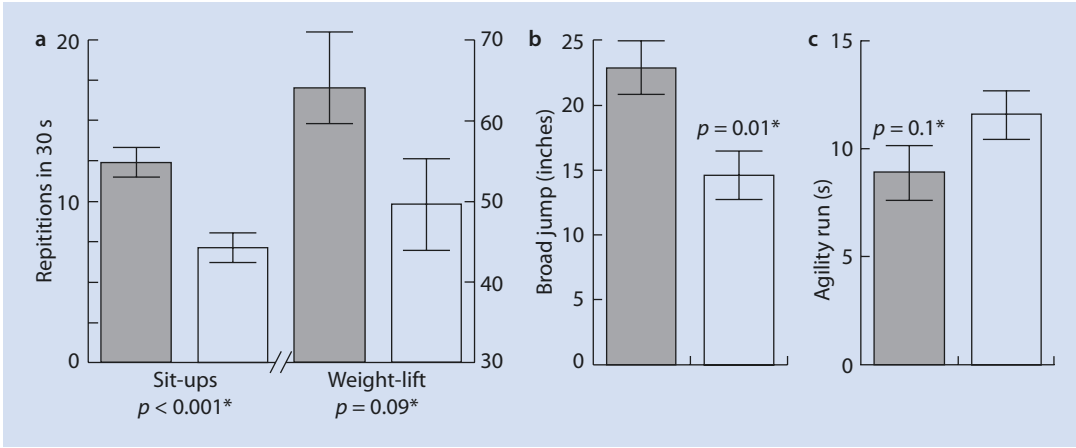

Fig. 5.4 Effect of growth hormone treatment on strength in PWS (hGH-treated (shaded) vs. untreated). **a** Demonstrates repetitions in 30 s, **b** demonstrates broad jump, and **c** demonstrates agility. * Age- and gender-matched analysis using ANCOVA

5.7.3 Motor Strength (Fig. 5.4)

This analysis of similar-aged children with PWS, one group treated with hGH for 6 years and the other naïve to hGH therapy, offers a unique assessment of the degree to which early-in-life hGH treatment alters the clinical course of this disorder. It also extends and tests the validity of findings of previous studies of hGH therapy in children under age 3 with PWS, none of which had a control group for longer than 12 months.

5.8 Effect of GH on Cognition

Cognitive impairment and developmental delay is a common feature seen in children with PWS [41]. Preliminary studies suggest that hGH may help prevent deterioration of certain cognitive skills in children with PWS. A prospective trial involved 50 prepubertal children with PWS (ages 3.5–14 years) who were randomized to receive either 2 years of hGH or no treatment. During the study period, hGH-treated subjects maintained consistent (albeit below normal) standard deviation scores (SDS) on tests of cognitive function (i.e., kept pace with non-PWS peers but did not catch up), while untreated children demonstrated deterioration in SDS in certain cognitive areas (abstract verbal reasoning −0.7 SDS [CI −1.3 to 0.03 SDS, $p = 0.04$] and vocabulary −0.7 SDS [CI −1.3 to 0.07 SDS, $p = 0.03$]) [42]. All study subjects were then enrolled in a longitudinal study of 4 more years of hGH therapy. When subjects were compared to their own baseline function, all children with PWS treated with hGH for 4 years showed significant improvement in verbal abstract reasoning +0.4 SDS (CI −0.1 to 0.7 SDS, $p = 0.01$) and visual-spatial skills +0.3 SDS (CI −0.07 to 0.6 SDS, $p = 0.01$); however, mean

vocabulary and total IQ testing results did not change significantly with hGH [42]. The important role of GH in cognitive function, brain plasticity, and learning merits further investigation. Presently, evidence suggests that hGH treatment does not normalize cognitive abilities of children with PWS but may reduce the disparity in cognitive disability compared to non-PWS peers that otherwise would increase over time.

5.9 Safety of hGH Treatment in PWS

5.9.1 Scoliosis

Scoliosis is common in patients with PWS, prompting concern whether hGH-induced acceleration of linear growth might influence the incidence or progression of scoliosis in PWS patients. Noncontrolled studies report that scoliosis frequency increases during long-term treatment with hGH, an expected finding in a group of growing children at high risk. In contrast, in controlled studies comparing hGH-treated and non-treated children with PWS over a 6-year time frame, there was no difference in the rate of development or severity of scoliosis between groups. In addition, when early evidence for scoliosis is carefully screened for at the time of hGH initiation, the new development of scoliosis in children with PWS appears unlikely. Interestingly, progression of scoliosis during hGH therapy has been associated with lower increases in paravertebral muscle volume measured by CT, suggesting muscle growth may prevent scoliosis progression. While this raises the question whether hGH-mediated increase in muscle mass and strength might actually decrease the occurrence or progression of scoliosis, there are no data yet to support this effect in PWS patients. Taken together, studies indicate that (1) monitoring for scoliosis in this at-risk population, whether treated with hGH or not, is important, and (2) hGH does not significantly increase the risk of scoliosis development.

5.9.2 Glucose Intolerance

Compared to similarly obese children without PWS, hGH-naïve PWS children and adolescents are relatively insulin sensitive. This may be partially attributed to the predominantly subcutaneous (rather than visceral) deposition of fat, but also to reduced counter-regulation by endogenous GH. As a result, there has been concern that hGH therapy could unmask and/or exacerbate glucose intolerance in PWS recipients. However, comparison of long-term hGH-treated and non-treated school-aged children with PWS showed no evidence of significantly higher fasting insulin, glucose, or HOMA-IR levels attributable to hGH treatment. Although hGH treatment has the theoretical potential to increase insulin resistance, clinically significant changes in measures of insulin resistance (e.g., fasting insulin) or more frequent development of impaired glucose tolerance has not been found in children with PWS receiving hGH.

5.9.3 Cardiorespiratory Compromise and Sudden Death

In 2002, the sudden death of a 6-year-old boy with PWS following 4 months of hGH therapy was reported [43]. This was followed by several cases of sudden unexpected death temporally associated with institution of hGH treatment in children with PWS being reported, including a larger review of 64 deaths of youth with PWS [43–48]. Importantly, the rate and causes of death in the 64 cases did not differ significantly between hGH-treated and untreated patients, and it was found obese youth with PWS and those with respiratory insufficiency or illnesses were at increased risk of death. Interestingly, the review demonstrated that 75% (21/28) of deaths in PWS youth treated with hGH occurred within 8 months of starting hGH, a pattern of clustering not seen in youth not treated with hGH. Concern regarding the temporal association of more frequent unexpected sudden death in PWS patients on hGH therapy has continued to drive investigation into whether a causal relationship may exist. A causative relationship between exposure to hGH and sudden death remains uncertain. Answering this question is made extremely complex by the tendency for individuals with PWS to demonstrate morbid obesity, obstructive sleep apnea (OSA), autonomic instability, reduced ventilator sensitivity to hypoxia and hypercarbia, and increased baseline rates of sudden unexplained death – all likely related to underlying hypothalamic dysfunction

and its complications. Factors supporting a causative relationship include the occurrence of most deaths in the first 3–8 months of hGH treatment and the known stimulatory effect of hGH on lymphoid tissue growth, which could increase airway obstruction. Factors arguing against such a relationship include likely prior underestimation of spontaneous mortality in PWS and the observation that hGH therapy for 6–12 months improves respiratory function and carbon dioxide sensitivity in treated subjects. On the other hand, children with PWS treated with hGH have demonstrated improvement in respiratory muscle strength response to carbon dioxide concentration, and nocturnal coupling of heart rate and blood pressure, all of which suggest improved cardiovascular and/or autonomic function [49, 50]. It has also been suggested that partial central adrenal insufficiency could be a contributing factor [51]. However, there remains uncertainty about the clinical consistency of these findings. The difficult question remains: how to incorporate awareness of the possibility of central adrenal insufficiency into clinical recommendations.

Taken together, these observations allow for the possibility of early risk for hGH-treatment-associated sudden death (particularly in early childhood when lymphoid tissue growth is pronounced) followed by long-term benefit. At the same time, cardiorespiratory complications from hGH do not develop in most PWS children receiving hGH, and it remains unresolved whether observed cases of sudden death are related to hGH therapy. Given this uncertainty, recommendations include (1) pre-hGH-treatment clinical and polysomnographic evaluation for sleep-related disordered breathing; (2) withholding of hGH until surgical and/or CPAP intervention and, if needed, weight reduction has corrected significant abnormalities detected; and (3) ongoing clinical monitoring for breathing changes or sleep disturbances during hGH therapy.

Even though the number of cases reported in excess of expected deaths remains small, these occurrences have prompted the inclusion of cautionary language in the drug prescribing information and altered the risk/benefit analysis for hGH therapy in a way distinctly different from other hGH treatment indications. In light of this potentially rare but serious risk of GH therapy, data supporting that such treatment changes the "natural history" of PWS not only in a statistically significant but also in a clinically significant and meaningful way is critical.

Case Study

A 4-month-old boy with Prader-Willi syndrome (PWS) is referred for endocrine evaluation.

History and Physical Examination

The child was born full term but spent 10 days in the NICU for low muscle tone and poor feeding. His mother reports that she has spent many hours trying to get him to nurse and has used multiple styles of nipples to bottle-feed. Testing for PWS was performed by methylation-specific PCR in the NICU and confirmed PWS. Family history is noncontributory. Vital signs are within normal limits. Length is 62 cm (15%), weight 5.7 kg (4%), and BP 90/45. Physical exam is pertinent for a quiet infant with low muscle tone lying on the examination table. His head showed slight bitemporal narrowing, almond-shaped eyes, slightly down-turned mouth, and soft anterior fontanelle. The genitalia showed a very shallow scrotum, with testes palpable but high in the scrotum, and a phallus measuring 2 cm in length. The remainder of the exam was normal.

What Endocrine Concerns Exist for This Patient?

Infants with PWS can demonstrate multiple hypothalamic abnormalities including central hypothyroidism, GnRH deficiency with possible hypogonadism, and growth hormone deficiency (GHD). For this reason determination of free T4 levels must be evaluated, as many newborn screening programs only evaluate for primary hypothyroidism (TSH only). GnRH deficiency can play a role in undervirilization of boys with PWS, including micropenis or undescended testicles. The presence of a very shallow scrotum in this child supports some duration or history of undescended testicles. During early infancy, the role of GH also plays a role in growth of the penis, as well as maintaining euglycemia, as a counter-regulatory hormone, but plays less of a role in linear growth. Monitoring of linear growth over time will provide important information about GH sufficiency. GH also plays an important role in accumulation of lean mass and adipose tissue. As stated previously, substantial evidence indicates that variable degrees of GH deficiency contribute to the body composition and growth phenotype in PWS.

Should hGH Therapy Be Considered at This Time?

The decision to initiate hGH therapy very early in life is often a difficult one. Whether the evidence supports *early initiation* of GH therapy is an important question as well. Infants and toddlers with PWS commonly demonstrate hypotonia, with poor suck and feeding, early failure to thrive, and delay in attainment of developmental milestones. Early abnormalities in body composition are present before the onset of characteristic hyperphagia and progressive obesity. Impaired physical function in PWS during childhood is most often related to body composition abnormalities (increased percent body fat and decreased muscle mass) and hypotonia, rather than to linear growth deficits. Multiple studies indicate that hGH therapy does improve, but cannot normalize, body composition and physical function. Analyses discussed previously in this chapter also confirm that treatment prior to 18 months of age increases mobility skill acquisition, may lessen deterioration of body composition, and accelerates motor development. Additionally, a comparison of 6–9-year-old children with PWS who had been treated with hGH for 6 years to similar-aged children with PWS who were naïve to hGH supports that hGH changes the natural history of PWS by improving body composition, motor function, height, and lipid profiles. However, it is important to recognize that every child is different, and benefits shown in large studies do not always translate to each individual.

Is hGH Therapy Safe in Children with PWS?

To more clearly justify hGH therapy in light of potentially rare but serious risk, it is important to critically assess whether such treatment changes the natural history of children with PWS in not only a statistically significant way but also in a clinically significant and meaningful way that exceeds potential risks. As a precaution, we recommend screening for evidence of central or peripheral apnea by performing a sleep study interpreted by a pediatric sleep specialist [47]. While a normal sleep study does not rule out any possibility of respiratory compromise, this allows for appropriate screening of higher risk and also allows for separate discussion of risks and parental consent. Typical dosing of hGH for children with PWS is 1 mg/m^2 body surface area. Monitoring of IGF-1 and IGFBP-3 levels is also important, as children with PWS have shown higher levels of IGF-1 on hGH treatment compared to non-PWS children [22].

What Other Therapies Should Be Considered?

hGH therapy should be part of a multidiscipline approach to treatment of PWS, including nutrition therapy and exercise/physical therapy. Children with PWS may develop hyperphagia with increased food seeking in early childhood around 3–5 years of age. Without intervention, hyperphagia can lead to marked obesity and an increased risk of associated complications, including diabetes, heart disease, decreased exercise tolerance, sleep apnea, and other problems. Food must be closely monitored or inaccessible between meals to decrease temptation. A child with PWS may always feel hungry, regardless of how much food they have eaten. Anecdotally, children whose parents adhere to a diet and exercise recommendations early on are less likely to become obese and better cope with their constant hunger. There is no evidence that hGH curbs appetite or prevents obesity, although it does influence whether weight gain can be from lean mass (muscle) or adipose tissue [52].

When Should hGH Therapy Be Discontinued?

While every patient is unique, and clinical decisions should be individualized, GH therapy throughout the linear growth years has demonstrated a strong clinical benefit. Currently the literature of metabolic benefits after cessation of linear growth is focused upon changes in lipid metabolism and body composition [53]. Similar to evaluating whether children with childhood GHD still require adult replacement of hGH, we recommend a trial off hGH for 6–9 months to determine if negative effects on lipid metabolism or body composition occurs before deciding if hGH therapy should be resumed after cessation of linear growth. During this time it remains important to counsel patients and families about nutrition and exercise guidance.

5.10 Summary

An abundance of evidence from controlled studies demonstrates that hGH treatment not only increases height but also improves body composition, physical function, and metabolic health in children with PWS. PWS represents one clinical scenario for which there is clear documentation of benefit from hGH therapy that extends far beyond height gain. Particularly when initiated early in life, hGH can significantly and favorably alter the natural history of PWS with low (but perhaps not negligible) risk of adverse effects. Potential hGH-mediated advancement in motor and cognitive development, while encouraging, requires further study. At the same time, questions remain about

necessity of continuing hGH therapy into adulthood and its cost-effectiveness. Whether treated with hGH or not, all individuals with PWS, given their complexities, deserve expert care to maximize health outcomes and quality of life, and hGH therapy should be one part of a multidiscipline approach to therapy.

Review Questions

1. Which of the following is not a clinical feature that suggests the possible diagnosis of Prader-Willi syndrome (PWS)?
   A. Neonatal hypotonia
   B. Hypogonadism
   C. Micrognathia
   D. Bitemporal narrowing
   E. Obesity with low IGF-1 levels
2. Which of the following is not an effect of hGH therapy in children with PWS?
   A. Stimulation of lipolysis
   B. Reduced resting energy expenditure
   C. Increased bone mineral density
   D. Increased physical strength
   E. Increase in HDL cholesterol
3. Which of the following is a potential adverse effect of hGH therapy in children with PWS?
   A. Increased scoliosis
   B. Increase in hemoglobin A1c levels
   C. Respiratory tract obstruction
   D. Increased skin-picking behaviors
   E. Increased food-seeking behaviors

Answers

1. C
2. B
3. C

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Growth Hormone Therapy in Children with Turner Syndrome, Noonan Syndrome, and SHOX Gene Mutations

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Key Points

- Turner syndrome, one of the most common chromosome anomalies in humans, represents an important cause of short stature and ovarian insufficiency in females.
- Turner syndrome is caused by loss of part or all of an X chromosome.
- Turner syndrome must be suspected in any female with short stature.
- Individuals with Turner syndrome are at increased risk for long-term cardiovascular morbidity, due to their predilection for congenital cardiovascular malformations.
- Noonan syndrome is part of a group of disorders known as RASopathies, caused by mutations in the RAS-MAPK signal transduction pathway.
- The most common mutation leading to Noonan syndrome is in the gene PTPN11.
- Cardiovascular abnormalities are also observed commonly in Noonan syndrome, with pulmonic stenosis the most common, followed by hypertrophic cardiomyopathy.
- Disorders caused by defects in the short stature homeobox-containing gene on the X chromosome (SHOX) result in short stature and growth failure.
- Haploinsufficiency of SHOX results in non-syndromic short stature and Leri-Weill dyschondrosteosis and is also the cause of short stature in Turner syndrome.
- Homozygous loss of SHOX expression leads to Langer mesomelic dysplasia, a rare condition characterized by severe short stature and skeletal dysplasia.
- Growth hormone therapy is approved for and improves growth in patients with Turner syndrome, Noonan syndrome, and SHOX haploinsufficiency.

6.1 Introduction and Background

In this chapter, we will discuss three relatively common disorders all associated with varying degrees of growth failure and short stature. Turner syndrome, Noonan syndrome, and SHOX gene deficiency will be reviewed sequentially, including a general description of each condition's clinical features and most important comorbidities. The remainder of the discussion will then focus on the treatment with recombinant human growth hormone of the varying degrees of growth failure in all three conditions.

6.2 Etiology, Clinical Presentation, and Diagnostic Evaluation of Turner Syndrome

6.2.1 Etiology

Turner syndrome (TS) is one of the most common sex chromosome anomalies in humans. It occurs in approximately 1 in 2000 female live births, irrespective of ethnic background [1]. Girls with TS have an abnormal or missing X chromosome, which may result in short stature, ovarian failure, cardiovascular abnormalities, neurocognitive problems, as well as an array of potential other health issues [2, 3].

Approximately 50% of girls with TS have the 45,X karyotype. It is believed that >99% of 45,X conceptions will not survive beyond 28 weeks gestation, mainly due to massive lymphatic obstruction and associated heart failure [4]. Twenty percent to 30% of patients may have a structural abnormality of the X chromosome: ring chromosome X, isochromosome of the long arm [i(X)q], or a partial deletion of the short arm (delXp). Thirty percent to 40% have mosaicism with at least two distinct cell lines (45,X/46,XX; 45,X/46,X,i(X); and 45,X/46,XY) [5]. Table 6.1 lists the most common TS karyotypes.

Most of the TS phenotype is caused by haploinsufficiency of certain genes normally expressed on both X chromosomes. To date, one such gene has been decisively identified: short stature homeobox-containing gene on the X chromosome or SHOX [6]. The short stature observed in TS is caused, in large part, by haploinsufficiency of SHOX gene expression in chondrocytes. Haploinsufficiency of certain pseudoautosomal genes (found on distal Xp) has been postulated to lead to the absence or hypoplasia of the peripheral lymphatics, resulting in generalized lymphedema, and/or a cystic hygroma (posterior cervical region mesenchyme lymph collection). A webbed neck, low upward sweeping hairline, and low-set

Table 6.1 Prevalence of TS karyotypes based on data from the Danish Cytogenetic Central Register

Karyotype	Prenatally diagnosed	Postnatally diagnosed
45,X	66%	40%
45,X/46,xx	23%	24%
45,X/46,X,i(Xq); 46,X,i(Xq); 45,X/46,X,i(Xq)/47,X,i(Xq),i(Xq)	3%	10%
45,X/46,X,del(X); 46,X,del(X)	3%	6%
45,X/46,xx/47,xxx; 45,X/47,xxx; 45,X/46,xx/47,xxx/48,Xxxx	3%	11%
45,X/46,X,r(X)	<1%	3%
45,X/46,XY and other karyotypes with Y chromosome material		5%

Data from Mortensen et al. [119]
Prenatal prevalence information was derived from amniocentesis and chorionic villus sampling

Box 6.1 Clinical Characteristics of Turner Syndrome (with Approximate Prevalence)

- Growth failure and short stature – height below the 5th percentile (95–100%)
- Delayed puberty and ovarian failure (~90%)
- Congenital heart disease, including bicuspid aortic valve (~30%), coarctation of the aorta (13%), partial anomalous venous return (10–18%), and aorta dilatation (≥20%)
- Renal malformations and hypertension (35–40% and 30%, respectively)
- Recurrent otitis media (>60%) and sensorineural hearing loss (25–50%; increasing numbers as patients age)
- Lymphedema at birth (>40%), which usually improves with age
- Dysmorphic phenotype, including short neck, low posterior hairline, neck webbing, micrognathia and retrognathia, broad chest, high arch to the palate, cubitus valgus, short 4th metacarpal and metatarsal bones, nail dystrophy, and multiple nevi (with getting older)
- Neurocognitive deficits (nonverbal learning disorder, difficulty with executive function), anxiety, attention-deficit disorder (20–30%)

prominent ears all result from a resolving cystic hygroma. Structural abnormalities of the heart and vascular system such as a bicuspid aortic valve and coarctation of the aorta are more common in TS girls with coexistent cystic hygroma, linking large dilated lymphatics with impaired cardiac outflow or aberrant intramyocardial lymphatic development [7]. Additional genes have been proposed as causative for specific TS characteristics: another pseudoautosomal gene may be involved in the neurodevelopmental problems of TS, because a small 8.3 Mb interval on distal Xp (Xp22.33) appears to be linked to the expression of the TS neurocognitive phenotype, with STS and NLGN4X the most likely candidates [8]. Genes on both Xp (such as BMP15) and Xq (such as FMR1 and FMR2) are important for maintenance of ovarian function and may very well be involved in the development of gonadal dysgenesis in TS [9].

6.2.2 Clinical Presentation

Many of the TS clinical features are listed in ▶ Box 6.1. The clinical presentation of TS varies widely, which influences the broad age range at which the diagnosis of TS is made (Fig. 6.1) [10, 11]. For the majority of patients diagnosed during prenatal life, karyotype screening was likely done for an older maternal age, or after finding an abnormal maternal serum test, and/or ultrasonography demonstrating a cystic hygroma, hydrops fetalis, or a major cardiac anomaly. Girls diagnosed in infancy almost always have lymphedema with/without neck webbing and other dysmorphic features [12]. In contrast, girls lacking classic phenotype hallmarks of TS are often not diagnosed until late childhood or adolescence when they are investigated for short stature and/or delayed puberty or even in adulthood when they develop premature ovarian failure.

6.2.2.1 Growth Failure and Short Stature

Short stature is the most common physical abnormality seen in TS, and poor linear growth/growth failure affects virtually all patients. Untreated TS women end up about 20 cm shorter than their peers (adult height about 3 standard deviations (SDs) below the mean) [13, 14]. Growth failure is due to (1) mild to moderate growth retardation in

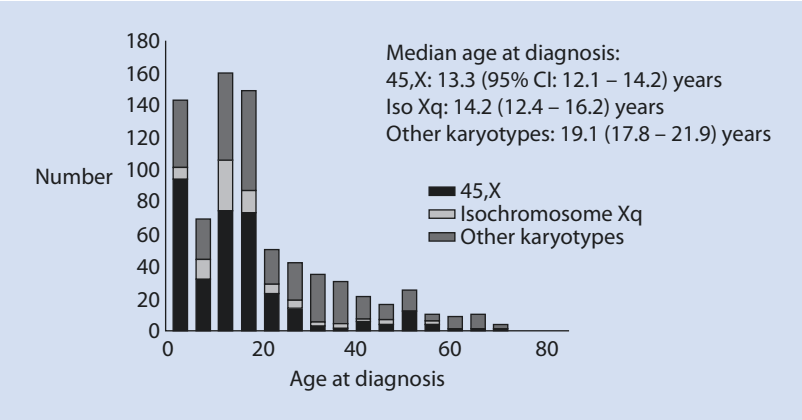

Fig. 6.1 Age at diagnosis for Turner syndrome and effect of karyotype; using data from the Danish Central Cytogenetic Register (From: Stochholm et al. [196]. Reprinted with permission from Oxford University Press)

utero, (2) slow growth during infancy, (3) slow growth during childhood, and (4) failure to experience a pubertal growth spurt (growth can be prolonged into the early 20s) [15–17]. Girls with TS are also, on average, −0.5 to −1.2 SDs below the mean for birth weight. Poor growth and weight gain in infancy may be exacerbated by poor feeding [18]. Patients with TS tend to be "stocky" because they have a greater relative reduction in body height than in body width and are often overweight. Developmental abnormalities of individual bones are responsible for many common findings such as short neck (cervical vertebral hypoplasia), cubitus valgus (radial head developmental abnormality), genu valgum, and short 4th metacarpals. Between 5 and 18 years of age, 20% have scoliosis (lateral curvature >10°), and 40% develop kyphosis [19]. A delayed bone age is found in the majority of patients with TS – although the degree of delay is not uniform among bones, a finding that could be due to the estrogen deficiency present in many patients early in childhood [20].

Osteoporosis and fractures are more frequent among women with TS [21]. Many girls have radiographic osteopenia and a coarse trabecular bone pattern, even in the prepubertal years. Although interpretation of studies of bone mineral density (BMD) in TS is difficult due to the measurement of areal instead of volumetric BMD, prepubertal girls with TS have decreased markers of bone formation, consistent with a low-bone turnover state and decreased bone deposition. There is a deficit in radial BMD, at largely cortical sites [22]. Osteopenia at predominantly trabecular sites develops during adolescence and progresses in adulthood. The pathogenesis of demineralization is unclear but is most likely an intrinsic bone defect exacerbated by suboptimal replacement of the estrogen deficiency in many patients [23].

6.2.2.2 Gonadal Failure

Although the majority of TS girls have gonadal failure and pubertal delay, some will enter puberty at the typical age. In a retrospective study of 522 TS patients 12 years and older, 32% of the girls with cell lines containing more than one X and 14% of classic 45,X patients had spontaneous breast development [24]. Sixteen percent further had spontaneous menarche occurring at a mean age of 13.2 ± 1.5 years. Although a number of patients developed secondary amenorrhea, some had regular menses for many years. Therefore, the diagnosis of TS should still be entertained in adolescents with unexplained short stature, even if they are menstruating, and should also be considered in the differential diagnosis for women experiencing premature ovarian failure [25]. Because there is this broad range of gonadal dysfunction in TS, it is difficult to predict which patient will enter puberty spontaneously and who will not. Follicle-stimulating hormone (FSH) has been used as a marker of ovarian integrity, because it follows a biphasic pattern in normal girls, with increased concentrations in infancy, decreased concentrations in childhood, and increased concentrations again early in puberty. This pattern is exaggerated in TS girls with ovarian dysfunction. The FSH values are increased in the first 2 years of life, decline gradually to reach lower values (often indistinguishable from those in normal girls) between 5 and 10 years of age, and rise again to castrate concentrations around the usual age for

puberty [26, 27]. Serum inhibin B and estradiol levels follow a similar biphasic age pattern with high values at 3 months of age, low values during the prepubertal years [28, 29], and increasing concentrations at puberty [30, 31]. Although all these measurements can be helpful to determine if a particular patient has ovarian failure, there exists a lot of overlap with normal females' values, irrespective of the karyotype. Measurement of gonadotropins such as FSH may have more limited diagnostic utility than previously considered. The measurement of anti-Müllerian hormone (AMH) may be more helpful, however. Serum AMH is detectable in 22% of all TS patients (10% in 45,X vs. 77% in 45,X/46,XX). Measurable AMH increases the likelihood of spontaneous puberty (odds ratio, 19.3) and menarche (odds ratio, 47.6) and is now considered a more sensitive marker of prepubertal follicular development than FSH [32, 33].

Girls with karyotypes containing Y chromosome material, such as in 45,X/46,XY, are at increased risk for developing gonadoblastoma [34], a gonadal tumor in which normal components of the ovary are present in varying amounts within circumscribed nests. A gonadoblastoma may produce sex steroids, which may lead to virilization or (occasionally) feminization, depending on the sex steroid produced. Although it is not a malignant tumor, the germ cell component may invade the ovarian stroma, resulting in a potentially malignant germinoma [35]. Rarely, a more malignant tumor, such as embryonal carcinoma or choriocarcinoma, may also develop in a gonadoblastoma. Using standard cytogenetic techniques, approximately 5% of patients with TS have Y chromosomal material, and of those, gonadoblastoma has been thought to develop in 15–25% [36].

Fertility is an area of great concern for women with TS [37]. Traditionally, assisted pregnancies using donor eggs have been the principal means by which women with TS achieved pregnancy. More recently the options for fertility preservation have included cryopreservation of ovarian tissue in young girls and oocyte freezing in adolescents or young women [38]. Studies have demonstrated a high risk of spontaneous abortion (25–40%), chromosomal abnormalities in the offspring (20%), and perinatal death (7%) [39]. When considering pregnancy outcome, spontaneous pregnancies are probably less associated with complications than pregnancies obtained after oocyte donation in TS patients, but the risk of fetal chromosomal abnormalities remains still high. When unassisted pregnancies occur, they are generally in patients with structural anomalies of the X chromosome, in which the Xq13-q26 region is spared, or in patients with a mosaic karyotype containing a 46, XX cell line.

6.2.2.3 Cardiovascular Abnormalities

Cardiovascular abnormalities are recognized as the most clinically significant pathology of TS patients. Some individuals with TS are diagnosed in the neonatal period or infancy secondary to cardiac lesions, such as coarctation of the aorta or hypoplastic left heart. It has long been recognized that individuals with TS are at increased risk for left-sided cardiac abnormalities, especially bicuspid aortic valve and coarctation of the aorta [40]. A study of 99 Danish women with TS (mean age = 37 ± 10 years) using both MRI and echocardiography revealed that common cardiovascular findings are an elongated transverse aortic arch (47%), bicuspid aortic valve (27%), dilation of the ascending aorta (20%), aortic coarctation (13%), aortic arch hypoplasia (2%), and aortic aneurysm (2%) [41]. A bicuspid aortic valve was functionally abnormal in many patients, causing aortic stenosis in 16% and regurgitation in 18%. This study and others have demonstrated that the arterial pathology is not limited to the aorta but also affects other intrathoracic arteries [42]. Another common structural cardiac abnormality is partial anomalous pulmonary venous return (PAPVR). In 1998, Mazzanti et al. detected PAPVR in 2.9% of TS patients, giving it the highest relative risk compared to heart defects in the general population [43]. Using cardiac MRI technology, that percentage appears to be much higher, and Kim et al. studied 51 patients with TS (median age, 18.4 years; range, 6–36 years) and detected PAPVR in 15.7% of that population [42]. Aortic dissection is estimated to occur in 1.4% of the TS population [44]. This devastating complication of TS usually occurs in adulthood but has occurred as young as 7 years. Although most cases have been associated with coarctation of the aorta, bicuspid aortic valve, hypertension, and/or aortic root dilatation, 10% does not have any of these risk factors [45–47]. Nonstructural abnormalities such as hypertension, conduction defects, or mitral valve prolapse are also more common than

in the general population, occurring in approximately 16% [40]. In a study of 62 TS patients, age 5.4–22.4 years, more than 30% were found to be mildly hypertensive, and over 50% had an abnormal diurnal blood pressure profile. The investigators were unable to correlate the presence of renal or cardiac abnormalities with hypertension. This may indicate the presence of an underlying vasculopathy inherent to the diagnosis of TS [48].

6.2.2.4 Neurocognitive and Psychosocial Problems

Individuals with TS are at increased risk for specific neurocognitive deficits and problems in psychosocial functioning. These include deficits in visual-spatial/perceptual abilities, nonverbal memory function, motor function, executive function, attention, and social skills [49, 50]. Abnormalities in cognitive function in TS are accompanied by structural differences in older children and adult's brains. For example, parietal lobes, parietal-occipital areas, and prefrontal areas, areas known to be associated with visual-spatial processing, are small when compared with controls [51]. Although their distribution of verbal IQs is relatively normal, lower full-scale IQs are more prevalent in this population due to lower scores on performance than verbal tasks [52]. These neurocognitive deficits put TS patients at a higher risk for educational problems. Rovet found that 48.2% of girls with TS versus only 20% of controls were recognized by their parents as having problems at school [53]. Mathematics is particularly problematic, and girls with TS score significantly lower than controls in overall arithmetic achievement [53, 54]. Although math is a consistent problem for girls with TS, hyperactivity, inattention, distractibility, and slowness may impair achievement in all educational disciplines. Many girls with TS repeat grades because of lagging cognitive and psychosocial skills. Although individuals with TS do not appear to be at an overall higher risk for psychiatric problems, there is evidence indicating that obsessive-compulsive tendencies and autism are more prevalent [55, 56].

6.2.2.5 Hearing Loss

Ear and hearing disorders are very common problems among girls and women with TS. The majority of patients suffer from conductive hearing loss secondary to recurrent otitis media during infancy and childhood. The high incidence of otitis media in this population (60–80%) seems to result from an abnormal anatomical relationship between the middle ear and the Eustachian tube [57, 58]. A short, more horizontally oriented Eustachian tube likely results in poor drainage and ventilation of the middle ear space and predisposes to more nasopharyngeal microorganisms invading the middle ear. Many girls therefore require tympanostomy tube placement, and some patients develop complications such as mastoiditis and cholesteatoma. In a study in which 56 girls with TS between the ages of 4 and 15 years were examined, 57% had eardrum pathology, such as effusion, myringosclerosis, atrophic scars, retraction pockets, and perforations. A conductive hearing loss was found in 43% of patients. In addition, a mid-frequency sensorineural hearing loss (SNHL) between 500 and 2000 Hz was present in 58% of the girls, four of whom already required hearing aids [58]. The presence of such a mid-frequency dip appears to be a strong predictor for future rapid hearing decline with potential social consequences [59]. The particular SNHL has been reported as early as age 5 years and appears to be progressive [60]. By their mid-40s, more than 90% of women with TS have a hearing loss of >20 dB, with greater than 25% requiring hearing aids.

6.2.2.6 Renal Abnormalities

Renal malformations occur in approximately 35–40% of individuals with TS [61, 62]. Of those with malformations, about half have abnormalities of the renal collecting system, and half have positional abnormalities, the most common being horseshoe kidney. Horseshoe kidney is more commonly associated with a 45,X karyotype, while collecting system malformations are more frequently associated with mosaic/structural X chromosome abnormalities [61]. Developmental abnormalities of the kidneys and collecting system predispose to urinary tract infections and possibly hypertension [62]. Vascular supply anomalies are observed with higher frequency [62].

6.2.2.7 Hypothyroidism and Other Autoimmune Disorders

Autoantibodies and autoimmune diseases are more common in individuals with TS than in the general population [63]. The most common autoimmune disorders in TS are Hashimoto thyroiditis, celiac disease, and inflammatory bowel

disease (IBD) [64]. In a study that evaluated 71 children with TS younger than 20 years of age, 15.5% were hypothyroid, 17% were positive for thyroid peroxidase and/or thyroglobulin antibodies, and 33.8% had thyromegaly [65]. The frequency of thyroid abnormalities increased with age, and no abnormalities were observed before 4 years of age.

A survey of 15,000 JRA patients from pediatric rheumatology centers in the USA, Europe, and Canada revealed 18 girls with a diagnosis of TS. This represents a prevalence at least six times greater than would be expected if the two conditions were only randomly associated. Patients had either polyarticular disease with early-onset and progressive disabilities or oligoarticular arthritis with a benign course [66]. The TS population may also be at increased risk for type 1 diabetes mellitus [21].

6.2.2.8 Gastrointestinal Disorders

Elevated liver enzymes are observed in patients with TS, who often remain asymptomatic. In a study of 218 adults with TS (mean age = 33 years), 36% had one or more liver enzyme concentrations higher than the reference level, the most prevalent being gamma-glutamyltransferase (GGT) [67]. After 5 years of follow-up, that percentage had risen to 59%. In a study of 27 individuals with TS who were biopsied for persistently elevated liver enzymes, 10 had marked architectural changes, including cirrhosis, nodular regenerative hyperplasia, and focal nodular hyperplasia, postulated to be caused by congenital abnormalities of the blood vessels. The remaining 17 individuals had nonalcoholic liver disease with steatosis, steatohepatitis, and steatofibrosis, most likely related to increased adiposity [68]. An autoimmune pathogenesis may also be operative in some cases because many have elevated antinuclear and/or anti-smooth muscle antibodies [69].

Celiac disease occurs in about 6% of those with TS, a prevalence nearly ten times that of the general population [70]. In another study, 18% of TS patients were noted to have celiac autoantibodies, and 26% of the antibody-positive patients had developed celiac disease (4.5% prevalence) [71]. Inflammatory bowel disease occurs in about 3% of females with TS [72], with Crohn's disease being as common as ulcerative colitis. Gastric and intestinal hemangiomas, telangiectasias, and phlebectasias are rare but can produce massive gastrointestinal bleeding when present [73, 74].

6.2.2.9 Obesity, Lipids, and Glucose Homeostasis

Individuals with TS have a modestly decreased life span. In a study of the Danish TS population, approximately 50% of all deaths were caused by cardiovascular disease, and these occurred 6–13 years earlier than expected [21]. Patients with TS were at increased risk for abnormalities constituting the metabolic syndrome including hypertension, dyslipidemia, type 2 diabetes, obesity, hyperinsulinemia, and hyperuricemia [21]. Body mass index (BMI) SDs begin to increase around the age of 9 years [75] and may exacerbate a tendency toward type 2 diabetes [76]. In one study of adult TS women (mean age = 42.5 years), visceral fat mass was increased, while trunk lean body mass (LBM), appendicular LBM, and skeletal muscle mass were decreased when compared to age-matched controls. Although this phenotype is associated with insulin resistance, glucose intolerance, and type 2 diabetes mellitus, studies in adults have pointed toward beta cell failure rather than insulin resistance as the primary defect in glucose homeostasis in TS [77, 78].

6.2.2.10 Lymphedema

Lymphedema is common in TS and begins in utero. Fetuses with 45,X TS may present with increased nuchal thickness alone on ultrasound or more generalized lymphedema [79]. Virtually all girls diagnosed during infancy have lymphedema secondary to maldevelopment of the lymphatic system. Lymphedema usually improves over the first few months of life but may worsen again with puberty or hormonal therapy. Older children and adults initially without clinically apparent lymphedema have been demonstrated to have hypoplastic superficial lymphatic vessels, explaining the first appearance of lymphedema in some individuals after infancy [80].

6.2.3 Diagnostic Evaluation

The diagnosis of TS requires that the patient has *both* the phenotypic characteristics and genotype consistent with TS. They must therefore have one or more clinical features (e.g., short stature, bicuspid aortic valve), as well as a deletion of the distal end of Xp (Xp11.2-p22) where the majority of genes associated with the TS phenotype appear to reside.

Most prenatal diagnoses of TS are made when karyotypes on amniotic fluid (less commonly by chorionic villous biopsy) are obtained for either an advanced maternal age, an abnormal maternal serum screen, or an abnormal fetal ultrasound. In the latter scenario, a karyotype is obtained for finding an increased nuchal thickness, a cystic hygroma, or a hypoplastic left heart. For fetuses diagnosed "incidentally," most will have a mosaic karyotype. Although those with 45,X/46,XX mosaicism can still have a more severe phenotype, many will be phenotypically quite normal at birth. All girls diagnosed prenatally should have a repeat karyotype performed after birth.

For those children diagnosed postnatally with a 45,X karyotype, at least 30 cells should be counted to explore for the possibility of mosaicism. This will allow for identification of at least 10% mosaicism with 95% confidence interval. If the 30-cell analysis fails to reveal mosaicism, fluorescent in situ hybridization (FISH) with X and Y centromere probes on at least 200 cells should be used to look further. FISH studies using specific DNA probes to the Y chromosome should also be performed when a small marker chromosome (a piece of chromosome material not otherwise identified) is identified, to determine if Y chromosome material is present. Polymerase chain reaction (PCR) has been used to detect Y chromosome material, but the false-positive rate is high. Therefore, if PCR is used, the finding should be confirmed with FISH. Genomic copy number microarray studies can be used to characterize genetic abnormalities but should not be used as a frontline screen for TS as low levels of mosaicism may be missed. In most cases, cytogenetic testing on blood lymphocytes is sufficient, but if TS cannot be confirmed on such testing and TS is still being considered, cytogenetic testing of skin fibroblasts should be considered.

A delay in diagnosis of TS is often the greatest obstacle to health care for girls with TS. In one study, the delay in diagnosis for those diagnosed in childhood or adolescence averaged more than 7 years (based on the presence of dysmorphic features and/or short stature). At the time of diagnosis, patients averaged 2.9 SD below the mean in height and had fallen below the 5th percentile for height an average of 5.3 years earlier [12]. In many girls with TS who have a delayed diagnosis, the TS phenotype is either absent or mild. This was the case when a systematic search for TS in 375 female children referred with growth retardation (less than −2 SD) and/or decreased height velocity identified 18 cases of TS, an incidence of 4.8% [81]. To facilitate timely diagnoses, Savendahl and Davenport have suggested guidelines for screening girls for TS [12]. These guidelines are presented and modified in ▶ Box 6.2.

Box 6.2 Screening Guidelines for Girls with Possible Turner Syndrome

Karyotype analysis for any girl with one or more of the following[a]:

- Unexplained growth failure/short stature
- Webbed neck
- Peripheral lymphedema
- Coarctation of the aorta
- Unexplained delayed puberty

Karyotype analysis for any girl with at least two or more of the following[a]:

- Nail dysplasia
- High arched palate
- Short 4th metacarpal
- Strabismus

Data from Savendahl and Davenport [12].
[a]Other suggestive features include a nonverbal learning disability, epicanthal folds, ptosis, cubitus valgus, multiple nevi, renal malformations, bicuspid aortic valve, recurrent otitis media, and need for eye glasses.

6.3 Outcomes and Possible Complications of Turner Syndrome

The clinical outcome of any TS patient strongly depends on the presence of associated comorbidities. Because these develop sometimes gradually, or do not present with overt symptoms, all patients with TS are recommended to undergo screening tests at diagnosis, to avoid the development of complications and institute early intervention/treatment. For example, upon diagnosis, all patients should be referred for detailed cardiovascular evaluation (to include electrocardiogram (ECG) and echocardiogram) by a cardiologist. Patients should be assessed for growth and pubertal development, undergo renal ultrasonography and a hearing test, and, after infancy, also be seen by ophthalmology. In childhood, screening for autoimmune thyroid disease (thyroid-stimulating

Table 6.2 Ongoing monitoring in Turner syndrome for the prevention/detection of comorbidities

All ages	Cardiology evaluation[a] (every 5–10 years) Blood pressure annually (consider 24-h Holter monitoring) Audiology and ENT (every 1–5 years)
School age	Thyroid and liver screening (annually) Celiac screen (every 2–5 years) Dental/orthodontic evaluation (as needed) Neurocognitive/educational and social evaluation (follow annual progress)
Older patients/ adults	Fasting lipids, glucose, insulin (annually) Thyroid and liver screening (annually) Celiac screen (as needed) Pubertal development/HRT/ counseling regarding (in)fertility Educational/vocational/psycho-sexual assessment

[a]Exam by cardiologist with expertise in congenital heart disease; blood pressure in all extremities; ECG; MRI and echocardiogram for older girls and adults; echocardiogram may suffice for younger patients

hormone, TSH; thyroxine, T4) and celiac disease (tissue transglutaminase, TTG; immunoglobulin A, IgA) and educational/psychosocial evaluation and orthodontic assessment (after age 7 years) are recommended. When the diagnosis is made in the second decade of life, assessment of liver enzyme status, fasting glucose, insulin, and lipids, BMD measurement, and evaluation of ovarian function are added to the screening tests.

Prevention of possible complications and detection of comorbidities in TS require lifelong vigilance. The recommendations for ongoing monitoring are based on expert consensus agreement and listed in Table 6.2 [2]. Additional expertise from reproductive endocrinology, gynecology, psychology, and psychiatry may be required depending on the individual patient. Given the multitude of health issues that can be encountered by TS patients, such preventative care is best offered through a multidisciplinary approach, which should also foster collaboration with TS support organizations. A particularly difficult period for care of the TS patient is the transition to adulthood, when the approach changes from care provision for a child/adolescent to an emerging adult patient. A successful transition to adult care takes a lot of time and effort and requires education of the TS patient to become more knowledgeable about her own health needs. Providers must use this opportunity to address the many medical and nonmedical aspects of TS health, including a comprehensive health evaluation. The communication and counseling should be tailored to the patient's maturity level. Important issues to discuss with the patient during the transition period include potential long-term complications of TS, especially cardiovascular disease and hearing loss, and need for ongoing monitoring and reproductive issues, including fertility options and potential risks of pregnancy. Strategies and resources for managing learning difficulties, which continue into adult life, include educational counseling and support and vocational counseling. The TS care team should specifically encourage the patient to engage with TS support groups, for emotional support and to help empower the patient to stay engaged with their health maintenance.

6.4 Growth Hormone Therapy in Turner Syndrome

Once the diagnosis of TS is made, growth should be assessed regularly. The use of a TS-specific growth chart will facilitate detection of concurrent problems that affect growth, such as hypothyroidism, and aid in the decision-making process for growth-promoting therapies. Growth charts for American girls [82] and Northern European girls [83] ages 0–3 years are available as well as growth charts for girls ages 2–18 years [84]. These growth data are applicable to girls with TS from the USA. As is the case for the normal population, there is a strong genetic component to each TS individual's growth pattern, and untreated TS girls are expected to follow a percentile *on the TS curves* throughout childhood and adolescence that is more or less commensurate with their midparental target percentile.

The objectives of hormonal therapies to promote growth in TS are to (1) attain a normal height for age early in childhood, (2) progress through puberty at a normal age, (3) attain a normal adult height to avoid stigmatization and physical restriction, and (4) avoid the adverse effects of therapy [85]. Clinical observation has suggested, although not unequivocally proven, that TS women treated with GH during childhood and achieving a height near or within the normal range face fewer obstacles, have higher self-esteem, and are more successful in social life and their careers [86].

The timing and administration of hormonal therapies for girls with TS are still evolving as experience is gained in their use. Growth hormone (GH) is the agent of choice. Clinical trials have demonstrated that GH improves adult height in girls with TS [87, 88]. When given in conjunction with GH therapy, anabolic steroids (such as oxandrolone) appear to have an additional beneficial effect [89–93]. However, when used alone, anabolic steroids increase short-term height velocity, but do not appear to improve adult height. Recent studies also suggest synergistic effects on growth of GH in combination with very low doses of estrogen [88].

6.4.1 Effects of GH on Linear Growth

Growth hormone therapy is considered the standard of care for girls with TS who have growth failure [2]. Girls with TS are not GH deficient, but supraphysiological GH doses improve statural growth. The demonstration that GH is effective in increasing adult height in TS was established in 2005 with the publication of the first randomized, controlled study of GH therapy to adult height, even though TS had been an approved indication for GH therapy many years before [87]. In that study, girls in the treatment group received GH (0.3 milligram per kilogram per week (mg/kg/week) divided into six doses/week). After a mean of 5.7 years, these girls averaged 7.2 cm taller than those in the control group. This improvement in adult height is roughly average for many studies in which height gain achieved by GH therapy was compared with the growth response of historical controls. Factors that determine the effect of GH on growth response include age at initiation of therapy (the earlier, the better), GH dose (the higher, the better), use of anabolic steroids (additive effect), and age at initiation of *feminizing doses* of estrogen (the later, the better) [94], with young age at GH initiation being the most important [87, 95]. A randomized, controlled 2-year "Toddler Turner" study of GH therapy in 89 girls with TS had GH therapy initiated between the ages of 9 months and 4 years (mean age, 2.0 ± 1.0 year) and showed that GH therapy is effective when commenced in early childhood [96]. After 2 years of therapy, the height of the GH-treated group was very close to average for the general population (±0.3 SD), and there was a between-group difference in height gain of 1.6 SDS (6.8 cm) with the TS girls who were not treated during that time. The girls who received GH therapy beginning at age 2 years ended up somewhat taller at near-adult height (NAH) than those who started GH therapy 2 years later (height SDs, −1.37 vs. −1.60, respectively; p = 0.590), and both attained a NAH approximately 10 cm above untreated historical controls ([97] = reference from ENDO 2016]). Early normalization of height has a number of potential benefits for girls with TS: negating the physical limitations of short stature, prevention of short stature-related underachievement, improvement in peer group integration, and the opportunity to initiate estrogen replacement at a physiologically appropriate age [96–98]. It is therefore recommended that GH treatment begins as soon as growth failure is demonstrated, even if this occurs before the patient falls below the 5th percentile [2].

GH therapy should be optimized for each individual, given its high cost and potential risk. In one study, TS patients were treated initially with a GH dose of 0.23 mg/kg/week, which was then doubled or tripled when the height velocity (HV) declined to less than twice the pretreatment HV. The estimated adult height benefit was 10.6 ± 3.8 cm compared to 5.2 ± 3.7 cm in the group who received a fixed dose of 0.3 mg/kg/week. In the group receiving incremental increases in GH dose, 83% attained heights in the normal range compared to only 29% in the fixed dose group [99]. Early work by Rosenfeld et al. demonstrated an additive effect of oxandrolone, an anabolic steroid that cannot be aromatized to estrogen, on adult height when compared with

historic controls [100]. In a multicenter, prospective, randomized trial in which patients began therapy at a mean age of 7–8 years and received treatment for a mean of 6 years, therapy with GH alone (n = 17) resulted in a height that was 8.4 ± 4.5 cm taller than the mean projected adult height at enrollment. Subjects receiving GH plus oxandrolone at a dose of 0.0625 mg/kg/day (n = 43) attained a mean height of 152.1 ± 5.9 cm or 10.3 ± 4.7 cm taller than their mean projected adult height [100]. Recently, Menke et al. examined the effect of oxandrolone in a randomized, placebo-controlled, double-blind, dose-response study in the Netherlands [89]. One hundred and thirty-three girls with TS were treated with GH combined with placebo, GH combined with oxandrolone in a low dose (0.03 mg/kg/day), or GH combined with oxandrolone at a conventional dose (0.06 mg/kg/day) from the age of 8 years. GH plus low-dose oxandrolone resulted in a greater height gain than the GH plus placebo group (9.5 ± 4.7 versus 7.2 ± 4.0 cm (p = 0.02)). However, height gain in the GH plus conventional-dose oxandrolone group was not significantly different from the placebo group. A recently published meta-analysis confirmed that oxandrolone has a positive effect on adult height in TS when combined with GH therapy [101]. It is recommended that oxandrolone treatment be started around 8–10 years of age at doses ranging between 0.03 and 0.05 mg/kg/day.

It has long been thought that adult stature is improved by delaying estrogen therapy as long as possible [102]. Chernausek et al. conducted a multicenter study in which 60 girls receiving GH therapy were randomized to initiate estrogen therapy (conjugated estrogens at a dose of 0.3 mg po q day) at either 12 or 15 years of age. The patients were all less than 11 years of age at study entry (mean, 9.5 years) and received 0.375 mg/kg/week of GH for approximately 6 years. Patients in whom estrogen treatment was delayed until age 15 years gained an average of 8.4 ± 4.3 cm over their projected height, whereas those starting estrogen at 12 years gained only 5.1 ± 3.6 cm. Growth was stimulated for approximately 2 years after the initiation of estrogen, but then declined as bone age advanced. Against these data, several studies now confirm that GH treatment leads to a normalization of adult height in most girls, even when puberty is induced at a normal pubertal age. The Dutch dose-response trial, for example, shows adult height in 60 TS girls who were treated via a randomized dose-response protocol. In this study, GH treatment was combined with low-dose estrogens at a relatively young mean age of 12.7 ± 0.7 years. Girls were randomly assigned to either receive (1) approximately 0.045 mg GH/kg/day or (2) this dose for 1 year followed by an increase to about 0.0675 mg GH/kg/day. A third cohort (3) then received the same amount of GH as the second group for the first 2 years of GH therapy, but was further increased to about 0.090 mg GH/kg/day, thereafter. After a mean duration of GH treatment of 8.6 ± 1.9 years, adult height was reached at a mean age of 15.8 ± 0.9 years. Adult height, expressed in centimeters or SD score, was 157.6 ± 6.5 or −1.6 ± 1.0 in the lowest dosage group, 162.9 ± 6.1 or −0.7 ± 1.0 in the second cohort, and 163.6 ± 6.0 or −0.6 ± 1.0 in those girls receiving the highest GH doses. The difference in adult height in centimeters, corrected for height SD score and age at the start of treatment, was significant between groups 1 and 2 and groups 1 and 3, but not between groups 2 and 3. Fifty of the 60 girls (83%) reached a normal adult height (adult SD score > −2) [103]. In the USA, the dose approved for treatment of short stature in TS is as high as 67 mcg/kg/day, although most patients are treated with a dose much below this. More recent studies also indicate that better height results can be obtained by starting estrogen therapy at 12 years of age than at ages 14 years and above [103, 104]. Most reassuring is a recently published randomized, placebo-controlled study to adult height of girls with TS ages 5–12.5 years who were randomized to four groups: double placebo, estrogen alone, GH alone, or GH and estrogen. Very low-dose ethinyl estradiol (or placebo) was given prior to age 12 years, after which all treatment groups received escalating feminizing estrogen doses. Growth hormone treatment (0.3 mg/kg/day divided into only *three doses per week*) alone increased adult height by approximately 5.0 cm over an average period of 7.2 years. Adult height was greater in the GH-estrogen group than the GH group by 0.32 + 0.17 SDS (2.1 cm) [105]. This modest growth benefit observed with the combination of ultralow-dose childhood estrogen replacement and GH, in addition to the above noted studies, indicates that the practice of delaying estrogen therapy should be abandoned for most TS patients.

6.4.2 Effect of GH on Body Proportions

As expected, there are differences in the response of specific bones to GH treatment. On average, untreated girls with TS have relatively large trunks, hands, and feet and broad shoulders and pelvis compared to height. It is the authors' impression that GH treatment can exacerbate this disproportionate growth of hands and feet but especially so in patients treated from early childhood onward until adult height is reached. On the other hand, GH modestly improves the disproportion between standing height and sitting height. There is no significant effect on the relative width of the shoulders and pelvis [106].

6.4.3 Effect of GH on Bone Mineral Density and on Craniofacial Development

Growth hormone therapy is likely to help support bone mineral accretion in TS girls and maintain prepubertal bone mineral density (BMD) [107]. Preliminary BMD data on patients after long-term GH therapy show an absence of osteopenia [108].

Growth hormone therapy in TS girls has not been demonstrated to have a significant effect on craniofacial growth [109], with the exception, perhaps, of an increase in the length of the mandible [110]. However, in these relatively short-term studies, the mean ages at initiation of GH therapy were 9 and 14 years. The growth of the cranial base is largely completed by age 6, whereas the synchondrosis of the mandible does not close until late adolescence. The effect of early-onset, prolonged, and/or high-dose GH therapy on craniofacial development is unknown. GH therapy also does not appear to affect the cadence of dental maturation.

6.4.4 Effect of GH on Psychosocial Function

There is abundant anecdotal evidence that GH therapy improves psychosocial function, one of the principal goals of this therapy. Unfortunately, few studies have formally addressed this important question, and controlled studies are unlikely to be done in the future. However, there are some data confirming the observations of physicians, families, and girls with TS that certain aspects of social interactions and behavior, but not cognition [111], are improved with GH therapy. In a study of 38 girls with TS treated for 2 years with GH, improvements were demonstrated in social and emotional functioning. The investigators reported that a quarter of the patients became more independent, happier, and socially involved [112]. In a study in which girls with TS were evaluated after 3 years of GH therapy, attention, social problems, and withdrawal were reported as improved [113]. In a Canadian study in which girls were randomized to either a GH or control group, analysis after 2 years revealed that there was a correlation with higher growth rate and the girls' perceptions of themselves as more intelligent, more attractive, having more friends and greater popularity, and experiencing less teasing than the untreated group [114]. However, the effect of GH therapy on adult quality of life (QoL) has been more difficult to demonstrate. A recent study of QoL compared 58 women with TS who had been treated with GH with 53 women with TS who had not been treated with GH. Except for less pain, no significant impact on QoL attributable to GH treatment could be found, despite the mean 5.1 cm increase in their adult height [115].

6.4.5 Safety of GH Therapy

Extensive post-marketing surveillance programs have documented that side effects of GH therapy in children are relatively rare. However, families should be fully informed about the potential risks associated with GH therapy, which may be more common in girls with TS than in other patient groups receiving GH. In the most extensive report to date, a study of 5220 girls with TS who received GH revealed higher incidences for disorders for which they are known or expected to be at higher baseline risk, scoliosis (0.39%), slipped capital femoral epiphysis (0.24%), diabetes mellitus (0.19%), and serious cardiovascular events (0.32%), than for non-TS patients receiving GH [116]. However, incidences were also increased for intracranial hypertension (0.23%), pancreatitis (0.06%), and new malignancies (0.11%). Other problems potentially caused or exacerbated by GH therapy but not captured in this report include lymphedema, carpal tunnel syndrome, and an increase in the number, size, and pigmentation degree of pigmented nevi. Limited reports have

not shown obvious adverse effects of GH on cardiac or aortic size or cardiovascular function [117, 118]. Of course, studies on the cardiovascular impact of GH in TS are going to be limited by a lack of pre-GH therapy phenotype characterization. Nevertheless, current evidence suggests that long-term GH therapy in TS does not induce an exaggerated trophic stimulus on cardiomyocytes, nor does it appear to increase aortic diameter compared with non-treated TS females [119].

Increased insulin resistance has been of considerable concern in this population already at higher risk for diabetes. Insulin sensitivity decreases, but there is generally no effect on glucose concentrations per se [120, 121]. In a study in which girls with TS were treated with GH for 7 years, the prevalence of impaired glucose tolerance was low; all hemoglobin A1c levels were normal, and none of the girls developed diabetes mellitus. Insulin concentrations decreased to values close to or equal to pretreatment values after discontinuation of GH treatment [122]. Although insulin resistance is increased during GH therapy, it may actually be improved after discontinuation due to the beneficial effects of GH therapy on body composition [123].

6.4.5.1 Safety of Anabolic Steroids

Side effects of anabolic steroids, used as adjunctive therapy to further improve growth, may include (a) virilization with development of acne, deepening of the voice, and growth of facial hair (and rarely clitoromegaly); (b) transient elevation of liver transaminases; (c) insulin resistance; and (d) premature skeletal maturation. It is known that mild virilization can occur when oxandrolone is used at a dose of 0.125 mg/kg/day [124]. Recently, virilizing effects were documented at a dose of 0.0625 mg/kg/day, a dose commonly used in clinical practice. In addition, slower breast development was reported at this dose also, as well as the lower dose of 0.03 mg/kg/day [89].

6.4.6 General Recommendations for Growth-Promoting Therapies

It is now clear that with early diagnosis and initiation of treatment, a normal adult height is a reasonable goal for most girls with TS. GH should be offered as a therapy for all girls with TS who are predicted to have a subnormal height. The predicted response to GH should be carefully reviewed with patients and their families to help limit unrealistic expectations of future height. Routine evaluation of GH secretory status in girls with TS is not warranted, because GH secretion in this group is similar to that of the normal population and GH secretory responses do not correlate with responses to exogenous GH [125]. Growth hormone therapy for this population requires somewhat higher dosing than used for GH-deficient patients. Standard GH therapy in the USA for TS is 0.375 mg/kg/week divided into seven doses (53–54 mcg GH/kg/day). Certain GH preparations have been approved by the USFDA to be administered up to 67 mcg/kg/day, and some patients will benefit from a dose titration toward that range. Although GH has been initiated at a mean age of 9–11 years in most studies, it is becoming clear that girls who begin GH therapy at an earlier age and receive GH for a longer period of time will experience a greater increment in height. Therefore, it is suggested that GH therapy be initiated once growth failure (decreasing height percentiles on the normal growth curve) is documented. Therapy should be continued until the individual reaches a satisfactory adult height or it is no longer beneficial (growth rate below 2–2.5 cm/year). One of the issues to consider when dosing TS girls with GH is the potential for overdosing. This can be observed in certain patients who are overweight and because GH is dosed based on body weight. Therefore, an alternative approach would be to dose GH using the patient's body surface area (BSA) – which is, at least in theory, for a variety of reasons more physiologic. Investigators analyzed serum IGF-I and adult height gain in two Dutch clinical trials in girls with TS that used BSA-based dosing and five Swedish clinical trials in girls with TS that used body weight (BW)-based dosing [126]. They found that (1) BSA-based dosing leads to stable serum IGF-I SDS over a period of 8–10 years, (2) BSA-based dosing leads to higher nominal doses in young girls (<9 years) and lower doses in adolescents than BW-based dosing, (3) adult height gain was greater on a BSA-based regimen than on a BW-based regimen in patients who start GH treatment before 8 years of age, and (4) the cumulative dose and cost were lower on a BSA-based regimen. Because of this, BSA-based dosing

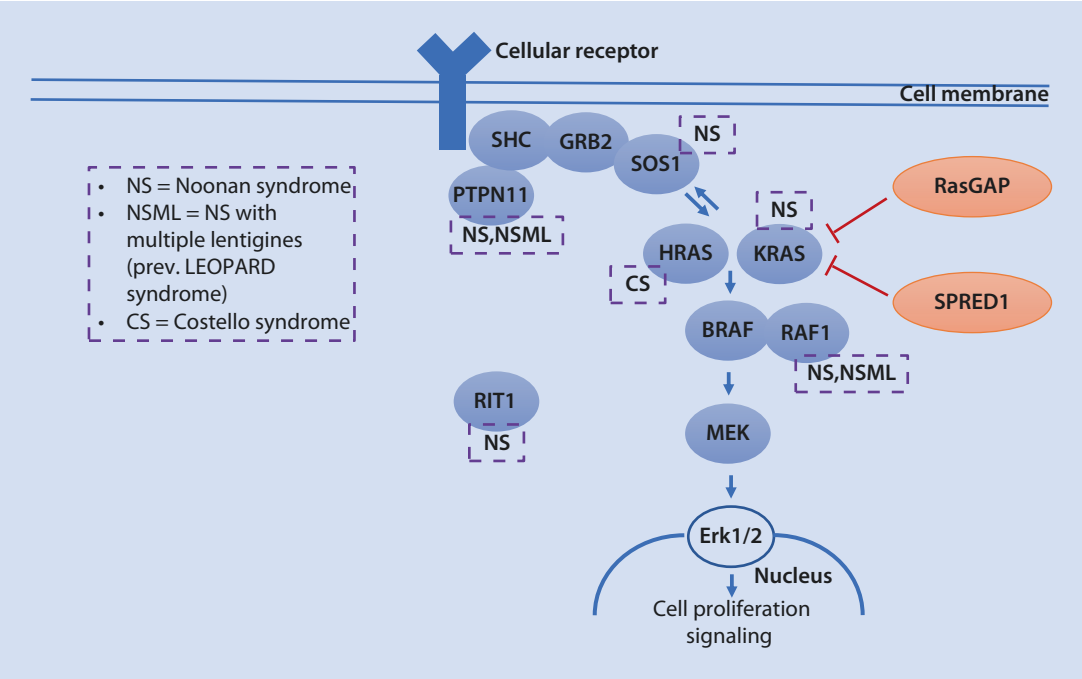

Fig. 6.2 RAS-MAPK signaling pathway. The *blue ovals* delineate components of the pathway, and the respective RASopathies that result from mutations therein are denoted in the *dashed boxes* [131]

could be more cost-effective than body weight-based dosing [126]. A dose of 0.048 mg GH/kg/day (0.34 mg GH/kg/week) is approximately equivalent to 1.33 mg GH/m^2/day.

Adjunctive therapy with oxandrolone at a dose of approximately 0.03 mg/kg/week can be considered at the age of 8 years and above. Ultralow-estrogen replacement in early childhood may improve growth but remains experimental at this point. Initiation of feminizing doses of estradiol should be begun during the time of normal puberty (approximately 12 years of age).

6.5 Etiology, Clinical Presentation, and Diagnostic Evaluation of Noonan Syndrome

6.5.1 Etiology

Noonan syndrome (NS) is an autosomal dominant disorder with a prevalence of 1 in 1000–2500. It is the second most common syndromic cause of congenital heart disease, second only to trisomy 21 [127]. The manifestations affect multiple organ systems, although the presentation is variable. Salient features include short stature, congenital heart defects, facial dysmorphisms, and mild intellectual disabilities.

Noonan syndrome is part of a group of disorders known as RASopathies, so called because they are caused by mutations in the RAS-MAPK (mitogen-activated protein kinase) signal transduction pathway (Fig. 6.2). The RASopathies, which have overlapping clinical characteristics, include NS, cardiofaciocutaneous syndrome, Noonan syndrome with multiple lentigines (NSML) (previously known as LEOPARD syndrome), Costello syndrome, and neurofibromatosis-Noonan syndrome. Mutations in different parts of this signal transduction cascade usually, although not in all cases, lead to a particular RASopathy.

The most common mutation leading to NS is in the gene *PTPN11*, encoding the tyrosine phosphatase SHP2, which positively regulates the RAS-MAPK pathway and which is involved in hematopoiesis, limb formation, and semilunar valve development [128]. Most mutations are de novo. When mutations are familially inherited, an advanced paternal age is associated [129, 130]. The second most common gene

mutation is *SOS1*, which encodes an activator of RAS [131]. Others include *RAF1*, *KRAS*, *RIT1*, *MAP2K1*, *BRAF*, and *NRAS*, in decreasing order of frequency, and phenotypic differences are observed depending on the gene affected (Tables 6.3 and 6.4). Roughly 20% of those diagnosed clinically as having NS have no identified mutation.

Table 6.3 Frequency of gene mutations causing Noonan syndrome

Affected gene	Chromosome locus	Frequency
PTPN11	12q24.13	50%
SOS1	2p22.1	10–13%
RAF1	3p25.1	3–17%
KRAS	12p12.1	<5%
RIT1	1q21.2	4–9%
MAP2K1	15q22.31	<2%
BRAF	7q34	<2%
NRAS	1p13.2	<1%

Data from Bezniakow et al. [128]

6.5.2 Clinical Presentation

6.5.2.1 Growth Failure and Short Stature

Mild to moderate postnatal growth failure is typical for NS patients, affecting upward of 70%, particularly in those with *PTPN11* mutations. Birth weight and length are usually normal, but growth failure can be observed shortly after birth. Although feeding difficulties are common in the first year of life, this does not seem to account for the early growth failure [132, 133, 137]. Starting at 2–4 years of age, height typically follows the 3rd percentile, with 83% of prepubertal NS children being affected by short stature. Thereafter, short stature is most common during the pubertal years, as a result of pubertal growth delay or attenuation. There is roughly a 2-year delay in the pubertal growth spurt, and the peak height velocity is lower than that of the general population, as can be seen in individuals with non-syndromic pubertal delay. Disproportionality is typically not observed. Mean adult height for men with NS is in the range of 161.0–169.8 cm and for NS women between 150.1 and 153.3 cm [133–136]. Attainment of adult height may be later than typical, given the frequent occurrence of pubertal (and bone age) delay. Therefore, short stature is common in prepubertal NS children, but in NS adults, short stature prevalence falls to 60% in

Table 6.4 Genotype and phenotype associations in Noonan syndrome for the four most commonly affected genes

Affected gene	Cardiovascular	Growth	Development	Other
PTPN11	Pulmonary stenosis more common	Short stature more severe; lower IGF-1		Bleeding diathesis, JMML, specific CNS tumors, and cryptorchidism more common
SOS1	Pulmonic stenosis more common; ASD less common	Short stature less severe	Fewer delays	
RAF1	Hypertrophic cardiomyopathy more common			
KRAS			More severe cognitive delay	

Data from Roberts et al. [127]

males and 50% in females due to a late pubertal catch-up growth.

Several growth factors [growth hormone (GH), fibroblast growth factors, and epidermal growth factor] stimulate the RAS-MAPK pathway, and the downstream targets include those involved in cellular proliferation, differentiation, survival, and metabolism. In those NS patients with *PTPN11*/SHP2 mutations, insulin-like growth factor 1 (IGF-1) release may be negatively affected via decreased signaling through the JAK2/STAT5B pathway and contribute to short stature. These patients often exhibit a relative GH resistance pattern, with elevated GH and lower IGF-I concentrations. Patients with mutations in genes further downstream in the RAS-MAPK pathway may have abnormal growth directly related to this abnormal signaling [137].

6.5.2.2 Cryptorchidism, Pubertal Delay, and Fertility

Undescended testes are found in 70–80% of boys with NS and are especially common in those boys with *PTPN11* mutations [138]. Delay in the age of onset of puberty in both boys and girls is seen in a significant number of patients. Thirty-three percent of boys with NS enter puberty after age 13.5 years, and 44% of girls enter puberty after age 13 years [133]. Female fertility is thought to be normal. However, both Sertoli and Leydig cell dysfunctions are common in adult males with NS, related to the role of *PTPN11* in normal testicular function and to damage to the testes by lack of descent [139]. Reproductive hormone concentrations differ in adulthood, but not in puberty, as compared to the general population, with higher luteinizing hormone (LH), follicle-stimulating hormone (FSH), testosterone, and estradiol and lower anti-Müllerian hormone (AMH) and inhibin B [140].

6.5.2.3 Cardiovascular Abnormalities

Fifty to ninety percent of NS patients have cardiovascular anomalies, thought to be pathogenically related to increased extracellular signal-regulated kinase ERK1 within the RAS-MAPK signaling pathway [131]. The spectrum of disease is wide. Pulmonic stenosis is the most frequent lesion, affecting 20–50% of patients, and disproportionately affects those with *SOS1* and *PTPN11* mutations, when the frequency is as high as 70%, but is also seen quite commonly with *RIT1* mutations [141]. Hypertrophic cardiomyopathy is the next most common lesion (20–30%), followed by secundum atrial septal defects (6–10%) [127]. Hypertrophic cardiomyopathy, which can be either congenital or acquired, tends to more frequently affect NS individuals with *RAF1* mutations and may also be more common in those individuals with *RIT1* mutations (75–85% and 50–70%, respectively) [141–144]. Most of these patients are diagnosed quite early, typically within the first 6 months of life [145, 146]. The likelihood of congestive heart failure in these patients is higher than in children with hypertrophic cardiomyopathy due to other causes, and mortality is high [147].

Electrocardiographic anomalies are detected in 90% of patients with NS, even in the context of an otherwise structurally normal heart. Common findings include right axis deviation with superior counterclockwise frontal QRS loop, superior or left axis deviation, or left anterior hemiblock or RSR' pattern in lead V1 [138].

6.5.2.4 Bleeding Diathesis

Coagulation defects are common in NS, with 50–89% having either a history of abnormal bleeding or abnormal hemostatic laboratory results and with 10–42% having both [148]. Bleeding diathesis and *PTPN11* mutations seem to be especially correlated, though individuals with other mutations (*KRAS*, *SOS1*, *RAF1*) have been reported to have coagulation defects as well.

6.5.2.5 Renal Abnormalities

Roughly 10% of NS patients have kidney anomalies, which typically do not require intervention. Dilatation of the renal pelvis is most common. More significant congenital anomalies are rare but reported, including duplex collecting systems, rotational anomalies, distal ureteric stenosis, renal hypoplasia, unilateral agenesis or ectopia, or bilateral cysts with scarring [127, 138].

6.5.2.6 Neurocognitive and Psychomotor Development

Intelligence quotient (IQ) scores below 70 are uncommon (6–23%), and most NS individuals will have IQ scores within the normal range [149, 150]. However, learning disabilities are common in NS children, and many require special education assistance [135, 149, 151]. These cognitive

deficits may be related directly to deficiencies in the RAS-MAPK pathway, the integrity of which is important for normal neurodevelopment [152]. Gross motor milestones may be delayed in NS, possibly related to joint hyperextensibility and hypotonia [138].

Articulation problems are also common, as are language delay, attention difficulties, and executive functioning problems [153, 154]. These are associated with reading and spelling difficulties. Hearing loss may underlie part of the language delay.

Psychopathology data in NS are scarce, and some studies have suggested higher rates of depression, but this is not a consistent finding among all reports [155–157].

6.5.2.7 Ocular and Otologic Manifestations

Ocular anomalies are almost universal in NS patients (>95%). The most common abnormalities are strabismus, nystagmus, myopia, or hypermetropia [158]. Corneal changes, such as prominence of nerves and cataracts, are often observed [159]. Orbital and lid anomalies include ptosis, epicanthal folds, sloping palpebral fissures, and hypertelorism.

External ear malformations affect the majority of NS patients, most commonly low set or posteriorly rotated. Hearing impairment can be congenital or acquired and affects about a third of NS patients and is mainly sensorineural in nature [160].

6.5.2.8 Malignancy

Somatic mutations in the RAS-MAPK pathway have been implicated in a variety of neoplasms. RASopathies are thought to increase the risk for neoplasia, and certain cancers occur more commonly. RASopathies have an estimated standardized incidence ratio of cancer 10.5 times that of the general population, with an NS-specific ratio of 8.1 [161]. A juvenile myelomonocytic leukemia (JMML)-like hematomalignancy has a higher incidence in NS (10%). This condition develops in early infancy (<2 months of age), but spontaneous remission has been reported [162]. There is likely also an association between having the *PTPN11* mutations and a higher risk for acute lymphoblastic leukemia [163]. A specific type of rare central nervous system tumor (dysembryoplastic neuroepithelial tumor) seems to be more common in NS, particularly in those patients with *PTPN11* mutations [164]. Multiple giant cell tumors of the bone have been described in individuals with *PTPN11* or *SOS1* mutations [127].

6.5.2.9 Lymphatic System

As in TS, lymphatic anomalies are common in NS. Many infants have evidence of this on prenatal ultrasound in the form of nuchal lucency, increased nuchal folds, cystic hygroma, and fetal edema. At birth, the majority have persistent evidence of lymphatic problems [165]. Neck webbing may be a remnant observed postnatally of such nuchal lymphedema in utero.

6.5.3 Diagnostic Evaluation

A clinical diagnosis is established on the basis of characteristic facial dysmorphisms (hypertelorism, downward eye slant with ptosis, low-set and posteriorly rotated ears) and the above-described organ system pathology (e.g., congenital heart disease). In order to risk-stratify for comorbidities, genetic testing is recommended. Initial testing would ideally entail a multigene panel that includes the known causative genetic mutations (◘ Table 6.3). If this is not feasible, sequential single-gene testing can be done, with investigation of the highest-frequency genes (i.e., *PTPN11*) first.

6.6 Outcomes and Possible Complications of Noonan Syndrome

A higher mortality, lower educational rate, and lower partnership have been reported in NS individuals when compared to the general population [135, 166]. The standardized mortality ratio is 3.00 (95% CI, 1.44–5.52). The majority of deaths are due to cardiac causes. Long-term data on the outcomes of cardiac disease in NS is scarce. Mild pulmonary stenosis is generally nonprogressive. Hypertrophic cardiomyopathy, which tends to present early in infancy, rarely develops beyond this age. However, mortality is higher in individuals with hypertrophic cardiomyopathy, with 22% of those children affected dying by 1 year of age [146, 167].

6.7 Growth Hormone Therapy in Noonan Syndrome

6.7.1 Effects of GH on Linear Growth

Starting in the 1980s, several small studies and case reports prompted advocacy for the use of recombinant human GH therapy in NS, with subsequent approval by the US Food and Drug Administration in 2007. Data regarding its efficacy and safety in this patient population have been limited and mixed.

Controlled trials regarding the use of GH in NS are scarce. The bulk of evidence for its use comes from case reports or case series and retrospective or uncontrolled studies. The majority of these do not report on adult height attainment. One controlled trial studied 32 NS children (23 treated, 9 untreated), in which GH therapy was started at a mean age of 7.4 ± 1.6 years (range, 4.8–13.7), while untreated children were 9.0 ± 4.1 years old (range, 4.1–14.8). Growth hormone dose was 0.047 mg/kg/day, and 19 of the study subjects completed 3 years of therapy. Baseline height was comparable between groups (−2.7 ± 0.4 standard deviation score (SDS) for the GH treated, −2.7 ± 0.6 SDS if untreated). After 3 years, height SDS increased to −1.9 ± 0.9 in the treated group versus −2.4 ± 0.7 in the control group. Height velocity in the treated group was higher than in the untreated patients in the first year of therapy, but in years two and three, the differences were not significant [168].

Few studies have followed children with NS to adult height. Osio et al. treated 18 NS subjects with a GH dose ranging from 0.03 to 0.06 mg/kg/day from an average age of 8.2 ± 3.0 years. The duration of therapy was 7.5 years. Patients were at a height SDS of −2.9 ± 0.4 at the start of therapy and gained 1.7 ± 0.9 SDS at the end of the study. Males had a slightly greater height gain than females, and the overall increase in height SDS corresponded to an absolute gain of 11 ± 6 cm [169]. Another study to follow NS children to adult height included 29 children (mean age, 11.0 ± 2.7 at the start of therapy) for a mean of 6.4 ± 2.3 years with a dose of 0.05 mg/kg/day. Height SDS went from a baseline of −2.8 ± 0.7 to an end of study SDS of −1.5 ± 0.8 (gain of +1.3 ± 0.75). Absolute mean gain was 8.6 ± 5 cm [170]. A third study reported near-adult height in 24 individuals with NS starting GH therapy at a median age of 10.17 years and with a baseline mean height SDS of −3.24, GH dose of 0.025–0.11 mg/kg/day. Mean duration of therapy was 7.6 years, and mean height SDS at adult height attainment was −2.41. Patients were noted to have an early response to GH therapy followed by attenuation in the response [171].

6.7.2 Effect of GH on Body Proportions

Disproportionality is not a typical feature of NS, and data suggests that GH therapy does not alter patients' body proportions [172].

6.7.3 Safety of GH Therapy

No studies reported major adverse events, including detrimental cardiovascular effects or an increase in or worsening of comorbidities. In the context of the cardiac hypertrophy and heart failure observed in acromegaly patients as a result of long-standing GH and IGF-I excess, concern was raised about the effect of GH therapy on cardiac health in NS. This has not been shown in studies that followed ventricular thickness in NS children on GH [168, 170, 173]. However, data regarding use in children with NS and existing hypertrophic cardiomyopathy is not available.

Neoplasia remains a concern in this patient group. There are no studies that allow a causative link to be established. However, there is a report of brain tumor growth during GH therapy after several years of treatment [164]. Caution is therefore recommended with the use of GH in this patient population, until additional information is reported.

6.7.4 General Recommendations for Growth-Promoting Therapy

Noonan syndrome patients are not universally short, and short stature, when present, is most pronounced in late childhood and in the early part of the second decade of life. This is important, because few studies have assessed the effect of GH therapy in NS on adult height. There is also a lack of consistency in terms of the effect of GH dose, timing of GH therapy, and GH responsive-

ness on attainment of adult height [130]. Many individuals with NS will have a delayed catch-up growth period that leads to a relatively normal adult height. Therefore, although GH is approved for use in NS by the USFDA, the recommendation to start NS patients on long-term GH therapy should be individualized. Should the practitioner recommend GH therapy, families should be counseled on the benefits, which may be limited, and potential risks. They should also be counseled on the lack of robust adult height data in this area, so that consent may be as complete as possible. Although no serious adverse events have been reported in terms of the effects of GH therapy on cardiovascular health and malignancy risk and GH therapy seems to be well-tolerated in the NS patient population, no conclusions about the long-term safety of GH in this patient group can be made. Long-term surveillance is necessary in those who have received GH therapy.

Growth hormone therapy should be considered in those NS patients not expected to reach a normal or near-normal adult height, keeping in mind the above caveats. As in girls with TS, GH therapy for this population often requires higher dosing than used for GH-deficient patients. In the USA, GH doses up to 66 mcg/kg/day (0.46 mg/kg/week) are recommended. However, patients are often started on doses ranging between 35 and 50 mcg/kg/day, followed by individualization of dosing. This can be done while targeting certain IGF-I concentration ranges (e.g., between +1 and +2 SDS), as well as based on treatment response age. Therapy should be continued until the individual reaches a satisfactory adult height and experiences near closure of the growth plates or GH is no longer beneficial (growth rate below 2.5 cm/year).

6.8 Etiology, Clinical Presentation, and Diagnostic Evaluation of SHOX Deficiency

6.8.1 Etiology

SHOX deficiency disorders are a frequent cause of short stature. These disorders are caused by defects in the short stature homeobox-containing gene on the X chromosome (*SHOX*). The protein product of the *SHOX* gene is thought to control chondrocyte apoptosis. Haploinsufficiency of

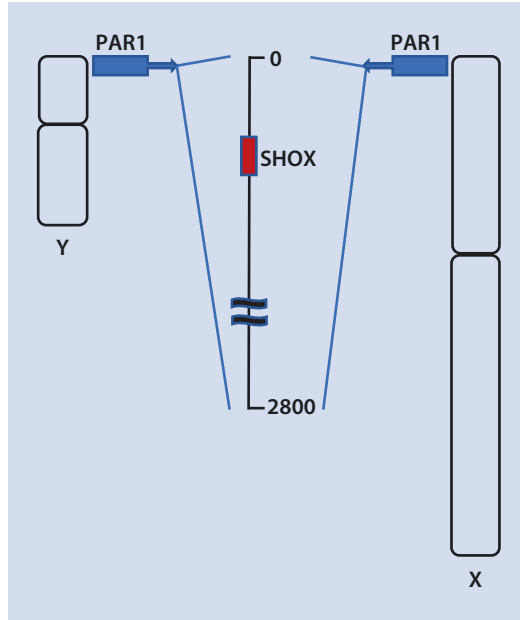

Fig. 6.3 SHOX gene diagram, showing its location on the distal short arm of the X and Y chromosomes within the pseudoautosomal region 1 (PAR1)

SHOX results in either non-syndromic short stature or Leri-Weill dyschondrosteosis (LWD) and is also the underlying etiology of the short stature noted in TS. Homozygous loss of *SHOX* expression leads to Langer mesomelic dysplasia, a rare disorder characterized by severe short stature and skeletal deformities [174].

The *SHOX* gene is located on the distal end of the short arm of both sex chromosomes (X and Y), in the telomeric part of the pseudoautosomal region 1 (PAR1) (Fig. 6.3) [175]. This region contains genes which escape X inactivation. Mutations in this gene are inherited in an autosomal dominant manner or may occur de novo. Around 80% of the described mutations involve gene deletions, either within the *SHOX* gene itself or downstream in the regulatory enhancer region. The remainder of the reported mutations is missense or nonsense mutations that result in faulty protein function, nuclear translocation, or dimerization. Microduplications have also been described in the context of short stature, thought to cause abnormal transactivation of *SHOX* gene expression [176–178].

SHOX haploinsufficiency underlies a spectrum of clinical severity. Individuals may be affected with varying degrees of isolated short stature or may have the full clinical picture of

LWD, which is characterized by short stature and typical skeletal deformities. *SHOX* haploinsufficiency accounts for 2–3% of non-syndromic short stature and 50–90% of LWD cases [175, 179, 180].

6.8.2 Clinical Presentation

6.8.2.1 Growth Failure and Short Stature

Reduced birth length is reported in children with SHOX deficiency, with height SDS ranging from −0.59 to −1.8 in a few small series [181–184]. In one of these series, 41% were noted to be small for gestational age. Thereafter, early childhood growth failure is noted. Middle childhood is characterized by a lack of catch-up growth, although severe growth failure is not described [56, 59, 60]. Pubertal growth was blunted in one small series, although other studies have not confirmed this [181]. Mean adult height is estimated to be −2.2 SD for LWD individuals [185, 186]. However, the variability around published estimates for adult height is large. Ascertainment bias of the studies may account for this, in addition to the inherent phenotypic variability. Individuals with the same mutation, even within the same family, have been noted to have any of non-syndromic short stature, LWD, and normal stature.

Langer mesomelic dysplasia results from *SHOX* nullizygosity and is predictably more severe in phenotype than LWD. Extreme short stature, with adult heights of −5.5 to 8.9 SDS below the mean, is typical. Bony anomalies include proximal tubular bone shortening, aplasia or hypoplasia of the ulna and fibula, and curvature and thickening of the radius and ulna. Madelung deformity is not typically seen.

6.8.2.2 Skeletal Deformities and Body Proportions

Mesomelia is often seen in individuals with SHOX deficiency, although this disproportionality may be absent in early childhood. However, as patients age, body proportions deviate from age-appropriate norms. Reduced arm span and increased sitting height-to-height ratio are the most reliable clinical indicators of SHOX deficiency (apart from increased BMI) and can aid in distinguishing SHOX deficiency from idiopathic short stature [179, 185, 187]. Disproportionality is noted to be less for those individuals with mutations affecting their *SHOX* enhancer region rather than a deletion or point mutation of the *SHOX* gene itself [188].

Madelung deformity is common in SHOX deficiency syndromes. It is estimated that half of LWD patients have Madelung deformity, which increases to 74–87% when radiographic criteria are used [184, 186]. It is thought to develop due to defective radial head growth, resulting in a relative overgrowth of the ulnar head. Shortening and bowing of the radius and volar/ulnar deviation of the hand may develop due to this restricted radial growth (◘ Fig. 6.4). Madelung deformity typically develops in mid to late childhood. Subtle radiographic signs may be seen in early childhood despite a lack of symptomatology and include lucency of the distal radius. Madelung deformity may evolve over time, resulting in limitations in forearm motion and pain [186].

Other skeletal anomalies have been described in individuals with SHOX deficiency, including scoliosis, high-arched palate, short fourth metacarpals, cubitus valgus, tibial bowing, and micrognathia [179].

Bone mineralization has been investigated in prepubertal subjects with SHOX deficiency. Total bone area was significantly increased with a relative decrease in cortical bone area and thinning of the cortex. These findings are of unclear significance but are also seen in patients with TS [189]. Increased body mass index has been noted in individuals with SHOX deficiency [190]. Segmental disproportionality is thought to underlie this finding, and in fact, a relative increase in limb circumference is described in this group, suggesting possible muscular hypertrophy [179].

6.8.3 Diagnostic Evaluation

Clinical suspicion of SHOX deficiency disorders should be high in the presence of mesomelic short stature, particularly if the family history supports an autosomal dominant mode of transmission. The presence of Madelung deformity or other previously discussed skeletal features is also suggestive.

Because deletions of 10 kb to 2.5 Mb are the most likely underlying etiology of *SHOX* haploinsufficiency, the initial diagnostic approach should

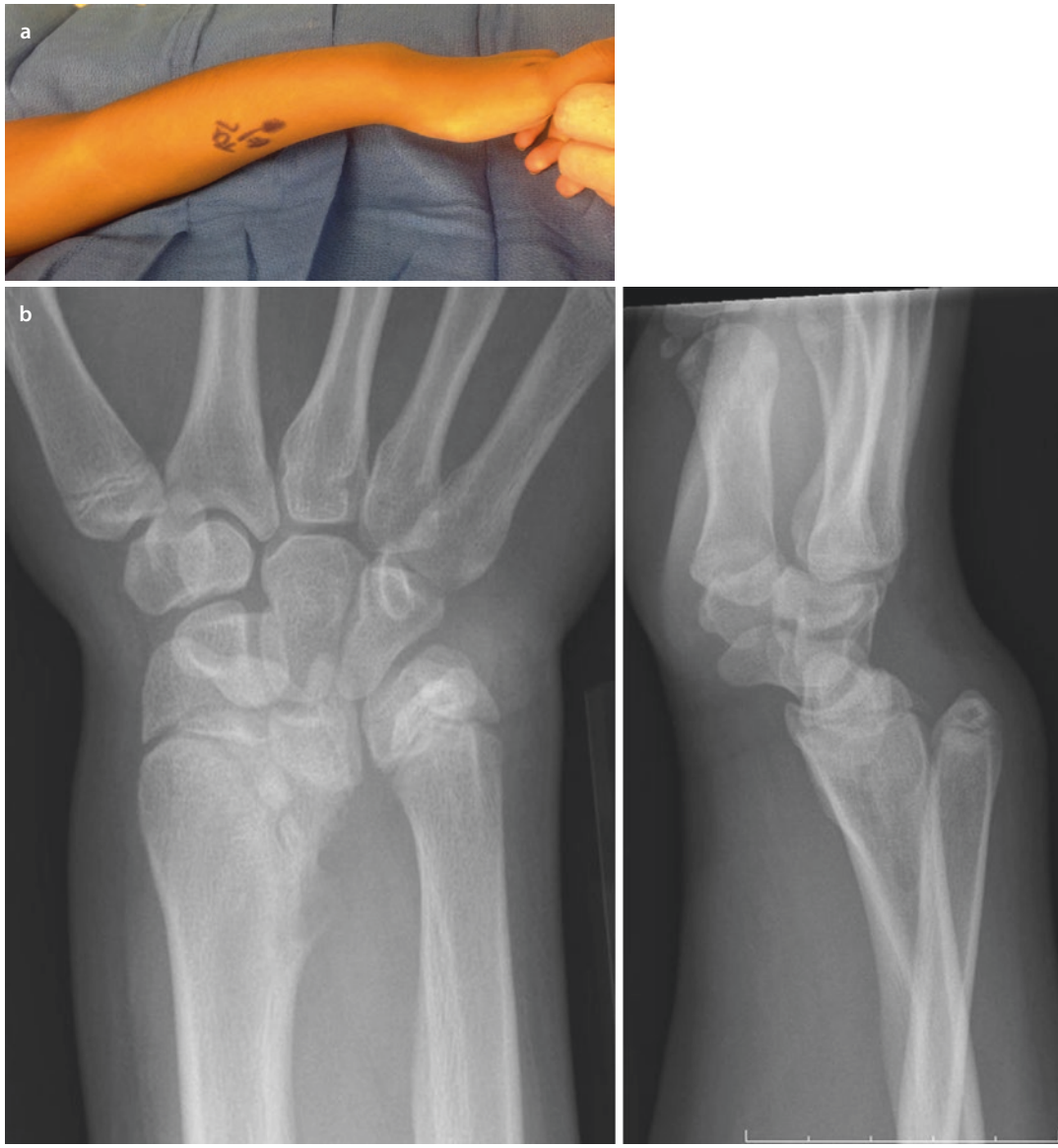

Fig. 6.4 Madelung deformity. **a** Bowing and shortening of the radius, ulnar head prominence. **b** Palmar and ulnar deviation of the carpal bones, dorsal subluxation of the ulnar head

assess copy number. Multiplex ligation-dependent probe amplification (MPLA) can detect copy number gains or losses of *SHOX* including its enhancer regions and is often used as the first line of testing. Often, this testing is confirmed and further characterized with an array of comparative genomic hybridization (CGH). Sequence analysis of the *SHOX* region should be employed if no deletion or duplication is identified with the previous method and suspicion for SHOX deficiency remains high.

6.9 Outcomes and Possible Complications of SHOX Deficiency

In addition to the main clinical features and outcomes of SHOX deficiency previously described, data from a small study yielded a greater proportion of these patients have abnormal responses on GH stimulation testing: 37.5% of SHOX deficiency patients (n = 16) were noted to have

impaired GH secretion (GH peak of <10 mcg/L in response to two separate stimuli). The significance of this finding is not clear, particularly given that short-term response to GH therapy was not different comparing those with normal and impaired GH secretion [191].

6.10 Growth Hormone Therapy in SHOX Deficiency

6.10.1 Effects of GH on Linear Growth

Patients with *SHOX* haploinsufficiency seem to respond to GH therapy similarly to TS individuals [192]. In a randomized prospective trial in which almost 2/3 of subjects attained adult height, patients with *SHOX* haploinsufficiency received GH for an average duration of 6 years. The patients attained a height SDS gain of +1.25 with an average GH dose of 0.37 mg/kg/week. In this same study, TS subjects treated with the same GH dose gained a comparable +1.46 SDS over 7.4 years duration of therapy. It was noted that subjects with SHOX deficiency had a pattern of steady increase in height SDS over the treatment duration, whereas TS subjects showed a rapid height SDS increase in the first 2 years of GH therapy, with plateauing thereafter [192].

Data comparing GH responsiveness for various mutations resulting in *SHOX* haploinsufficiency are sparse. However, it has been reported that individuals with *SHOX* enhancer defects have a growth response to GH therapy that is greater than in those with a *SHOX* gene defect. First-year height velocity and increase in height SDS were greater in the former group. Nevertheless, these parameters did not differ in the second through fourth years on GH between groups [62].

The effect on linear growth in peripubertal SHOX deficiency of the combination of GH and GnRH analog has been reported in one small study. Those who received this combination (for a duration of between 2 and 4.9 years) saw a height SDS gain of +0.6. This is in contrast to those who did not receive either medication who lost height (−1.2 SDS). It should be noted that, at baseline, those who did not receive treatment were similarly aged but taller than those who did receive treatment [193].

6.10.2 Effect of GH on Body Proportions

In a study following changes in radiographic findings, patients with SHOX deficiency who were treated with GH for 2 years were not found to have a greater degree of bony changes than those not treated with GH. Measures included hand/wrist, forearm, and lower leg indices (including 4th and 5th metacarpal shortening; radial, ulnar, and tibial bowing; elbow deformities; ulnar variance; tibial tuberosity anomalies; and hypertrophy of the medial femoral condyle) [194].

6.10.3 Safety of GH Therapy

Although there is a relative paucity of data, no significant adverse events have been reported with the use of GH treatment in SHOX deficiency patients. Enlargement of hands and feet and worsening of scoliosis were noted as GH-related effects in a randomized, controlled study. The authors also noted several potentially GH-related adverse events, including arthralgias, hypothyroidism, and benign neoplasias [192].

This same study followed bone age changes, which were described as advancing slightly more rapidly than chronologic age during the first 3 years of GH therapy, with subsequent stabilization.

6.10.4 General Recommendations for Growth-Promoting Therapy

As can be expected from the paucity of data regarding the use of GH in patients with SHOX deficiency, fully developed guidelines have not been proposed. Current evidence supports that GH is safe for most patients with short stature related to SHOX deficiency. As previously discussed, efficacy may vary for differing mutations, but in general, GH is thought to increase adult height in these patients. Given the similarity in response to GH of SHOX deficiency patients to TS patients, a similar approach may be taken. Therefore, longer duration of therapy is likely beneficial to augmenting adult height. Dosing is typically recommended at 50 mcg/kg/

day (0.35 mg/kg/week) and should be titrated for individual growth response and radiographic (bone age) and biochemical (IGF-I) data. For short peripubertal children with SHOX deficiency, addition of an agent for pubertal suppression could be considered, although data for this combination of therapy is not very strong.

Case Study

A 7-year-old boy is referred to a pediatric endocrinology clinic for evaluation of short stature. Upon review of his medical history, it is noted that he was born full term with an appropriate birth weight and length. He had feeding difficulties in the first year of life, thought to be contributory to his early failure to thrive and poor linear growth. However, by age 3, growth failure was noted to be less severe, and he maintained a height at −2.5 SDS. Otherwise, his health history had been notable for mild degree of pulmonic stenosis not necessitating surgical intervention. He also had early intervention for speech delays but was believed to have normal intelligence. Family history yielded that his father was 165 cm tall. Paternal grandparents were noted to be 180 cm (grandfather) and 167 cm (grandmother) tall. Mother was noted to be 165 cm tall.

He was followed for several years after initial screening evaluation showed normal growth hormone secretion, thyroid status, no generalized inflammation, and a normal karyotype. However, between the ages of 10 and 11 years, he had further growth failure to a height SDS of −3. Examination at that time revealed proportionate short stature in a prepubertal male. Mild hypertelorism was noted, along with mildly low-set ears. Bone age at 11 years of age was delayed to 9 years. Noonan syndrome was suspected, and genetic testing confirmed a mutation in the PTPN11 gene. He was followed over the next several years, and testicular growth began at age 13.5 years, followed by attenuated pubertal growth with a peak growth velocity of 6 cm/year, after which he attained an adult height of 166 cm at age 20 years.

This case illustrates a couple of key points regarding Noonan syndrome, its growth pattern, and the decision to intervene with GH therapy. First, the classic growth pattern seen in children with Noonan syndrome is described (normal birth weight, early growth failure followed by height velocity stabilization, then further failure due to pubertal delay, and catch-up growth with attenuated growth spurt). Second, short stature is most prominent in the late prepubertal years, but patients may not always require intervention to reach a normal adult height. A referral to pediatric endocrinology is recommended, however, to help with the decision-making regarding growth hormone therapy in these patients, as several studies have shown improvements in statural growth in patients with Noonan syndrome [195].

6.11 Summary

In this chapter, we discussed three common disorders associated with varying degrees of growth failure and short stature: TS, NS, and SHOX gene deficiency. Each of these conditions is associated with a variety of comorbidities. Growth hormone therapy is beneficial for improving the growth deficiency in each disorder, with the evidence for improving adult height being the strongest for patients with TS. However, patients with NS and SHOX haploinsufficiency also improve their growth with GH therapy, although there exists significant variability in the treatment response. Recommendations for GH therapy, monitoring, and treatment individualization vary for each disorder.

Review Questions

1. A 12-year-old girl's height plots out significantly below the 3rd percentile. A review of her prior growth pattern shows a decrease over the last 3 years from the 25th percentile, which she followed throughout childhood, to where she is now. Her midparental height is at the 75th percentile curve. She is otherwise healthy but has some learning issues since 3rd grade, especially reporting difficulties with mathematics. She had pubarche 2 years ago.

   Her physical exam shows proportionate short stature and Tanner stage I for breast development.

Of the following laboratory evaluations, which will most likely lead to the patient's diagnosis?

A. Tissue transglutaminase IgA
B. Complete blood count
C. Anti-Müllerian hormone
D. Insulin-like growth factor 1
E. Free thyroxine

2. A 13-year-old girl is being evaluated for lower abdominal pain. Pelvic ultrasonography revealed a relatively small left ovary and a right-sided adnexal mass. Her exam shows facial acne and Tanner stage III pubic hair growth, and she has dark hairs on all her extremities. As part of her work-up, karyotype analysis reveals this girl has a 45,X genotype plus a marker chromosome.

Of the following statements, which one is correct?

A. She is at an increased risk for virilization.
B. She is unlikely to develop gonadal failure.
C. She cannot feminize because she has a gonadoblastoma.
D. She will respond less well when treated with GH therapy.
E. She requires a right unilateral gonadectomy.

3. A newborn boy is noted to have undescended testes and pulmonic stenosis, along with a wide neck and low-set ears. Over the next several months, he is noted to have growth failure. Noonan syndrome is suspected, and genetic testing is sent. Which is most likely to be the underlying genetic etiology and mechanism?

A. RAF1, maternal transmission
B. SOS1, paternal transmission
C. SOS1, de novo mutation
D. PTPN11, de novo mutation

4. A 9-year-old girl is noted to have growth failure over several years, having been born at a normal length. Weight is preserved. Her father is of normal height, but her mother is noted to be short at 4′8″ and did not attain an adult height appropriate for her genetic background. On exam, she has dental crowding and has a stocky appearance. The patient is noted to have a shorter than expected arm span and an increased sitting height-to-height ratio. Testing showed:

Normal blood count and electrolytes.
Karyotype = 46,XX
IGF-I = 220 ng/mL
T4 = 8.7 mcg/dL
TSH = 4.5 mcIU/mL
Skeletal age = 8.5 years
Spine x-ray = 10° thoracic levoscoliosis
SHOX haploinsufficiency is suspected.

Which feature on this girls' exam is the most reliable indicator of a defects in the *SHOX* gene?

A. Scoliosis
B. Micrognathia
C. Disproportionate growth
D. Lower limb muscular hypertrophy

Answers

1. A
2. C
3. D
4. C

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Management of Adults with Childhood-Onset Growth Hormone Deficiency

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Key Points

- GH plays important physiological roles, primarily mediated by circulating and locally produced insulin-like growth factor 1 (IGF-1), including linear growth during childhood and maturation and maintenance of body composition during the transition period and adulthood. Untreated GHD can therefore have a negative impact on individuals throughout their life span. Issues related to linear growth are obviously more important in the pediatric population, whereas body composition and quality of life (QoL) gradually become more important in adults.
- GHD can occur in pediatric patients (childhood-onset GHD, CO-GHD) or in adults (adult-onset GHD, AO-GHD). Isolated idiopathic GHD is the most common cause of CO-GHD, and most patients revert to a normal endogenous GH secretion during transition and young adulthood. AO-GHD is mostly due to a secondary damage of the hypothalamic-pituitary area and is commonly associated with multiple pituitary hormone deficiencies (MPHD). About 15–20% of AO-GHD cases derive from a CO-GHD persisting during transition and adulthood.
- After children on GH replacement therapy (GHRT) achieve near-adult height, GH should be stopped, and they should be retested to confirm a persistent GHD. Serum IGF-1 measurement can be enough in the minority of patients who are highly likely to remain GHD. All other patients require GH provocative testing.
- Patients with persistent CO-GHD during transition and adulthood should be treated to attain full body and bone maturation and to improve their QoL and some cardiovascular risk factors (chiefly the lipid profile). GHRT is generally safe, and GH dosing has to be individualized according to patient's characteristics.

7.1 Introduction and Background Information

While in patients with CO-GHD growth is the major endpoint of therapy during childhood, a number of diagnostic and therapeutic challenges exist once the pediatric indication for treatment ends. This period of going from the pediatric indication to the adult indication has been referred to as the transition period. Not all children entering transition have persistent GHD. These are predominantly in the "idiopathic" CO-GHD category, while the patients with anatomic pituitary damage represent those who more commonly remain GH deficient. Diagnosis, treatment, and follow-up of patients with persistent GHD during transition and adulthood is the subject of this chapter.

7.1.1 What Is the Frequency of CO-GHD?

GHD – either isolated or associated with other deficits (MPHD) – may present during childhood (CO-GHD) or during adulthood (AO-GHD). Approximately 6000 new cases of adults with GHD are diagnosed each year in the United States, with an incidence rate of about 2 per 100,000 people/year [1, 2], and with 15–20% of those cases representing pediatric cases coming from patients transitioning to the adult indication.

7.1.2 What Is the Transition Period?

The transition period is the life phase between puberty and adulthood. It starts at late puberty (mid to late teens) and ends 6–7 years after achieving final height (early to mid-twenties) [3]. Up to transition, the role of GHRT in GHD patients is primarily to promote linear growth. After near-adult height is attained, the aims of GHRT will include full body maturation, metabolism control, and QoL.

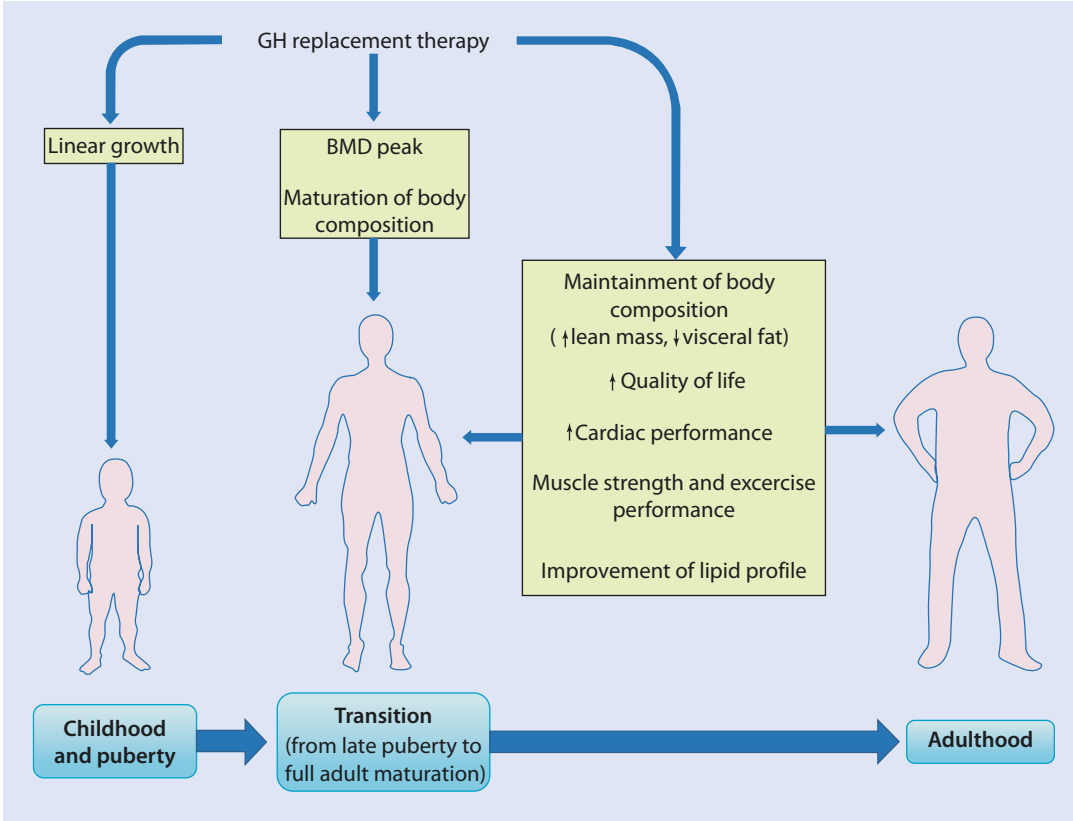


Fig. 7.1 Goals of GHRT during lifetime. Physiological endogenous GH secretion varies during lifetime, gradually rising during the prepubertal phase, reaching its peak at puberty, and steadily decreasing thereafter. During childhood and puberty, the foremost aim of GHRT is linear growth and attainment of final height. During transition and young adulthood, exogenous GH promotes full body maturation (especially to gain an optimal lean mass/fat mass ratio) and bone mineral accretion. During this time, other issues become gradually important, such as muscle strength, cardiac performance, some biochemical parameters (chiefly lipid profile), and QoL, the latter being more evident in older patients. *Abbreviations*: BMD bone mineral density, GHD GH deficient, GHRT GH replacement therapy, QoL quality of life

7.1.3 What Are the Aims of GH Replacement Therapy Throughout Life?

In the past, GHRT in patients with CO-GHD was stopped after achieving final height, defined as a growth velocity <2 cm/year. During the past two decades, a growing body of evidence concerning the beneficial effects of continuing GHRT in transition and adult patients with persistent GHD and of starting GHRT in those with AO-GHD has led to the increasing usage of recombinant human GH (rhGH) in these groups of patients. GHRT has been proven to positively affect the attainment and maintenance of adult body composition, bone mineral density (BMD), metabolic profile (including several biochemical parameters, such as the lipid profile), and QoL. Figure 7.1 reports the different goals of GHRT throughout life.

7.1.4 What Are the Differences Between CO-GHD and AO-GHD?

The various causes of GHD in children differ dramatically from etiologies of GHD in adults (see Fig. 7.2). Most adults, for example, have readily recognizable causes of GHD (usually in the setting of MPHD) due to obvious insults such as

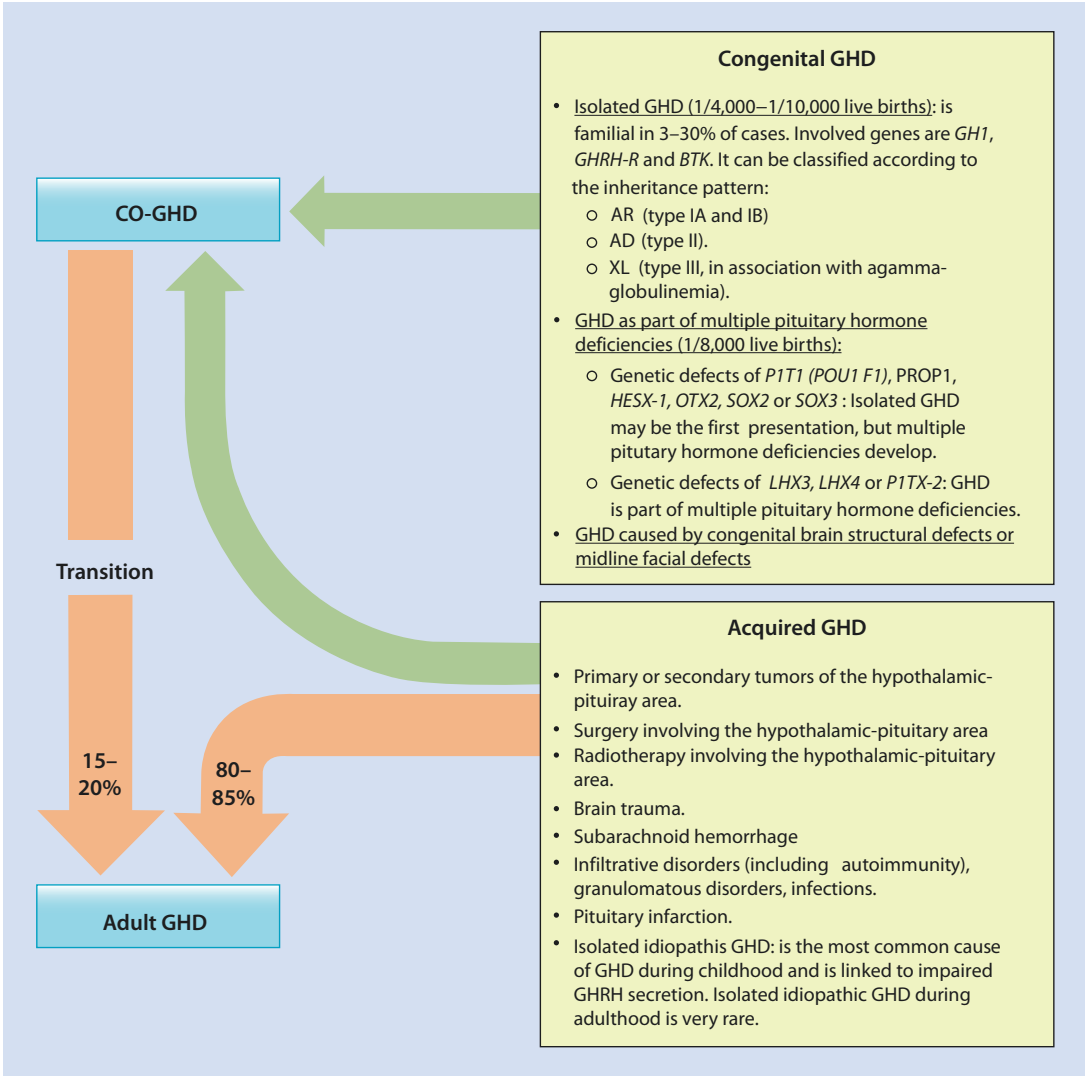


Fig. 7.2 Causes of GHD. Childhood-onset GHD (CO-GHD) can result either from congenital and acquired etiologies. Isolated idiopathic GHD is the most common cause in this category. GHD in adults (AO-GHD) is more frequently associated with multiple pituitary hormones deficiency (MPHD) and is acquired in 80–85% of cases. About 15–20% of adults with GHD represent the progression of persistent CO-GHD through transition and young adulthood. Many of the cases previously thought to be idiopathic (both in the adult and in the children subset) have now been recognized as due to head trauma. *Abbreviations*: AD autosomal dominant, AO-GHD adult-onset GH deficiency, AR autosomal recessive, BTK Bruton tyrosine kinase, CO-GHD childhood-onset GH deficiency, GHD GH deficiency, GHRH GH-releasing hormone, GHRH-R growth-hormone-releasing hormone receptor, HESX-1 homeobox expressed in ES cells 1 (also known as homeobox protein ANF), LHX LIM/homeobox protein, MPHD multiple pituitary hormone deficiencies, OTX2 orthodenticle homeobox 2, PITX-2 paired-like homeodomain transcription factor 2 (also known as pituitary homeobox 2), POU1F1 POU domain class 1 transcription factor 1 (also known as GH factor 1 or pituitary-specific positive transcription factor 1, Pit1), PROP1 PROP paired-like homeobox 1 (also known as prophet of Pit1), SOX SRY-related HMG-box, X X-linked

anatomic abnormalities, genetic causes, a tumor of the hypothalamic-pituitary area, surgery to remove the tumor, or radiation as part of the therapy of that tumor. The cause of GHD in children is more often idiopathic, isolated, and discovered because of poor growth. Table 7.1 reports the main differences between CO-GHD and AO-GHD [4–6].

Table 7.1 Differences between CO-GHD and AO-GHD

CO-GHD	AO-GHD
Isolated GHD is more frequent	GHD is more frequently associated with MPHD
Final adult height is normal but is approximately <1 SDS in comparison to mean normal population (subjects are usually <2–4% shorter than normal)	Normal final adult height
If GH replacement therapy is stopped after final adult is reached but GHD persists later in life, GHD adults usually have <16–20% height-normalized lean body mass, fat mass, and BMD	At initial diagnosis, GHD adults usually have normal lean body mass, fat mass, and BMD
Adults with a history of CO-GHD usually present with lower BMI and WHR	Adults with AO-GHD usually present with higher BMI and WHR
Adults with a history of CO-GHD usually have lower levels of IGF-1 and IGFBP3 (after withholding GH replacement therapy). IGF-1 response to rhGH may be blunted	Adults with AO-GHD usually have higher levels of IGF-1 and IGFBP3 (before starting GH replacement therapy). IGF-1 response to rhGH is generally acceptable
Adults with a history of CO-GHD usually have higher HDL levels	Adults with AO-GHD usually have lower HDL levels at initial diagnosis
Adults with a history of CO-GHD usually have higher QoL scores	Adults with AO-GHD usually have lower QoL scores at initial diagnosis

Abbreviations: *AO-GHD* adult-onset GH deficiency, *BMD* bone mineral density, *BMI* body mass index, *CO-GHD* childhood-onset GH deficiency, *GHD* GH deficiency, *HDL* high-density lipoprotein, *IGFBP3* insulin-like growth factor-binding protein 3, *MPHD* multiple pituitary hormone deficiencies, *rhGH* recombinant human GH, *QoL* quality of life, *WHR* waist-hip ratio

7.1.5 Is Counseling Important for Caregivers and Patients During the Transition Period?

The stage for informing the patient and his or her family about the possibility of continuing GHRT during adulthood must remain with the pediatrician taking care of the patient during the pediatric treatment period. "Transitioning" the patient to the adult indication requires consultation even before the pediatric indication is completed, so that the patient and family can come to an informed decision to continue or discontinue GHRT, addressing the patient – if necessary – to an adult endocrinologist with an expertise on hypopituitarism and GHD. There is no consensus about the ideal time for shifting the patient from pediatric to adult services. Some authors suggest transitioning the patient around the age of 19, concurrently to other changes in the patient's life (e.g., the end of high school) [7–9].

An analysis of the Hypopituitary Control and Complications Study (HypoCCS) showed that many patients stop GHRT during transition and do not undergo retesting, missing an important window of treatment [10]. Another retrospective study on 112 patients with CO-GHD indicated that 83% of patients stopped GHRT before transitioning from pediatric to adult care (median age at discontinuation was 16.3 years), but the first reevaluation by an adult endocrinologist occurred much later in life (median age at first visit: 19.4 years), despite a very high rate of persistent GHD [7]. Finally, another study on patients with chronic conditions transitioning to adult healthcare services highlighted that approximately 25% of patients are lost to follow-up [9]. In the latter cohort, the longer the period between the last pediatric visit and the first appointment at the adult clinic, the higher the dropout rate.

There are several reasons to plan carefully the shift of GHD patients from pediatric to adult endocrinology services. As described below, in most cases, CO-GHD must be confirmed during transition in order to identify those patients with persistent GHD who will require long-term GHRT. Retesting requires withholding GH for several weeks: the diagnosis of persistent GHD

should be posed rapidly and treatment (if necessary) restarted as expeditiously as possible in order to avoid a long gap without GH in a phase that is essential to attain full body maturation. Moreover, the longer the period without GH, the higher the risk of losing the patient to follow-up, with likely detrimental long-term effects [11].

7.2 Etiology

Figure 7.2 reports the causes of GHD in children and adults. A comprehensive review of the etiologies of GHD and hypopituitarism in pediatric patients can be found in ▶ Chap. 1.

In the pediatric population, the main cause of GHD is sporadic isolated GHD, thought to be related to impaired GH-releasing hormone (GHRH) secretion (idiopathic GHD) [12]. Between 3% and 30% of isolated GHD is familial, suggesting the presence of underlying genetic disorders [13]. In adults, pituitary tumors, craniopharyngioma, and other non-pituitary brain tumors (and related treatments) account for most cases of AO-GHD, although in the past 10 years an increased rate of idiopathic GHD and other non-common causes of GHD have been reported [14]. Head trauma and aneurysmal subarachnoid hemorrhage may account for a subset of GHD cases higher than previously suspected [15–17]. It has been appreciated that traumatic brain injury-induced GHD might occur in seemingly insignificant head trauma by history but still result in elements of hypopituitarism.

7.3 Clinical Presentation: Which Patients Remain Persistently GHD After Childhood?

Patients can be classified in high-, moderate-, and low-risk categories according to the cause of GHD and the likelihood of having persistent GHD during transition and adulthood. Table 7.2 reports several causes of GHD and the behavior of GH secretion according to etiology [13, 16, 18–33]. Patients with persistent CO-GHD during adulthood generally receive diagnosis at an earlier age and achieve final height earlier during adolescence when compared to those with transient GHD, and this is particularly evident in patients having structural and organic causes of GHD [26]. On the contrary, most of the cases of idiopathic, isolated CO-GHD without structural hypothalamic-pituitary abnormalities will recover normal GH secretion during transition and young adulthood [34].

7.4 Diagnostic Evaluation

The proven benefits of continuing GHRT during transition and adulthood in patients with persistent CO-GHD have challenged the practice of stopping treatment at the end of linear growth. This has led to extensive research in the field, in the attempt to optimize indications on how to manage patients with a suspect of persistent CO-GHD. Table 7.3 reports the indications of consensus guidelines regarding if, when, and how to retest patients with a likelihood of persistent CO-GHD [24, 35–37]. All guidelines agree on the necessity to retest almost all patients during transition after stopping GHRT for at least 4 weeks. Biochemical reevaluation can be performed by either serum IGF-1 evaluation or provocative testing, depending on the probability of persistent GHD. Furthermore, a cautious follow-up should be encouraged for patients with discordant laboratory results during transition and young adulthood and those at risk for developing late-onset GHD (e.g., cranial irradiation).

7.4.1 When Should Testing for Persistent GHD After Childhood Be Performed?

The ideal timing to retest patients with CO-GHD is controversial. A reevaluation could be performed during mid-puberty, especially in those patients having a low risk of persistent GHD [38]. The hypothesis is that pubertal sexual hormones can enhance endogenous GH secretion [39]: early retesting would reduce the duration of unnecessary GHRT, but we believe that this approach is less useful in patients highly likely to remain GHD (e.g., MPHD, structural and organic causes of GHD). Delayed retesting at the end of transition (early to mid-twenties) is not advisable because patients with transient GHD will receive years of unnecessary treatment. Most authors agree that biochemical reevaluation should be performed when near-adult height is achieved (defined by

Table 7.2 Causes of CO-GHD and behavior of GH secretion during transition and adulthood

Isolated CO-GHD	>2/3 of patients can have a normal response if retested for GHD during transition and adulthood. Subjects with idiopathic CO-GHD and no structural pituitary abnormalities are more likely to be GH sufficient at retesting. Several causes have been advocated in patients with a diagnosis of CO-GHD and normal GH responses at provocative tests during transition and adulthood:
	Partial GHD: It is sufficient to cause short stature during childhood but might not meet criteria for AO-GHD diagnosis
	Transient GHD (?)
	Lack of reproducibility of provocative tests: Inter- and intra-individual variability; changes of diagnostic criteria over time
	GH neurosecretory dysfunction: Some patients can show a normal response to provocative tests, but they can have an impaired spontaneous GH secretion
	No sexual priming in peripubertal children: False-positive results to GH provocative tests can occur in patients with pubertal delay if no sexual priming is performed before testing[a]
	Patients presenting with a low GH peak during provocative tests at initial diagnosis are more likely to remain GHD. Likewise, children with a partial GHD have a higher chance of becoming GH sufficient later in life
	Patients diagnosed with CO-GHD earlier in life are more likely to remain GHD
	CO-GHD patients reaching final adult height at earlier age are more likely to remain GHD
Genetic causes of CO-GHD	They can cause isolated GHD or, more frequently, MPHD
	GHD is permanent
	GH-E32A and GH-R183H mutations causing type II, autosomal dominant, isolated GHD: Cases of normal response to GH provocative testing have been reported in patients treated with rhGH during childhood and retested at transition. This effect was transient and patients became GH deficient later in life[b]
Structural causes of CO-GHD (congenital anomalies with abnormal pituitary at MRI)	More than 95% of patients have persistent GHD
	Those with isolated GHD may reverse to normal
Organic causes of CO-GHD (e.g., surgery) and MPHD (≥ 3 axes affected)	More than 95% of patients have persistent GHD
Brain trauma	10–60% of children and adults develop hypopituitarism after brain trauma
	GH, FSH, and LH are the most frequently affected hormones. There is apparently no relationship between trauma entity and the risk of GHD
	GHD may be transient (especially in children)

Table 7.2 (continued)

Irradiation	Risk of GHD is higher:
	For a lower age at the time of irradiation
	For higher radiation doses (risk usually starts for doses >18 Gy; >50% of patients develop GHD for a total amount of radiation >40 Gy)
	GHD can occur years after irradiation exposure: patients with normal GH response need a long-term follow-up and should be retested for 5–10 years after completion of radiotherapy
	When GHD develops, it is generally permanent, although cases of transient GHD have been reported
	Radiation therapy may cause precocious puberty (particularly in females) and concurrent GHD. In this case growth velocity may appear normal, despite impairment of GH secretion
Ectopic posterior pituitary	If the ectopic posterior pituitary is located at the median eminence, GHD is very likely to be permanent
	If the ectopic posterior pituitary is located along the pituitary stalk, a spontaneous resolution of GHD is possible
	Patients with an ectopic posterior pituitary can have non-concordant laboratory results (normal stimulated GH peak but low IGF-1), requiring long-term monitoring
Autoimmune GHD	The presence of PA may be associated with hypopituitarism (autoimmune etiology). Isolated GHD can develop in this setting
	Children with GHD and a high PA title are more likely to remain GHD during transition and adulthood
	Patients with GHD and a middle PA title may resolve GHD throughout life, but they can develop isolated hypogonadotropic hypogonadism

[a]Sex hormones (particularly estrogens) stimulate endogenous GH secretion, and children with pathological responses to GH testing before puberty may become GH sufficient as puberty progresses. Hence, some pediatric endocrinologists give oral estrogens (both in females and in males) or intramuscular testosterone (only in males) a few days before GH testing in order to discriminate between patients with pubertal delay and those with true GHD. This could be particularly useful in patients with an intermediate GH response during provocative tests, as sex steroid priming might reduce the rate of false-positive results. There is currently no consensus regarding the use of (and the way to use) sex steroid priming in peripubertal children before performing a GH stimulation test
[b]GH-E32A and GH-R183H mutations cause type II, autosomal dominant, isolated GHD. Patients with heterozygous mutation will produce both wild-type GH (22-kDa isoform) and a mutant GH: a truncated, nonfunctioning 17.5-kDa isoform for the GH-E32A mutation and an isoform causing prolonged retention into secretory granules of somatotropes for the GH-R183H mutation. Mutated GH exerts a dominant-negative effect on normal GH production, presumably because of constant endogenous GHRH stimulation with detrimental effects on wild-type GH secretion. Giving exogenous rhGH can remove the GHRH stimulus and restore normal somatotropic function, which can lead to a normal response of GH at retesting
Abbreviations: *AO-GHD* adult-onset GH deficiency, *CO-GHD* childhood-onset GH deficiency, *FSH* follicle-stimulating hormone, *GHD* GH deficiency, *GHRH* GH-releasing hormone, *Gy* gray, *kDa* kilodalton, *LH* luteinizing hormone, *MPHD* multiple pituitary hormone deficiencies, *MRI*, magnetic resonance imaging, *PA* pituitary antibodies, *rhGH* recombinant human GH

Table 7.3 Consensus guidelines: if, when, and how to retest patients with CO-GHD during transition and adulthood

Endocrine Society (2011)	Patients with CO-GHD who are candidates for GH therapy should be retested for GHD after adult height achievement (transition from pediatric to adult care)
	A low IGF-1 after stopping GH replacement therapy for at least 1 month is sufficient to prove persistent GHD in patients with CO-GHD and multiple pituitary hormone deficiencies (three or more axes) if they have:
	A radiologically confirmed congenital anomaly in the sellar or suprasellar region
	Acquired hypothalamic-pituitary disease (e.g., craniopharyngioma)
	Previous surgery for lesions affecting the hypothalamic-pituitary area
	Previous high-dose irradiation of the hypothalamic-pituitary area
	Known mutations causing GHD
	Irreversible lesions/damage of the pituitary gland causing three or more pituitary hormone deficits
	Provocative testing is required in all other causes of CO-GHD. GH replacement therapy should be stopped for at least 1 month before retesting:
	The ITT and the GHRH-arginine test show enough sensitivity and specificity to establish the diagnosis of GHD. If the ITT and the GHRH-arginine test cannot be performed, the glucagon stimulation test is a feasible option
	GHRH-arginine test may be misleading in patients with recent (<10 years), hypothalamic causes of suspected GHD (e.g., irradiation)
	Multiple sampling of GH levels would be useful to assess the diagnosis of GHD, but it isn't a feasible option in routine clinical practice
	If deficiencies of other pituitary hormones are present, they should be corrected before testing for GHD
American Association of Clinical Endocrinologists (2009)	GH should be only given to patients with clinical features of adult GHD and a biochemically proven diagnosis. Patients with CO-GHD should be retested for GHD after final height achievement. At least 1 month of GH withholding is necessary before retesting. If dynamic testing is required, ITT should be the preferred option (if feasible): The GHRH-arginine test and the glucagon test are reliable alternatives
	For children receiving GH treatment for conditions other than GHD (e.g., Turner's syndrome and idiopathic short stature), there is no proven benefit to continuing GH treatment during adulthood. These patients should not be tested for GHD when adult height is achieved
	Transition patients with an organic hypothalamic-pituitary disease, three or more pituitary hormone deficiencies, and a low IGF-1 (<2.5 percentile of the age- and sex-adjusted range) do not require dynamic testing and can start GH replacement therapy. Organic diseases include known mutations causing GHD, embryopathic/congenital defects, and irreversible hypothalamic-pituitary structural lesions
	Transition patients with an organic disease, up to two pituitary hormone deficiencies and a low IGF-1 (<50 percentile of the age- and sex-adjusted range) require a provocative test (ITT or GHRH-arginine test)
	Patients with idiopathic CO-GHD or GHD of possible hypothalamic origin should be evaluated as follows:
	If there is a low suspicion of GHD and IGF-1 is normal (≥ 0 SDS of the age- and sex-adjusted range), observe the patient
	If the likelihood of GHD is high (e.g., multiple pituitary hormone deficiencies are present) and IGF-1 is low (<0 SDS of the age- and sex-adjusted range), perform a provocative testing (ITT, GHRH-arginine test, arginine test, or glucagon test). If response to GHRH-arginine is normal and suspicion is still high, proceed to ITT, glucagon, or arginine test

Table 7.3 (continued)

GH research society (2007)	Patients with CO-GHD should be retested for GHD after final height achievement
	For children receiving GH treatment for conditions other than GHD (e.g., Turner's syndrome and small for gestational age), there is no proven benefit to continuing GH treatment during adulthood. These patients should not be tested for GHD when adult height is achieved
	A GH stimulation test is not required in:
	Patients with three or more pituitary hormone deficiencies and IGF-1 below the reference range (after at least 1 month off GH treatment)
	Patients with genetic mutations associated with GHD (e.g., *POU1F1*, *PROP-1*, *HESX-1*, *LHX-3*, *LHX-4*, *GH-1*, *GHRH-R* genes)
	A GH stimulation test is required in all other patients with CO-GHD (after withholding GH replacement therapy for at least 1 month). ITT should be the preferred option (if feasible): The GHRH-arginine test and the glucagon test are reliable alternatives. The use of recombinant 22 kDa GH calibrator (IRP 98/574) is recommended in all GH assays
	Consider a second reinvestigation at the completion of the somatic growth (around age 25 years):
	Patients with idiopathic CO-GHD. This may affect the decision to continue a long-term GH replacement therapy during adulthood
	Patients with discordant results of provocative GH testing during transition (e.g., normal GH on stimulation but low IGF-1), who didn't receive GH therapy
	In patients who received pituitary irradiation and with a normal response to the GHRH-arginine test, ITT should also be performed
	Plan long-term follow-up of patients who received pituitary irradiation or with infiltrative/inflammatory lesions of the hypothalamic-pituitary area, as GHD may develop many years after the initial insult
European Society for Paediatric Endocrinology (2005)	In patients with severe congenital or acquired panhypopituitarism (four or five hormone deficiencies), GH replacement therapy can be continued without interruption during transition
	All other patients need reevaluation during transition to confirm GHD. After withholding GH replacement therapy for at least 1 month, patients should be divided as follows:
	High likelihood of severe GHD (severe CO-GHD with or without two or three additional pituitary hormone deficits, severe GHD due to structural hypothalamic-pituitary abnormalities, patients treated for brain tumors or who received high-dose cranial irradiation): Measure IGF-1. If it is ≤ -2 SDS, no provocative test is needed. If IGF-1 is > -2 SDS, a GH stimulation test should be performed
	Low likelihood of severe GHD (all other patients, including idiopathic CO-GHD, either isolated or with one additional hormone deficit): Measure IGF-1 and perform a GH stimulation test
	ITT should be the test of choice. Arginine or glucagon tests are viable alternatives. The GHRH-arginine test may be unreliable in patients with hypothalamic GHD
	If deficiencies of other pituitary hormones are present, they should be corrected before testing for GHD
	Plan follow-up for:
	Patients with discordant results of provocative GH testing during transition (e.g., normal GH on stimulation but low IGF-1)
	Patients at risk for late-onset GHD (e.g., history of cranial irradiation)

Abbreviations: *AO-GHD* adult-onset GH deficiency, *CO-GHD* childhood-onset GH deficiency, *GHD* GH deficiency, *GHRH* GH-releasing hormone, *GHRH-R* growth-hormone-releasing hormone receptor, *HESX-1* homeobox expressed in ES cells 1 (also known as homeobox protein ANF), *IGF-1* insulin-like growth factor 1, *IRP* International Reference Preparation, *LHX* LIM/homeobox protein, *POU1F1* POU domain class 1 transcription factor 1 (also known as GH factor 1 or pituitary-specific positive transcription factor 1, Pit1), *PROP1* PROP paired-like homeobox 1 (also known as prophet of Pit1), *SDS* standard deviation score

growth velocity <2 cm/year) and bone age is of 16–17 years in males and 14–15 years in females [21, 40, 41]. This is presumably the best compromise in order not to miss an important window of treatment in those with persistent GHD and to avoid unnecessary treatment in those who restore normal endogenous GH secretion.

7.4.2 How to Test for Persistent GHD After Childhood?

GHRT has to be stopped before retesting to remove the negative feedback of exogenous GH on endogenous secretion. An interval of 1–3 months without GHRT is widely considered adequate.

It is important to remember that other replacement therapies must be optimized in patients with MPHD before retesting: poorly controlled hypothyroidism, in particular, can lower IGF-1 levels and cause blunted GH responses at dynamic testing [42].

7.4.2.1 Basal Evaluation

Hartman et al. have suggested that – with appropriate clinical history of pituitary disease – if the IGF-1 serum concentration is <84 ng/ml (utilizing the Esoterix IGF-1 assay) or the pituitary damage causes the loss of 3 or more pituitary hormones, there is a 98% chance the patient is GH deficient making provocative GH testing optional [25]. The value of 84 ng/ml corresponds to an IGF-1 approximately 3 standard deviations (SD) below sex- and age-matched controls for patients older than 28 years. The authors highlight, however, that even lower SD IGF-1 values are required to obtain such a high positive predictive value in younger patients. Finally, they observed that IGF-1 below 2 SD in comparison to sex- and age-matched controls predicts GHD in only 83% of cases, which is inadequate to avoid dynamic testing [25]. Maghnie et al. have confirmed these observations, reporting that a sex- and aged-corrected IGF-1 below 2 SD has a sensitivity of only 62%, with a consistent overlap between controls, transition patients with isolated GHD, and those with MPHD [43].

Therefore, we can conclude that a low basal IGF-1 can have a diagnostic value per se in cases of very likely persistent GHD (three or more pituitary hormone deficiencies plus congenital anomalies, organic causes, iatrogenic damage, or genetic mutations associated with GHD). A very low IGF-1 (3 SD below sex- and age-matched controls) can have a diagnostic significance, as long as other causes of low IGF-1 are excluded, such as hypothyroidism, liver disease, renal failure, malnutrition, and therapy with oral estrogens. All other conditions require a provocative testing to confirm a persistent CO-GHD.

7.4.2.2 Provocative Testing

As in children, there is no "foolproof magic" stimulation test during transition. Table 7.4 reports the recommended cutoffs of GH provocative tests according to consensus guidelines [24, 35–37]. The 22 kDa recombinant GH isoform (international standard IS 98/574) is currently recommended to standardize GH assays [44].

The insulin tolerance test (ITT) is still the gold standard. Insulin is given intravenously (usually 0.1–0.15 units/kg of body weight), and sampling of blood for glucose and GH is done before and 15, 30, 60, 90, and 120 min after the injection. The glucose should go below 50 mg/dL, and patients should have symptoms of hypoglycemia: this is a proper hypoglycemic stimulus for endogenous GH and other pituitary hormones secretion. The test should be stopped (and glucose given intravenously) if severe neuroglycopenic symptoms develop or glycemia falls to <35 mg/dL. The ITT requires close monitoring, and it may be dangerous in patients with a history of cardiovascular, cerebrovascular, or seizure disorders.

Where GHRH is available, the combined arginine + GHRH test is a viable option to evaluate patients during transition. About One μg/kg of GHRH is given intravenously at the beginning of the test, and 0.5 g/kg (maximum 30 g) of arginine is infused over 30 min: blood samples for GH should be taken 30, 60, 90, and 120 min after the start of arginine infusion. This test has very high sensitivity and specificity both in children and in adults [45] but is deeply influenced by body mass index (BMI): the higher the BMI, the lower the GH peak, and therefore BMI-related cutoffs should be used [46]. Moreover, arginine + GHRH test poses some problems in patients with idiopathic CO-GHD and those with neurosecretory GHD, which might be observed after cranial irradiation that was performed within 10 years. In these two diagnoses, the pituitary fails much later than the hypothalamus causing a deficiency in hypothalamic GHRH with an intact

Table 7.4 Consensus guidelines: GH cutoffs during provocative testing

	ITT	GHRH-arginine test	Glucagon test	Arginine test
Endocrine Society (2011)	GH <4.1 μg/L Cutoff for stimulated GH may be higher in adolescents and young adults (6.1 μg/L)	GH <5.1 μg/L. Consider using different cutoffs according to BMI: BMI <25 kg/m^2: GH <11.5 μg/L BMI 25–30 kg/m^2: GH <8.0 μg/L BMI >30 kg/m^2: GH <4.2 μg/L Cutoffs for stimulated GH may be higher in adolescents and young adults (5.6 μg/L or 19.0 μg/L, according to different studies)	GH <2.5–3 μg/L	Not recommended
American Association of Clinical Endocrinologists (2009)	GH <5.0 μg/L	Use different cutoffs according to BMI: BMI <25 kg/m^2: GH <11.0 μg/L BMI 25–30 kg/m^2: GH <8.0 μg/L BMI >30 kg/m^2: GH <4.0 μg/L	GH <3.0 μg/L	GH <0.4 μg/L
GH research society (2007)	In adults: GH <3.0 μg/L During transition: GH <6.0 μg/L	Use different cutoffs according to BMI: BMI <25 kg/m^2: GH <11.0 μg/L BMI 25–30 kg/m^2: GH <8.0 μg/L BMI >30 kg/m^2: GH <4.0 μg/L	GH <3.0 μg/L	Not recommended in adults. May be used in nonobese adolescents
European Society for Paediatric Endocrinology (2005)	In adults: GH <3.0 μg/L During transition: GH <5.0 μg/L Evaluate long-term follow-up in transition patients with GH ≥5.0 but <5.0 μg/L, because of the risk of an evolving endocrinopathy	Not given	Not given	Not given

Abbreviations: *BMI* body mass index, *GHRH* GH-releasing hormone, *ITT* insulin tolerance test

or functioning pituitary. Supplying GHRH in the test can result in a false-negative response, as it stimulates only the pituitary secretory capacity: the results of arginine + GHRH testing may be misleading within 10 years of pituitary irradiation [47, 48].

The current unavailability of GHRH to use in the arginine + GHRH test in some countries (e.g., the United States) makes an alternative test necessary. After ITT the next test suggested is the glucagon stimulation test. The dose of glucagon is 1 mg given intramuscular or subcutaneously (or 1.5 mg if the patient is 90 kg in weight or heavier). The sampling for GH during the glucagon test is every 30 min for 4 h [49]: the test may cause discomfort (nausea, vomiting, headache, sweating) and late-onset hypoglycemia. The body of literature about glucagon stimulation test is smaller than for ITT and the arginine + GHRH test: further refinement of this test may uncover some categories (overweight patients) where a different cutoff may be necessary, as has been confirmed with the arginine + GHRH test [46, 50].

In summary, more stringent testing of patients with idiopathic CO-GHD is necessary. To this end, ITT, glucagon test, and arginine + GHRH (where

available) are suggested to provide convincing evidence of persistent GHD. The usage of more than one test may be considered in case of borderline or non-concordant results (e.g., discrepancy between basal IGF-1 and stimulated GH) or when clinical history poses some challenges (e.g., recent brain irradiation). Less stringent tests such as levodopa, clonidine, or arginine alone are currently not suggested, since they have low sensitivity and specificity for GHD diagnosis [51].

7.5 Outcomes and Possible Complications

GHRT is recommended if GHD is confirmed during the transition period, in order to complete somatic development, obtain full skeletal maturation, and promote increase of lean mass. Retesting for GHD and – if confirmed – starting GHRT should be done as quickly as possible [24, 37]. In adolescents who decline the offer to continue GHRT, monitoring of GH-dependent endpoints is required. Evidence of subsequent deterioration constitutes the basis for reassessment of the decision to reinstate GHRT [35].

7.5.1 Why Should We Replace Young Adults with GH?

The indications for restarting GHRT in persistently GHD children who have completed growth targets and are in transition to adulthood are similar to those for adults who have developed the deficiency later in life. GH deficiency has been associated with decreased QoL and an increase in bone fracture rates [52–54]. Other reasons include abnormal risk factors for accelerated atherogenesis, including increased cholesterol and decreased high-density lipoprotein (HDL) cholesterol. Although these findings are compelling reasons to treat young adults who have completed vertical growth, the major impetus stems from the issues surrounding bone health. Table 7.5 reports the outcomes of GHRT in transition patients and adults with GHD [55–82]. Despite the beneficial effects of GHRT on several cardiovascular risk factors (reduced visceral fat, and better lipid profile), there is no clear evidence that this replacement therapy decreases the mortality rate in transition and adult hypopituitary patients [83].

7.5.2 Is It Possible to Predict the Clinical Response to GHRT in Adults?

During childhood, linear growth is the primary outcome of GHRT. It is an objective parameter easily assessed by pediatric endocrinologists. During transition and adulthood, other parameters become important: body composition (including BMI and waist circumference), BMD, lipid profile, and QoL. However, if we consider them separately, none of these measurements shows enough sensitivity and specificity to properly assess the clinical response to GHRT, and physicians mainly use IGF-1 as a biochemical marker of response. Nonetheless, whether an increase of IGF-1 levels reflects a positive clinical response is still a matter of debate.

An analysis of the KIMS International Metabolic Database [84] showed that the worse the initial values of some endpoints (QoL, low-density lipoprotein-cholesterol, and waist-hip ratio), the better the response of the same variable. Additionally, a later analysis of the same database highlighted a sexual dimorphism in the clinical response, with greater responses of BMI in men and of QoL in women [85]. On the wake of these observations, Schneider et al. [86] identified lower IGF-1 values, higher total cholesterol levels, higher waist circumference, and worse QoL (using the QoL-AGHDA questionnaire) at baseline evaluation as the best predictors of the clinical response to 2 years of GHRT. These authors suggest that, given a biochemically proven GHD during adulthood, the basal and 6-month evaluation of the aforementioned parameters may aid the clinician in the decision of starting or continuing GHRT. This replacement therapy is long-term and expensive and exposes the patient to potential side effects. Therefore, it is imperative to identify which adult patients are more likely to be good responders. Radovick et al. [21] suggest to individualize indications to treat patients with persistent CO-GHD during transition on the basis of the diagnosis (isolated GHD vs. MPHD) and of the risk of metabolic complications (considering BMD and body composition). These authors suggest confirming treatment in transitioning patients with MPHD if the risk/benefit ratio is favorable and the patients agrees to continue daily injections. On the contrary, in

Table 7.5 Effects of GHRT on several clinical, biochemical, and radiological outcomes during transition and adulthood

BMD	In normal subjects BMD continues to increase during transition and young adulthood, after the final adult height has been achieved (BMD peak, defined as a T-score > −1 when compared to the average adult bone mass, is usually attained by age 25–30 years in men and age 20–25 years in women)
	Most authors have shown that GHRT in CO-GHD patients is useful during transition to obtain a normal BMD peak and bone maturation, which are protective factors for the development of osteoporosis. However, some studies didn't reveal any benefit of GHRT on BMD during transition, questioning the protective role of rhGH against osteoporosis and fragility fractures during adulthood
	Beneficial effect of GHRT on bone appears to be influenced by gender, age, and treatment duration, being more pronounced in younger patients and in men, especially after 1 year of treatment and in those with lower baseline BMD and more severe GHD at diagnosis. GH stimulates both bone formation and resorption, which can cause BMD to decline during the first months after initiation of GHRT; however, after 12–18 months of treatment, the anabolic effects of GH on bone become evident
	The duration of the interval between rhGH discontinuation and re-initiation in patients with CO-GHD and persistent GHD directly predicts lower BMD. This highlights the importance to carefully plan retesting during transition and to restart GHRT as expeditiously as possible when GHD is confirmed
	Adult women with CO-GHD who didn't receive GHRT during childhood are more likely to have lower BMD in the femoral neck, especially when the temporal gap between hypothalamic-pituitary insult and the start of GHRT is extended
	Some authors have reported that, by using appropriate size corrections, bone density is normal in children and adults with isolated or idiopathic CO-GHD, who don't show an increased risk of fragility fractures. This latter risk seems to be increased only in adult patients with organic causes of hypopituitarism
Maturation and maintenance of body composition	GH is required to achieve and maintain full adult body composition
	Stopping GHRT when near-adult height is reached but GHD persists, causes total body fat (especially visceral fat) to increase and lean mass to decrease during transition and adulthood. On the contrary, continuing GHRT during transition and adulthood improves lean mass/fat mass body ratio, and this beneficial effect seems to be more pronounced in males. Some authors, however, didn't report significant changes of fat and lean mass in untreated transition patients with persistent CO-GHD
	When comparing patients with CO-GHD, either isolated or associated with MPHD, it has been reported that associated pituitary deficiencies predict higher BMI and waist circumference, but GHRT improves these parameters to the same extent in isolated and associated GHD
	In a cohort of GHD patients on long-term GHRT (at least 10 years), waist circumference and BMI increased in comparison to baseline[a]
	Untreated GHD during transition is associated with lower muscle cross-sectional area and decreased muscle strength. However, this latter observation wasn't confirmed by other authors in transition patients
QoL	Untreated GHD during transition and adulthood is associated with decreased QoL, mainly issues related to cognitive function, fatigue, well-being, and mood. However, data regarding transition are conflicting
	rhGH withholding at final height in young adults with CO-GHD can correlate with worse psychosocial outcomes, especially in those with MPHD, those who started GHRT after 12 years of age, and those who were shorter at first evaluation
	A longer discontinuation of GHRT during transition and young adulthood in patients with persistent, non-idiopathic CO-GHD leads to poorer QoL scores

Table 7.5 (continued)

	MPHD predict worse QoL in comparison to isolated GHD, but GHRT improves these parameters to the same extent in isolated and associated GHD
	Current evidence doesn't provide enough data to establish whether restarting GHRT during transition in patients with persistent CO-GHD might have significant and favorable effects on QoL
Lipid profile and other cardiovascular risk factors	An abnormal lipid profile has been observed in transition and adult patients with persistent, untreated CO-GHD. Reported alterations include ↓HDL and ↓TG; ↑LDL and ↑lipoprotein(a); ↑TG, ↑LDL, and ↑total/HDL cholesterol ratio; ↑total cholesterol and ↑LDL/HDL ratio; and ↓HDL and ↑LDL/HDL ratio
	In a cohort of GHD patients on long-term GHRT (at least 10 years), total cholesterol and LDL were reduced, and HDL were increased in comparison to baseline[a]
	Some authors reported no differences in lipid profile between treated and untreated transition patients, especially those with isolated or idiopathic CO-GHD
	↑fibrinogen has been observed in adolescents with untreated GHD
	Increased diastolic blood pressure has been reported in transition patients with persistent CO-GHD who stopped GHRT
	Conflicting results have been reported regarding the potential effect of GHRT on IMT. Higher IMT has been described in Japanese adults with a history of CO-GHD and after at least 3 years of rhGH discontinuation, whereas other authors didn't find any alteration of IMT in adolescents with CO-GH during and after discontinuation of GHRT
Glucose metabolism	↑insulin sensitivity has been described after rhGH withholding at final height. This was evident both in patients with persistent GHD during transition and adulthood and in those who restored a normal GH secretion
	↓insulin sensitivity has been reported in transition and adult patients with persistent CO-GHD after restarting GHRT, although contrasting results were reported by other authors
	Several studies suggest that restarting GHRT doesn't significantly affect insulin resistance, insulin sensitivity, and HbA1c levels in the long term, although an increase in insulin circulating levels has been reported
	In a cohort of GHD patients on long-term GHRT (at least 10 years), fasting glucose levels were increased in comparison to baseline. The prevalence of metabolic syndrome was increased as well (especially in males)[a]
	Current evidence doesn't provide enough data to establish a relationship between rhGH and the risk of type 2 diabetes. However, it should be noted that untreated GHD can predispose to diabetes per se (increased BMI and visceral fat)
Cardiac structure and performance	↓LVM has been reported in adolescents with persistent CO-GHD after 6 months of rhGH discontinuation, which can improve after GHRT re-initiation
	Some studies did not report substantial modifications of cardiac structure and function despite GHRT

[a]The study by Claessen KM et al. [71] provides interesting insights regarding the long-term effects of GHRT, as included patients were evaluated along at least 10 years of follow-up. The authors conclude that the prevalence of metabolic syndrome increases during long-term GHRT (mainly due to the development of abdominal obesity, hypertriglyceridemia, and hyperglycemia), despite an improvement of cholesterol profile (total cholesterol, HDL, and LDL): the effects of these changes on overall cardiovascular risk and mortality is currently unknown. However, it must be noted that most of the enrolled patients in this study had MPHD: therefore, it is difficult to assess to which extent GHRT plays a role in these observations in comparison to other replacement therapies. Finally, 38% of subjects in this cohort received cranial radiotherapy: irradiation is a risk factor for metabolic syndrome per se, perhaps because of the long-term effects of hypothalamic radiation-induced damage

Abbreviations: *BMD* bone mineral density, *BMI* body mass index, *CO-GHD* childhood-onset GH deficiency, *GHD* GH deficiency, *GHRT* GH replacement therapy, *HbA1c* hemoglobin A1c (glycosylated hemoglobin), *HDL* high-density lipoprotein, *IMT* intima-media thickness, *LDL* low-density lipoprotein, *LVM* left ventricular mass, *MPHD* multiple pituitary hormone deficiencies, *QoL* quality of life, *rhGH* recombinant human GH, *TG* triglycerides, ↑ increased, ↓ decreased

persistent isolated GHD GHRT is encouraged in those with an abnormal metabolic profile (low BMD, high fat mass, low lean body mass). Low-risk patients, however, should be followed up carefully, as to detect early alterations of these parameters or a worsening of the QoL and discuss once again the possibility to restart GHRT.

7.5.3 Are There Complications and Side Effects Possibly Related to GHRT in Adults?

GHRT is usually considered safe in adults. However, several potential adverse effects must be taken into consideration before starting GHRT and during follow-up. A comprehensive review of the possible complications and side effects in pediatric patients on GHRT can be found in ▶ Chap. 1.

7.5.3.1 Mortality

GHD patients may be a category with an intrinsic higher mortality risk, related to the underlying condition causing GHD (many individuals having a history of treated brain neoplasms, previous cranial irradiation, or subarachnoid hemorrhage). Hence, it is very difficult to assess whether GHRT can play a role in affecting all-cause or cause-specific mortality. The "Safety and Appropriateness of Growth hormone treatments in Europe (SAGhE)" is a study involving approximately 25,000 European patients who received GHRT in childhood during the 1980s and 1990s. The aim of the consortium is to assess long-term safety of GHRT in these patients, comparing their mortality and cancer risk to the general population. A preliminary report of the French SAGhE study raised concerns regarding the safety of GHRT in about 6000 patients who received GH for idiopathic isolated GHD, neurosecretory dysfunction, and idiopathic short stature or born short for gestational age [87]. Despite these categories are generally regarded as "low risk" for mortality when compared to the general population, the French SAGhE study found an increase of 33% of all-cause mortality in this cohort of patients, especially in those who received >50 μg/kg of daily GH. Bone tumors and cerebral hemorrhage were the main causes of mortality, while the overall risk of cancer-related deaths was not increased. However, a report of the SAGhE study from the Netherlands, Belgium, and Sweden and an analysis of the Hypopituitary Control and Complications Study (HypoCCS) did not confirm these findings [10, 88]. Consequently, we can conclude that existing evidence does not support an association between GHRT and mortality risk [89]. Prospective, placebo-controlled studies involving healthy subjects would be necessary to address this point, but this is not an ethically feasible option.

7.5.3.2 Cancer Risk and Tumor Regrowth

GH and IGF-1 exert anabolic and anti-apoptotic effects promoting cellular growth and proliferation. Concerns have consequently been raised regarding the possible role of GHRT in increasing cancer risk and tumor regrowth.

The Pediatric Endocrine Society has recently performed a review of existing literature on this topic, focusing on children treated with GH and on cancer risk during treatment or throughout long-term surveillance after GHRT withholding [90]. Authors concluded that GHRT is safe in children without intrinsic risk factors for malignancy (chiefly those with idiopathic CO-GHD), and it is not associated with the development of new primary cancer. On the other hand, the existing body of evidence cannot exclude a relationship between increased cancer risk (second neoplasms) in childhood cancer survivors receiving GHRT [89]. These conclusions underline the necessity to discuss this aspect with patients and their caregivers, to assess the risk/benefit ratio in each case and to plan a long-term follow-up in patients with a history of pediatric malignancy. On the other hand, the risk of primary tumor regrowth in childhood cancer survivors receiving GHRT should not be a concern if the neoplasm has been successfully treated and there are no signs of active malignancy. There is no consensus regarding the optimal timing between anti-cancer treatment completion and the start of GHRT, and this should be decided on an individual basis. Evidence regarding the cancer risk in adults receiving GHRT are less robust, but publications suggest that there is no effect of GHRT on the risk of new primary neoplasms [89]. Data about recurrence of primary cancers and the development of second neoplasms in adult cancer survivors are inconclusive. A recent joint meeting of the European Society of Paediatric Endocrinology, the GH Research Society, and the Pediatric Endocrine Society "did not support cancer surveillance

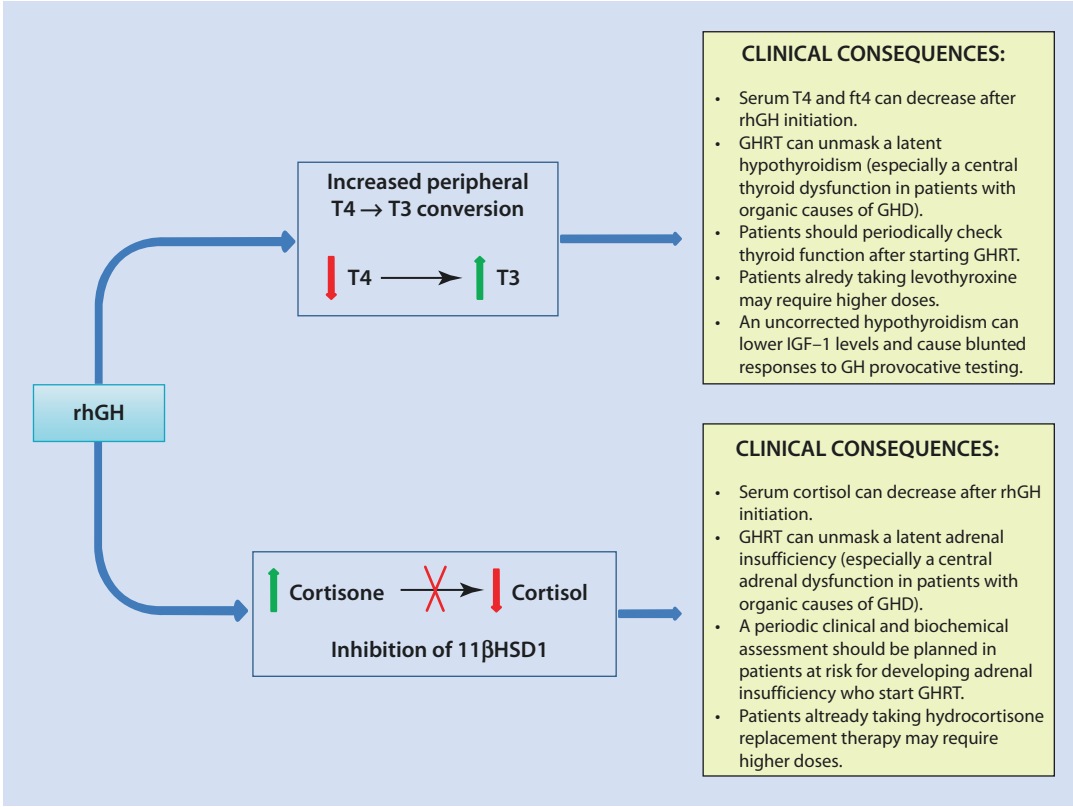


Fig. 7.3 Possible effects of GHRT on thyroid and adrenal function. GH increases peripheral T4 to T3 conversion and reduces the enzymatic activity of 11βHSD1. These actions may unmask latent hypothyroidism or adrenal insufficiency or lead to higher requirements of levothyroxine or hydrocortisone. *Abbreviations*: ↑ increased, ↓ decreased, CBG corticosteroid-binding globulin, fT4 free thyroxine, GHD GH deficiency, GHRT GH replacement therapy, IGF-1 insulin-like growth factor 1, MPHD multiple pituitary hormone deficiencies, rhGH recombinant human GH, T3 total triiodothyronine, T4 total thyroxine, TRH thyrotropin-releasing hormone, 11βHSD1 11β-hydroxysteroid dehydrogenase type 1

beyond local standard practice in patients (children and adults) currently treated with GH nor in those previously treated with GH (including those with a previous malignancy)" [89].

7.5.3.3 Glucose Metabolism and Diabetes Mellitus

GHRT can exert complex influences on insulin sensitivity. On one hand, GH is an insulin counter-regulatory hormone promoting gluconeogenesis in the liver and reducing glucose uptake by hepatocytes. On the other hand, GHD is associated with altered body composition (unfavorable lean mass/fat mass ratio) that can lead to insulin resistance and predispose to impairment of glucose metabolism.

Diabetes mellitus is not an absolute contraindication to GHRT. However, if the patient has diabetes and GH therapy is started, the glycemic control may worsen before it becomes better because of the positive effect on body composition [7]. Likewise, adults predisposed to diabetes (e.g., positive family history, personal history of gestational diabetes, metabolic syndrome) may experience a worsening of blood glucose control [91]. This suggests the importance of enhancing surveillance of individuals at risk for developing diabetes mellitus after the start of GHD. Finally, it is advisable to start GHRT with lower doses in these categories of patients.

7.5.3.4 Adrenal and Thyroid Function

Exogenous GH may affect adrenal and thyroid axes by increasing thyroxine and cortisol catabolism (see Fig. 7.3), which should be periodically checked during follow-up and after rhGH dose

titrations [92, 93]. Patients who received brain radiotherapy are chiefly at risk, as pituitary insufficiency may occur several years after the insult and GHRT might unmask central hypothyroidism and adrenal insufficiency during long-term follow-up.

7.5.3.5 Other Possible Side Effects

Adverse events reported in adults on GHRT include arthralgia, fluid retention (peripheral edema), paresthesias (especially carpal tunnel syndrome), and reduced concentration [94]. These side effects are dose-dependent and are generally transient. Starting treatment with low doses of GH, gradually titrated upwards, reduces the incidence of these side effects.

7.5.4 Are There Contraindications for Continuing GHRT?

Before reinstating GHRT during transition and adulthood, it may be important to consider the contraindications to GHRT that might exist or have developed since stopping GH therapy. The first and most important would be the development of an active malignancy. This should be obvious by the history, but the clinician should be aware of this possibility. Some patients may have had a central nervous system tumor that was irradiated in childhood. This should be reexamined by an appropriate MRI image of the brain. The tumor may have recurred, or more likely, a new tumor may have developed because of irradiation to the area. Developing a second central nervous system tumor is a known risk after cranial irradiation.

Another contraindication to GHRT is the presence of active proliferative or severe nonproliferative diabetic retinopathy, given the proven effects of the GH/IGF-1 axis on the retina in patients with diabetes [95].

Pregnancy is not an absolute contraindication to GH therapy [96]. The placenta produces chorionic somatomammotropin during the third trimester (or even earlier) and, therefore, GH is not necessary during the last 3 months of pregnancy [89]. A feasible treatment for pregnant GHD patient is to give two-thirds the dose in the first trimester, one-third in the second, and none in the third trimester. This usually keeps IGF-1 within the normal range.

7.6 Treatment

7.6.1 How to Treat Patients with Persistent CO-GHD During Transition and Adulthood?

rhGH is given by subcutaneous daily injections (typically at nighttime). Long-acting GH preparations are currently under investigation and may become a therapeutic advance in the near future [97].

Physiological endogenous GH secretion varies during lifetime, peaking at puberty and steadily decreasing thereafter. rhGH dosing should mimic this pattern, taking age and life phase into consideration. Other aspects, including gender, estrogen status, and comorbidities, have to be considered as well. Oral estrogens cause liver resistance to GH [98]. Because of this, we usually recommend transdermal estrogens in young hypopituitary women. The aim of GHRT should be to find the ideal rhGH dose that provides an optimal biochemical and clinical response avoiding side effects. Most authors agree that rhGH dosing should not be weight-based, as this strategy leads to a higher incidence of adverse events: clinicians should start GHRT with low doses, gradually titrating upward [99]. There is no difference between CO-GHD and AO-GHD in the indications of rhGH doses and adjustments, although IGF-1 response in CO-GHD may be blunted. ◘ Table 7.6 reports the indications of consensus guidelines regarding rhGH dosing [24, 35–37], while ◘ Fig. 7.4 highlights the factors potentially affecting the GH/IGF-1 axis and the response to rhGH in treated patients with GHD, focusing on the role of obesity in normal patients and those with GHD.

7.6.2 How to Monitor GHRT During Transition and Adulthood?

The indications of consensus guidelines regarding rhGH dose titration and follow-up of patients receiving GHRT are reported in ◘ Table 7.7 [24, 35–37]. Successful monitoring of the patient receiving GH therapy requires an awareness of side effects, not only of GH excess but also of symptoms associated with starting rhGH and/or

Table 7.6 Consensus guidelines: how to treat patients during transition and adulthood

Endocrine Society (2011)	Start treating with low doses of GH:
	Patients <30 years: 400–500 μg/day (patients during transition may require higher doses)
	Patients 30–60 years: 200–300 μg/day
	Titrate GH dosing according to clinical response, side effects, and IGF-1 levels. Weight-based regimens are discouraged. If necessary, increase daily dosing by 100–200 μg/day every 1–2 months. Take gender, estrogen status, and age into consideration when deciding the GH dosing:
	GH requirements are higher in some transition and young adult patients
	GH requirements are lower in older patients
	Women usually require higher GH doses to achieve the same IGF-1 response of men, especially if they are on concomitant oral estrogen. GH dosage may need to be lowered if women who stop oral estrogens or are switched to transdermal formulations
American Association of Clinical Endocrinologists (2009)	Start treating with low doses of GH:
	Patients <30 years: 400–500 μg/day. Patients during transition may require higher doses. When restarting GH replacement therapy during transition, the initial dose should be approximately 50% of the dose between the pediatric and the adult ones
	Patients 30–60 years: 200–300 μg/day
	Patients >60 years: 100–200 μg/day
	Patients with diabetes or prone to glucose intolerance (obese subjects or those with personal history of gestational diabetes or family history of diabetes): 100–200 μg/day
	GH dosing should be individualized in all patients and should not be weight-based. Titrate the dose upward until reaching the minimal dose that normalizes IGF-1 without causing unacceptable side effects. Increase daily dosing by 100–200 μg/day every 1–2 months based on clinical response, IGF-1, adverse effects, and individual considerations:
	Women on oral estrogens or with an intact hypothalamic-pituitary-gonadal axis require higher GH doses to achieve the same clinical and biochemical response of men
	Consider using longer time intervals and smaller dose increments in older subjects
	If patients are not compliant to daily injections, consider providing the weekly GH dose in three to four administrations per week
GH research society (2007)	Start treating with low doses of GH:
	Transition period: Intermediate dosed between the pediatric doses and the adult ones
	Young women: 300 μg/day
	Young men: 200 μg/day
	Older individuals: 100 μg/day
	GH dosing should be individualized in all patients and should not be weight-based. Dose escalation should be gradual, individualized, and guided by clinical and biochemical response
European Society for Paediatric Endocrinology (2005)	Start treating with low doses of GH:
	Transition period: 200–500 μg/day
	Women usually require higher doses of GH, especially if receiving oral estrogens

Abbreviations: *AO-GHD* adult-onset GH deficiency, *CO-GHD* childhood-onset GH deficiency, *GHD* GH deficiency, *IGF*-1 insulin-like growth factor 1

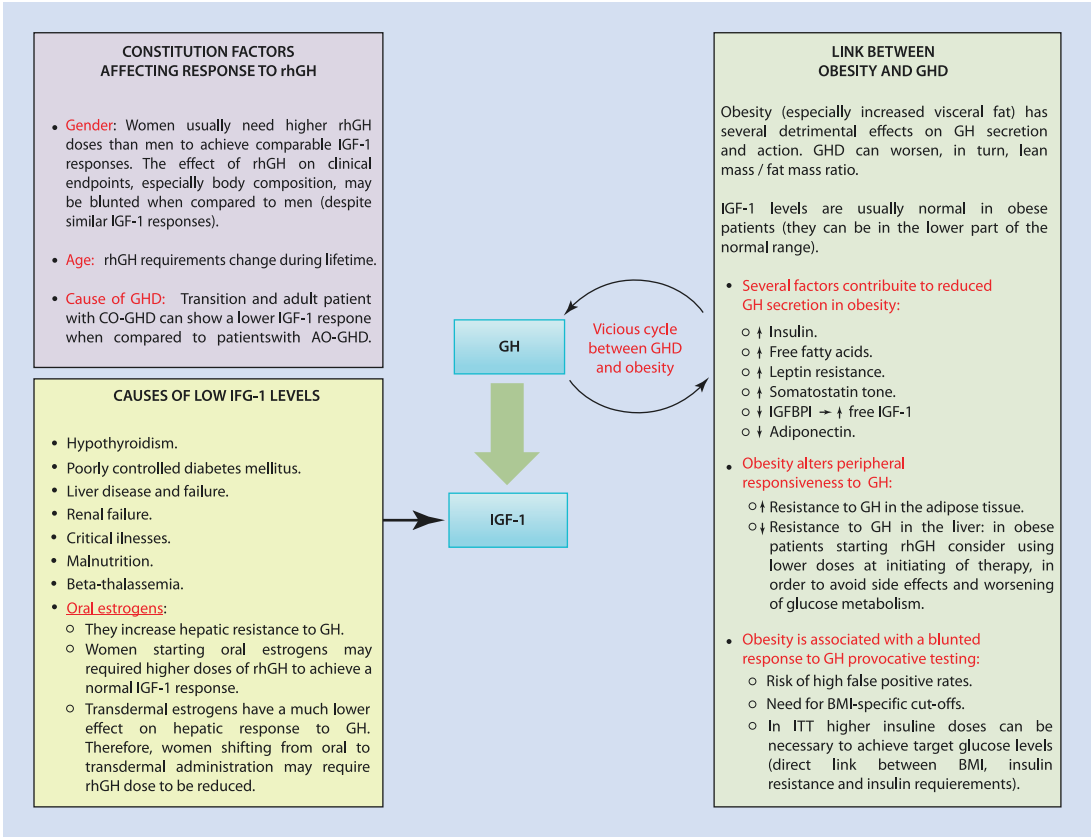


Fig. 7.4 Interactions between obesity and GH/IGF-1 axis and factors affecting GH provocative testing, IGF-1 levels, and response to GHRT. This figure reports modifiable and non-modifiable factors that can affect GH/IGF-1 axis. Premenopausal women and those on oral estrogen therapy generally have higher GH requirements, as estrogens cause a post-receptor inhibition of GH effects in hepatocytes. On the contrary, androgens and androgen replacement therapy do not affect GH action. *Abbreviations*: AO-GHD adult-onset GH deficiency, BMI body mass index, CO-GHD childhood-onset GH deficiency, GHD GH deficiency, IGF-1 insulin-like growth factor 1, IGFBP1 insulin-like growth factor-binding protein 1, ITT insulin tolerance test, rhGH, recombinant human GH

Table 7.7 Consensus guidelines: dose titration and follow-up of patients on GHRT

Endocrine Society (2011)	Patients should be monitored at 1–2 months intervals during dose titration and at 6-month intervals thereafter. Monitoring should include:
	Assessment of adverse effects
	Overall clinical evaluation: Clinical benefits of GH replacement therapy may not be evident for more than 6 months after starting treatment. Clinical examination should include the measurement of waist circumference and QoL questionnaires
	Serum IGF-1 levels: The commonly used target for IGF-1 is the upper half of the age-adjusted range. IGF-1 responses may be lower in patients with CO-GHD when compared to AO-GHD
	Annual check of fasting glucose and the lipid profile. Patients with diabetes mellitus may require adjustments in antidiabetic therapy
	Periodic control of thyroid and adrenal function. In hypopituitary patients, adjustments of thyroid hormone replacement therapy may be necessary after starting GH
	DXA scanning to assess BMD before starting treatment. If BMD is abnormal at baseline, repeat DXA every 1.5–2 years

(continued)

Table 7.7 (continued)

American Association of Clinical Endocrinologists (2009)	Patients should be monitored at 1–2 months intervals during dose titration. After achieving maintenance GH dose, patients should be monitored at 6–12 months intervals. Monitoring should include:
	Assessment of adverse effects
	Overall clinical evaluation, including measurement of blood pressure, waist circumference, heart rate, waist-to-hip-ratio, and signs and symptoms of adrenal insufficiency. Specific QoL questionnaires should be administered before starting GH replacement therapy and every 6–12 months thereafter
	Serum IGF-1 levels: The ideal target is the middle of the age- and sex-adjusted range. Consider a trial of higher GH doses to determine whether this provides further benefit as long as the serum IGF-1 levels remain within the normal range and the patient doesn't experience side effects
	Fasting glucose levels, glycated hemoglobin, and lipid profile
	Serum-free thyroxine, morning serum cortisol (and cosyntropin stimulation test, if necessary), serum testosterone. In hypopituitary patients, adjustments of thyroid hormone, glucocorticoid, and testosterone replacement therapy may be necessary after starting GH
	Electrocardiogram (echocardiogram and carotid ultrasound should be considered in selected cases)
	DXA scanning to assess BMD before starting treatment. If BMD is abnormal at baseline, repeat DXA every 2–3 years
	Periodic magnetic resonance imaging in patients with pituitary microadenomas or postsurgery residual pituitary tumors
	If no apparent or objective benefits of treatment are achieved after at least 2 years of GH replacement therapy, consider withholding treatment
GH research society (2007)	Titrate GH dosing according to serum IGF-1 levels:
	IGF-1 should be kept below the age- and sex-adjusted upper limit of normal range (including those patients with proved GHD, who have normal IGF-I levels at baseline)
	After GH dose titration, monitor IGF-1 no sooner than 6 weeks
	After achieving maintenance GH dose, monitor IGF-1 at least yearly
	In hypopituitary patients, adjustments of thyroid hormone and glucocorticoid replacement therapy may be necessary after starting GH
	QoL assessment is important but does not necessarily require a questionnaire
	Perform pituitary imaging before GH initiation in patients who successfully underwent treatment for tumors of the hypothalamic-pituitary area. In those with residual tumors, a regular imaging should be planned during treatment. GH replacement therapy does not impose a need for intensifying follow-up
European Society for Paediatric Endocrinology (2005)	Titrate GH dosing according to serum IGF-1 levels:
	IGF-1 should be kept within the age- and sex-adjusted upper normal range
	IGF-1 should be monitored every 6 months
	Patients should be monitored at least yearly. Monitoring should include:
	Height, weight, BMI, and waist and hip circumference
	Fasting plasma glucose, insulin, and glycated hemoglobin. Consider glucose tolerance testing in obese subjects and those with a family history of diabetes
	Annual magnetic resonance imaging for at least 3 years should be planned after treatment for intracranial malignancy. Patients with treated craniopharyngioma or pituitary adenoma should be reimaged at a frequency determined by the perceived risk of regrowth of the tumor
	Bone densitometry and lipid profile at 2–5-year intervals

Abbreviations: *BMD* bone mineral density, *BMI* body mass index, *DXA* dual-energy X-ray absorptiometry, *GHD* GH deficiency, *IGF*-1 insulin-like growth factor 1, *QoL* quality of life

raising a dose. Patients can frequently have transient adverse symptoms (usually days 10–14) after starting or raising a dose. These consist of muscle or joint pain and disappear by days 21–28 after initiating or raising the dose. If symptoms persist after this period of time, the dose is considered excessive and should be reduced to the next lower tolerated dose.

Serum IGF-1 concentrations should be followed at 4–6-week intervals until the plateau or maintenance dose is reached and then every 6–12 months. The serum IGF-1 is more of a safety guide than an absolute concentration that defines the target dose. If the IGF-1 exceeds the normal age- and sex-specific range, the dose should be reduced. An IGF-1 in the mid-normal range is the target, but it should be recognized that there is no "magic level." If the patient has a good clinical response to GHRT but suboptimal IGF-1 levels, the current dosage should be maintained and not increased. In most adults, the maintenance dose is reached by pushing the dose to tolerance and the development of symptoms of excess, then backing off to a tolerable level. In following this format, the serum IGF-1 concentration is seldom exceeded.

Lipid concentrations may be obtained yearly. However, if there are no lipid abnormalities at baseline, there is no need to repeat these since they will only improve and not deteriorate. Blood glucose should be obtained and followed at 6-month intervals to make sure that the patient does not develop hyperglycemia. The index of suspicion should be greatest in obese patients with a family history of type 2 diabetes.

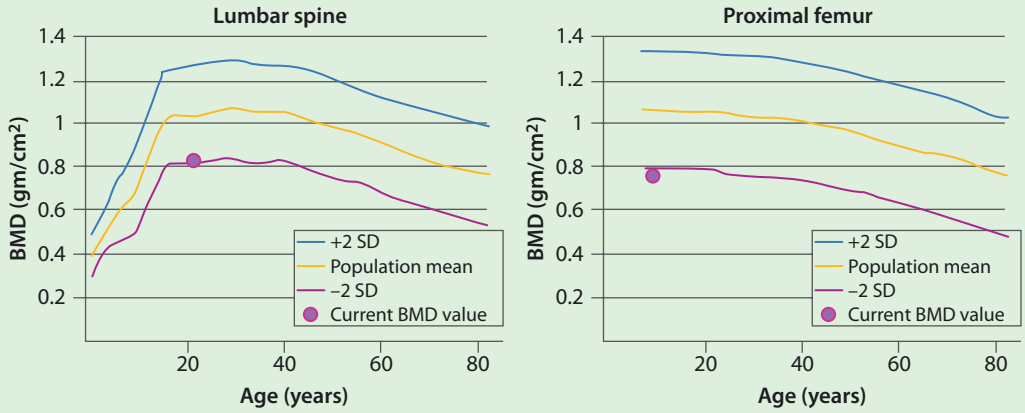
Case Study

21-Year-Old Woman with Idiopathic CO-GHD

A 21-year-old woman is brought into your office by her parents for a second opinion of persistent GHD after stopping GH at age 17. Her history is that she started GH at age 12 after GHD was diagnosed by her pediatric endocrinologist. The presentation at age 12 was poor growth. She was diagnosed as isolated idiopathic GHD after finding a low IGF-1 and poor GH response to insulin-induced hypoglycemia. She received GH from age 12 until age 17 when she stopped growing. She was 5′5″ when she stopped GH and has remained at that height since. Although she started college at age 18, she dropped out after 1 year due to lack of interest and inability to concentrate. Her parents noticed a drop in energy and social interests after stopping GH. She was seen by another endocrinologist who found her to have normal thyroid, adrenal, and gonadal function. She was given an arginine + GHRH stimulation test, and the results obtained showed a peak value of 9 ng/mL at 90 min (Table 7.8). She was told that she was not GH deficient because her BMI was 28 kg/m^2 and therefore did not meet the normal cut point of <8 ng/mL for arginine + GHRH when BMI is between 25 and 30 kg/m^2. She has gained 9 kg since stopping GH, and most of that, she states, has been in her waist area. She states that she has difficulty concentrating at her school work and not enough energy to study. Her weight is 77 kg and her BMI is 28 kg/m^2. Her bone density reveals a *z*-score of −2.3 in her hip and −2.0 in her spine (Fig. 7.5), and she is 33% fat. You consider that she might have had a false-negative test response to arginine + GHRH since she has an idiopathic cause of GHD and may lack hypothalamic GHRH. It is decided to use a test which activates the entire hypothalamic/pituitary unit and choose both insulin tolerance testing (Table 7.9) and glucagon testing (Table 7.10) (two tests) to prove GHD in face of the normal responses to arginine + GHRH. As can be seen, she has a normal response to arginine/GHRH based upon her BMI but fails both the insulin test (cut point <4.1 ng/mL) and glucagon (cut point <3 ng/mL). It is decided to restart GH replacement. The dose selected is 50% of her pediatric dose of 2 mg a day. Over the next 12 months, her dose is titrated to her maintenance dose of 2 mg/day (see Table 7.11). She has a return of her energy and concentration power and does well in school. She also loses 9 kg and her waist circumference drops 10 cm. This case illustrates that some patients with idiopathic CO-GHD will remain

Table 7.8 GHRH + arginine stimulation test for a 21-year-old CO-GHD patient with a BMI of 28 kg/m^2

Time after starting arginine + GHRH (min)	GH (ng/mL)
0	0.1
30	0.4
60	9.0
90	8.0
120	4.0



	Bone mineral density (gm/cm²)	*T*–Score (SD from young adult normal mean)
Lumbar spinr	0.83	–2.0
Proximal femur	0.66	–2.3

Fig. 7.5 Bone mineral density study of a 21-year-old woman with CO-GHD taken before restarting GH. *Abbreviations*: BMD bone mineral density, SD standard deviations

Table 7.9 ITT for a 21-year-old CO-GHD patient who had a normal response to arginine + GHRH

Time (min)	ACTH (pg/mL)	Glucose (mg/dL)	GH (ng/mL)	Cortisol (μg/dL)
0	28	88	0.1	19.5
20	22	23	0.4	18.7
22	–	23	–	18.7
35	–	36	–	–
40	–	55	1.2	42.2
60	42	90	1.2	23.7
90	22	87	0.3	21.6

GH deficient but that testing with arginine + GHRH might result in false-negative results. If the patient was to test positive with arginine + GHRH, it would be accepted that she was GHD, but if she passed and looked normal, it could be that she was only missing the hypothalamic hormone GHRH, and testing using the hypothalamic hormone GHRH or supplying the "missing link" might give a normal response. This case also points out the consequences of GHD in this age group which is predominantly to impact bones and cognition.

Table 7.10 Glucagon stimulation test for a 21-year-old CO-GHD patient who had a normal response to arginine + GHRH

Time (min)	Glucose (mg/dL)	GH (ng/mL)	Cortisol (μg/dL)
0	96	0.6	7.1
30	163	0.5	6
60	176	0.5	10.1
90	143	2.3	14
120	116	2.9	18.1
150	90	2	20.5
180	70	1.4	15.4
210	58	1.3	16.7
240	68	1.2	25.7

A 20-Year-Old Young Man with a Craniopharyngioma

A 20-year-old man is sent to you by a neurosurgeon for evaluation of his endocrine status. He had normal growth and development but has recently been found to have a craniopharyngioma identified because of the development of visual field abnormalities. He underwent pituitary surgery to remove the

Table 7.11 Dose titration for a 21-year-old CO-GHD patient transitioning to the adult indication

Date	mcg/d	IGF-1 (NL 180–780 mg/ml)
August 2008	800	60
October 2009	1200	130
January 2010	1600	204
March 2010	2000	300
May 2010	2400	480

tumor. Subsequent to the surgery, he was found to be GH deficient. After a year of replacement therapy with testosterone, thyroid hormone, and hydrocortisone and stable MRI image of the sellar area, he returns for follow-up care. On physical exam he weighed 148 kg and was 170 cm tall. His IGF-1 concentration was undetectable, and he has no GH response to arginine stimulation testing (all GH concentrations <0.1 ng/ml). His fasting insulin was 48 IU/mL and simultaneous glucose 115 mg/dL. Because of concerns about his glucose status, GH therapy was started cautiously. He was started on 0.3 mg s.c. daily. Immediately he began to have polyuria and polydipsia. This progressed to moderate ketoacidosis over a 1-week period. Because of this rather dramatic and sudden appearance of type 2 diabetes, he did not want to restart GH therapy for fear of going into ketoacidosis again. Very shortly thereafter he required oral hypoglycemic agents to control his blood glucose. This case represents the extreme of aggravation of diabetes after beginning GH therapy or exposing latent diabetes after starting GH therapy. Physicians should be aware of this category of patient when beginning GH therapy, and in this category we begin with a small dose of 0.1 mg per day to avoid aggravation of his insulin resistance.

19-Year-Old Woman with Autoimmune Hypophysitis

This 19-year-old woman was referred for evaluation of persistent fatigue despite normalization of free T4 and TSH for primary hypothyroidism. Because of the known association of autoimmune thyroid disease and pituitary autoimmunity, an IGF-1 concentration was obtained which was low, i.e., 60 ng/ml (nl: 182–780 ng/ml). Her periods were regular. She was proven to have GH deficiency on the basis of a poor response of GH to insulin-induced hypoglycemia (peak GH 3.3 ng/ml) but had normal cortisol response to hypoglycemia. Her hrGH dose was titrated to a mid-range IGF-1 concentration to a total dose of 2.4 mg/day, which she tolerated without side effects. This young lady represents dosing experience with patients in their late teen and early 20s. She has required and tolerated rather large doses of GH to normalize her IGF-1 concentration and to achieve sufficient lipolysis. She did not have side effects of GH therapy, and her maintenance dose was established by titrating to mid- to high-normal IGF-1 concentrations.

7.7 Summary

The treatment of adults who have been GHD as children is an emerging clinical science. As for other chronic conditions, the correct management of persistent GHD during the delicate phase of transition is crucial. Patients and their families should receive adequate support when transferred from pediatric to adult endocrine services. Until quite recently, the main target of GHRT in patients with CO-GHD was the attainment of full linear growth, and the treatment was stopped as soon as near-adult height was achieved. However, during the last 20 years, the literature has questioned this practice, as the discontinuation of the replacement therapy during adulthood in those with a persistent GHD can cause detrimental effects on body composition, bone health, cardiovascular system, and QoL. The first and foremost important rule is to confirm the patient is persistently deficient, especially in patients who carry the diagnosis of idiopathic GHD. A number of issues surround therapy including dosing and monitoring. As time passes, there will be more young adult patients seeking therapy for their GH deficiency. These situations include the trend for pediatricians to suggest continuing GH after the pediatric indication and adult endocrinologists who are familiar with the care of this group of patients and the patients themselves seeking a solution to their symptoms.

Review Questions

1. Which is the most frequent cause of GH deficiency in adults?
   A. Persistent idiopathic childhood-onset GH deficiency.
   B. Acquired hypothalamic-pituitary damage.
   C. Craniopharyngioma.
   D. Adult idiopathic GH deficiency.

2. Does body mass index affect the GHRH + arginine stimulation test for GH in normal individuals?
   A. A higher body mass index is associated with a lower peak of stimulated GH.
   B. A lower body mass index is associated with a lower peak of stimulated GH.
   C. Body mass index does not affect the GHRH + arginine stimulation test.
   D. Body mass index alters the response to the GHRH + arginine stimulation test only in those with morbid obesity (>40 kg/m^2).
3. Can basal IGF-1 be used in all patients to confirm a persistent GH deficiency during the transition period?
   A. Yes, a low IGF-1 (<2 standard deviations in comparison to age- and sex-matched controls) predicts a deficit in almost all patients.
   B. No, a low IGF-1 (<2 standard deviations in comparison to age- and sex-matched controls) shows high sensibility and specificity only in patients older than 28 years.
   C. No, a low IGF-1 (<3 standard deviations in comparison to age- and sex-matched controls) shows high sensibility and specificity only in patients older than 28 years.
   D. No, a low IGF-1 (<3 standard deviations in comparison to age- and sex-matched controls) shows high sensibility and specificity only in patients younger than 28 years.
4. Do transdermal estrogens affect exogenous GH administration?
   A. Yes, they enhance hepatic responsiveness to GH.
   B. Yes, they reduce hepatic responsiveness to GH.
   C. Yes, they promote peripheral IGF-1 degradation.
   D. No, they do not influence GH metabolism.

Answers

1. B
2. A
3. C
4. D

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Skeletal Dysplasias

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Key Points

- Skeletal dysplasias are genetic disorders that affect the development of the skeletal system. There are currently 436 described skeletal dysplasia syndromes.
- The presence of a skeletal dysplasia should be considered in any child presenting with short stature, particularly if skeletal disproportion is identified.
- Bisphosphonate treatment of osteogenesis imperfecta often falls to the pediatric endocrinologist.
- Study of skeletal dysplasias will help us to better understand the mechanisms that regulate liner growth.

8.1 Introduction and Background Information

Skeletal dysplasias (or more appropriately, the osteochondrodysplasias) are genetic disorders that affect the development of the skeletal and cartilaginous tissues. They are of interest to the pediatric endocrinologist not only because most have an impact on linear growth causing short stature (and occasionally tall stature) but also for what these disorders teach us about the mechanisms and regulation of growth.

As of 2015, there were 436 described skeletal dysplasias that were associated with one of 364 different genes [1], with more associations being made routinely [2]. Although each individual disorder is relatively rare, as a group, the incidence of any skeletal dysplasia is roughly 1 in 5000 births [3, 4]. Making the definitive diagnosis in a child with skeletal dysplasia is important in order to screen for and treat the problems particular to each condition. Diagnosis and management generally require a team approach involving the geneticist, orthopedist, and radiologist. Yet it is often to pediatric endocrinology that these patients are first referred, due to the growth abnormalities. It is therefore incumbent on the endocrinologist to understand the basics of this field and to recognize when a skeletal dysplasia may be present.

The study of skeletal dysplasias dates back to the earliest descriptions of disease, due to their obvious anatomical abnormalities. These roots persist in the Greek and Latin anatomical descriptions that characterize the field. While adding certain elegance ("achondroplasia" just sounds better than "absence of cartilage growth"), it can make learning about the skeletal dysplasias somewhat intimidating. Table 8.1 defines many of the terms commonly used in the field, and Fig. 8.1 shows their application to the anatomy of the long bone.

Table 8.1 The vocabulary of skeletal dysplasias

Acromelia	Shortening of the terminal parts of the limbs (hands and feet) in relation to the upper and middle limb segments
Atlantoaxial instability	Abnormal extra motion occurring between the first and second cervical vertebrae (C1 and C2)
Cervical	Relating to the vertebrae found at the level of the neck
Chondro-	Relating to cartilage
Cubitus valgus	Deformity of the elbow in which it cannot be fully extended; also called "increase carrying angle"
Diaphysis	The middle or shaft part of a long bone
Diaphyseal	Relating to the diaphysis
Dysplasia	An abnormality of development of a body tissue or organ

Table 8.1 (continued)

Endochondral bone formation	The type of bone formation that occurs at the growth plates of the long bones
Epiphysis	The ends of the bone of the long bones; the part of the bone that is beyond the growth plate
Epiphyseal	Relating to the epiphysis
Genu valgus	Bowing of the leg inward, also called knock-knee
Genu varus	Bowing of the leg outward, also called bow-legged
Growth plate	The cartilage layer at the ends of the long bones where linear bone growth occurs
Hypoplasia	Underdevelopment of body tissue or organ
Kyphosis	An outward curvature of the spine in the sagittal plane
Lordosis	An inward curvature of the spine in the sagittal plane
Lumbar	Relating to the vertebrae found at the level of the lower back
Membranous bone formation	The type of bone formation that occurs within a membrane of connective tissue, resulting in bones shaped like plates such as in the skull or the scapulae
Mesomelia	Shortening of the middle parts of the limbs (forearm and foreleg) in relation to the upper and terminal segments
Metaphysis	The widening region of the long bone in which the epiphysis and diaphysis meet; the part of the middle of the bone that is adjacent to the growth plate
Metaphyseal	Relating to the metaphysis
Micromelia	A symmetric shortness of the limbs
Odontoid process	Normal bony peg of the second cervical vertebrae that allows the neck to rotate
Ossification	Process by which cartilage calcifies and changes into bone
Osteo-	Relating to bone
Osteotomy	Surgical cutting of the bone as a realignment procedure
Physis	The growth plate
Physeal	Relating to the growth plate
-plasia	Relating to the form or structure of a body tissue or organ
Rhizomelia	Shortening of the upper parts of the limbs (upper arm and thigh) in relation to the middle and terminal segments
Scoliosis	A lateral curvature of the normally straight vertical line of the spine
Spondylo-	Relating to the spine
Thoracic	Relating to the vertebrae found at the level of the ribs and chest
-trophy	Relating to growth

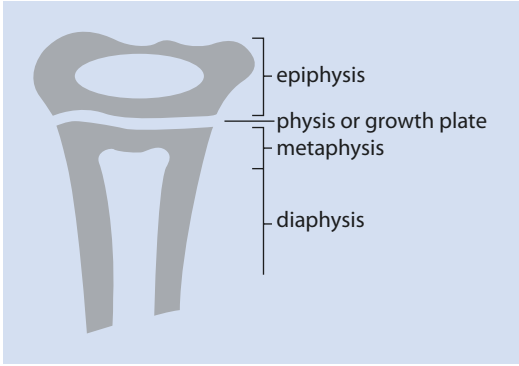

Fig. 8.1 Anatomy and nomenclature of the long bones

8.2 Etiology

Skeletal dysplasias are caused by abnormalities in genes that are important in the regulation of skeletal tissues. As such, they are congenital, and many syndromes can be diagnosed prenatally or in the neonatal period. However, some genes have subtle effects or play a role only in later development and/or growth, and the phenotypic features appear later in life. The past decade has seen great advancement in identifying the causative gene in the skeletal dysplasias [1]. This has aided greatly in the classification of the skeletal dysplasias, as well as our understanding of the regulation of skeletal tissues.

8.3 Clinical Presentation

When a child presents for evaluation of short stature or tall stature, the possibility of a skeletal dysplasia should always be a consideration. It is unusual for patients with the more severe forms (such as achondroplasia) to be referred to an endocrinologist; the presence of a skeletal dysplasia is obvious clinically, and these patients are generally referred to the geneticist for evaluation. However, there are a number of skeletal dysplasias where the primary clinical feature is short stature while other features are absent or subtle. Tall stature can also be a symptom of a skeletal dysplasia where the evaluation starts with the endocrinologist. A family history, anthropometric measurements, a careful physical exam, and the knowledge of what to look for are the basis for detecting a skeletal dysplasia in these situations.

Skeletal dysplasias are of genetic origin, and the majority is caused by single gene abnormalities. Hence, a thorough family history may provide valuable information regarding these syndromes. The most common syndromes have a dominant mode of inheritance, including achondroplasia, hypochondroplasia, Léri-Weill osteodyschondrosteosis, and osteogenesis imperfecta. Except in cases of de novo mutations, children with these syndromes will have a parent with the same problems. It is not uncommon that an affected parent has never been diagnosed with the syndrome as the clinical features may be subtle, such as in hypochondroplasia or Léri-Weill osteodyschondrosteosis. With the newly available genetic tools, it is now routine that a diagnosis made in a child leads to the diagnosis being made in a parent and grandparent. Obtaining parental heights can assist with the evaluation. Reported parental heights are notoriously inaccurate; in our clinic, we measure parents' heights whenever possible.

Endocrine causes of short or tall stature generally affect the skeleton as a whole, and these patients have normal skeletal proportions. However, the genes associated with skeletal development generally affect different parts of the skeleton with varying impact. Hence, a major indication for the presence of a skeletal dysplasia is body disproportion. Any child being evaluated for short stature should have an upper-to-lower segment ratio calculated, an arm span measured, and a head circumference measured. The lower segment measurement is done by having the child stand with his or her back against the wall and heels together, flat on the ground, and touching the wall. The upper border of the symphysis pubis is located by palpation, and a mark is made on the wall level to this point. The measurement from the floor to the mark is the height of the lower segment. The upper segment is determined by subtracting the lower segment from the measured height and the upper-to-lower segment ratio determined. The ratio varies with age, with the limbs growing somewhat faster than the spine during puberty. There is also a racial effect; African-Americans have relatively longer limbs and a lower upper-to-lower segment ratio. Age- and race-specific reference ranges are available (Fig. 8.2). A patient with ratio that is greater than the 95th percentile has disproportionately short legs and suggests a short-limbed skeletal

Fig. 8.2 Upper-to-lower segment ratio. The upper-to-lower segment ratio in childhood is shown for Caucasians (*left panel*, $n = 1015$) and African-Americans (*right panel*, $n = 1089$). African-Americans tend to have relatively longer limbs and a lower upper-to-lower segment ratio (The original figure is from McKusick [5], used by permission of Elsevier. Figure is reproduced from Hall et al. [6], used by permission of Oxford University Press)

dysplasia. A patient with short stature and an upper-to-lower segment ratio less than the 5th percentile suggests a disproportionately short spine, possibly due to a spondylodysplasia and/or scoliosis. Conversely, a patient with tall stature and ratio that is less than the 5th percentile has disproportionately long legs, suggesting an overgrowth syndrome such a Marfan syndrome. A related measurement is the sitting height and the sitting-to-standing height ratio. For this measurement, the child is placed on a flat stool with his or her back against the wall. The child's thighs should be parallel to the floor. A mark is made at the top of the head. The distance from the floor to the mark minus the height of the stool gives the seated height. Reference ranges are available [7].

An arm span measurement is also helpful. It is determined by having the patient stand against a wall with his or her shoulders touching the wall, with the arms spread outwards. Marks are made at the tip of the middle finger of each hand and the distance between them measured. If the patient cannot stand, a rigid pole can be placed behind the neck and the arms stretched along the pole and the finger tips marked. As with the upper-to-lower segment ratio, the arm span/height difference increases during puberty and is higher in African-Americans (Fig. 8.3). An arm span/height difference that is less than the 5th percentile suggests disproportionately short arms, while a difference greater than the 95th percentile suggests a short spine. Either suggests the possibility of a skeletal dysplasia. Children with an excessive arm span/height difference should also be evaluated for scoliosis. Occasionally, it is taught that an arm span that is 4 cm less than or greater than the standing height is suggestive of skeletal disproportion [7]. However, the changes with pubertal status and racial differences make this simplistic guideline inappropriate. More appropriate guidelines are presented in Table 8.2. An arm-span-to-height ratio of >1.05 is used in the revised Ghent nosology for Marfan syndrome [9].

A head circumference (occipital-frontal circumference, OFC) is also important for detecting body disproportion. An OFC within the expected range in a child with severe short stature could suggest a skeletal dysplasia, although other diagnoses must also be considered.

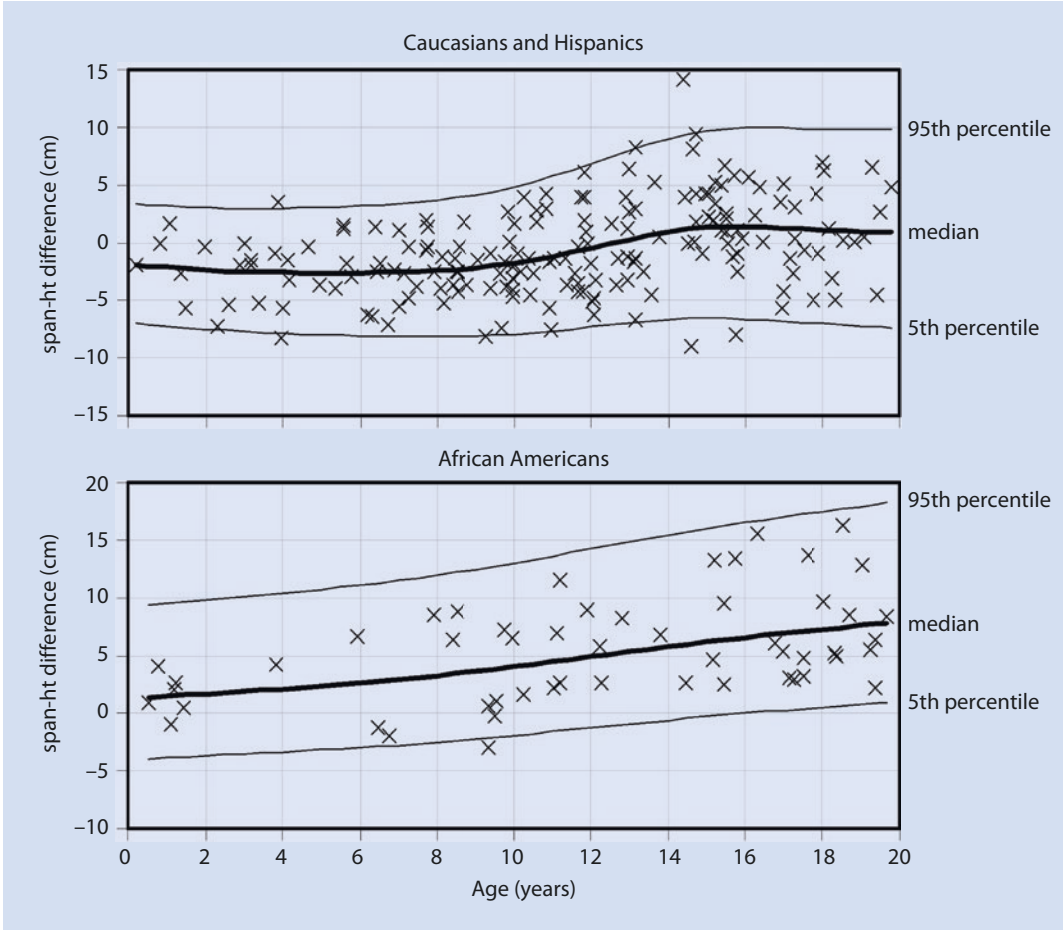

Fig. 8.3 Arm span/height difference. Arm span/height difference for healthy children in Florida was determined. Arm span was measured as described in the text. Height was determined by stadiometer. Curves were fitted using the LMS method [8] and the median, 5th, and 95th percentile curves shown. Data for Caucasian and Hispanic children are shown (*top panel*, $n = 178$). Consistent with upper-to-lower segment ratio data, African-American children tend to have relatively longer limbs and a higher arm span/height difference (*bottom panel*, $n = 53$)

Table 8.2 Reference range for arm span/height difference

Arm span/height difference				
	Caucasian		African-American	
	Prepubertal (cm)	Pubertal[a] (cm)	Prepubertal (cm)	Pubertal[a] (cm)
5th percentile	−7.2	−5.7	−2	2.4
Median	−2.5	0.4	1.6	6.3
95th percentile	1.8	6.7	8.5	14.4

[a]For girls, breast Tanner stage 2 or higher; for boys genitalia Tanner stage 2 or higher

A thorough physical examination focused on the skeletal system is important. Observation of the child while standing will give the clinician an idea of body proportion. As a rule of thumb, the hands should rest level with the mid thigh. Examination of the skull should note the presence and position of the fontanelles and the presence of asymmetry, frontal bossing, and hypo- or hypertelorism. A highly arched palate is common in a number of skeletal dysplasias. The presence of a short neck, kyphosis, or scoliosis may suggest an abnormality of the spine. Examination of the arms and legs may show disproportionate shortening of the upper or lower arm or leg. Limb segment measurements can be done. A guide such as that by Gripp et al. [7] provides measurement techniques and reference ranges. Bowing of a limb is an important finding, as is limitation in joint mobility. Prominent radial and/or ulnar heads at the wrist may indicate Madelung deformity, seen in *SHOX* haploinsufficiency disorders (Léri-Weil osteodyschondrosteosis or Turner syndrome). Close examination of the hands and feet for shortening of some or all of the metatarsals, metacarpals, and phalanges should also be done. Extra or missing digits, syndactyly, and camptodactyly are also important potential findings.

8.4 Diagnostic Evaluation

Once a skeletal dysplasia is suspected, more in-depth evaluation is called for. The diagnostic evaluation of a child suspected of having a skeletal dysplasia requires a thorough history including a three-generation family history. Attention should be paid to signs and symptoms such as early-onset arthritis or joint pain, fractures, joint replacement surgeries, dental problems, and hearing or vision abnormalities. The physical examination should include specific measurements as discussed above to assess for disproportion, including signs of disproportion within the limb. Ranges of motion throughout are important to assess as both increased and decreased flexibility are associated with various dysplasias. Lastly, a skeletal survey should be undertaken to look for diagnostic clues. There is not a standard dysplasia survey, as various experts and institutions do things differently. Our preferred evaluation includes the following: an AP image from pelvis to floor, standing if possible, otherwise supine (this type of image allows for anatomic alignment of the lower extremities to be assessed and disproportion within the limb to be assessed); AP and lateral images of the thoracolumbar spine (to assess for scoliosis and any vertebral body abnormalities); an AP image of the arm from the shoulder to wrist; and an AP image of the hands. If osteogenesis imperfecta or another fragility condition is being entertained, AP and lateral skull images are done to assess mineralization and for Wormian bones. If any spine abnormalities are present either on clinical examination or x-ray, lateral flexion and extension views are recommended to assess for cervical spine instability. Important consideration should be given to having these images reviewed by a radiologist familiar with skeletal dysplasias in children. If no such radiologist is available locally, assistance is available at the University of California, Los Angeles, International Skeletal Dysplasia Registry (► www.ortho.ucla.edu/isdr) [10] and the European Skeletal Dysplasia Network (► www.esdn.org) [11] which can review patient images and provide diagnostic possibilities. Assistance might also be available through regional skeletal dysplasia programs.

Once the evaluation is completed, the information gathered from the history and physical and radiographic examinations can be reviewed to try and reach a specific diagnosis. If a specific or limited differential diagnosis can be reached, molecular testing may be available to confirm a diagnosis. GeneTests (► www.genetests.org) is an NIH-funded database which can be used to identify clinical and research laboratories that provide genetic testing for a wide range of skeletal dysplasias. Advances in genetic diagnostic techniques including third-generation sequencing, multiple gene panel sequencing, and whole-exome sequencing have diagnosis confirmation now possible in the majority of skeletal dysplasia syndromes [2].

8.5 Common Syndromes

8.5.1 Achondroplasia

Achondroplasia (MIM 100800) is the most common skeletal dysplasia with incidence estimates ranging from 1 in 15,000 to 1 in 26,000 births

[12, 13]. The average adult height for men is 131 cm, with a range of 118 to 144 cm (3′10″ to 4′9″, SD score − 8.3 to −4.6), and for women, 123 cm with a range of 113 to 137 cm (3′8″ to 4′6″, SD score − 7.7 to −4.0) [14]. Individuals with achondroplasia have average intelligence. The clinical features of achondroplasia are distinctive enough that diagnosis can be made thru clinical and radiographic means, and the diagnosis is usually made at birth [15]. Due to advances in late-term prenatal ultrasound and the detection of limb shortening, achondroplasia can be diagnosed prenatally. Classic findings include rhizomelic (upper arm and thigh) limb shortening, relatively long and narrow torso, and large head with frontal bossing. As a result of the defect in endochondral bone formation, the skull base and mid-face are affected resulting in mid-face hypoplasia, foramen magnum stenosis, and abnormal Eustachian tube anatomy. There is ligamentous laxity in most joints, although typically the elbows cannot be fully extended. The fingers are short and broad, giving rise to a stubby appearance. In infancy and early childhood, the third and fourth fingers do not fully oppose giving rise to a trident appearance. Key radiographic characteristics in the infantile period include squared iliac wings with flat acetabula, a radiolucent aspect of the proximal femoral metaphyses, fibulae which tend to be longer than tibiae, platyspondyly with anterior wedging of the lumbar vertebral bodies, and lumbar interpediculate distance narrowing.

Achondroplasia is caused by a mutation in the fibroblast growth factor receptor-3 gene (*FGFR3*) [16]. Mutations which change the amino acid glycine to arginine at position 380 (Gly380Arg) of the FGFR-3 protein account for greater than 98% of all reported cases of achondroplasia. This typical G380R gain-of-function mutation results in constitutive activation of the receptor. In growth plate chondrocytes, this receptor activates the signal transducers and activators of transcription (STAT1) pathway, which inhibits chondrocyte proliferation, and the mitogen-activated protein kinase (MAPK) pathway, which inhibits both proliferation and chondrocytic differentiation. The net result of inhibited chondrocyte proliferation and differentiation is poor bone growth [17–19]. Achondroplasia is inherited in an autosomal dominant manner, but about 85% of patients with achondroplasia represent new mutations. Given this high rate of new mutations and the incidence of achondroplasia, the base pair of codon 380 in *FGFR3* has the highest known rate of mutation in man. New mutations typically arise from the father during sperm formation, and paternal age greater than 35 years has been found to be a risk factor [20, 21]. A recent hypothesis is that sperm containing a *FGFR3* mutation have a selective advantage, and as men age, more *FGFR3* mutant sperm are present.

Routine care consists of management of the orthopedic issues (such as leg bowing), hydrocephalus, foramen magnum stenosis, and ear infections from the Eustachian tube abnormalities [20]. There is no specific growth treatment for achondroplasia. A number of small studies have reported on the use of recombinant human growth hormone (rhGH) in achondroplasia for up to 6 years [22–24]. Studies generally show an improvement in height velocity during the first year of treatment and an average net gain in height SD score of 1.0 to 1.6 (8 to 14 cm) [25]. The body disproportion is reported to be worsened [23] or unchanged [22, 24] by treatment, and no unusual adverse effects have been reported. Because of the small gains relative to the profound short stature, rhGH treatment is generally not recommended for the treatment of achondroplasia.

C-type natriuretic peptide (CNP) is a small peptide that acts in a paracrine manner in the growth plate to regulate growth (see acromesomelic dysplasia, type Maroteaux, below). One mechanism of this is through inhibiting intracellular signaling of the MAPK pathway [26]. In achondroplastic mice, overexpression of CNP [27], as well as exogenous administration of CNP [28], rescues the skeletal abnormalities, leading to the proposal of CNP as a possible future treatment in humans [19, 28]. An analog of CNP with prolonged half-life in the blood [29] is currently in clinical trials in children with achondroplasia. In studies in transgenic mice with the achondroplasia mutation, the existing drugs of meclizine [30] and lovastatin [31] have shown effectiveness in improving growth and may provide future treatment possibilities. Other approaches that show promise in mice models include using tyrosine kinase inhibitors [32], a soluble FGFR3 to reduce available FGFs [33], and using an FGFR3 monoclonal antibody that downregulates FGFR3 signaling, including FGFR3 with achondroplasia mutation.

8.5.2 Hypochondroplasia

Hypochondroplasia (MIM 146000) is a common skeletal dysplasia with incidence estimates ranging from 1 in 15,000 to 1 in 40,000 births [34]. The adult range height is 132 to 165 cm (4′4″ to 5′5″, SD score − 6.3 to −1.7) in men and 127 to 150 cm (4′2″ to 4′11″, SD score − 5.6 to −2.0) in women. Approximately 10% of people with hypochondroplasia have learning problems. As with achondroplasia, some children can be diagnosed prenatally and others at birth. However, most children with hypochondroplasia are diagnosed in early childhood. Individuals at the mild end of the hypochondroplasia spectrum may overlap with average-sized individuals making it difficult to establish a clinical diagnosis. Short stature with rhizomelic limb shortening is a cardinal feature. In one study, in subjects with hypochondroplasia with height SD scores of less than −2, 80% had a sitting height-to-height ratio SD score of greater than 2.5. This was compared to only 4.3% of healthy people of the same stature, and this has been proposed as a screening criterion [35]. In some patients, the first sign of the condition may be a failure to achieve the normal pubertal growth spurt. The head circumference is average or slight macrocephaly may be present. The facial features are usually normal, and the classic features of achondroplasia (frontal bossing and mid-face hypoplasia) are not present. Ligamentous laxity can be present in most joints although typically the elbows cannot be fully extended. The fingers can be short and broad, giving rise to a stubby appearance. However, they do not have the typical trident appearance of achondroplasia. Key radiographic features include narrowed lumbar interpedicular distance, squared shortened ilia, short femoral necks, mild metaphyseal flaring, and shortened phalanges [36].

Like achondroplasia, hypochondroplasia is caused by mutations in *FGFR3* [34]. While the mutation in achondroplasia is essentially always caused by the same mutation, there is greater variability of the type of mutations in hypochondroplasia. The lysine for asparagine substitution at codon 540 (Asn540Lys) is the most common gain of function mutation to cause hypochondroplasia, but several others have been described. A second gene has been implicated but has not yet identified [37]. When there is an established clinical and radiographic diagnosis of hypochondroplasia, *FGFR3* mutations can be identified in about 90% of individuals [37]. Because there is phenotypical overlap between achondroplasia and hypochondroplasia, and between hypochondroplasia and idiopathic short stature, genetic confirmation of the diagnosis is recommended [37].

As with achondroplasia, rhGH seems to have some effect in hypochondroplasia, although studies are small and short term [38–41]. The largest and longest-term study [42] showed that, while improving growth velocity and height SD score, rhGH also worsened the skeletal disproportion. A meta-analysis of published studies concluded there was a positive effect of rhGH on height [43]. Surgical limb lengthening procedures (distraction osteogenesis) have been reported in adults with hypochondroplasia [44, 45] to improve height and skeletal disproportion but are by no means a routine practice at this time. Since the activating mutations of FGFR3 cause of most cases of hypochondroplasia as they do for achondroplasia, we predict that the drug interventions being explored for achondroplasia will likely also prove effective in hypochondroplasia.

8.5.3 Multiple Epiphyseal Dysplasia

Multiple epiphyseal dysplasia (MED) is a relatively common disorder with a prevalence of at least 1 in 20,000 [46]. MED is a heterogeneous disorder of bone and cartilage development that results in small irregular epiphyses. Proportionate short or average stature is present, as are frequently painful joints, and possibly a mild myopathy. MED is not recognizable at birth and is typically recognized after 2 years of age and in some cases not until early adulthood. Typically adults will grow to be between 145 and 168 cm (4′9″ and 5′6″). MED may present as a delay in walking. Initial complaints usually include joint stiffness, pain, contractures, or limping. In some forms of MED, the fingers and toes are short and stubby, especially the thumb. Minor flexion contractures of the knees and elbows can be present. Joint pain which could be fluctuating or episodic in childhood develops in adolescence or early adulthood. Genu valgus or varus may develop.

Key radiographic characteristics of MED include irregular epiphyses usually at the hips, knees, ankles, wrists, and hands. Bones of the pelvis, spinal column, and skull are typically normal. In middle to late childhood, the epiphyses are

either flat or small. An important diagnostic sign is the epiphyses of distal tibias which are laterally malformed to produce a sloping wedge-shaped articular surface. This may be more apparent later in life. A bipartite (split) patella can be seen and, if present, shortening of the long bones is mild.

Multiple epiphyseal dysplasia is a genetically heterogeneous condition which can be inherited in either an autosomal dominant or autosomal recessive manner. Dominant forms of MED are more common. The most common dominant genetic cause of MED is from mutations in the gene encoding cartilage oligomeric matrix protein (*COMP*) (type 1, MIM 132400). Other dominant forms are caused by mutations in any three of the type IX collagen genes (*COL9A1*, type 6, MIM 120210; *COL9A2*, type 2, MIM 600204; *COL9A3*, type 3, MIM 600969) or the matrilin-3 gene (*MATN3*, type 5, MIM 607078). These proteins are extracellular matrix components found in growth plate cartilage. The autosomal recessive form of MED is caused by mutations in the diastrophic dysplasia sulfate transporter (*DTDST*, type 4, MIM 226900). The precise percentage of MED patients with identifiable mutations varies, but it is clear that mutations cannot be found in all patients.

There are no published studies of the use of rhGH in MED. Its effectiveness on height growth and body disproportion is unknown.

8.5.4 Léri-Weill Dyschondrosteosis

Léri-Weill dyschondrosteosis (LWD, MIM 127300) is a moderately severe form of dwarfism, characterized by short stature, mesomelia (shortening of the forearms and lower legs), and a characteristic finding at the wrist known as Madelung deformity. Stature in genetically proven cases is variable, with height SD scores ranging from −4.6 to 0.6 [47], a range that overlaps the healthy population. The short stature is of early childhood onset [48], with little change in height SD score during puberty [47]. The short stature is disproportionate and can be identified through a high upper-to-lower segment ratio (SD scores 2.9 ± 3.4) and low arm span/height difference (−5.1 ± 3.0 cm) [48]. As with height, there is overlap with the healthy population. Madelung deformity results from presence of physeal bar in the ulnar aspect of the growth plate of the distal radius, causing asymmetric growth [49]. This results in bowing of the radius and tilting of the articular surface of the radius toward the ulna and palm (Fig. 8.4). The resulting anterior displacement of the wrist gives the characteristic "bayonet" appearance of the wrist. Subluxation of the ulnar or radial head makes it a prominent feature on the dorsal wrist. The deformity is detectable in young children but can be subtle [48]. It progresses until the growth plate fuses, making it more prominent in adolescents and adults. Wrist extension and supination can be limited. Madelung deformity is a variable feature of LWD; it is detectable by exam or x-rays in 53% of young children [48] and up to 88% in adults [47]. Women are more likely to be affected and are generally more severely affected. Other features of LWD include shortening of the 4th metacarpals, high-arched palate, cubitus valgus and limited mobility of the elbows, bowed tibias, and scoliosis.

The majority of cases of LWD results from heterozygous deletion or inactivating mutations of the "short-stature homeobox-containing gene" or *SHOX* [51, 52]. Microdeletions of *SHOX* enhancer regions have also been implicated [53]. The *SHOX* protein is a transcription factor that is expressed in epiphyseal growth plates [54], but its precise role is still being elucidated. *SHOX* is located on the distal ends of the short arms of both the X and Y chromosomes, in the pseudoautosomal regions. Although *SHOX* is located on the X chromosome, LWD is not transmitted in an X-linked inheritance pattern but in an autosomal dominant-like pattern. Variable penetrance is seen, resulting in the wide spectrum of severity. Because of the variability of the phenotype, the prevalence of LWD is unknown. Homozygous mutations of *SHOX* are the cause of Langer mesomelic dysplasia (MIM 249700), a profoundly severe form of short-limbed dwarfism. Genetic testing for *SHOX* deletions and mutations is now available through several commercial laboratories.

Large-scale deletions of the X chromosome can result in loss of *SHOX*, giving rise to LWD as part of a contiguous gene syndrome that may (in boys) include a number of X-linked syndromes, including ichthyosis, learning/behavioral difficulties, Kallmann syndrome, chondrodysplasia punctata, and skeletal deformities with short stature [55]. By virtue of a missing X chromosome, girls with Turner syndrome have only a single *SHOX* gene. Although

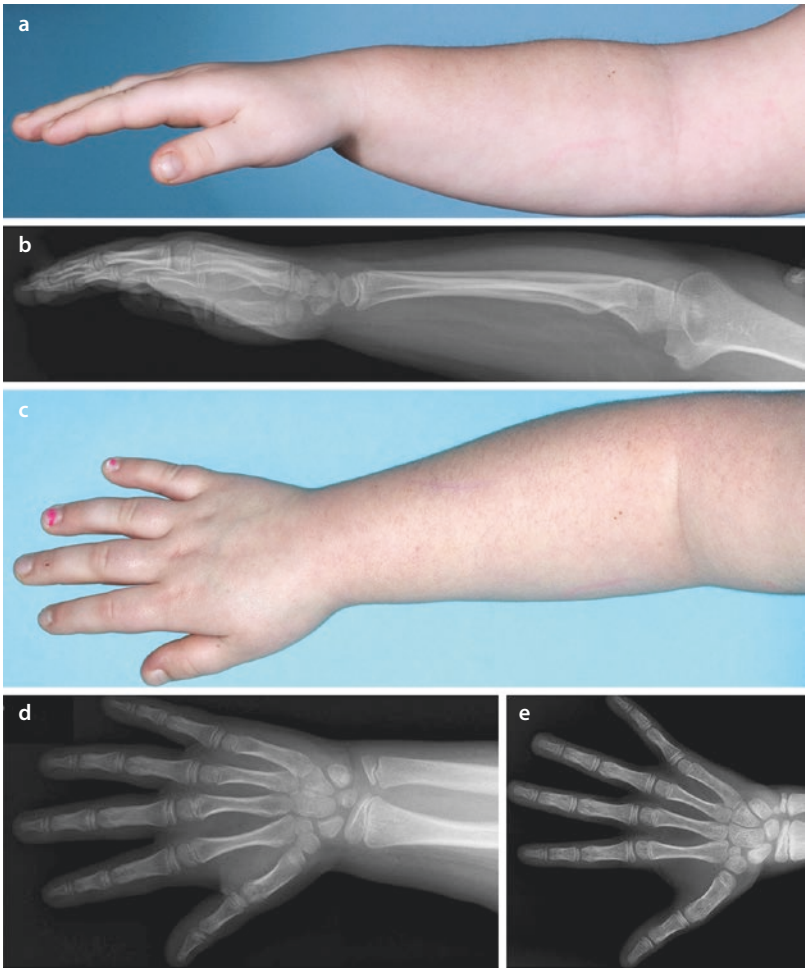

Fig. 8.4 Madelung deformity in a prepubertal child. An 8-year-old girl with Léri-Weil dyschondrosteosis (LWD) due to a partial deletion of Xp demonstrates Madelung deformity. The deformity is the result of the presence of physeal bar in the ulnar aspect of the growth plate of the distal radius. In prepubertal children, the findings can be subtle [49]. *Panels* **a** and **b** show the volar displacement of the wrist, giving a subtle "bayonet" appearance with prominence of the ulnar and radial heads on the back of the wrist. This patient also has cubitus valgus ("increased carrying angle"). *Panels* **c** and the AP x-ray (*Panel* **d**) shows modest bowing of the radius and tilting of the radial articular surface. The film also shows mild shortening of the 4th metacarpal. A normal wrist of the same bone age is shown in *Panel* **e** for comparison. In older and/or more severe cases of Madelung deformity, other findings can include lucency in the radial head, a triangular-shaped radial epiphysis with fusion of the ulnar half of the growth plate, wedging of the carpal bones, and subluxation of the radial/ulnar articulation [47, 50]

not traditionally diagnosed as having LWD, these girls phenotypically often have the findings of LWD, and it is believed that the loss of the *SHOX* copy explains all or almost all of the short stature associated with Turner syndrome. Madelung deformity is seen in Turner syndrome, but at a lower prevalence than in LWD (25% vs. 53%) [48].

In a recent study of prepubertal children with idiopathic short stature (height less than the 3rd percentile or height less than the 10th percentile with height velocity less than the 25th percentile), of 740 unrelated subjects, 23 had a *SHOX* deletion, 5 had an intragenic *SHOX* mutation, and 8 had deletions proximal to *SHOX* [53]. This represents an incidence of 4.9% of *SHOX*-related abnormalities in children with idiopathic short stature. In the absence of other features, these children are not generally diagnosed with LWD, but rather with "*SHOX* haploinsufficiency," a designation that includes LWD and Turner syndrome.

Recombinant human growth hormone has been shown to be effective in *SHOX* haploinsufficiency disorders. Use of rhGH in girls with Turner syndrome is now a routine care, and one study demonstrated a near final adult-height improvement over predicted height SD score of 1.3 ± 0.6 [56]. Trials in LWD are more limited but suggest an equally good response [57]. Both Turner syndrome and *SHOX* haploinsufficiency are FDA-approved indications for rhGH treatment [58].

8.5.5 Osteogenesis Imperfecta

Osteogenesis imperfecta (OI) is a heterogeneous group of disorders characterized by bone fragility and low bone mass predisposing to fracture. OI is estimated to occur in 1 in 10,000 to 15,000 live births. Extra-skeletal manifestations associated with OI can include blue sclera, dentinogenesis imperfecta, excess joint and skin laxity, and hearing loss. The severity of OI is widely variable ranging from in utero fractures and perinatal lethality to very mild forms without fractures. Given the range of severity which exists in OI, classification and categorization of patients can be useful to assess prognosis and determine potential therapeutic options. The most widely used classification scheme was developed by Sillence et al. [59] and distinguishes four types of OI. Type I is the mild form of OI, type II is perinatal lethal, type III is severely deforming, and type IV is moderately deforming. Subsequent molecular and histological evaluations have given rise to at least an additional ten types [60]. While some experts have proposed expanding the original Sillence classification with each causative gene receiving its own type numbers, other authors continue to utilize a clinically based approach and decouple the Sillence classification from the causative gene and adding only OI Type V because it is clinically distinguishable [1, 61, 62]. Table 8.3 shows this classification schema. Typically, patients with the moderate and severe forms of OI are readily diagnosed. Type I OI is the most common form and can be more difficult to diagnose. In this type, fractures are uncommon at birth and tend to begin with ambulation. The frequency of fractures then commonly decreases after puberty. Vertebral fractures can occur and lead to scoliosis. When long-bone fractures do occur, they heal without deformity. These individuals also have normal heights or mild short stature and typically have blue sclera (~90%) and ligamentous laxity (~50%). Dentinogenesis imperfecta (~10%) and hearing loss (~15%) are less common [63]. In a large cohort of 598 individuals with a clinical diagnosis of OI, pathogenic mutations were identified in 97–98% of affected individuals [64]. Of the 256 patients with a diagnosis of OI Type I, disease-causing variants were detected in 248 (97%). All mutations found in OI Type I were dominant and exclusively affected type I collagen

Table 8.3 Classification of osteogenesis imperfecta types

Type	Gene	MIM	Locus	Protein product	Inheritance
Nondeforming					
I	*COL1A1*	166200	17q21.33	Collagen, type I, alpha-1 chain	AD
	COL1A2	166200	7q21.3	Collagen, type I, alpha-2 chain	AD
	PLS3	300910	Xq23	Plastin 3	XL
Perinatal lethal					
II	*COL1A1*	166220	17q21.33	Collagen, type I, alpha-1 chain	AD
	COL1A2	166220	7q21.3	Collagen, type I, alpha-2 chain	AD
	CRTAP	610682	3p22.3	Cartilage-associated protein	AR
	P3H1	610915	1p34.2	Prolyl 3-hydroxylase 1	AR
	PPIB	259440	15q22.31	Peptidyl-prolyl isomerase B (cyclophilin B)	AR

Table 8.3 (continued)

Type	Gene	MIM	Locus	Protein product	Inheritance
Progressively deforming					
III	*COL1A1*	259420	17q21.33	Collagen, type I, alpha-1 chain	AD
	COL1A2	259420	7q21.3	Collagen, type I, alpha-2 chain	AD
	CRTAP	610682	3p22.3	Cartilage-associated protein	AR
	P3H1	610915	1p34.2	Prolyl 3-hydroxylase 1	AR
	PPIB	259440	15q22.31	Peptidyl-prolyl isomerase B (cyclophilin B)	AR
	SERPINH1	613848	11q13.5	Serpin peptidase inhibitor, clade H, member 1	AR
	BMP1	614856	8p21.3	Bone morphogenetic protein 1	AR
	FKBP10	610968	17q21.2	FK506-binding protein 10	AR
	PLOD2	609220	3q24	Procollagen-lysine, 2-oxoglutarate 5-dioxygenase 2	AR
	SERPINF1	613982	17p13.3	Serpin peptidase inhibitor, clade F, member 1	AR
	SP7	613849	12q13.13	Transcription Factor Sp7 (Osterix)	AR
	WNT1	615220	12q13.12	Wingless-type MMTV integration site family, 1	AR
	TMEM38B	615066	9q31.1	Transmembrane protein 38B	AR
	CREB3L1		11q11.2	cAMP response element-binding protein 3-like 1	AR
	SEC24D		4q26	SEC24-related gene family, Member D	
Moderate form					
IV	*COL1A1*	166220	17q21.33	Collagen, type I, alpha-1 chain	AD
	COL1A2	166220	7q21.3	Collagen, type I, alpha-2 chain	AD
	CRTAP	610682	3p22.3	Cartilage-associated protein	AR
	PPIB	259440	15q22.31	Peptidyl-prolyl isomerase B (cyclophilin B)	AR
	FKBP10	610968	17q21.2	FK506-binding protein 10	AR
	SERPINF1	613982	17p13.3	Serpin peptidase inhibitor, clade F, member 1	AR
	WNT1	615220	12q13.12	Wingless-type MMTV integration site family, 1	AD
	SP7	613849	12q13.13	Transcription Factor Sp7 (Osterix)	AR
Calcification of the interosseous membrane and/or hyperplastic callus					
V	*IFITM5*	610967	11p15.5	Interferon-induced transmembrane protein 5	AD

Table modified from Van Dijk and Sillence [62]

genes (*COL1A1* or *COL1A2*). In the 342 patients with a diagnosis of moderate and severe OI (Types II, III, and IV), disease-causing variants were detected in 337 (99%). Dominant mutations causing OI were found in *COL1A1*/*COL1A2* (77%) and *IFITM5* (9%). Recessive mutations were observed in 12% of individuals with moderate to severe OI, most commonly *SERPINF1* and *CRTAP*. Thus, about 20% of individuals with moderate to severe OI had mutations in genes other than *COL1A1*/*COL1A2* [64]. Patients with Type I OI tend to have quantitative defects in their type I collagen protein arising from large-scale deletions or nonsense mutations ("functional null" alleles) in *COL1A1* or *COL1A2*. Patients with OI Types II, III, and IV often have missense mutations that alter glycine residues in either *COL1A1* or *COL1A2* or mutations in genes which affect collagen production and processing [61].

The clinical diagnosis of OI is based on history and clinical examination, bone mineral density determination by DXA, findings on radiographs, and bone and mineral biochemistry. Once OI is suspected, it is recommended that genetic testing be done not only to confirm the diagnosis but also to determine the recurrence risks. Identifying the type of OI is important in predicting the course of the disease as well as identifying other problems that are specific to certain types. For instance, patients with OI Type V are at risk for hyperplastic callus formation, radial head dislocation, and abnormalities in the cranio-cervical junction [65]. Several commercial laboratories now offer OI sequencing panels (see ▶ GeneTests.org).

The most effective treatment strategies for OI are multidisciplinary, consisting of general medical management to maximize bone health by optimizing vitamin D status and calcium intake while minimizing the morbidity of fractures and pain, orthopedic management of fractures and skeletal deformities, and physical/occupational therapy to maximize strength and function. Other important caregivers are dentists for the treatment of the complications of dentinogenesis imperfecta and the otolaryngologist for the treatment of hearing loss [66–68]. The last two decades have seen an increasing use of bisphosphonates in the treatment of OI and is now routinely utilized for moderate to severe forms. It often falls to the pediatric endocrinologist to manage this treatment. Bisphosphonates are analogs of pyrophosphate that bind to bone mineral and inhibit osteoclast function, reducing bone resorption. Glorieaux et al. [69] reported on the use of pamidronate (a bisphosphonate given by IV) in OI in an uncontrolled study and noted a decrease in fracture frequency, improvement in bone mineral density, and improvement of chronic bone pain. A longer-term study [70] confirmed the results and in addition showed an improvement in linear growth and weight gain in more severely affected individuals (OI Types III and IV). Other studies describe similar improvement but also report a case of nonunion of a tibial fracture [71] and delayed fracture healing [72]. Two larger review studies including a Cochrane review have been completed. In the first review, 10 trials (519 children) [73] and the second 14 trials (819 participants) [74] were included. The studies were, for the most part, at a low risk of bias. The Cochrane review [74] found that for oral bisphosphonates, data versus placebo could not be aggregated. They reported in two studies there appeared to be a reduction in fractures, but no differences were reported in the remaining three trials which commented on fracture incidence. Both reviews state that all studies investigating lumbar spine bone mineral density indicated a statistically significant increase in at least one time point a result of bisphosphonate. There was general agreement that treatment with oral or intravenous bisphosphonates in children with OI results in an increase in bone mineral density. Although most studies observed a significant decrease in fracture incidence and bone pain, the varied study designs did not allow this to be shown conclusively. The medications in general do appear to be safe and well tolerated [73, 74].

At our center, we use the protocol reported by Glorieaux et al. [69], namely, pamidronate diluted in normal saline (0.1 mg:1 cc NS at maximum concentration), infused over 4 h, 0.5–1 mg/kg (depending upon patient age) each day for 3 consecutive days, cycled every 8 to 16 weeks (depending upon patient age). Some centers delay infusions after osteotomies [75], although this is somewhat controversial. Infusions are generally not delayed after fractures. The primary adverse effect is a febrile acute-phase reaction that is common after the first dose. On the first day of the first infusion, we decrease the dose to ¼ of the typical dose and ½ of the typical dose on days 2 and 3 to decrease this effect. Premedication with acetaminophen or ibuprofen before each dose can also be helpful. Transient hypocalcemia after an infusion is seen

on occasion but is rarely of clinical significance with pamidronate. We perform pretreatment assessment and normalization of vitamin D status and calcium intake to lessen this potential effect. Osteonecrosis of the jaw following dental procedures is a rare but serious complication of bisphosphonate therapy in adults. This is of particular concern in children with OI associated with dentinogenesis imperfecta, who may require multiple dental procedures. However, no cases have been reported in children with OI, and a retrospective survey failed to identify any cases [76, 77].

Zoledronic acid is another IV bisphosphonate that is increasingly being used for the treatment of OI. The advantage of zoledronic acid over pamidronate is that the infusion is given over 30 min (vs. 4 h for pamidronate) and is given as a single infusion (vs. daily for 3 days for pamidronate). Small randomized studies have shown results are comparable for both drugs [78, 79], although larger and long-term studies have not been published. Our experience is that zoledronic acid more frequently causes significant hypocalcemia and acute-phase reactions than pamidronate. Until longer-term data is available, our center will continue to use pamidronate.

There is debate in the field on how long to treat OI patients with bisphosphonates. Bisphosphonates remain bound to bone for decades after administration, and, although long-term studies to date do not suggest any very long-term adverse effects, there remains some uncertainty. It seems prudent to limit the exposure of these children as much as possible to these drugs. An observational study of 23 children with OI where pamidronate was stopped after at least 3 years of treatment showed a rapid decrease in bone mineral density in the radial diaphysis [80]. It has been suggested that this might create regions of localized bone weakness at the junction of the bone laid down during the treatment and bone laid down after the treatment [80]. Some centers are now decreasing the bisphosphonate dose by half once the bone mineral density *z*-score by DXA has improved to >−2.0 [65], although this approach has not been rigorously tested.

There is interest in the use of rhGH in patients with OI, both to treat growth failure associated with the more severe forms, but also because of its anabolic effect on bone. Growth hormone deficiency is not part of OI, although there may be a blunted IGF-1 release in response to rhGH [81]. In short-term trials, rhGH improves both linear growth and bone mineral density [82, 83], and a long-term randomized controlled study of rhGH is ongoing (▶ ClinicalTrials.gov Identifier NCT00001305).

8.6 Uncommon Syndromes: What They Teach Us About Growth

8.6.1 Jansen-Type Metaphyseal Chondrodysplasia and Blomstrand Chondrodysplasia

Jansen-type metaphyseal chondrodysplasia (MIM 156400) is a very rare autosomal dominant form of dwarfism characterized by short, bowed limbs and brachydactyly. Affected neonates can have abnormalities and rib fractures or choanal atresia that may require respiratory support. However, neonates may appear normal. Postnatal growth failure becomes obvious within 1 to 2 years of age. X-rays show a rachitic-type picture with normal-appearing epiphysis, widened growth plates, and severely disordered metaphyses with splaying, cupping, and fraying. They also show osteopenia and subperiosteal bone resorption suggestive of hyperparathyroidism. About half of patients with this syndrome have hypercalcemia, along with hypercalciuria, hypophosphatemia, hyperphosphaturia, and increased $1,25(OH)_2$ vitamin D levels, a pattern strongly suggestive of hyperparathyroidism. However, parathyroid hormone (PTH) and PTH-related protein (PTHrP) levels are low normal or suppressed [84]. In 1995, it was reported that Jansen-type metaphyseal chondrodysplasia was caused by *activating* mutations of the parathyroid hormone receptor (*PTH1R*) [85].

In 1985, Blomstrand et al. [86] described a neonate that died within hours of birth with a form of short-limbed dwarfism with features of advanced skeletal maturation (Blomstrand chondrodysplasia, MIM 215045). This is a very rare autosomal recessive form of dwarfism that is generally natally lethal, although milder forms have been described [87]. The fetuses/neonates showed severely shortened limbs, polyhydramnios, hydrops fetalis, coarctation of the aorta, and hypoplastic lungs. X-rays consistently showed dense bones with premature mineralization of the teeth and all of the bones in the hands and feet [88]. In many ways, Blomstrand chondrodysplasia showed the opposite features of Jansen-type metaphyseal chondrodysplasia, despite both being

short-limbed forms of dwarfism. This symmetry was confirmed when Blomstrand chondrodysplasia was found to be caused by *inactivating* mutations of the parathyroid hormone receptor [89].

The parathyroid hormone receptor (parathyroid hormone 1 receptor, PTH1R, gene *PTH1R*) is a G-protein-coupled receptor whose ligands are both PTH and PTHrP. In renal tubular cells, osteoblasts, and osteoclasts, PTH1R mediates the calcium-regulatory properties of PTH and is the source of the hypercalcemia found in Jansen-type metaphyseal chondrodysplasia. However, in growth plate chondrocytes, PTH1R mediates the growth-regulatory properties of PTHrP and gives rise to the dwarfism features of both syndromes.

In the 1990s, through an elegant series of experiments conducted in a number of laboratories in chicks and rodents, a major regulatory mechanism of the growth plate was described involving Indian hedgehog and PTHrP [90]. Briefly, differentiated chondrocytes within the hypertrophic zone of the growth plate produce a paracrine signaling peptide called Indian hedgehog, which diffuses through the growth plate. Indian hedgehog is detected by perichondrium and periarticular cells, as well as chondrocytes in the proliferative and pre-hypertrophic zone. In response, these cells produce PTHrP, which inhibits the differentiation of pre-hypertrophic chondrocytes and prevents their entry into the hypertrophic phase. The reduced number of hypertrophic chondrocytes results in reduced Indian hedgehog production, closing the feedback loop. Through this feedback mechanism, the number of chondrocytes entering the hypertrophic phase of differentiation is regulated. Several other signaling pathways, including transforming growth factor-β(beta) (TGF-β(beta)) [91] and bone morphogenetic proteins (BMP) [92], are intermediates in, and modulators of, this feedback loop. The discovery of the disruption of this feedback loop as the cause of Jansen-type metaphyseal chondrodysplasia and Blomstrand chondrodysplasia proved the presence and importance of this regulatory mechanism in human growth.

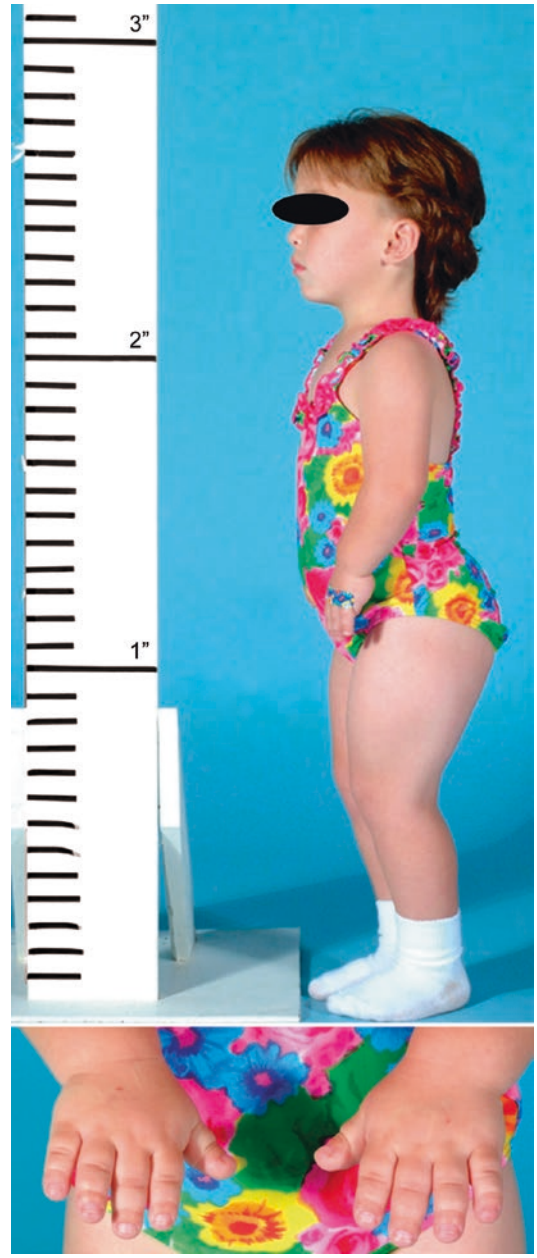

Fig. 8.5 A patient with acromesomelic dysplasia, type Maroteaux (AMDM). An 8-year-old girl with AMDM demonstrates severe short stature (height SD score of −8.5) with disproportionately short forearms/lower legs and severe shortening of the bones in the hands and feet. Her sitting height-to-standing height ratio was 0.58 (SD score of +3.7) and her arm span/height difference was −17.8 cm (SD score of −5.9). She also has Madelung deformity of both wrists

8.6.2 Acromesomelic Dysplasia, Type Maroteaux

Acromesomelic dysplasia, type Maroteaux (AMDM, MIM 201250), is an autosomal recessive form of dwarfism characterized by severe body disproportion with shortening of the forearms and lower legs and especially the bones in the hands and feet (Fig. 8.5) [93]. Infants with AMDM usually appear

normal at birth, although short birth length and subtle disproportion have been observed [94]. Generally, these children are identified after their linear growth slows at 1–2 years of age. Adults with this syndrome have height SD scores ranging from −5 to −10 [95]. It is a rare syndrome, with a prevalence of roughly 1:2,000,000 [96]. In 2004, the cause of AMDM was found to be homozygous inactivating mutations in the gene that encodes for natriuretic peptide receptor B (NPR-B, gene *NPR2*) [95]. The ligand for NPR-B is C-type natriuretic peptide (CNP). This observation was the first to identify CNP as an important regulator of human growth [97]. Both CNP and NPR-B are synthesized in the growth plate; CNP acts through a paracrine regulatory mechanism. Rodent and in vitro studies have now shown that CNP stimulates growth plate chondrocyte differentiation and hypertrophy, in part through inhibiting MAPK pathway signaling [26]. As such, CNP is a counter-regulatory mechanism to the Indian hedgehog/PTHrP pathway. This effect has led to the exploration of CNP as a specific treatment for achondroplasia [19].

It was observed that the parents and heterozygous carrier siblings of patients with AMDM were shorter than the general population [95, 96, 98]. Heterozygous carriers of *NPR2* mutations have on average height SD scores that are 1.4 shorter than noncarrier family members, but with no body disproportion or other detectable abnormalities [96]. Heterozygous inactivating mutations in *NPR2* have been identified in 1–3% of children with idiopathic short stature [99, 100], making this a not uncommon cause of idiopathic short stature.

Case Studies

Case 1 is a 10-year and 4-month-old girl referred for evaluation of short stature. Her length dropped from the 32nd percentile at birth to the 5th percentile by 18 months of age. Since that time, she has been growing along the 3rd percentile curve. She is otherwise healthy with no other medical problems. Past medical history showed she was the product of an uncomplicated pregnancy and born at term with a birth weight of 2.80 kg (*z*-score of −1.0, 16th percentile) and a length of 48.3 cm (*z*-score of −0.5, 32nd percentile). Her mother is 155 cm (*z*-score of −1.3, 10th percentile) and her father is 165 cm (*z*-score − 1.7, 4th percentile) and has quite short arms. Her physical exam shows a height of 127.0 cm (*z*-score − 1.9, 3rd percentile), weight of 38.0 kg (*z*-score 0.5, 68th percentile), and a BMI of 23.5 (*z*-score 1.7, 95th percentile). She has obvious short arms, with an arm span of 119.0 cm and an arm span/height difference of 8.0 cm (*z*-score of −2.0, 3rd percentile). Her upper-to-lower segment ratio is 1.02 (*z*-score of 1.8, 4th percentile). Her physical exam is significant for cubitus valgus, slight genu valgus, prominent radial heads with a step-off at the wrists, and brachydactyly. She did not have neck webbing, lymphedema, shield chest, nor a heart murmur. A left hand/wrist x-ray showed a bone age that is concordant with her chronologic age, but also tilting of the radial and ulnar heads, with abnormal distal radial and ulnar physes, consistent with Madelung deformity (Fig. 8.6). A full skeletal survey showed the cubitus valgus and was otherwise unremarkable. Laboratories were remarkable for a normal female karyotype (46XX). Sequencing of *SHOX* showed a heterozygous mutation of c.3G > A, p,Met1Ile.

This case illustrates the need for pediatric endocrinologist's understanding of skeletal dysplasias. This girl presents with the common complaint of short stature. The patient has skeletal disproportion that is not severe and has some characteristic skeletal findings, including cubitus valgus and Madelung deformity. The first consideration should be the diagnosis of Turner syndrome. However, this girl has none of the non-skeletal features of Turner syndrome and finding that her father has very similar findings makes Turner syndrome unlikely. Léri-Weill dyschondrosteosis then becomes the leading diagnostic possibility, either from a *SHOX* mutation or from a *SHOX* deletion inherited from her father. This family proved to have a point mutation that eliminated the start codon of the SHOX gene, eliminating all translation from the mRNA, and confirms the diagnosis. This patient is currently on human growth hormone treatment.

Case 2 is a 15-year and 9-month-old boy, referred for short stature. Previous growth charts are unavailable, but he has always been the shortest boy in his class, and the difference between him and his peers had increased in the previous 1–2 years. He is otherwise healthy with no other medical problems. Past medical history showed he was the product of an uncomplicated pregnancy and born at 40 weeks of gestation with a birth weight of 4.48 kg (*z*-score of 2.0, 98th percentile). Birth length is unknown. His mother is 163 cm (*z*-score of −0.1, 48th percentile) and her father is 157 cm (*z*-score − 2.8, 0.3rd percentile). The paternal grandfather is also short. The physical exam shows a height of 156.1 cm (*z*-score − 2.1, 2nd percentile), weight of 67.1 kg (*z*-score 0.6, 73rd percentile), and a BMI of 27.5 (*z*-score 1.7, 95th percentile). He has an arm span of

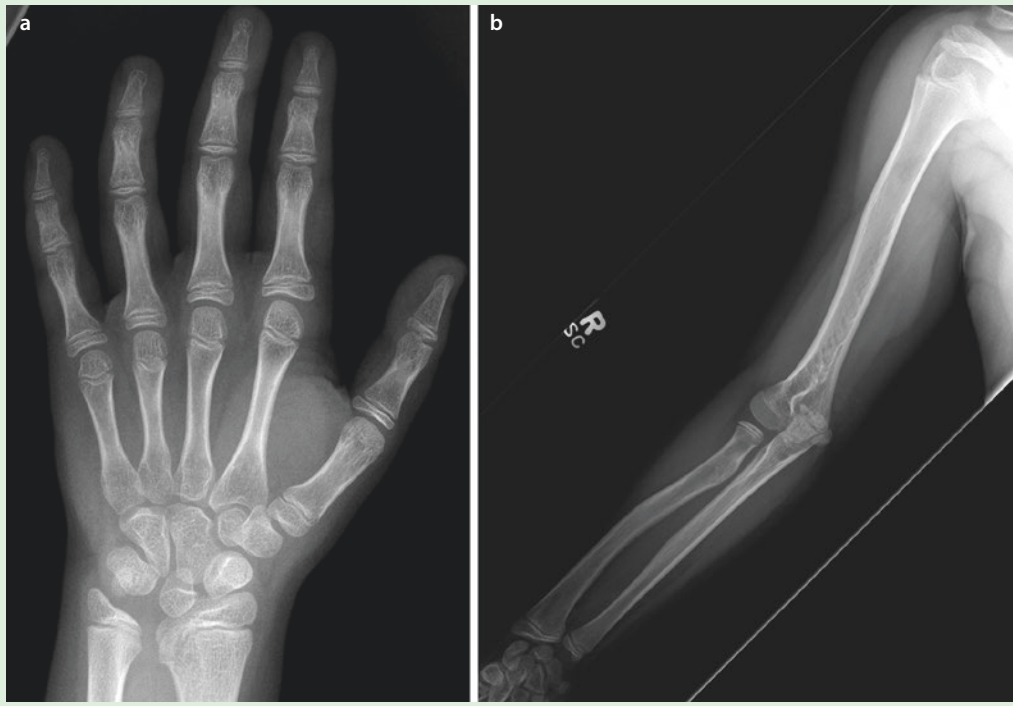

Fig. 8.6 X-rays for case 1, a 10-year-old girl with short stature. *Panel a* shows the left hand/wrist film obtained for a bone age. The growth plates of the radial and ulna heads are abnormal, particularly at the medial borders. These abnormal growth plates cause the articular surfaces of these bones to grow at an angle toward each other, creating a "V" configuration between them. This also causes bowing of the radius. These are the findings of Madelung deformity. *Panel b* shows the outward angulation at the elbow (cubitus valgus), as well as the bowing of the radius

155.8 cm and an arm span/height difference of −0.3 cm (*z*-score of −0.4, 35th percentile). His upper-to-lower segment ratio is 1.09 (*z*-score of 3.5, >99.9th percentile). His physical exam shows skeletal disproportion, with his fingers falling to the level of his hips when standing. His upper arms and thighs are clearly short compared to his forearms and lower leg. His forehead is prominent, but he has no other dysmorphic facial features. His hands and fingers are short and broad. A left hand/wrist x-ray showed a bone age of 16–9/12 at a chronologic age of 15–8/12 (*z*-score 0.9) and no bone abnormalities. Laboratories were unremarkable. Sequencing of *FGFR3* showed a heterozygous mutation of c.1620C > A, p.Asn540Lys.

This young man has hypochondroplasia. As in case 1, the skeletal disproportion is the key in recognizing the presence of a skeletal dysplasia, leading to prompt diagnosis. In both the cases, there is a significant family history of short stature (three generations in case 2), but the correct diagnosis had not been previously recognized. For case 2, his bone age suggested that human growth hormone treatment would not be effective and was not attempted.

8.7 Summary

There are 436 syndromes of skeletal dysplasia. Most are readily recognized as such, but in a number, the effect on height is relatively minor and other signs and/or symptoms are absent or subtle. It is not uncommon for these to present to the pediatric endocrinologist for evaluation of short stature. It is incumbent that the pediatric endocrinologist to be able to recognize when a child might have a skeletal dysplasia, to be able to diagnose the most common syndromes, and to understand what treatment options are available.

In the words of William Harvey (1578–1657), "Nature is nowhere accustomed more openly to display her secret mysteries than in cases where she shows traces of her workings

apart from the beaten path; nor is there any better way to advance the proper practice of medicine than to give our minds to the discovery of the usual law of nature by careful investigation of cases of rarer forms of disease" [101]. Children with skeletal dysplasias on occasion require the diagnostic and therapeutic skills of the pediatric endocrinologist. In return, these patients give us the opportunity to learn more about human growth and the human skeleton through "careful investigation" of these rare forms of disease.

Review Questions

1. You have been asked to see an adopted 18-month-old male infant with a recent tibia fracture which occurred following a low-energy fall. He is noted to have blue sclera and you have a clinical suspicion that he has a mild form of OI. You would like to proceed with molecular testing to confirm the diagnosis. The most effective genetic testing should be:
   A. *CRTAP*, *P3H1*, and *PPIB* panel
   B. *COL1A1* and *COL1A2* sequencing
   C. *IFITM5* sequencing
   D. Whole-exome sequencing
2. A 7-year-old girl presents with short stature, with a height on the 2nd percentile. She has no dysmorphic features and a normal upper-to-lower segment ratio and arm span/height difference. A karyotype is normal, 46XX. Has *SHOX* haploinsufficiency been ruled out.
   A. True
   B. False
3. A 12-year-old boy presents with short stature, with a height on the 4th percentile. He has skeletal disproportion with a high upper-to-lower segment ratio and low arm span/height difference. His mother also has short stature, also with short-appearing arms. You suspect he has a skeletal dysplasia. The most appropriate next step in the evaluation is:

   A. Radiographic skeletal survey
   B. Referral to genetics
   C. Send genetic testing for *SHOX* and *FGFR3*
   D. Whole-exome sequencing

Answers

1. (B) For mild osteogenesis imperfecta, the most common causes by far are mutations or deletions of one of the type I collagen genes.
2. (B) In children with "idiopathic" short stature, as many as 5% will have a *SHOX* microdeletion, point mutation, or mutation in a regulatory region.
3. (A) A skeletal survey should be able to differentiate the types of skeletal dysplasias by looking for characteristic features such as Madelung deformity and cubitus valgus for Léri-Weill dyschondrosteosis or rhizomelic shortening and spinal and pelvic changes for hypochondroplasia.

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Growth Hormone Excess and Other Conditions of Overgrowth

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Key Points

- Most common cause of tall stature is familial.
- Pituitary gigantism is extremely rare but is the most common cause of GH excess.
- IGF-1 levels should be interpreted keeping age, gender, and Tanner stage in mind.
- Pituitary adenomas causing GH excess at a young age are aggressive and often require a multimodal therapeutic approach – surgical, medical, and radiation therapy.

9.1 Introduction and Background

Tall stature refers to height or length that is 2 SDs greater than the mean for the reference population. The race and ethnicity of the reference population should ideally be considered when considering a diagnosis of tall stature. Since stature is normally distributed, at least 2.5% of the population would be tall by definition. Although there are no data to suggest that girls are more likely to have tall stature than boys, girls are more likely to seek medical attention due to societal perception. Assessment of stature should take into account the child's age, weight, body proportions, pubertal onset and progression, height velocity, and familial stature.

9.2 Etiology

The approach to overgrowth or tall stature can be categorized based on the time of onset of growth acceleration (Table 9.1).

Table 9.1 Causes of overgrowth or tall stature can be categorized based on the time of onset of accelerated growth

Causes	Proposed mechanism	Clinical presentation	Adult height
Intrauterine/infantile overgrowth			
Maternal diabetes mellitus	Maternal hyperglycemia causes fetal hyperglycemia leading to hyperinsulinemia and increased growth	Large for gestational age, neonatal hypoglycemia, organomegaly	Normal
Cerebral gigantism (Sotos syndrome)	Mostly sporadic autosomal dominant inheritance: deletion of NSD1 gene located on chromosome 5	Rapid growth in early childhood, macrocephaly, frontal bossing, high-arched palate, poor coordination and mental retardation, expressive language delay, large hands, increased arm span. Most have an advanced bone age	Near normal
Beckwith Wiedemann syndrome	Dysregulation of imprinted genes on chromosome 11p	Polyhydramnios, placentomegaly, macroglossia, hemihyperplasia, neonatal hypoglycemia (mild and transient), hypothyroidism, hyperlipidemia, anterior abdominal wall defects (omphalocele, umbilical hernia), embryonal tumors such as Wilms's tumor and hepatoblastoma	Upper range of normal for the family
Childhood/adolescent overgrowth			
Normal variation			
Familial	Genetic variation	No dysmorphic features, high normal height velocity, normal bone age, family history of tall stature	Approximates target height

(continued)

Table 9.1 (continued)

Causes	Proposed mechanism	Clinical presentation	Adult height
Endocrine disorders			
Growth hormone excess (pituitary gigantism)	Oversecretion of GH caused by a pituitary/ hypothalamic/ ectopic mass-secreting GH or GHRH	Tall stature (if onset before epiphyseal closure), coarse facial features, myalgias, skin tags, other features dependent on other hormonal excesses/deficiencies	Tall
Precocious puberty (central or peripheral)	Stimulation of growth from exposure to sex steroids followed by early cessation of statural growth from early epiphyseal fusion	Secondary sex characteristic development which may be isosexual or contrasexual depending on the cause, advanced bone age	Shorter than target height
Hyperthyroidism	Stimulatory effect of thyroid hormones on GH axis which leads to increased height velocity along with rapid advancement of bone age	Associated signs of hyperthyroidism and the primary etiology	Initial acceleration of growth but eventually short if untreated
Sex hormone deficiency or receptor insensitivity	Delay in epiphyseal closure with continued growth	Hypogonadism, eunuchoid body proportions	Tall
Aromatase deficiency	Loss-of-function mutations in Cyp19A on chromosome 15 causing congenital estrogen deficiency	Tall stature, osteopenia/osteoporosis; tall stature even in the setting of growth hormone deficiency	Tall
Glucocorticoid resistance	Increased adrenal androgen production from elevated ACTH	Hirsutism and virilization in females and isosexual precocious puberty in males	Near normal
Congenital lipodystrophy	Autosomal recessive; mutations of AGPAT2 and BSCL2	Insulin resistance, near absence of adipose tissue	Near normal
Nonendocrine disorders			
Obesity	Excess calories	Advanced bone age, pubertal onset is usually earlier than in children who are normal weight	Near normal
Marfan syndrome	Mutations in fibrillin gene	Autosomal dominant; low upper segment to lower segment ratio, arachnodactyly, ectopia lentis, dilation of the aortic root	Tall
Homocystinuria	Deficiency of the enzyme cystathionine synthase	Similar to Marfan syndrome with lens subluxation, mental retardation, thromboembolic disease with elevations in urinary homocysteine and methionine	Tall
Klinefelter syndrome (47XXY)	Sex chromosome aneuploidy (one or more supplementary X chromosome)	Tall boys with relatively long legs, learning disability, microorchidism, gynecomastia	Tall

Table 9.1 (continued)

Causes	Proposed mechanism	Clinical presentation	Adult height
Melanocortin 4 receptor (MC4R) mutation	Mutations in MC4R receptor	Most common cause of monogenic obesity, associated with frequent and taller GH pulses (unlike exogenous obesity) [1]	Tall
47XYY	Aneuploidy of Y chromosome	Mild motor and language development delay, radioulnar synostosis, clinodactyly, and sometimes attention deficit hyperactivity disorder (ADHD) and autism spectrum disorders	Tall
Triple X syndrome	Triple SHOX gene effect	Insulin resistance and behavioral disorders	Normal or tall
Fragile X syndrome	FMR1 CGG repeats	Mental retardation, macroorchidism, X-linked disorder	Tall

9.3 Approach to a Child with Tall Stature

Keeping in mind the various causes of tall stature, we suggest the following approach to a tall child.

1. *Confirm the diagnosis of tall stature*: The current height should be greater than 2 SDs for the sex- and age-matched reference population, keeping race and ethnicity in mind. This should be interpreted in the context of predicted adult height derived from the heights of the biological parents. While benign familial tall stature is associated with tall parents, the clinician should be mindful that having a tall parent or parents does not rule out associated pathology as there could be pathologic familial or genetic causes of tall stature.
2. *Consider the pubertal stage*: Early puberty may result in growth acceleration and result in current height being greater than in peers who have not yet entered puberty. Physical examination and bone age should be able to aid with this diagnosis. Further workup should elucidate the cause of precocious puberty.
3. *Ascertain the timing of accelerated growth rate*: If the increased growth velocity in a younger child is present since birth, some of the syndromes of intrauterine growth acceleration, such as maternal diabetes and Beckwith Wiedemann, should be considered. Once a child reaches its own genetic potential on the growth curve, which usually occurs around 2 years of age, he/she typically tracks along the corresponding growth percentile. If there is a sudden acceleration in growth velocity and deviation from that curve, it is important to rule out conditions such as hyperthyroidism, growth hormone excess, obesity, and constitutional advancement of growth.
4. *Anthropometry including head circumference*: Patients with Marfan and Klinefelter syndromes typically have longer limbs and hence have a lower upper/lower segment ratio and longer arm span than standing height. Macrocephaly is a feature of Sotos and Weaver syndromes. Body mass index (BMI) is helpful in diagnosing obesity, and greater severity and early onset of obesity may suggest the need to look for monogenic causes of obesity.
5. *Look for any syndromic features*: A careful history should explore any behavioral or developmental concerns which may be associated with syndromes such as Sotos, triple X, or fragile X or conditions such as homocystinuria. Appropriate genetic testing should be undertaken, and karyotype or plasma homocysteine levels assessed. A medical history of ocular issues and cardiac defects may provide clues to the diagnosis of Marfan syndrome. Hypogonadism or macroorchidism may suggest Klinefelter or fragile X syndromes, respectively.

6. *Estimate the adult height of the child*: Estimation of adult height in the setting of tall parents or one tall parent is not entirely reliable. There are data to suggest that the Bayley-Pinneau (BP) method overestimates, while the Tanner-Whitehouse (TW) method underestimates the predicted adult height in constitutionally tall children [2]. If the child's height prediction in the range of target adult height and there are no syndromic features, he/she most likely has familial short stature. If the child grows, or is projected to grow, beyond than the target height range without any syndromic features or growth acceleration, hypoestrogenism or estrogen resistance leading to delayed epiphyseal closure should be considered.

9.4 Growth-Decreasing Therapy

Children and parents who find the predicted height unacceptable may seek treatment. However, there is little evidence that tall stature causes social or emotional challenges in adolescents or adults. Such therapy should be undertaken after an evaluation for hormonal causes of tall stature, as the latter may be treated by treating the underlying condition. Growth-reducing therapy is not commonly sought in patients with syndromic tall stature.

9.4.1 Sex Steroid Treatment

Estrogen in high doses, such as 100–300 mcg of ethinyl estradiol daily, has been used in the past to accelerate growth plate maturation causing early epiphyseal fusion. The efficacy in reducing final height is better when treatment is initiated at a lower bone age [2]. The mean height reduction is about 1.6 cm [3]. Long-term use of high-dose estrogen is linked with decreased fertility and increased incidence of imminent ovarian failure [4, 5]. There is also concern for a higher risk of breast cancer [6] and thromboembolic episodes.

Testosterone treatment in boys is less effective than estrogen treatment in achieving final height reduction. It leads to early epiphyseal closure after aromatization to estrogen. There are studies showing equal efficacy of 250 mg and 500 mg of testosterone enanthate given intramuscularly every 2 weeks [7]. There are no known adverse effects on fertility or cancer risk in adolescents treated with high doses of testosterone. However, high testosterone levels may result in polycythemia and acne and cause aggressive behavior.

9.4.2 Surgery

The most common surgical procedure used to decrease growth is bilateral percutaneous epiphysiodesis of the distal femur and proximal tibia and fibula. In this operation, the growth plates of the long bones of the legs are obliterated by needling to prevent more linear growth. Since this procedure decreases limb growth, it can potentially alter final body proportions [8]. Unlike hormonal treatment, it can be used efficaciously at an advanced bone age. Complications are rare but include surgical scars, neurovascular damage, exostosis, or asymmetrical fusion leading to asymmetric limb length [9].

9.5 Growth Hormone Excess

Growth hormone excess is a very rare disorder of childhood. If it occurs in a growing child before epiphyseal fusion, it leads to tall stature causing gigantism. However, after epiphyseal fusion, GH excess has no effect on height and instead causes overgrowth of bone leading to acromegaly. Growth hormone excess can occur from a variety of sources – pituitary GH secretion from a tumor or somatotroph hyperplasia, hypothalamic GH-releasing hormone (GHRH) secretion, ectopic source of GH or GHRH (extremely rare in children), or decrease in somatostatin inhibition [10].

9.6 Incidence

This is a very rare disorder. The incidence of childhood pituitary tumors is about 0.1 in a million person-years, and less than 10% of these are GH-secreting adenomas [11, 12]. In a recent claims-based approach in the United States, which analyzed disease incidence based on acromegaly-based insurance claims, the annual incidence of GH excess in children, from 2008 to 2012, was three to eight cases per million person-years. There was no significant difference between incidence based on gender and the incidence increased with increasing age [13].

Table 9.2 Syndromes associated with gigantism/GH excess

Syndrome	Gene	Associated features
Multiple endocrine neoplasia type 1 (MEN 1)	*MENIN*	Parathyroid, anterior pituitary, and pancreatic neuroendocrine tumors
Multiple endocrine neoplasia type 4 (MEN 4) [16]	Cyclin-dependent kinase inhibitor (*CDKN1B*)	Parathyroid, anterior pituitary, testicular, cervical, thyroid, adrenal, and renal tumors
Familial isolated pituitary adenoma (FIPA)	Aryl hydrocarbon receptor-interacting protein (*AIP*)	At least two members of the same family with pituitary tumor
McCune-Albright syndrome	*GNAS1*	Fibrous dysplasia, cafe-au-lait spots, precocious puberty; sometimes hyperthyroidism or hypercortisolemia
Carney complex	*PPKAR1A*	Skin pigmentation, cardiac myxomas, Cushing's syndrome with pigmented nodular adrenocortical disease (PPNAD)
X-linked acrogigantism (X-LAG)	GPR101	Median onset of gigantism is early, about 12 months [17]
Paraganglioma, pheochromocytoma, and pituitary adenoma association (3PA)	Succinate dehydrogenase (*SDH A-D*)	Most cases are familial

9.7 Clinical Presentation

GH excess in childhood presents with accelerated growth. However syndromes associated with GH excess (Table 9.2) should also be considered, and the child should be assessed for symptoms associated with those syndromes. Because tall stature is often accepted by society as a favorable physical attribute, there may be delays in seeking medical help for this condition. In children, like adults, the average delay in diagnosis is about 5 years [14, 15]. The lag between initial symptoms and diagnosis is shorter in females than males [15].

9.7.1 Symptoms Related to GH Excess

The usual presentation of growth hormone excess before epiphyseal fusion is increased growth velocity and tall stature. In older adolescents, musculoskeletal symptoms of GH excess, i.e., arthralgias, wrist pain, large feet and hands, coarsening of facial features, prognathism, macrocephaly, and kyphoscoliosis, may be seen. Sleep apnea from mucosal and lymphoid hypertrophy may occur. The complications of GH excess such as hypertension, cardiomyopathy, glucose intolerance, or frank diabetes mellitus are less often seen in this younger age group.

9.7.2 Symptoms Related to the Tumor and Its Other Secretions

The signs and symptoms of a sellar mass may include headache, visual field deficits, and increased intracranial pressure. Sometimes tumors co-secrete GH and prolactin (mammosomatotrophs, as these cells have common embryonic origin), which can be associated with galactorrhea. Hyperprolactinemia may also occur from stalk compression in the absence of tumor secretion of prolactin.

9.7.3 Symptoms Related to Co-existing Pituitary Deficiencies

Oligomenorrhea or primary amenorrhea may occur when the tumor impacts the secretion of gonadotropins. Adrenal insufficiency and central hypothyroidism may be present and need to be ruled out. Diabetes insipidus is rarely seen at presentation.

9.8 Diagnostic Evaluation

Tall stature accompanied by increased growth velocity and other musculoskeletal features should raise suspicion of GH excess which should be investigated as outlined below. Furthermore, if GH excess is detected, presence of co-existing syndromes (Table 9.2) should be considered.

9.8.1 Biochemical Testing

IGF-1 level: This is the initial screening test for anyone in whom GH excess is suspected. IGF-1 is a composite measure of basal GH secretion [18] and has a log-linear relationship with cumulative GH levels [19]. IGF-1 levels should be interpreted keeping age and Tanner stage in mind. These levels can be altered (typically lowered) with malnutrition, hypothyroidism, hepatic or renal dysfunction, or oral estrogen use. An elevated IGF-1 level in the setting of normal GH levels should be followed carefully as this may be evidence of early disease [20]. There is significant interassay variability in IGF-1 quantification, and the same assay should be used over time when monitoring a given patient.

IGFBP-3 level: Studies suggest that IGFBP-3 is a sensitive marker of somatotroph function, and the levels are concordant with glucose-induced GH suppression and with IGF-I levels [21].

Random GH level: The use of random GH level to diagnose GH excess is not reliable as GH secretion is pulsatile in both normal and adenomatous pituitaries.

Oral glucose challenge test: This is considered the gold standard for the diagnosis of GH excess. This test assesses the ability of a glucose load to suppress GH levels. After administration of a 1.75 g/kg (maximum of 75 g) oral glucose load, there is an increase in insulin secretion, which leads to a decrease in IGFBP-1 levels, thereby causing an elevation in free IGF-1 levels. This should lead to suppression of GH in 30–120 min. A nadir GH level (determined by sensitive radioimmunoassay) of higher than 1 mcg/L is considered suggestive of GH excess [22]. GH suppression is affected by age, gender, BMI status, and the presence of diabetes.

Visual field testing: This is recommended in all patients where radiographic imaging suggests compression of the optic chiasm and should be periodically monitored. Occasionally impingement of the cranial nerves in the cavernous sinus can also lead to visual defects.

Radiologic investigation: Pituitary MRI is recommended to determine tumor size, location, and invasiveness. We recommend an MRI using the pituitary protocol, i.e., 2 mm slices to get a detailed look of the pituitary and for increased sensitivity in detecting microadenomas. Computed tomography is acceptable where MRI is not available. The former has the advantage of detecting calcifications in patients with a large sellar/suprasellar mass, which may represent a craniopharyngioma rather than a pituitary adenoma.

9.8.2 Evaluating Comorbidities

- Hormone evaluation for other pituitary hormones should be undertaken. Prolactin, thyroid hormone, and 8 AM cortisol levels should be checked.
- Diabetes mellitus should be ruled out.
- A sleep study must be considered, especially if there are symptoms of snoring and daytime somnolence.
- Cardiac valvular heart disease (particularly left sided), hypertrophic cardiomyopathy, and endothelial dysfunction are seen in older patients with GH excess. They are rarely seen in a young population but must be ruled out if there are any concerning signs and symptoms as this could also increase operative risk.

9.9 Treatment

The goal of the treatment is to (i) remove or shrink the tumor, (ii) normalize GH secretion and IGF-1 levels while preserving pituitary function to avoid long-term complications, and (iii) decrease the morbidity and mortality associated with GH excess. As with most pituitary tumors, younger age at presentation is associated with more aggressive tumors [23]. Giant GH-secreting pituitary tumors are invasive and need a multimodal treatment approach to control GH excess and tumor growth [24]. Surgery is the mainstay of therapy, but adjuvant medical and radiation therapy are often needed. There is debate in the literature about the relative importance of normalization of

IGF-1 versus nadir GH levels after glucose suppression in predicting better outcomes [25–27]. Of note, much of the data comes from older patients with acromegaly and should be interpreted with that in mind.

9.9.1 Medical Therapy

Medical therapy is sometimes used preoperatively to reduce IGF-1 levels and for tumor shrinkage for better surgical outcomes. It is also needed postoperatively for biochemical control if remission is not obtained with surgical and radiation therapy.

9.9.1.1 Somatostatin Analogs (SSTA)

There are two analogs available in the market – long-acting release octreotide (LAR) given intramuscularly and lanreotide depot/autogel given subcutaneously. They have greater affinity for somatostatin type 2 (sst2) and type 5 (sst5) receptors found in the pituitary gland, pancreas, and GH-secreting neoplasms of the pituitary gland and a lesser affinity for somatostatin receptors 1, 3, and 4. Both preparations are equally effective in reducing IGF-1 levels [28].

Pasireotide is another somatostatin analog with enhanced binding to more somatostatin receptors. Pasireotide binds to somatostatin receptors (sst_{1-5}), with high affinity for the sst_1, sst_2, and sst_3 subtypes and highest affinity for the sst_5 subtype. Its diverse receptor binding allows for its use in both Cushing's disease and conditions of GH excess. In vitro studies suggest similar decreases in GH levels with first-generation SSTAs and pasireotide [29].

The response to SSTA correlates with expression of sst2 receptors [30], but receptor expression is not routinely assessed clinically. Smaller tumors, lower baseline IGF-1, and densely granulated tumors which appear hypointense on T2-weighted MRI images show a better response to SSRA [31, 32]. IGF-1 normalization is achieved in both drug naive and postoperative patients in about 20–35% of patients, and about 50% reduction in tumor volume is obtained in about 59% of patients [33, 34].

9.9.1.2 Dosing and Side Effects

Lanreotide depot (starting dose of 60–120 mg every 4 weeks depending on IGF-1 level) and long-acting octreotide (10–40 mg every 4 weeks) can be given and should be slowly titrated up until a normal IGF-1 level is attained. Abdominal cramps, flatulence, and diarrhea can be severe and leading to discontinuation of treatment. Since the medications are depot preparations and these side effects can be severe, a trial of short-acting preparations is recommended to establish tolerance before starting treatment with the long-acting preparation. Long-acting pasireotide is also available and can be used at a dose of 20–60 mg every 4 weeks. Pasireotide may cause hyperglycemia in more than half of all patients [35].

9.9.1.3 Dopamine Agonists

Dopamine agonists are usually used alone (for mild IGF-1 elevations) or in conjunction with a SSTA, and the combination may be effective in lowering the IGF-1 level further [36]. The response to dopamine agonists is independent of the baseline prolactin levels or histochemical staining of the tumor for prolactin [37, 38]. The efficacy of cabergoline appears to decrease with its prolonged use [38]. The valvular side effects associated with cumulative cabergoline use do not seem to be exaggerated in patients with GH excess [39].

9.9.1.4 Growth Hormone Receptor Antagonist (Pegvisomant)

Pegvisomant is a GH receptor antagonist that blocks the peripheral production of IGF-1 in a dose-dependent manner. As pegvisomant does not inhibit GH secretion, GH levels continue to be elevated during pegvisomant administration. It may thus lead to increase in adenoma size, and initial radiological monitoring should be considered [40–42]. The combination of pegvisomant with an SSTA and dopamine agonist is more effective than either drug used alone [43]. Pegvisomant is administered as daily subcutaneous injections, which may have an impact on adherence to therapy. Common side effects include local discomfort and lipohypertrophy. Elevations in transaminases are seen in a very small subset of patients, and the medication should be withheld if the elevation is greater than three times the upper limit of the normal range [44]. Liver function tests should be checked monthly for the first 6 months after initiating pegvisomant and then every 6 months if tolerated well.

9.9.2 Surgical Therapy

This is the treatment of choice for small and large tumors that are resectable and those that pose a risk to visual loss. The cure rate depends on the size of the tumor and the expertise of the surgeon [45]. When operated in a center with high volume of surgery by an experienced pituitary surgeon, the initial remission rate for microadenomas can be more than 85% and almost 50% for macroadenomas [46]. Tumor invasion into the cavernous sinus decreases the remission rate [47]. There is an immediate lowering of GH levels with successful surgery; however the decline in IGF-1 may sometimes be delayed due to the differential half-life of its binding proteins. Hence the IGF-1 level assessed about 12 weeks after surgery may be a better reflection of surgical success [48, 49].

9.9.3 Radiation Therapy

This serves as adjuvant therapy when there is residual tumor after surgery. Radiotherapy leads to a decrease in tumor size and reduces IGF-1 concentrations in the majority of patients [50]. However, radiation effects may take a few years to manifest, and hence this is used in situations where both surgery and medical therapy are not effective in treating the condition, and not as a primary therapy [51]. Stereotactic radiotherapy aims to deliver radiation in a more precise manner and may have fewer complications [52]. The increased availability and use of proton beam radiation therapy allow for more targeted delivery of radiation to the tumor with less radiation scatter and hence fewer side effects of radiation. Patients who receive radiation should be monitored for other pituitary deficiencies, which may evolve over the next decade.

9.10 Outcomes and Possible Complications

Many of the complications associated with long-term exposure to GH excess are not witnessed by pediatric endocrinologists. However, there is increased morbidity and mortality associated with this disorder in adults.

9.10.1 Cure and Remission

Data regarding the cure rate after surgery (and not needing any medical therapy) are difficult to interpret due to the variable duration of follow-up and different biochemical cutoffs used to define cure. The early remission rate after surgery is about 80% in microadenomas and less than 50% with macroadenomas [53, 54]. About 40% of patients who achieve remission postoperatively have normal GH levels 16 years after surgery [54].

9.10.2 Mortality

Mortality with prolonged GH excess is higher than in the general population [55]. The main cause of mortality a decade ago was cardiovascular mortality; however, more neoplastic processes contributing to mortality have been reported in recent years [56, 57]. Mortality is also linked with higher GH and IGF-1 levels at the end of life [57]. With the advent of better medical therapies, the proportion of patients with biochemically controlled disease has increased leading to improved outcomes [58].

9.10.3 Panhypopituitarism

This may be seen from residual tumor compression or surgical and radiation treatment. This needs monitoring at least on an annual basis.

9.10.4 Cardiovascular

GH excess is associated with cardiomyopathy – biventricular hypertrophy, hypertension, valvulopathies, and endothelial dysfunction [59]. Hypertension is seen in almost half of the patients [60]. Reversal of the cardiac defects can be achieved with appropriate biochemical control and is dependent on patient age, disease duration, and other metabolic comorbidities [61].

9.10.5 Respiratory and Sleep Disorders

The anatomical changes in soft tissues, mucosa, lymphoid tissue, and the skeleton including tongue swelling, rib cage deformity, and changes

in lung volume and elasticity result in disordered sleep and decreased respiratory reserve. Sleep apnea may persist after recovery from GH excess, and hence polysomnography should be considered in high-risk patients with overweight and diabetes [62].

9.10.6 Glucose Metabolism

Abnormalities in glucose metabolism can range from impaired glucose tolerance to impaired fasting glucose to frank diabetes, and the prevalence is higher than that seen in general population [63]. Chronic GH excess leads to insulin resistance at the hepatic and peripheral tissue level (muscle and adipose tissue) [64]. Some of the therapies, particularly pasireotide, are known to worsen glucose tolerance. Hyperglycemia may persist even after GH excess is cured [65].

9.10.7 Osteopathy

There is increased recognition of the higher prevalence of vertebral fractures in patients with GH excess, which correlates with disease activity [66]. More research is necessary to predict fracture risk in this population, which happens in the setting of normal bone mineral density [67].

9.10.8 Neoplasia

Benign and malignant neoplasms are more common in GH excess patients compared to an age-matched population. Colonic polyps are the most common type of tumor seen with GH excess [68]. There is a fivefold increased risk of thyroid cancer [69]. The increased risk of colon, breast, and prostate cancer in acromegaly as compared to general population is currently a matter of debate [70–72]. While some groups think that acromegaly increases the risk of these neoplasms, others doubt this supposition as cancer-related mortality is lower in acromegaly compared with the general population [73, 74]. Nonetheless, until proven otherwise, systematic screening for a neoplastic disease is advised in older patients.

9.10.9 Quality of Life (QoL)

QoL is impaired in patients with GH excess despite improvement in the disease state. However, biochemical control is shown to improve QoL [75]. Other factors, including obesity, depression, fatigue, and metabolic complications should be taken into account when addressing QoL in this population.

Case Study

A 15-year-old girl presented with coarse facial features and tall stature. Review of her growth charts revealed that her height percentiles trended upward after the age of 8 years (when she was still prepubertal). She did not report any headaches, nausea, or vision complaints and did not have any obvious mucosal or skin lesions or renal calculi. She was still premenarcheal but thought that she started breast development about 5 years ago. She required CPAP at night for sleep apnea and reported decreased exercise tolerance. Her maternal grandmother died from "brain cancer" and further details could not be retrieved. There was no history of tall stature in the family. Her midparental height was 165 cm (5 ft 5 inches).

Anthropometry: Height, 191.3 cm (75.3 inches; +4.27 SD); weight, 85.3 kg (188 Lb; +2.03 SD); US/LS ratio, 1.06 (appropriate for age). Vitals were normal.

Pertinent examination findings: She was a tall girl with coarse facial features who looked very different from her mother. There was no skin or mucosal findings. She had no organomegaly or joint laxity. Visual field examination on confrontation was normal. She was Tanner stage 5 on breast examination.

Biochemical testing: TSH 1.20 uU/mL (0.40–5.00), free T4 1.0 ng/dL (0.9–1.8); FSH 2.4 IU/L, LH 0.2 IU/L, estradiol <20 pg/mL, ACTH 25 pg/mL (6–76), AM cortisol 4.2 mcg/dL, prolactin 22.3 ng/ml (0.1–23.3), IGF-1846 ng/mL (218–659 for age and Tanner stage, Z-score 3.2), HGBA1C 5.50%

An oral glucose tolerance test was performed and showed a nadir GH level of 35.9 ng/mL. She passed her cosyntropin stimulation test.

Imaging: MRI revealed a sellar/suprasellar mass lesion measuring up to 39 × 42 × 44 mm with bilateral cavernous sinus invasion. The optic chiasm was compressed and displaced along the antero-superior aspect of the mass. There was encasement of the cavernous internal carotid arteries bilaterally.

Visual field testing: She had a significant temporal defect in the left eye with good visual acuity and no afferent pupillary defect.

9.10.10 What Is the Next Step in Management?

She was started on a somatostatin analog (Somatuline) to attempt tumor shrinkage for better surgical outcome. She was closely monitored for visual deterioration, and surgical resection was performed 2 months after the start of Somatuline treatment. Her IGF-1 level continued to be elevated following surgery.

Due to her family history of "brain cancer," we sent genetic testing for *AIP* and *MENIN* genes, both of which were negative.

9.10.11 What Additional Therapy Would You Consider?

She was started on cabergoline at a dose of 0.25 mg biweekly and oral contraceptives (OCPs). She also received radiation for the residual tumor and continually elevated IGF-1 levels (in the 700 s) (normal range 218–659 ng/mL for age and Tanner stage). Her cabergoline dose was further increased to 0.5 mg every other day, and her IGF-1finally normalized.

Over the course of next 6 months, her mother reported that the patient had episodes of increased rage and crying, and she started getting in trouble with her teachers at school. She also developed extreme fatigue and sleepiness during the day and was awake at night. This was very debilitating and led to her missing a lot of school.

9.10.12 What Other Investigations or Medication Changes Would You Consider?

- Tests to rule out hypopituitarism were repeated.
  She subsequently developed central hypothyroidism and secondary adrenal insufficiency and was placed on appropriate supplementation.
- Sleep apnea and depression were ruled out.
  She had discontinued CPAP at night and was no longer snoring. She did not appear sad to her mother or her friends.
- Cabergoline dose was decreased.
  Emotional liability, anger, and sleep disturbances are occasionally seen with cabergoline particularly in this age group. Her rage episodes improved after reducing the cabergoline dose.
- She was referred to a sleep specialist who diagnosed her with narcolepsy clinically, which was confirmed by a multiple sleep latency test (MSLT).
  Her reports of episodes of sudden feeling that she could not walk anymore and had to sit down instantly were suggestive of loss of motor tone associated with narcolepsy. There are some reports of narcolepsy after radiation treatment. She was started on a stimulant, and her symptoms of fatigue improved with improved sleep.

9.11 Summary

- Tall stature is a rare presentation in the pediatric endocrinologists' practice because of the societal acceptability of tallness.
- There are many syndromes that lead to overgrowth pre- and postnatally, although familial tall stature is the most common.
- Most common cause of GH excess is pituitary over secretion of GH from a tumor or hyperplasia which can be associated with other syndromes or occur in isolation.
- Most GH-secreting tumors at a young age are macroadenomas and are difficult to cure. Surgery is the mainstay of therapy.
- Management requires a multifaceted approach of surgery, medications, and radiation therapy to keep IGF-1 levels in the normal range.
- There are numerous advances in medical treatments available to treat GH excess, and these are used in combination or as monotherapy, depending on patient tolerability.
- There is significant assay variability in IGF-1 measurements, and the same assay should thus be used in monitoring treatment in a given patient.

Review Questions

1. What is the most common cause of GH excess in adolescents?
   A. Hypothalamic hamartoma
   B. Somatotropinoma
   C. McCune-Albright syndrome
   D. Idiopathic

2. What is the most likely genetic diagnosis in the following scenario?

   An 18-month-old girl presents with accelerated height velocity of 8 cm/yr., and her current height is 4 SD above the mean for the population. Her parents report that she crossed height percentiles before she turned 6 months old. She has widely spaced teeth, and her MRI reveals a large macroadenoma and high levels of IGF-1.

   A. *GNAS* mutation
   B. Imprinting on chromosome 15p
   C. Microduplication on chromosome Xq
   D. Triple X chromosome
3. A 17-year-old male with GH excess had a transsphenoidal resection of a macroadenoma that infiltrated the cavernous sinus and is currently on lanreotide every 3 weeks and cabergoline biweekly. His IGF-1 continues to be elevated. What is the next best step?

   A. Initiate pasireotide
   B. Repeat TSS
   C. Initiate testosterone
   D. Initiate pegvisomant

Answers

1. A
2. C
3. D

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Hypothalamic and Pituitary Disorders

Diabetes Insipidus

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Key Points

- Diabetes insipidus can result either from vasopressin deficiency, usually due to inadequate secretion of vasopressin from the posterior pituitary gland (central diabetes insipidus), or from an impaired renal response to the antidiuretic effect of vasopressin (nephrogenic diabetes insipidus).
- The clinical hallmarks of diabetes insipidus are polyuria of inappropriately dilute urine and hyperosmolarity. The diagnosis of diabetes insipidus usually can be confirmed using either a water deprivation test or a saline infusion test. In some clinical settings, formal testing may not be required if a patient has hypernatremia in the presence of dilute polyuria.
- Adequate free water intake is the first line of therapy for all cases of diabetes insipidus. Vasopressin and its analog desmopressin (dDAVP) are the specific therapies for central diabetes insipidus. Treatment of nephrogenic diabetes insipidus typically depends upon reversal of the underlying cause, but pharmacological treatment may be partly successful.
- Treating infants and postoperative patients with diabetes insipidus can be particularly challenging and will require additional management strategies and special care.

10.1 Introduction

Diabetes insipidus is a syndrome of dysregulated free water balance resulting from vasopressin deficiency or insensitivity of the kidney to vasopressin action. In the absence of vasopressin-mediated urinary concentration, there is increased excretion (polyuria) of dilute urine. The loss of free water leads to increased thirst and water intake (polydipsia). If the thirst is not quenched, the progressive free water deficit leads to a hyperosmolar state characterized by plasma hypernatremia. Diabetes insipidus may be categorized as central (or neurogenic), when due to vasopressin deficiency, or nephrogenic, when the result of diminished renal responsiveness to the antidiuretic action of vasopressin. Central diabetes insipidus can be treated with vasopressin or vasopressin analogs such as desmopressin. Treatment of nephrogenic diabetes insipidus typically depends upon reversal of the underlying cause, but pharmacological treatment may be partly successful.

10.2 Normal Physiology of Water Balance

Vasopressin is the mammalian antidiuretic hormone and regulator of free water balance and plasma osmolality. Vasopressin regulates plasma sodium concentration but does not control total body sodium content and thus has little effect on total body volume. Vasopressin is synthesized in neurons of the hypothalamus and then undergoes axonal transport through the pituitary stalk to the nerve endings that form the posterior pituitary gland. Regulated vasopressin secretion from the posterior pituitary occurs in response to physiological stimuli, such as hyperosmolality and volume depletion [1]. In the kidney, circulating vasopressin can bind to V2 vasopressin receptors located on the basolateral surface of epithelial cells in the distal tubule and collecting duct of the nephron. V2 vasopressin receptor activation drives synthesis and translocation of water channels (aquaporin 2) to the luminal surface of the epithelial cells. These channels facilitate reabsorption of water from luminal fluid into the tubular cell [2]. Other water channels (aquaporin 3 and aquaporin 4) that are constitutively present in the basolateral membrane transport water from within the tubular cell to the circulation [3]. The overall effect of the tubular reabsorption of water is to concentrate the urine and conserve total body water. Plasma osmolality normally is regulated within a narrow range of approximately 280–295 mosm/kg [1, 4]. After water deprivation, increased plasma osmolality stimulates release of vasopressin from the posterior pituitary. Vasopressin-mediated urine concentration increases urine osmolality to greater than plasma osmolality, and with maximal urinary concentration, urinary osmolality can be as high as 1000 mosm/kg. If the action of circulating vasopressin is not sufficient to maintain appropriate free water balance, a further increase in plasma osmolality stimulates thirst, which is the behavioral drive for the intake of additional free water. With

sufficient free water intake, plasma osmolality is maintained in the normal range [5]. However, if thirst is impaired or water is not available, continued dehydration will result in the development of hyperosmolarity.

10.3 Clinical Presentation

The clinical hallmarks of diabetes insipidus are polyuria of inappropriately dilute urine and hyperosmolarity. Polyuria can be defined as a urine output of greater than 2 l/m^2 per day or approximately 40 ml/kg/day [6] and may be due to either a solute diuresis or water diuresis [7]. A solute diuresis can result from an excess excretion of either inorganic or organic solute. For example, after the intravenous administration of large volumes of saline, glomerular filtration of the excess sodium will produce a solute diuresis as the excess sodium is excreted in the urine. Most diuretics produce a diuresis by increasing distal delivery of isotonic tubular filtrate to increase the volume of urine output. Excess delivery of other inorganic solutes, such as ammonia or bicarbonate, also can induce a solute diuresis. Glucose will produce polyuria if plasma levels are sufficiently high (typically >180 mg/dl) so that the rate of glomerular filtration overwhelms the tubular reabsorption of glucose. Other organic solutes, such as mannitol, can be filtered but do not undergo tubular reabsorption and will produce a solute diuresis [7]. Therefore, a solute diuresis will result in copious production of urine, but the presence of solute typically produces in non-dilute urine with urine osmolality greater than or equal to plasma osmolality.

A water diuresis is characterized by production of large volume of dilute urine with an osmolality that is less than plasma osmolality and typically less than 200 mosm/kg. A water diuresis can occur in response to a large water load such as the intentional intake of excess free water (polydipsia) [7]. Primary polydipsia may be related to a pathophysiological disorder of thirst secondary to disruption of thirst regulation in the hypothalamus (dipsogenic polydipsia) [8]. More typically, primary polydipsia is a volitional act with water drunk in a volume in excess of the needs of the body to maintain a normo-osmolar state. Primary polydipsia may occur from habit or in response to social cues but when severe is usually related to a psychiatric disturbance (psychogenic polydipsia). Patients with non-dipsogenic polydipsia do not have increased thirst per se, but patients with psychogenic polydipsia have compulsive drinking that remits with resolution of psychiatric symptoms [5]. In contrast to primary polydipsia, patients with diabetes insipidus excrete dilute, hypo-osmolar urine due to impaired urinary concentrating ability, and the resulting increased thirst and secondary polydipsia are an appropriate physiological response to the loss of free water.

Hypernatremia is the most commonly measured manifestation of a hyperosmolar state. Sodium in combination with an equimolar amount of anions accounts for most of the measurable and effective osmotic load of plasma. A free water deficit that results in a hyperosmolar state will produce hypernatremia, and therefore the plasma sodium level frequently serves as a clinical surrogate for osmolality. Hypernatremia can result from either sodium excess or free water deficit [9]. Most circumstances of excess sodium intake occur in situations where the individual has little control of intake. Examples of clinical situations with sodium overload include excess administration of hypertonic intravenous fluids and excessive oral ingestion of hypertonic fluids such as seawater or hypertonic infant formula [10]. Hypernatremia more commonly is the result of a free water deficit. Water deprivation with persistent insensible losses leads to a free water deficit that will cause a progressive increase in plasma osmolality. The normal response to hyperosmolality is increased secretion of vasopressin that acts on the kidney to concentrate the urine and facilitate free water conservation. After loss of both salt and water, impaired access to water or a relatively greater loss of water can lead to hypernatremia even if total body sodium is also depleted. Thus, a diuresis can produce both hypernatremia and a decrease in blood volume. Diabetes insipidus is characterized by a defect in renal free water conservation. Patients with diabetes insipidus develop increased thirst and polydipsia to prevent development of hyperosmolality, but if free water intake is impaired, hyperosmolality and hypernatremia will develop.

Diabetes insipidus may occur acutely or may present as a more chronic condition. Non-traumatic central diabetes insipidus and most cases of nephrogenic diabetes insipidus present as chronic conditions. Less commonly,

hypothalamic or pituitary damage can lead to the acute onset of diabetes insipidus. The classic triphasic response has been described after injury to the pituitary or neurohypophysis [11]. This is of particular note when managing the postoperative care of patients after surgery of the pituitary or hypothalamus. Within the first 12–48 h after acute trauma, vasopressin secretion may be severely impaired and result in diabetes insipidus. If the damage is severe enough to produce axonal degeneration of vasopressin-secreting neurons, there will be unregulated release of vasopressin to the peripheral circulation. This can result in inappropriate antidiuresis (SIADH) and may lead to development of hyponatremia between 5 and 12 days after pituitary damage. If the trauma is so severe as to cause death of vasopressinergic neurons, then prolonged diabetes insipidus may ensue. Only some phases of this response may be clinically evident after acute damage to the pituitary or pituitary stalk, with no more than 10% of patients exhibiting all three phases [11].

The effect of diabetes insipidus on growth and development of children depends upon the age at which the disease becomes clinically apparent. With untreated diabetes insipidus, increased fluid intake will alter caloric intake. Children who drink water in preference to food or who have anorexia related to hypernatremia may show growth delay due to chronic derangement of water balance and caloric malnutrition [12]. Conversely, intake of large quantities of sweetened beverages in response to the increased thirst of diabetes insipidus can markedly increase caloric intake and lead to obesity. Nursing infants can receive both caloric and free water intake from breast milk or formula. Chronic water deprivation in infants can lead to failure to thrive, irritability, constipation, and even fever [13]. However, increased formula intake in response to increased thirst will provide calories in excess of needs and may result in the development of obesity in infants with diabetes insipidus [14].

10.4 Causes of Diabetes Insipidus

Diabetes insipidus results from an inadequate level of vasopressin or an impaired renal response to circulating vasopressin. Inadequate levels of vasopressin are nearly always associated with impaired pituitary secretion of vasopressin and can result from three main mechanisms: congenital deficiency of vasopressin, physical destruction of vasopressin-secreting neurons, or the presence of an infiltrative or inflammatory process that inhibits vasopressin synthesis, transport, or secretion. In nearly half of children presenting with pediatric central diabetes insipidus, the underlying cause may not be apparent at the time of diagnosis [15], but in most cases, it will become apparent within a few years of diagnosis [16].

Causes of Central Diabetes Insipidus

Congenital:
- Developmental defects: septo-optic dysplasia, holoprosencephaly, other midline defects
- Inherited: familial diabetes insipidus (vasopressin mutations), Wolfram (DIDMOAD) syndrome

Pituitary injury:
- Head trauma
- Suprasellar tumors: craniopharyngioma, germinoma
- Pituitary macroadenoma
- Surgery
- Vascular: cerebral aneurysm, intracranial hemorrhage, sickle cell disease

Infiltrative and inflammatory disorders:
- Langerhans histiocytosis
- Granulomatous processes: sarcoidosis, Wegener's granulomatosis, syphilis
- Neoplasm: CNS lymphoma, leukemia, metastatic carcinoma (breast)
- Infection: bacterial meningitis, tubercular meningitis, viral encephalitis
- Autoimmune hypophysitis

Vasopressin deficiency can occur with a variety of congenital disorders, such as septo-optic dysplasia and holoprosencephaly, which disrupt the normal development of the pituitary gland and other midline structures [16–18]. Diabetes insipidus is part of Wolfram's (DIDMOAD) syndrome, which is characterized by central diabetes insipidus, diabetes mellitus, optic atrophy, and sensorineural deafness resulting from mutation of the *wolframin* gene [19].

Familial diabetes insipidus is inherited as an autosomal dominant syndrome of vasopressin deficiency [20]. Infants are normal at birth, but between ages 2 and 10 years, they develop vasopressin deficiency and diabetes insipidus. The few

reported autopsies in individuals with this disorder have suggested that there may be degeneration of vasopressin-secreting neurons [21], but this has not been confirmed. More than sixty different mutations have been identified within the vasopressin pre-prohormone [22–24]. Most of these mutations are located in regions of the vasopressin precursor that encode the signal peptide or vasopressin-associated neurophysin. Vasopressin deficiency is inherited as an autosomal recessive disorder with one identified point mutation within the vasopressin peptide sequence, which results in leucine-vasopressin that has a limited ability to activate the vasopressin receptor in the kidney [25].

Destruction of the pituitary gland, pituitary stalk, or hypothalamus can cause diabetes insipidus [13, 15, 16, 26, 27]. Head trauma can cause transection of the pituitary stalk to produce diabetes insipidus. The children with severe head trauma, the incidence of central diabetes insipidus, may approach 20% [28]. However, the more common causes of pituitary destruction are tumors of the pituitary, hypothalamus, or surrounding structures. Suprasellar tumors such as craniopharyngioma and germinoma may present with diabetes insipidus. Surgical resection of pituitary or hypothalamic masses can cause temporary or permanent impairment of vasopressin secretion if there is damage to the pituitary gland or stalk. Radiation of the hypothalamus or pituitary can disrupt anterior pituitary function but only rarely has been reported to cause vasopressin deficiency.

A wide variety of infiltrative and infectious disorders have been associated with the development of central diabetes insipidus [13, 15, 26–31]. Infiltration of the pituitary stalk can disrupt transport of vasopressin to the posterior pituitary. Germinomas, sarcoidosis, and Langerhans cell histiocytosis are the most commonly reported causes of diabetes insipidus due to infiltration of the pituitary gland or stalk. Acute bacterial meningitis and chronic meningeal processes such as tuberculosis and CNS lymphoma also can lead to central diabetes insipidus. "Idiopathic" central diabetes insipidus may represent a stalk lesion that is too small to visualize on MRI. Although more common in adults, lymphocytic hypophysitis with involvement of the pituitary stalk has been reported in children [32, 33]. One report has suggested a relationship between a prior viral infection and the onset of idiopathic diabetes insipidus [15].

Diabetes insipidus occasionally may present during pregnancy, particularly in individuals with a preexisting partial defect of vasopressin secretion. Circulating peptidases, synthesized in the placenta, can participate in the degradation of vasopressin [15]. If the pituitary is unable to respond with an appropriate increase in vasopressin production and synthesis, the patient may develop diabetes insipidus. This syndrome should resolve after delivery, but occurrence of diabetes insipidus during pregnancy may be evidence of an underlying partial diabetes insipidus and indicate a need for further evaluation of water balance regulation and vasopressin action in the postpartum period [34].

When the renal response to vasopressin is impaired, an individual develops nephrogenic diabetes insipidus. Inherited defects associated with nephrogenic diabetes insipidus have been identified in the V2 vasopressin receptor and in aquaporin 2, the water channel regulated by vasopressin [35]. Most mutations associated with abnormal V2 receptor function are inherited as X-linked recessive disorders and impair the receptor response to vasopressin [36] by decreasing vasopressin binding or downstream signaling [37]. Interestingly, gain of function mutations at the same codon has been associated with the chronic nephrogenic syndrome of inappropriate antidiuretic hormone action [38]. Mutations of aquaporin 2 that are associated with nephrogenic diabetes insipidus are autosomal recessive. Most functional studies of these mutations have shown them to impair intracellular transport and subsequent vasopressin-mediated translocation of the aquaporin into the apical membrane of the renal tubular cell [39, 40]. Some of these mutations may impair the water channel function of the aquaporin [41] or prevent formation of aquaporin tetramers in the cell membrane [42].

Acquired nephrogenic diabetes insipidus typically is not as severe as inherited forms and usually is related to underlying renal tubular or interstitial damage. Medullary or interstitial damage may affect water balance, not by inhibiting vasopressin action, but by disruption of the medullary gradient, which can prevent urinary concentration greater than plasma osmolality. Thus, interstitial kidney disease can produce a relative vasopressin resistance [43]. A wide variety of agents and processes have been associated with

development of nephrogenic diabetes insipidus. Drugs inhibit vasopressin action and exert their effect through a variety of mechanisms [44]. For example, some antibiotics, including demeclocycline, blunt vasopressin response by impairing post-receptor signaling of the V2 receptor [44].

Reported Causes of Nephrogenic Diabetes Insipidus

Congenital

- Inherited: mutations in V2 receptor gene or aquaporin 2 gene
- Renal malformations: congenital hydronephrosis, polycystic kidney disease

Acquired

- Electrolyte disorders: hypokalemia, hypercalcemia
- Renal diseases: obstructive uropathy, chronic pyelonephritis, polycystic kidney disease
- Systemic diseases: sickle cell disease, amyloidosis, multiple myeloma, sarcoidosis

Drugs

- Lithium salts
- Methoxyflurane
- Alcohol
- Demeclocycline and other tetracyclines
- Anti-infection agents: foscarnet, amphotericin, methicillin, gentamicin
- Antineoplastic agents: cyclophosphamide, platinum-based agents, isophosphamide, vinblastine
- Hypoglycemic agents: acetohexamide, glyburide, tolazamide
- Others: phenytoin, colchicine, barbiturates

Nearly half of all cases of drug-induced nephrogenic diabetes insipidus are related to the long-term use of lithium salts [45], which may inhibit post-receptor activation and thereby decrease transcription of aquaporin mRNA, aquaporin synthesis, and translocation of aquaporin into the apical membrane of tubular cells. In individuals receiving chronic lithium therapy, the reported prevalence of lithium-induced diabetes insipidus varies between 20% and 70% and may depend upon the dose and duration of therapy. The differentiation of acute and chronic lithium injury remains unclear. Short-term exposure to lithium may impair urine concentrating ability in more than one-half of individuals. With discontinuation of lithium, renal function returns to normal. However, with prolonged exposure to lithium, irreversible changes occur with permanent renal tubule insensitivity to vasopressin and resulting impairment of urine concentration and free water preservation [46].

10.5 Diagnosis

The hallmarks of diabetes insipidus, polyuria, and hyperosmolality can present with varying degrees of severity, and each can be caused by a wide variety of other conditions. Thus, the diagnosis of diabetes insipidus requires sufficient evaluation to characterize the polyuria and hyperosmolarity and to exclude other conditions that could present with similar findings. It is important to confirm the diagnosis of diabetes insipidus before pursuing an extensive evaluation to determine the etiology or initiating therapy in an individual patient.

The diagnosis of diabetes insipidus depends upon confirmation of disrupted free water balance by characterizing the polyuria and the potential hyperosmolar state. Other causes of polyuria, such as primary polydipsia or an osmotic diuresis, must be excluded by clinical evaluation and laboratory analysis. For example, the hyperglycemia of diabetes mellitus can produce polyuria and eventually result in hypernatremia. An individual with polydipsia and plasma sodium level that is low or low normal (and not near the upper range of normal) more likely has primary polydipsia and does not have diabetes insipidus. Conversely, in an individual with diabetes insipidus, polydipsia is driven by the free water deficit and resulting hyperosmolality, and it is unlikely that plasma sodium levels will be low.

The manner of diagnosing diabetes insipidus depends upon the presentation and clinical setting. A patient that slowly develops diabetes insipidus as an outpatient may be able to sufficiently increase oral intake of free water to maintain a normal plasma osmolality. This individual may present with complaints of excessive thirst and frequent urination. One clinical clue that these symptoms are not due to primary polydipsia may be bed-wetting or frequent nocturia with high levels of urine output occurring during periods of decreased water intake. Patients with well-compensated diabetes insipidus are at risk for decompensation if they develop an acute medical illness or are otherwise limited in free water

intake. In the same way, an individual developing acute diabetes insipidus after pituitary surgery or head trauma may not be able to respond to the need for increased free water intake and quickly will develop a hyperosmolar state.

The diagnosis of diabetes insipidus can be confirmed by observing the response to water deprivation. The normal response to a free water deficit and mild increase in plasma osmolality is increased vasopressin secretion, which acts on the renal tubules to conserve free water and maintain plasma osmolality in the normal range. By contrast, in an individual with diabetes insipidus, impaired free water conservation is accompanied by persistent excretion of an inappropriate volume of dilute urine. In the absence of increased water intake, this leads to a free water deficit and the development of hypernatremia.

Diagnostic Testing for Diabetes Insipidus (Summary)

1. Basal testing:
    1. Diabetes insipidus unlikely:
        - Serum osmolality <270 mosm/kg
        - Urine osmolality >600 mosm/kg
        - Urine output <1 L/m^2
    2. Diabetes insipidus likely:
        - Serum osmolality >300 mosm/kg (or serum sodium >150 meq/L)
        - Urine osmolality <300 mosm/kg
2. Water deprivation study:
    1. Water deprivation stage:
        1. Precede by overnight fast (if will be tolerated and if indicated by clinical circumstances)
        2. Continue complete water deprivation until:
            1. Loss of >5% of basal body weight
            2. Plasma osmolality >300 mosm/kg
            3. Urine osmolality >600 mosm/kg
        3. Also discontinue if signs of hemodynamic compromise (heart rate, blood rate)
    2. Vasopressin administration stage
        1. Parenteral administration of vasopressin or dDAVP
            1. Vasopressin (Pitressin) 1 mcg/m^2
            2. Desmopressin (dDAVP) 0.1 mcg/kg (max 4 mcg)
        2. Differential response to vasopressin
            1. Central diabetes insipidus:
                - Decrease in hourly urine output
                - Urine osmolality increases by 50%
            2. Nephrogenic diabetes insipidus:
                - No decrease in urine output
                - No increase in urine osmolality
3. Saline infusion test
    1. Infusion
        1. Consider prior water load to suppress vasopressin secretion
        2. 3% saline at 0.1 ml/kg/h for up to 3 h or until plasma osmolality >300 mosm/kg
    2. Response
        1. Urine output decreases, and urine osmolality increases when plasma osmolality reaches vasopressin secretory threshold
        2. Analyze relationship between plasma osmolality, urine osmolality, and plasma/urine vasopressin levels using appropriate nomograms [4, 5, 50, 52]

The possibility of diabetes insipidus may be raised if a patient has a marked polyuria after head trauma or a surgical procedure in which the pituitary could be damaged. If access to ad-lib water intake is limited, excretion of inappropriately dilute urine (urine osmolality less than plasma osmolality) will lead to continued free water loss and development of hypernatremia. Careful assessment of documented fluid balance (I + O's) in the operating room and postoperative period and measurement of plasma and urine concentration will help in determining if persistent polyuria is driven by prior fluid overload or is due to the development of diabetes insipidus. Development of hypernatremia with inappropriately dilute urine should be confirmed with laboratory measurement of plasma and urine osmolality. In the absence of hypernatremia, postoperative polyuria is more likely to represent a diuresis in response to intravenous fluid administered during or after surgery. Appropriate management of individuals with postoperative diuresis and possible diabetes insipidus should include serial measurement of plasma sodium and urine-specific gravity every few hours until the diuresis resolves.

In an individual with a likely cause for diabetes insipidus and acute development of dilute polyuria and hypernatremia, a clinical diagnosis of diabetes insipidus may be made. If this patient has hypernatremia in the presence of dilute urine, then a formal water deprivation may not be required for the diagnosis of diabetes insipidus. In subjects with a clinical diagnosis of acute central diabetes insipidus, a therapeutic trial of desmopressin may be an appropriate diagnostic maneuver. However, pitfalls to this approach include the

presence of a concurrent cause of polyuria and hypernatremia. For example, an osmotic diuresis following administration of mannitol during a neurosurgical procedure may produce polyuria and possibly mild hypernatremia if water access if impaired. Other medical problems may obscure the diagnosis of diabetes insipidus. For example, in patients with severe hypothalamic or pituitary destruction, centrally mediated cortisol or thyroid hormone deficiency may impair free water clearance [11].

In individuals where the diagnosis of diabetes insipidus is not well documented, a formal diagnostic test must be performed. One of the most common tests to confirm the diagnosis of diabetes insipidus is the water deprivation test [6, 13, 29, 47]. The water deprivation test should be performed in a clinical setting that provides adequate monitoring of the patient. This is particularly important when studying young children. The water deprivation test is rarely appropriate for the evaluation of infants. As outlined in the Diagnostic Testing For Diabetes Insipidus Summary above, the goal of the water deprivation test is to deprive the individual of sufficient free water so that vasopressin, if present, will be released and act on the kidney to promote urinary concentration. In the absence of vasopressin, free water deprivation will permit continued excretion of dilute urine, leading to a free water deficit and development of hyperosmolality. Subjects can be prepared for the formal water deprivation test by an overnight fast of food and water. It is important to ensure that the duration of overnight avoidance of food and fluid does not exceed the maximum duration the child can normally go without fluid intake. This decreases the osmotic load to the kidneys and begins the process of water deprivation. However, depending upon the clinical circumstances and age, some patients may require close observation during the entire period of deprivation. Up to 14 h of water deprivation may be required to complete an informative study in a patient with mild symptoms [13].

Once the diagnosis of diabetes insipidus is confirmed, the response to administration of vasopressin (or preferably, a vasopressin analog such as desmopressin) can demonstrate whether the diabetes insipidus is due to vasopressin deficiency or an impaired renal response to vasopressin [6, 13, 29, 47]. After vasopressin administration, patients with complete central diabetes insipidus typically have a greater than 50% increase in urinary osmolality. However, a urine osmolality greater than 600 mosm/kg is also an appropriate response and may be seen in cases of partial diabetes insipidus. Individuals with primary polydipsia should retain the ability to concentrate urine to greater than 600 mosm/kg and may demonstrate little additional response after desmopressin administration. Typically, when vasopressin or desmopressin is administered to patients with nephrogenic diabetes insipidus, the urine osmolality will not increase greater than 400 mosm/kg and usually remains less than plasma osmolality [29].

In some cases, the results of the formal water deprivation test may be inconclusive [5, 47]. With a partial central deficiency of vasopressin, there may be some measurable response to water deprivation, but urinary concentration may not be normal. In cases of long-standing central diabetes insipidus, the response to exogenous vasopressin administration may be impaired due to washout of the renal medullary gradient. Patients without diabetes insipidus, including those with primary polydipsia, should maximally concentrate urine with adequate water deprivation and thus may not have a significant additional response to exogenous vasopressin. Therefore, endpoints need to be set for concluding a water deprivation study [6, 13, 29, 47]. There are three: (1) persistent inappropriately low urinary osmolality despite a 3% loss of body weight, (2) hyperosmolarity and hypernatremia with an inappropriately low urinary osmolality, or (3) appropriate urinary concentration (greater than 600 mosm/kg). Urine osmolality may appear to plateau at a submaximal concentration (<600 mosm/kg) without development of plasma hyperosmolarity. However, if the patient has not shown signs of volume deficiency, then the water deprivation should be continued to determine if further concentration of urine to greater than 600 mosm/kg can be achieved. A common error that leads to an inconclusive test is ending the test after the patient has lost a certain percentage of body weight without regard for the clinical circumstances. In patients who are fluid replete or overloaded before the test (as in patients with primary polydipsia), the serum osmolality may not have risen enough to reach the threshold for vasopressin secretion at the end of the test. It is thus useful to also use increase in heart rate and other clinical criteria to assess the fluid status to

decide when to end the test. In some cases, it may be appropriate to use a therapeutic trial of desmopressin for a week. If the patient responds to therapy, this may confirm the diagnosis of central diabetes insipidus. Alternatively, if further diagnostic testing is desired, the week of antidiuretic therapy should facilitate recovery of the concentrating gradient in the kidney, which can normalize the response to a test dose of desmopressin.

Other diagnostic tests may be needed to confirm the diagnosis of diabetes insipidus. Typically, urine or plasma vasopressin levels are not readily available. They usually are not required for the diagnosis of diabetes insipidus, but, in selected clinical circumstances, a vasopressin level may be helpful [5, 47, 48]. A plasma vasopressin level assayed after water deprivation can distinguish between central and nephrogenic diabetes insipidus [48], particularly in cases where there is only a partial defect in vasopressin secretion or action [5, 47]. To be most informative, plasma vasopressin must be evaluated as a function of plasma osmolality [47]. Vasopressin levels can be increased by hypotension, smoking, and nausea, and these stimuli should be avoided during testing for diabetes insipidus [15, 47]. Concurrent plasma osmolality and vasopressin levels obtained during a saline infusion also may help identify a partial defect in vasopressin secretion or may be useful when trying to study a patient that has a high likelihood of both central and nephrogenic diabetes insipidus.

In some patients, the water deprivation cannot be performed because of hemodynamic instability or difficulty in getting cooperation with water deprivation [49]. For example, infants may not tolerate an extended fast. In these circumstances, the saline infusion test can be used to evaluate for possible diabetes insipidus [5, 47, 48]. A solution of 3% sodium chloride infused over 2–3 h at a dose of 0.1 cm^3/kg/h will provide a hyperosmolar stimulus to vasopressin secretion [5, 49]. When the plasma osmolality threshold for vasopressin secretion is reached, plasma and urinary vasopressin levels will increase, and urinary osmolality will increase abruptly in response to increased vasopressin action on the kidney. Some authors suggest a water load (20 ml/kg of 5% dextrose intravenous over 2 h) prior to the saline infusion to ensure that vasopressin levels are suppressed at the beginning of the saline infusion test [49]. Comparison of plasma vasopressin levels with corresponding plasma osmolality measurements can be used to determine if there is an appropriate relationship in the regulation of vasopressin secretion [5, 29, 49]. This test also is useful in identifying patients with normal vasopressin secretory ability, but an altered osmotic threshold for the release of vasopressin [29].

Interpretation of the saline infusion test may be confounded by a number of issues. The vasopressin peptide is highly labile and can degrade if the blood sample is not collected, processed, and stored correctly. Blood samples should be kept on ice and carefully processed immediately after the blood is obtained, and the plasma kept frozen until assayed [50]. Vasopressin levels rarely are assayed in hospital labs, and, thus, the samples must be sent to a reference laboratory, with a resulting increase turnaround time for receiving test results. Clinical laboratories typically do not measure plasma osmolality with high precision, and this may further complicate the interpretation of the saline infusion test [5].

10.6 Determining the Etiology of Diabetes Insipidus

Once the diagnosis of diabetes insipidus is made, then efforts can be made to further identify the underlying cause if it is not clear from the clinical presentation. Patients with central diabetes insipidus should undergo imaging of the pituitary and hypothalamus. Unless a large intracranial mass is suspected, computed tomography (CT) is of little use in determining the cause of diabetes insipidus. Magnetic resonance imaging (MRI) allows a more detailed study of the neurohypophysis, including the pituitary and the pituitary stalk [51]. An anterior pituitary microadenoma will not cause diabetes insipidus. Early studies demonstrated a relationship between intact neurohypophyseal function and posterior pituitary hyperintensity on T1-weighted MRI [52, 53]. Loss of this pituitary "bright spot" suggested loss of vasopressin-secreting neurons or deficient vasopressin production and became to be considered diagnostic of diabetes insipidus [15]. Typically, the posterior pituitary bright spot is diminished or absent in both forms of diabetes insipidus, presumably because of decreased vasopressin synthesis in central disease and as a result of increased vasopressin release in nephrogenic

disease [53–55]. However, loss of the pituitary bright spot is not a specific finding of diabetes insipidus, as a bright spot may not be seen in up to one-fifth of normal individuals [51] and may persist even in cases of central diabetes insipidus [56, 57].

Careful attention to the pituitary stalk may reveal a lesion disrupting vasopressin transport and secretion [57]. Further evaluation of such a lesion will depend upon the clinical history of the patient. A previous diagnosis of a process, such as sarcoidosis that can cause pituitary stalk infiltration, may indicate that watchful observation, while treating the underlying process, is appropriate. Other tests may be needed to identify a systemic illness that may explain the infiltrative process. In rare circumstances, biopsy of the stalk lesion may be needed to rule out a diagnosis such as central nervous system lymphoma. However, this step should be undertaken with due consideration and guidance from experienced endocrinology and neurosurgery consultants, as the biopsy is likely to cause permanent damage to the pituitary stalk. If no lesion can be identified, then other causes, such as an inherited disorder or hypophysitis, should be considered. If no cause for diabetes insipidus can be identified, then the patient should be followed and reassessed regularly. For example, germinomas may disrupt pituitary function and cause diabetes insipidus many years before they are apparent on MRI of the pituitary [27, 30]. Follow-up should include periodic imaging for evidence of a growing mass and repeat assessment of anterior pituitary function, as stalk lesions also may disrupt anterior pituitary function [15, 29].

Patients with nephrogenic diabetes insipidus should be evaluated to exclude an electrolyte disorder, such as hypercalcemia or hypokalemia, which may contribute to renal insensitivity to vasopressin. Even in the absence of mechanical urinary outlet obstruction, diagnostic imaging such as CT or ultrasound may reveal hydronephrosis as a result of the high flow of urine in the ureters. This seems to be more common in children and may represent functional urinary obstruction as result of the high urinary flow rate compared to the relative size of the urinary outflow system [58]. Treatment of the diabetes insipidus should help reverse this hydronephrosis.

With a family history of diabetes insipidus, genetic studies may be appropriate to confirm the cause of diabetes insipidus in an individual patient (22–24). Genetic studies also should be performed in an individual in whom there is no other apparent mechanism to cause diabetes insipidus. Identification of a genetic cause will eliminate the need for more invasive diagnostic evaluation and may be important if symptoms of diabetes insipidus appear in other family members.

10.7 Treatment and Management of Central Diabetes Insipidus

Adequate free water intake is the first line of therapy for all cases of diabetes insipidus. Patients with an intact thirst mechanism will appropriately regulate plasma osmolality if allowed free access to water. If the patient is unable to drink by mouth, then intravenous administration of free water in the form of hypotonic fluids should be used to prevent development of a hyperosmolar state. If the patient has severe hypernatremia, intravenous administration of hypotonic fluid should be used to replenish the free water deficit.

Vasopressin and analogs such as desmopressin are the specific therapy for central diabetes insipidus [59] (Table 10.1). Because vasopressin must be administered parenterally and has a relatively short half-life, it is not an ideal drug for long-term treatment of diabetes insipidus. However, these same characteristics occasionally make it useful for short-term treatment of acute onset diabetes insipidus and for use in diagnostic testing.

The synthetic vasopressin analogue desmopressin (dDAVP) is now the standard therapy for central diabetes insipidus [60, 61]. Desmopressin has two molecular alterations compared to native vasopressin: deamidation of the amino terminal cysteine and replacement of arginine-8 with d-arginine. These two alterations result in a compound with a prolonged half-life of antidiuretic activity and elimination of the pressor activity characteristic of native vasopressin. Desmopressin can be administered parenterally but also can be given by the nasal or oral route. Because of diminished delivery through the nasal or gastric mucosa and proteolysis by mucosal and gastric enzymes, these non-parenteral routes require higher doses of desmopressin than required with intravenous or subcutaneous administration (Table 10.1).

Table 10.1 Vasopressin therapy for the treatment of central diabetes insipidus

Drug	Route	Conc.	Adult dose	Duration
Synthetic vasopressin (Pitressin)	IM/SQ	20 U/ml	2–10 U	2–8 h
	IV	20 U/ml	0.25–3 U/kg/h	2–8 h
Desmopressin acetate (dDAVP)	IM/SQ	4 mcg/ml	1–4 mcg/day (divided doses) 0.02–0.1 mcg/kg/dose in young children	6–12 h
	Rhinal tube	100 mcg/ml	5–40 mcg/day	12–24 h
	Nasal spray	100 mcg/ml	10–40 mcg/day (10 mcg/spray)	12–24 h
	Oral	100 mcg/tab	100–800 mcg/day (50–300 mcg BID/TID)	8–12 h
	Sublingual	60, 120, 240 mcg/tab	120–720 mcg/day (60–120 mcg TID)	8–12 h

Nasal administration of desmopressin can be accomplished using a rhinal tube or nasal spray. To use the rhinal tube, the patient draws the dose of desmopressin into the flexible plastic rhinal tube, places one end of the tube into the nose, and uses the mouth to blow through the tube to puff the medicine into the nose. Use of the rhinal tube requires that the patient has the dexterity and understanding to follow this technique, although parents can assist children with tube placement and providing the puff of air. Some authors suggest diluting the desmopressin 1:10 with saline to facilitate administration of small doses by rhinal tube [26, 31]. Nasal administration of desmopressin also can be performed with a spray pump that administers a premeasured dose of 10 mcg desmopressin per spray. However, utility of this form of nasal desmopressin can be limited in pediatrics, as the fixed dose of the spray precludes small adjustments of dose, and children may require doses smaller than 10 mcg. Nasal absorption of desmopressin can be affected by upper respiratory congestion. It is, however, useful as an alternative to oral desmopressin in patients with diabetes insipidus who have nausea and vomiting due to illnesses such as gastroenteritis or in those patients who are unable to take fluids/food orally before anesthesia for a minor procedure.

Most patients with chronic central diabetes insipidus are treated with an oral formulation of desmopressin. As most of an orally administered desmopressin dose is degraded before it can be absorbed, the oral dose is 10–20-fold greater than an equivalent nasal dose. Because of variation among individuals in the duration of action of desmopressin, the appropriate dose and frequency of administration must be determined for each individual patient. Although some patients may require only one dose per day, most find management of polyuria and polydipsia easier when taking oral desmopressin two to three times a day. When initiating desmopressin therapy, it may be useful to start with one bedtime dose and then titrate the size and frequency of dosage based on the patient's response to therapy.

Administration of vasopressin or desmopressin requires careful attention to free water intake to prevent the development of hyponatremia. Oral intake of fluids may be driven by stimuli other than thirst, such as social cues and habitual drinking ingrained during a period of untreated diabetes insipidus. Because of this it is important to avoid excess restriction in urine output [31]. Providing a daily period of "breakthrough" with mild polyuria as the effect of the exogenous vasopressin decreases may be a convenient way to ensure that there is no excessive antidiuresis with an accumulation of excess free water and progressive development of hyponatremia [59].

In patients treated with desmopressin, oral intake of fluids must be driven by and regulated by thirst. Management of diabetes insipidus in a patient with an impaired thirst mechanism requires special attention to fluid balance. Daily measurement of intake and output as well as body weight may be needed to maintain fluid balance. Frequent monitoring of plasma sodium levels should be used to provide early identification of problems with water balance. Thus, management of diabetes insipidus in these individuals requires special vigilance by the patient, family, and physician.

10.8 Postoperative Management of Diabetes Insipidus

Perioperative management of diabetes insipidus requires careful attention to fluid balance as assessed by intake and output, daily weight, and laboratory tests such as serum sodium and urine osmolality [11, 59]. Careful measurement of urine volume and concentration may be facilitated by continuing the use of an indwelling urinary catheter for the first 1–2 days after major surgery. In patients with preexisting diabetes insipidus, continuing desmopressin therapy will help in the maintenance of water balance. Care must be coordinated with other members of the healthcare team to ensure that fluid balance is carefully managed to prevent hyponatremia due to excess intravenous fluid administration.

Many approaches have been suggested for the management of acute postoperative diabetes insipidus. The first line of treatment remains adequate free water administration to prevent hyponatremia. Some clinicians prefer to use only fluids, while others initiate desmopressin therapy to help fluid balance and to improve patient comfort by decreasing thirst and decreasing the need to void. After transsphenoidal pituitary surgery, parenteral administration will need to be used because of the difficulty of nasal administration of desmopressin. Parenterally administered desmopressin also is preferred as it has a shorter duration of action and decreases the chance of hyponatremia developing in response to excess fluid intake. An intravenous infusion of vasopressin at a low dose (0.08–1.10 mU/kg) can be used in the immediate perioperative period or during other procedures, such as administration of chemotherapy, which have the potential to disrupt free water balance [62].

Once a postoperative patient is taking oral fluids, fluid balance may be regulated by thirst. Depending upon the likely extent of pituitary and hypothalamic damage, the clinician must be sure that thirst is intact and that the patient is not responding to other cues, such as mouth dryness. Acute pituitary damage is likely to be accompanied by some or all of the classic triphasic response [11], and patients can be at risk for development of severe hyponatremia if desmopressin is continued into the period of SIADH or if a patient drinks to excess during therapy. Therefore, the decision as to whether to use desmopressin in the immediate postoperative period may depend upon the clinician's assessment as to the severity of polyuria, the likelihood that the patient will have permanent diabetes insipidus, the presence or the absence of an intact thirst drive, other medical conditions that may be affected by hypernatremia (or hyponatremia), and patient comfort and convenience. Although symptoms may resolve, there still should be close monitoring of urine output volume, urinary osmolality (or specific gravity, which can be performed at the bedside), and plasma sodium levels to ensure that there is adequate, but not excessive, therapy. When patients are receiving intermittent desmopressin therapy, the onset of polyuria of dilute urine indicates the need for the next dose of desmopressin. Each subsequent dose of desmopressin should not be delayed until the patient again develops hypernatremia. However, patients should not be treated on an arbitrary fixed schedule, as breakthrough prevents the development of hyponatremia that may result with accumulation of excess free water [59].

10.9 Diabetes Insipidus in Infants

Infants are a special situation in the management of diabetes insipidus, as fluid intake is linked to caloric intake and usually is regulated by parents or other caregivers. In infants, an obligatory high oral fluid requirement combined with vasopressin treatment can cause free water accumulation and hyponatremia. For this reason, infants with central diabetes insipidus are often managed with fluid therapy alone. The use of breast milk or a low-solute formula (e.g., Similac 60/40) provides a lower renal solute load, which can reduce the urine volume by 20–30%. Some infants may benefit from the addition of a thiazide diuretic that can increase urine osmolality and decrease urine output [18]. Chlorothiazide is available as an oral suspension and can be given to infants with central and nephrogenic diabetes insipidus at a dose of 5–10 mg/kg per day, usually divided into doses administered two or three times a day. As an alternative, hydrochlorothiazide (starting dose of 1–2 mg/kg/day) may be more widely available but will require a pharmacy to compound as an oral suspension. The combination of low solute formula and a thiazide diuretic can

significantly reduce the amount of free water supplementation needed in infants with either form of diabetes insipidus to about 20–30 ml for every 120–160 ml of formula [63]. Serum calcium should be monitoring in infants being treated with a thiazide diuretic [18]. In some circumstances, infants with central diabetes insipidus can be treated successfully with once-daily subcutaneous injections of desmopressin (initial dose 0.002–0.1 pg/kg once daily; the dose can be increased and given twice daily if necessary) until they have transitioned to solid food. Desmopressin therapy in infants through the subcutaneous route has been associated with far fewer episodes of hyponatremia and hypernatremia than the intranasal and the oral routes [64]. In general, however, many infants with central diabetes insipidus can be treated successfully with a combination of low-solute formula (or breast milk) and sufficient free water to maintain a normo-osmolar state. This can be accomplished by careful attention to intake and output and calculation of the volume of formula needed to meet the infant's caloric needs. If an infant is breastfeeding, it may be easier to have the mother use a breast pump so that the volume of milk can be measured accurately. Additional free water then is given to maintain water balance and normal plasma osmolality.

10.10 Management of Nephrogenic Diabetes Insipidus

Treatment of nephrogenic diabetes insipidus can be a challenging endeavor. Discontinuing or using a decreased dose of the causative drug may lead to remission of the diabetes insipidus. However, this must be done in consultation with appropriate specialists who can help in management of the underlying disease and in identification of other agents that may be used without the development of diabetes insipidus. For example, use of other neuropsychiatric agents, such as valproic acid or carbamazepine, may permit a dose reduction or discontinuation of lithium. However, some clinical circumstances require continuation of the causative agent and subsequent management of the resulting diabetes insipidus.

Decreasing the solute load to the kidney with a low-salt and low-protein diet will decrease the total urine volume and limit the degree of polyuria associated with nephrogenic diabetes insipidus. Some patients with partial nephrogenic diabetes insipidus may respond to high doses of desmopressin [5]. A variety of agents, including nonsteroidal anti-inflammatory agents and diuretics, have been reported to improve the symptoms of diabetes insipidus. Indomethacin can decrease polyuria and polydipsia, while other agents such as ibuprofen appear to be much less effective [65]. Diuretics, such as hydrochlorothiazide and amiloride (Midamor), probably ameliorate diabetes insipidus by producing mild chronic volume depletion. This leads to increased volume reabsorption in the proximal tubule of the kidney, which results in decreased distal delivery of filtrate and an overall decrease in urine volume. Combined therapy with hydrochlorothiazide and amiloride has been reported to be successful [66]. In patients with lithium-induced diabetes insipidus, amiloride may decrease entry of lithium into tubular cells and thereby decrease the effect of lithium on vasopressin action. In some patients, amiloride therapy will provide complete resolution of lithium-induced nephrogenic diabetes insipidus [46]. Amiloride may not be available in many community pharmacies but should be available from a hospital pharmacy. Individuals with nephrogenic diabetes insipidus can be managed with a combination of hydrochlorothiazide, a nonsteroidal agent such as indomethacin, and high-dose desmopressin, but most patients have only partial remission of symptoms and require increased free water replacement to maintain normo-osmolality.

The use of diuretics for the treatment of diabetes insipidus is not risk-free. The persistent decrease in extracellular volume caused by diuretic therapy puts the patient at risk of hypovolemia and severe dehydration, particularly during an episode of febrile illness or water deprivation. Thiazide diuretics may cause hypokalemia, which can further impair renal responsiveness to vasopressin. Subjects with concurrent lithium-induced diabetes insipidus and hyperparathyroidism are particularly susceptible to water deprivation, as dehydration can precipitate hypercalcemia and, in turn, hypercalcemia can further exacerbate the diabetes insipidus. In patients treated with diuretics for lithium-induced diabetes insipidus, the resulting volume depletion and the effects on

tubular function can decrease lithium clearance and may lead to increased plasma lithium levels.

Management of possible drug-induced nephrogenic diabetes insipidus should begin prior to the development of symptoms such as polyuria and polydipsia. When teenagers and young adults start lithium therapy, they should be informed of the possible development of diabetes insipidus and instructed to monitor urine volume. Progressive development of polyuria may be one indication to reevaluate the need for chronic lithium therapy and reconsideration of substituting other therapies for lithium.

10.11 Lifelong Management of Diabetes Insipidus

With attention to water balance and appropriate therapy, diabetes insipidus can be well controlled and have minimal impact on quality of life. Treatment of diabetes insipidus decreases sleep disruption and facilitates full participation in school and daily activities. Treatment of diabetes insipidus has been reported to improve school performance and behavior and to allow normal growth [7, 12]. Thus, with appropriate treatment, diabetes insipidus should not impair physical or mental development [67]. Patients and families should understand that even short periods of noncompliance with therapy could lead to serious medical complications. However, intercurrent illness or stress can disrupt management of diabetes insipidus even in a well-controlled patient. Patients and caregivers should be instructed to closely follow water intake and urine output and to obtain daily weights during febrile or gastrointestinal illness. Evaluation of any change in mental status should include measurement of serum sodium to exclude hypernatremia due to exacerbation of the diabetes insipidus or hyponatremia secondary to water intoxication. In emergency situations, if a patient or family is unable to communicate the history of diabetes insipidus, severe derangement in water balance could occur before the diagnosis of diabetes insipidus is recognized by emergency personnel or health providers unfamiliar with the patient. Thus, patients should be encouraged to wear a medical alert bracelet or other form of identification that provides a clear indication that they have diabetes insipidus.

10.12 Summary

Diabetes insipidus is a syndrome of dysregulated free water balance, and its clinical hallmarks are polyuria of inappropriately dilute urine and hyperosmolarity. Making an accurate diagnosis of diabetes insipidus requires a thorough understanding of the physiologic mechanisms that regulate free water balance. Once the diagnosis of either central diabetes insipidus or nephrogenic diabetes insipidus is made, most patients will require further evaluation to determine the etiology of the disease. Adequate free water intake is the first line of therapy for all cases of diabetes insipidus. Vasopressin and analogs such as desmopressin are the specific therapy for central diabetes insipidus. The treatment of nephrogenic diabetes insipidus typically depends upon reversal of the underlying cause, although pharmacological treatment may be partly successful. Treating infants and postoperative patients with diabetes insipidus can be particularly challenging and will require additional management strategies and special care.

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Management of Acute and Late Endocrine Effects Following Childhood Cancer Treatment

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Key Points

- As survival rates increase for pediatric cancer patients, the number of childhood cancer survivors (CCS) who suffer from a chronic health condition, including endocrine abnormalities, also increases.
- The type and degree of endocrine abnormality vary widely by cancer type, treatment, age of onset, type of therapy, and gender but can involve any endocrine pathway.
- There are established guidelines that describe surveillance and screening for endocrine abnormalities in CCS.

11.1 Introduction

Recent advances in the treatment of pediatric cancers have resulted in increasing numbers of children surviving their malignancy, with current survival rates greater than 80%. There are approximately 375,000 adult survivors of childhood cancer (1 in 530 adults aged 20–39 years) in the United States [1]. Therapeutic options consist of a combination of multi-agent chemotherapy, surgery, radiotherapy, and bone marrow or stem cell transplantation. Unfortunately, decreasing mortality from malignancy comes at the cost of increased morbidity resulting from acute and late effects of treatment. The St. Jude Lifetime Cohort Study reported that by 45 years of age, 95.5% of CSS will suffer from at least one chronic health condition [2]. Another study found that 40% of CCS developed at least one chronic health condition within 15 years of cancer diagnosis [3]. Additionally, endocrine disorders affect over 50% of CCS following chemotherapy and radiotherapy [4–6]. Degree of risk is related to age at cancer diagnosis, cancer type, and therapeutic interventions and may occur soon after treatment or may not develop for many years after cure. Therefore, lifelong follow-up of survivors, which includes screening for endocrine dysfunction, is recommended to ensure early diagnosis, timely institution of appropriate treatment, and counseling when needed. This chapter will describe the acute and late endocrine effects of treatment for childhood cancer by endocrine system and chronology of onset, with a discussion of the pathophysiology, diagnosis, and treatment for each system involved.

11.2 Acute Effects of Treatment

11.2.1 Preoperative Considerations

The first step in treating pediatric brain and spinal cord tumors is surgery. Depending on the severity of patient presentation, emergent surgical intervention may be needed. Tumors of the hypothalamic region or pituitary gland, such as craniopharyngiomas or germinomas, may present with diabetes insipidus (DI) or anterior pituitary dysfunction prior to surgery. Tumors in the regions of the hypothalamus and pituitary gland have been associated with adrenocorticotropic hormone (ACTH) insufficiency in approximately 30% of patients at diagnosis [7–9]. Coexisting adrenal insufficiency may mask the symptoms of DI due to impaired free water clearance. In these patients, upon institution of glucocorticoid replacement, polyuria may develop and unmask the diagnosis of DI. Therefore, pre- and postoperative assessment of the CRH-ACTH-cortisol axis (HPA) is imperative. Occult ACTH deficiency may result in adrenal crisis during the perioperative or postoperative period. If preoperative testing is unfeasible, empiric coverage with stress-dose glucocorticoids should be provided for safety. Steroids in doses that exceed stress-dose glucocorticoid-equivalent doses may be used by neurosurgeons during surgery to decrease brain swelling. Coordination of care regarding steroid use in the intra- and postoperative period is needed. Stimulation testing will identify patients who will require stress-dose steroids for future surgical procedures and diagnostic imaging that requires general anesthesia. Of note, in contrast to patients with primary adrenal insufficiency, brain tumor survivors still have an intact angiotensin-aldosterone pathway; therefore, replacement with mineralocorticoid is not needed.

11.2.2 Diabetes Insipidus (DI), Syndrome of Inappropriate Antidiuretic Hormone Secretion (SIADH), and Cerebral Salt Wasting (CSW)

Antidiuretic hormone (ADH) is synthesized in the magnocellular neurons of the hypothalamic paraventricular and supraoptic nuclei and then transported via axons to the posterior pituitary for storage pending release into circulation. DI may be a presenting symptom of Langerhans cell histiocytosis or germinoma [10]. Additionally, intraoperative damage to ADH neurons during hypothalamic-pituitary surgery is one of the most common causes of central DI. DI in the immediate postoperative period may be only the first phase of the "triple-phase response" [11]. During the first phase, which commonly lasts up to 48 h postoperatively, the patient develops symptoms of DI consisting of high volume output of dilute urine, hypovolemia, and hypernatremia. Local edema at the surgical site and interruption of normal ADH secretion are thought to be causative. High urine output following surgery may also occur secondary to postsurgical diuresis as a result of intraoperative fluid overload. This can be distinguished from DI by measuring urine and serum osmolality and evaluating for ability to concentrate urine. The duration of this first phase is variable, so intravenous vasopressin infusion should be used. This formulation allows for quick titration and discontinuation of vasopressin effect, compared to oral or intranasal formulations, in the event that the patient progresses to the second phase of the triple-phase response: syndrome of inappropriate antidiuretic hormone secretion (SIADH).

The cause of the second phase is inappropriate antidiuretic hormone secretion due to retrograde degeneration of the ADH neurons that had been surgically interrupted. This unregulated release of ADH results in inappropriately decreased urinary output with high urinary osmolality (>200 mOsm/kg), hypervolemia, and modestly elevated urinary sodium concentrations (greater than 20–30 meq/liter). Because these patients are volume expanded, excess salt administration is not effective in raising serum sodium levels and may worsen water retention. This phase of SIADH may last up to 10 days as dying neurons release ADH. Fluid restriction is recommended for treatment.

Finally, if more than 90% of ADH cells are damaged, patients develop the third phase of permanent DI. At this point, oral or intranasal administration of ADH analogs (desmopressin) provides safe and effective options for DI management. For infants with central DI, management using low renal solute formula (free water therapy), sometimes in combination with thiazide diuretic, has been shown to be effective [12]. Given that a majority of their nutrition is in liquid form as breast milk or formula, the use of desmopressin may predispose infants to water intoxication and hyponatremia. In older children, the presence of an intact thirst mechanism should be assessed. A set fluid intake goal may be needed for children who do not have an intact thirst mechanism in order to prevent hypernatremia. Children with intact thirst can be instructed to drink when thirsty to maintain appropriate sodium levels.

Cerebral salt wasting (CSW) presents with hyponatremia, clinical evidence of dehydration, inappropriately concentrated urine, and renal sodium wasting, often in the setting of acute head injury, bleed, or surgery. While the patient's hyponatremia may be initially mistaken for SIADH, careful clinical evaluation of the patient will show dehydration, opposed to a fluid overloaded state. The onset of SIADH tends to be within 48–72 h postoperatively, whereas CSW typically has a later onset during the seventh to tenth postoperative day [13]. It is important to make this differentiation because the treatment plan differs greatly between SIADH and CSW. While fluid restriction is instituted for patients with SIADH, this would further exacerbate the underlying process in CSW. Instead, administration of intravenous saline and sodium chloride supplementation is indicated to help restore intravascular volume and replace ongoing renal sodium losses of CSW.

The most probable mechanism behind the development of CSW involves disruption of neural input to the kidney along with the stimulated release of natriuretic factors [14, 15]. This leads to increased urinary sodium excretion and causes

a decrease in effective arterial blood volume. This state of dehydration leads to baroreceptor stimulation, resulting in appropriate ADH release and urinary concentration. There have also been several case reports of renal salt wasting with the administration of cisplatin [16–19]. A recent report described a pediatric patient with a suprasellar primitive neuroectodermal tumor who had resultant central DI following surgical debulking. The patient then developed hyponatremia, natriuresis, and decreased urine output following cisplatin administration. Additionally, there are case reports of CSW following infection after hematopoietic stem cell transplantation. These case reports were not associated with diuretic treatment or acute therapy with chemotherapy known to cause hyponatremia (i.e., cyclophosphamide) [20].

In a retrospective review of 159 patients with suprasellar tumors, DI was the most commonly observed disorder of water and electrolyte balance [21]. SIADH was also observed in this cohort of patients but rarely persisted more than 1 month after treatment. CSW was present after treatment in 3.6% (six patients); four of the patients were still in need of salt supplementation 33–43 months after treatment. Of note, three of these patients had an additional thyroid-stimulating hormone (TSH) deficiency, and two had ACTH deficiency, highlighting the importance of evaluation of anterior pituitary hormone abnormalities that may contribute to water or electrolyte imbalances.

11.2.3 Chemotherapy

During treatment for malignancy, malnutrition and cachexia are common side effects. Thus, weight loss, organ dysfunction, and wasting may occur without proper nutritional support. Gastrointestinal side effects are common with both irradiation and chemotherapy, including emesis, diarrhea, malabsorption, stomatitis, and esophagitis. Tumor-induced cytokine production initiates a cascade of metabolic events including glycogenolysis, lipolysis, proteolysis, increased resting energy expenditure, and gluconeogenesis [22]. Due to the increased complications associated with parenteral nutrition, an enteral route should be attempted first using elemental or partially digested formulas [22]. Total parenteral nutrition (TPN) alone or in combination with enteral feeding has been shown to reverse malnutrition, improve immunologic status, improve muscle function, and improve survival [23].

However, it should be noted that pediatric patients with acute lymphoblastic leukemia (ALL) can experience significant weight gain in early cancer treatment which may persist after treatment has been completed [24–27]. This weight gain may be secondary to decreased physical activity and to treatment, particularly steroids [26]. Several studies have assessed the effects of exercise programs during and following cancer treatment [28]. However, study sizes were low, and the benefits of such interventions (body composition, flexibility, cardiorespiratory fitness, muscle strength, and health-related quality of life) also needed to be compared to the risk and effects of fatigue, level of daily activity, and adverse events.

High-dose glucocorticoid treatment is the cornerstone of therapy for childhood ALL. It is given intermittently throughout the duration of treatment over a 2–3-year period. Additionally, high-dose steroid therapy may be used postoperatively in the treatment of brain tumors. Glucocorticoids exacerbate cachexia by their counter-regulatory effects on protein metabolism, leading to muscle wasting and further decrease in activity level. The metabolic effects of glucocorticoids also include decreased peripheral insulin sensitivity [29], increased hepatic glucose production [30], and islet cell toxicity and apoptosis [31, 32]. Concomitant use of medications that act as pancreatic toxins contributes to the development of glucocorticoid-induced diabetes. L-asparaginase, which is often combined with glucocorticoids in the treatment of ALL, has toxic effects on the beta cell and has been associated with the development of transient or persistent diabetes [33]. Medication-induced diabetes may also be associated with increased risk of metabolic syndrome in cancer survivors [34]. Body mass index (BMI), fasting insulin, and insulin resistance can increase during the maintenance phase of ALL therapy, which includes glucocorticoid therapy. Biomarkers of obesity – leptin and adiponectin – were found to increase and decrease, respectively, during maintenance therapy, suggesting that steroids play a likely role in the development of metabolic syndrome in ALL survivors [35]. Similarly, a study of 49 CCS found that 37% had high leptin levels and 41% had low total adiponectin levels. Leptin and adiponectin

are associated with components of metabolic syndrome such as BMI, high systolic BP, and higher triglyceride concentrations [36].

Considering the myriad of metabolic influences of glucocorticoids, with their predominant effect on peripheral glucose uptake, it is not surprising that glucocorticoid-induced hyperglycemia is largely a postprandial phenomenon. Various types of treatments for type 2 diabetes mellitus have been utilized or proposed for steroid-induced diabetes. These include sulfonylureas [37], phenylalanine derivatives [38], alpha-glucosidase inhibitors [39], thiazolidinediones [40, 41], and insulin [42, 43]. Unfortunately, there are very few reports in the adult literature that assess the effectiveness of oral agents and none in the pediatric population. With the limited information presently at hand, short- and/or intermediate-acting insulin preparations present the best therapeutic option for glucocorticoid-induced diabetes, in part, because their limited duration of action reduces the risk of hypoglycemia.

It has been established that when glucocorticoid therapy has been used for brief periods, less than 10 days, therapy can be discontinued without taper without adverse event [44]. However, during longer courses of glucocorticoids, the HPA axis may be suppressed and unable to respond to acute stress for up to 1 year following discontinuation of high-dose steroid treatment. Therefore, it is important to administer higher doses of glucocorticoid during times of stress such as surgery, general anesthesia, intercurrent febrile illness, or emesis.

More recently, immune checkpoint inhibitors, such as ipilimumab, which are used in the treatment of typically adult cancers like melanoma, have been found to be associated with hypophysitis with or without hypopituitarism, thyroid dysfunction, and, in rare cases, adrenalitis [45, 46].

Alkylating agents commonly used to treat pediatric cancers, such as cisplatin, thiotepa, cyclophosphamide, ifosfamide, and carboplatin, form covalent linkages to phosphate, amino, sulfhydryl, hydroxyl, carboxyl, and imidazole groups, thus disturbing fundamental mechanisms of cell proliferation. This class of drugs has been implicated in the development of nephrogenic DI through the downregulation of *AQP2* expression [47]. The treatment of drug-induced nephrogenic DI is first centered on removal of the offending agent. However, when this is not possible, thiazide diuretics are commonly used [48]. Thiazide diuretics reduce the glomerular filtration rate and enhance urinary sodium excretion at the expense of water [12, 49, 50]. The end result is increased proximal tubular sodium and water reabsorption.

11.3 Late Effects of Treatment

11.3.1 Growth Failure/Growth Hormone Deficiency (GHD)

Impaired growth resulting in reduced adult height is one of the most common endocrine issues in CCS [51]. Causes are multifactorial and include growth hormone deficiency (GHD), central precocious puberty (CPP), hypothyroidism, and spinal irradiation. For patients exposed to craniospinal and total body irradiation, truncal short stature may occur with decreased skeletal response to growth hormone (GH) therapy [52–55]. Even without radiation exposure, linear growth in survivors may be affected by growth plate exposure to chemotherapy, which is currently under investigation [56, 57]. In cancer survivors, adult height SDS has been found to be lower than height SDS at initiation of cancer treatment [58–60].

GHD can occur in CCS as the result of direct tumor invasion, debulking surgery, or irradiation therapy in the hypothalamic-pituitary region. Most commonly, GHD occurs after cranial irradiation but can result after exposure to total body irradiation (TBI) and spinal radiation therapy [61]. GH is the most vulnerable anterior pituitary hormone and is often the only anterior pituitary deficit to develop after cranial irradiation [62–64]. Frequently, the actual site of irradiation damage is the hypothalamus, which is more sensitive to irradiation than the pituitary. Low doses of irradiation can affect the hypothalamus (18 Gy of conventional fractionated radiotherapy) [65, 66], while higher doses of radiation are required to produce damage directly to the pituitary gland.

GHD after treatment with irradiation to the hypothalamic-pituitary region is both dose- and time-dependent, with the highest risk associated with greater doses of radiation and a longer time interval from treatment [53]. Radiation doses above 30 Gy led to blunted GH responses to stimulation testing in almost all pediatric

patients within 2–5 years following cranial irradiation [53]. Age at treatment has been shown to be inversely proportional to the development of multiple pituitary hormone deficiencies. Young children, compared to adults, have shown greater vulnerability to developing isolated GHD after treatment with TBI doses as low as 10 Gy [65–70].

Studies in adult survivors of brain tumors show maintenance of tonic (non-pulsatile) GH secretion, pulsatile quality of GH secretion, and diurnal variation but noted marked dampening of the pulse amplitude [71]. The preservation of basal GH secretion is hypothesized to be due to decreased IGF-I-dependent negative feedback. Additionally, radiation-induced reduction of somatostatin secretion has been postulated to result in greater tonic GH release from the remaining somatotrophs [72]. The decreased GH pulse amplitude has been shown to be secondary to decreased hypothalamic growth hormone-releasing hormone (GHRH) secretion along with diminished somatotroph number [72, 73]. While it appears that the hypothalamic-pituitary unit and the regulation of GH secretion remain intact, even following cranial radiation exposure during childhood, it is clear that poor growth during childhood may be one of the only objective signs clinicians have regarding GHD in this patient population [71, 74, 75].

Establishing the diagnosis of GHD can be challenging and should be made in the context of both clinical findings and laboratory results. For patients who received craniospinal irradiation, upper and lower body segment disproportion may serve as an early indicator of radiation-induced skeletal injury. Monitoring for a decrease in growth velocity is one of the most sensitive indicators of GHD in cancer survivors [76]. Radiation exposure has been associated with a specific form of GHD called growth hormone neurosecretory dysfunction (GHNSD) [77–81]. These patients have a preserved peak GH response on stimulation testing, despite decreased endogenous GH secretion [82]. During puberty, patients with GHNSD will fail to mount an appropriate acceleration in growth velocity due to a lack of increased GH secretion [83, 84].

No gold standard has been established for diagnostic testing for GHD in children following cranial irradiation. IGF-I and insulin-like growth factor binding protein-3 (IGFBP-3) levels are routinely used in clinical practice as surrogate markers of GH secretion during the investigation of short stature. However, IGF-I and IGFBP-3 levels are less reliable indicators of GH status following cranial irradiation and in cases of hypothalamic-pituitary tumors. In these patients, normal levels of IGF-I and IGFBP-3 (above −2 SD) can be seen in the setting of abnormal growth velocity and GHD [85, 86]. Further, because GHD is so common following irradiation to the hypothalamus and/or pituitary, the need for failing two provocative tests to diagnose GHD has been questioned in such patients whose growth patterns suggest GHD. In their published guidelines on pediatric GH treatment, the Growth Hormone Research Society advocated needing only one failed stimulation test to make the diagnosis [87]. However, the Pediatric Endocrine Society (formerly named in honor of Lawson Wilkins; 2003) concluded that GH stimulation tests are optional in a child with growth failure who has evidence of additional pituitary hormone deficiencies or in patients with a history of surgery or irradiation in the region of the hypothalamus and pituitary [88].

GH replacement therapy has been shown to increase growth velocity and adult height in CCS with GHD [56, 89]. However, survivors treated with spinal radiation doses exceeding 20 Gy respond less robustly to GH therapy and are at risk for developing disproportionate growth of the limbs in comparison to the trunk [90, 91]. This becomes most apparent during the time of the pubertal growth spurt [92]. Further, it is important to monitor patients for the development or progression of existing scoliosis during GH treatment; GH treatment has been associated with worsening of pre-existing kyphosis and scoliosis that may require orthopedic intervention [93].

Children with a prior history of malignancy constitute approximately 20% of all pediatric patients treated with GH [88]. The mitogenic properties of GH and IGF-1 have prompted concerns regarding the safety of GH treatment in survivors of malignancy. Several studies using data from the Childhood Cancer Survivor Study (CCSS) and St. Jude Children's Research Hospital indicate that GH treatment does not increase tumor recurrence in persons successfully treated for a primary lesion [94–96]. Studies assessing the risk of tumor recurrence, specifically in the brain tumor survivor population treated with GH have consistently reported no increased

risk associated with GH treatment [95, 97–100]. However, the 2003 guidelines published by the Pediatric Endocrine Society caution to wait at least 1 year after completion of tumor therapy with no evidence of further tumor growth before initiating GH therapy in this group of children [88]. Patients with craniopharyngiomas, a benign tumor, may be treated with GH once the tumor has been adequately controlled or stabilized [88].

All cancer survivors are at risk for developing a second neoplasm. In a report from the CCSS including 361 GH-treated individuals, including 122 survivors of acute leukemia and 43 survivors of soft tissue sarcomas [95], data suggested that GH treatment may slightly increase the risk of a secondary solid tumor, particularly in acute leukemia survivors. Meningiomas were the most common second neoplasms that were observed in survivors treated with GH who were also exposed to cranial irradiation as part of their treatment protocol [101]. An updated analysis from the CCSS, adjusted for CNS radiation dose and duration of follow-up after radiation therapy, found no increase in the rates of meningioma, glioma, or any subsequent CNS neoplasm associated with GH treatment [102]. Although not found to be associated with GH treatment, meningiomas, gliomas, and other CNS neoplasms occurred in 4.7% of GH-treated survivors and 1.7% of other cancer survivors treated with cranial radiation therapy [102]. Therefore, ongoing surveillance of such patients for second malignancies is important. The Genentech National Cooperative Growth Study (NCGS), a registry containing 20 years of GH safety data covering approximately 55,000 patients and nearly 200,000 patient-years of GH, concurred that GH exposure does not increase risk for new malignancy in children without risk factors but may slightly increase or hasten the onset of second neoplasms in patients previously treated for cancer [103].

Summarizing evidence available through February 2014, a report from the Pediatric Endocrine Society Drug and Therapeutics Committee concluded that GH can be used to treat GH-deficient CCS who are in remission with the understanding that GH therapy may increase their risk for second neoplasms [94]. Although there is no sufficient evidence to support titrating GH therapy to maintain normal IGF-I levels to modify the risk of malignancy, maintaining IGF-I levels at supraphysiologic levels has not been known to increase height outcomes, and the potential risks are not well studied. Additionally, it is recommended that if the patient has a known tumor predisposition due to genetic or other medical conditions, the patient and caregivers should be informed that the risks of developing cancer associated with GH treatment have not been adequately studied in these populations [94].

While radiation-induced GHD is usually permanent, the need to reevaluate patients after reaching adult height for continued treatment remains controversial [87, 104–106]. Adult GHD is an established indication for replacement therapy and provides the potential benefits of decreasing adiposity, improving plasma lipids, increasing bone density, and improving quality of life [107–110]. In CCS, peak GH level <7ug/L on clonidine and arginine stimulation testing has been associated with greater adiposity, unfavorable lipid levels, and decreased insulin sensitivity compared to sibling controls [111]. According to the 2009 guidelines published by the American Association of Clinical Endocrinologists, cancer survivors with irreversible hypothalamic-pituitary structural lesions, evidence of panhypopituitarism, and serum IGF-I levels below the age- and sex-appropriate reference range when off GH therapy are deemed to be GH deficient and do not require further GH stimulation testing [112]. When transitioning to adult GH therapy, the starting dose of GH should be approximately 50% of the dose between the pediatric doses required for growth and the adult dose. After initiating GH therapy, physicians should follow patients at 1–2-month intervals, at which time the daily GH dose should be increased by 0.1–0.2 mg based on clinical response, serum IGF-I levels, side effects, and individual considerations [112]. When maintenance doses are achieved, serum IGF-I, fasting glucose levels, hemoglobin A1c, blood pressure, BMI, waist circumference, and waist-to-hip ratio should be assessed every 6–12 months. In survivors with a history of cranial irradiation, 6–12-month monitoring should also include testing of the other anterior pituitary hormone functions, fasting lipid panel, and overall clinical status. Children treated with TBI who are also on GH therapy may be at increased risk of slipped capital femoral epiphysis (SCFE) compared to children treated with GH therapy for idiopathic GHD [113]. In one retrospective chart review, SCFE presented as atypical valgus SCFE or bilaterally

SCFE at an incidence of 35.9 per 1000 person-years, a 211-fold greater rate than what is reported in children treated for idiopathic GHD [113].

11.3.2 Thyroid

Thyroid abnormalities are common in CCS. Although primary subclinical hypothyroidism is the most common thyroid abnormality, clinical hypothyroidism, hyperthyroidism, benign nodules, and thyroid malignancies can also occur after head and neck irradiation or total body irradiation before transplant [114–118]. Given the impact of thyroid status on growth and development, it is important to recognize these disorders early and initiate appropriate treatment. Additionally, untreated hypothyroidism may also further impact fertility of cancer survivors [119]. Irradiation to the head and neck containing fractionated doses greater than 18 Gy can result in direct damage to the gland, resulting in primary hypothyroidism [120, 121]. A recent study evaluating survivors of medulloblastoma provided some evidence that treatment with proton radiotherapy may result in reduced risk of hypothyroidism and need for any endocrine replacement therapy than treatment with photon radiotherapy [122]. While most patients will develop primary hypothyroidism 2–4 years after radiation therapy, gland failure may not present for up to 25 years following radiation treatment [123]. Exposure to radiolabeled agents such as 131I-metaiodobenzylguanidine (MIBG) [124, 125] and 131I-labeled monoclonal antibody for neuroblastoma [126] treatment has been shown to result in primary hypothyroidism in up to 50% of cases despite prophylactic treatment with potassium iodide during therapy.

Given the variability in time required to develop hypothyroidism after radiation exposure, annual screening of survivors is recommended or more frequently if symptomatic. Unfortunately, the symptoms of hypothyroidism are nondescript and may be mistaken for the gastrointestinal side effects of radiation exposure and fatigue that this population experiences on a day-to-day basis. The most sensitive indicator of central hypothyroidism is a blunted or absent nocturnal surge of TSH. However, given the challenge of obtaining nocturnal surge studies, clinicians must rely upon measurement of TSH and free thyroxine (T4) levels. The TSH may be inappropriately low or normal in the presence of a low free T4 level. In patients with TBI exposure or direct neck exposure to radiation therapy, laboratory examination will be consistent with primary hypothyroidism with an elevated TSH and low free T4 levels.

The goal of thyroid hormone replacement therapy is to support normal growth and development. This is achieved by maintaining the free T4 in the upper half of normal [127]. Since TSH levels are unreliable in patients with secondary hypothyroidism, it does not need to be monitored during therapy.

Direct or scatter radiation exposure to the thyroid is a significant risk factor for the development of benign and malignant thyroid lesions [128]. Thyroid cancers account for about 10% of subsequent malignancies in CCS [129]. The greatest risk for the development of thyroid cancer appears to involve children treated before 10 years of age and/or with doses in the range of 20–39.9 Gy [129]. Individuals treated with higher radiation have an elevated risk compared to the general population but decreased compared to those who received less radiation to the thyroid. This decrease is attributable to the cell-killing effect of higher doses of radiation [118, 130, 131]. In general, thyroid cancers in patients treated with radiation behave in a nonaggressive fashion, similar to what is observed in de novo thyroid cancers among younger individuals [131]. The etiology of thyroid cancer in this population is thought to be secondary to radiation-induced rearrangements of oncogenes *RET-PTC* [132, 133]. Recent studies evaluating adult survivors of childhood cancer found an increased risk of secondary thyroid malignancy in subjects who did not receive radiation but did have exposure to alkylating agents [118, 130].

The Children's Oncology Group Long-Term Follow-Up Guidelines for Survivors of Childhood, Adolescent, and Young Adult Cancers (COG-LTFU) recommended annual thyroid palpation on physical examination to assess for nodules [134]. However, small or posterior nodules may not be appreciated by palpation. Although there are no formal guidelines regarding the use of thyroid ultrasonography for regular screening for secondary thyroid malignancies, several studies have investigated the utility of using thyroid ultrasound surveillance [117, 135–137]. One group used thyroid ultrasound 5 years after radiation

and repeated the study every 3 years if negative. Of 197 cancer survivors who had received radiation to the thyroid gland, 74 patients (37.5%) developed thyroid nodules, and fine-needle aspiration (FNA) was performed in 35. FNA was suspicious or diagnostic for malignancy in 11 patients, and a follicular lesion was diagnosed in 9 [135]. The mean time since completion of treatment to diagnosis of a secondary thyroid cancer was 13.08 years in this cohort. Another study showed that thyroid ultrasound detected thyroid nodules in 46/78 patients. Fourteen patients underwent FNA, and 5 patients were diagnosed with papillary carcinoma; average duration since initial radiation was 18.45 years [136].

11.3.3 Gonadal Axis

11.3.3.1 Precocious Puberty

Precocious puberty is defined as the onset of thelarche before the age of 8 years in females and testicular enlargement before the age of 9 years in males. Cranial irradiation, particularly to the hypothalamic region, has been associated with the development of central precocious puberty (CPP) at both lower doses for leukemia treatment (18–35 Gy) and higher doses for brain tumor treatment (>35 Gy) [138]. The mechanism for CPP following irradiation is hypothesized to involve dysregulation of cortical influences on the hypothalamus and a release of the inhibitory GABAnergic tone [139, 140].

Risk factors associated with the development of CPP following hypothalamic irradiation include younger age at treatment, female sex, and increased BMI. The observed difference between the sexes has been postulated to reflect gender differences in the interaction between higher centers in the central nervous system (CNS) and hypothalamic function [73]. It is thought that the CNS restraint on puberty is generally more easily disrupted in girls than in boys. Thus, girls more often develop CPP than boys following lower-dose irradiation (16–24 Gy), while higher radiation doses (25–50 Gy) lead to CPP in both sexes equally [66, 141].

Testicular volume may be a less reliable indicator of pubertal onset when assessing boys who have received chemotherapy and radiation. Damage to the seminiferous tubules during treatment may result in atrophic testes that are incapable of enlarging during pubertal progression. Therefore, looking for other secondary sexual characteristics on physical examination is necessary. Increased growth velocity is a well-established hallmark of pubertal development. However, a caveat for the survivor population is that they may have a blunted or absent growth spurt due to concomitant hormone deficiencies or they may have obesity-related acceleration of linear growth.

Skeletal maturation (bone age) can be assessed using the standard X-ray taken of the left hand and wrist and compared to a series of normative films [142]. Bone age advancement greater than 2 standard deviations from the mean for the patient's chronological age is consistent with precocious puberty. Gonadotropin levels best distinguish CPP from peripheral causes of sexual precocity. Because pubertal gonadotropin secretion is pulsatile, gonadotropin-releasing hormone (GnRH) or GnRH agonist stimulation tests are often needed to capture peak levels. A robust luteinizing hormone (LH) response indicates a pubertal pattern. Elevated plasma estradiol levels in girls and testosterone levels in boys are also indicative of pubertal progression. In girls, physical exam findings consistent with estrogen stimulation such as color change of the vaginal mucosa, increased physiologic vaginal discharge, and uterine growth on the pelvic ultrasound are supportive in the diagnosis of CPP.

Standard treatment of CPP consists of depot parenteral preparations of GnRH agonists, usually administered as monthly or quarterly intramuscular injections [143], or annual subdermal implants placed under local anesthesia [144]. In general, this class of drugs is effective in retarding progression of secondary sexual characteristics, preventing menses, slowing bone age advancement, and increasing adult height [145]. The injectable form is supported by more long-term efficacy and safety data, while the implantable approach may facilitate adherence by eliminating the need for monthly injections.

Aromatase inhibitors were developed as adjuvant therapy for estrogen-responsive breast cancer in postmenopausal women. The primary aim of therapy in pediatrics is attenuation of the effects of estrogen on growth, skeletal maturation, and secondary sexual development [146]. Use in children and adolescents is still limited and off-label.

11.3.3.2 Delayed Puberty

Delayed puberty can result from primary gonadal injury or deficiency of central activating signals (hypogonadotropic hypogonadism). High-dose cranial irradiation (>40 Gy) is associated with hypogonadotropic hypogonadism within the context of combined hormonal pituitary deficiencies [147–152]. Cancer survivors with a history of exposure to radiotherapy, including abdominal/pelvic, TBI, and craniospinal, and/or alkylating agents are at increased risk of gonadal failure [153–156]. Additionally, there have been case reports of gonadal failure after treatment with ^{131}I–metaiodobenzylguanidine for neuroblastoma thought to be secondary from scatter radiation [157]. Dosages of alkylating agents and radiation affect this risk. COG-LTFU recommends screening patients at risk of hypogonadism by asking about onset and tempo of puberty, menstrual history, and Tanner stage annually until sexual maturity [158, 159].

Ovarian function can be affected in postpubertal girls at doses as low as 5 Gy, and higher radiation dose (i.e., 10 Gy) is associated with impaired ovarian function in prepubertal girls [160, 161]. This is related to the decrease in oocyte pool with age. Survivors who lose ovarian function during cancer therapy or within 5 years of completion are classified as having acute ovarian failure (AOF). During childhood and adolescence, doses in the range of 10–30 Gy have been noted to cause AOF in the majority of patients [162–165]. Further, doses of ovarian irradiation less than 10 Gy are capable of inducing AOF in patients who have additional risk factors, namely, concomitant exposure to alkylating agents and older age at diagnosis [165]. Some studies have shown that ovarian dysfunction can be transient and may not require hormone replacement [154].

Survivors who retain ovarian function after the completion of cancer treatment may go on to experience menopause before age 40 years and are classified as having premature ovarian failure, formally known as premature menopause [166, 167]. Exposure to alkylating agents can also decrease ovarian reserve, which can be demonstrated by lower anti-Mullerian hormone (AMH) levels, lower ovarian surface area, and higher follicle-stimulating hormone (FSH) [168]. In a study of 706 female survivors of childhood solid tumors, risk factors for nonsurgical menopause included exposure to alkylating agents, increasing radiation dose to the ovaries, procarbazine dose, cyclophosphamide dose, and unilateral oophorectomy [168]. Compared with patients treated before the onset of puberty without alkylating agents or radiation to the ovaries, survivors treated after the onset of pubertal period with alkylating agents alone or even with a low radiation dose to the ovaries had, respectively, a 9- and 29-fold higher rate of nonsurgical menopause at a given age [168]. Among female survivors of CNS tumors following treatment with CSI/abdominal radiotherapy and alkylating agents, the cumulative incidence of premature ovarian failure 6 years after radiotherapy was 83% in one observed cohort; recovery of ovarian function was observed in 39% [152]. The median estimated RT doses were 5.6 Gy (range, 0.7–30.5 Gy) and 6.1 Gy (range, 0.6–31.9 Gy) to the right and left ovaries, respectively. There was no significant correlation between dose of CSI or cumulative dose of cyclophosphamide and the incidence of POI [152].

Studies have shown that AMH levels decrease during cancer treatment and may be useful in determining ovarian follicle reserve and predicting onset of menopause [169–172]. Even when cancer survivors have regular normal menstrual cycles, AMH levels can be low and reveal apparent depletion of ovarian reserve. Lower AMH levels were associated with increased age at diagnosis and increasing dose of alkylating agents [171]. There is also evidence that obesity and insulin resistance are associated with gonadal damage, demonstrated in one study by lower AMH levels and lower follicle count on ultrasound [173]. Although not established, AMH levels may help guide advice regarding remaining reproductive life span and fertility interventions in clinical settings [174].

In males, testicular radiation, gonadotoxic chemotherapy, orchiectomy, cranial surgery, or central radiation involving the hypothalamic-pituitary-gonadal (HPG) axis can result in primary or secondary hypoandrogenism. This may manifest clinically as delayed or arrested puberty. Pubertal status has been found to be a risk factor for radiation-induced primary hypoandrogenism; a dose of 24 Gy was found to induce hypoandrogenism when survivors were treated before puberty compared to a testicular dose of 30 Gy when survivors were treated postpuberty [158]. Following radiation therapy directed at the testes, there can be a disproportionate elevation of FSH

over LH, signaling a relative increase in damage to the sperm-producing Sertoli cells compared to the Leydig cells. Leydig cells are comparatively more resistant to treatment toxicity than testicular germ cells, so survivors may have azoospermia but adequate androgen levels [175–177]. The COG-LTFU recommends annual assessment of pubertal development until sexual maturity using Tanner staging, with testicular volume determined by Prader orchidometer, for survivors treated with gonadotoxic therapy before the onset of puberty, keeping in mind that testicular volume may be decreased in patients who have received gonadotoxic therapy [134]. Young men treated with gonadotoxic therapies after puberty should be assessed annually for symptoms of androgen deficiency: decreased libido, decreased spontaneous erections, gynecomastia, loss of body hair, decreased muscle bulk or testicular volume, and hot flashes [159].

In both males and females, diagnosis of hypogonadism is based on laboratory testing, which includes LH, FSH, and morning testosterone or estradiol level, depending on sex, in combination with bone age radiography to assess skeletal maturity until epiphyseal fusion. When primary gonadal failure is suspected, standard gonadotropic levels may be ordered because a significant elevation is expected. However, when evaluating a patient with suspected hypogonadotropic hypogonadism, ultrasensitive (ICMA) gonadotropin levels should be obtained to increase sensitivity. Male fertility and sperm production can be assessed through sperm analysis.

Treatment for female hypogonadism is a balancing act of inducing secondary sexual characteristics, promoting growth without accelerating fusion of the growth plates, and promoting proper accrual of bone mass. Induction of thelarche using unopposed estrogen is a gradual course of dose escalation until menarche is achieved. After that point (or no more than 2 years if no spontaneous bleeding occurs), progesterone is added to promote cycling. To increase convenience, a combination estrogen-progestin oral contraceptive pill can be introduced.

Testosterone replacement is the primary treatment for male hypogonadism. The goals of therapy are to support normal pubertal development, increase sexual function, and help build bone density. Initially, testosterone replacement doses are low and build every 6 months to reach full adult replacement over a 3-year period. Multiple formulations are available and require intramuscular injection, gel, or patch application [178]. Testosterone esters are injected into the muscle every 2–4 weeks at a dose up to 200 mg. Patches must be changed daily and are applied dermally on the back, thigh, or upper arm. Gel is applied to a covered area of skin daily at a dose of 50–100 mg. Patients should be instructed to wash their hands immediately after application and to avoid contact of the exposed area with shared towels, clothing, and other people to prevent unintentional dosing of household contacts. Testosterone therapy cannot overcome damage to the spermatogenic cells.

11.3.3.3 Fertility Preservation and Sexual Function

The ability to have genetically related children is an important issue for patients surviving cancer [179]. A report from the Childhood Cancer Survivor Study, which followed both male and female survivors of childhood cancer treated with chemotherapy alone between 1970 and 1999, found that survivors had a lower likelihood of siring or having a pregnancy than their siblings [180]. Surgical, medical, and technological advances have enabled the medical community to provide fertility options for patients with cancer and cancer survivors. The American Society of Clinical Oncology (ASCO) recommends discussion of the impact of cancer treatment on fertility and options of fertility preservation in the course of cancer diagnosis and treatment [181]. For children, the ASCO guidelines recommend using established methods of fertility preservation (semen cryopreservation and oocyte cryopreservation) for postpubertal children, presenting information on additional investigational methods available for children, and referring experimental protocols when available [181].

In females, fertility can be impaired by factors that not only injure the gametes but also impede conception, implantation, and carriage of term pregnancy. Direct uterine effects after abdominal irradiation in young girls included irreversible changes in uterine musculature and blood flow, leading to future spontaneous pregnancy loss and intrauterine growth retardation of fetuses [178, 182]. In addition, alkylating agents and radiation dose of >30 Gy or ovarian/uterine radiation doses of ≥5 Gy are associated with a decreased likelihood of pregnancy [159]. In the CCSS, the

likelihood of pregnancy for female survivors was decreased for hypothalamic-pituitary doses above 30 Gy [183].

Compared to healthy siblings, young male survivors observed in the CCSS were less likely to sire a pregnancy after treatment with alkylating agents, bleomycin, surgical excision of any organ of the genital tract, and radiation to the testes >7.5Gy [184, 185]. Exposure to alkylating agents without radiation to the testes was found to be significantly associated with an increased risk for azoospermia and oligospermia in a cohort of male patients from the St. Jude Lifetime Cohort [186]. Impaired spermatogenesis was found to be unlikely when the cyclophosphamide equivalent dose was less than 4000 mg/m^2. In a large Norwegian national cohort study, male cancer survivors had reduced paternity and were more likely to use assisted reproductive technology compared to non-cancer subjects, particularly in survivors of testicular cancer, brain tumors, lymphoma, leukemia, and bone tumors, and when diagnosed with cancer before 15 years of age [187].

To reduce the cytotoxic effects of radiation and chemotherapy, some investigators have attempted to render the germinal epithelium quiescent by creating an artificial prepubescent state using a GnRH agonist [188]. Several recent retrospective and randomized controlled trials have reported positive results in the use of a GnRH agonist when receiving chemotherapy, showing preservation of the cyclic ovarian function, increase in pregnancy rate, and decrease in amenorrhea in GnRH agonist-treated patients compared to those who received chemotherapy alone [189]. However, there are no current consensus guidelines on the use of GnRH agonist to preserve fertility during chemotherapy. Although the success rate on fertility of oophoropexy to move the ovaries out of the radiation field varies from 50% to 90% due to several patient and treatment factors, this has been a technique used to preserve fertility [183, 190, 191]. However, the uterus may still be damaged by radiation, which may decrease the chances of a term pregnancy and increase IUGR.

Cryopreservation of ovarian or testicular tissue is an experimental strategy for preserving future fertility or gonadal function that is available to both pre- and postpubertal females and males [192, 193]. However, a risk of malignant cells present in the preserved tissue, particularly in leukemia patients, should be considered [194]. Freezing unfertilized oocytes has had some success but with significant limitations and only a small number of reported pregnancies. In vitro fertilization with frozen embryos has an approximately 20% success rate for pregnancy per cycle [195]. This process is complicated by the fact that many pediatric patients are young and do not have a partner to provide sperm and cancer treatment often cannot be delayed to entertain the IVF process. For men, semen cryopreservation is an option if ejaculation can be achieved. In one study, the live birth rate of pregnancies resulting from cryopreserved sperm of cancer survivors was 62%, which was significantly higher than the control normospermic non-cancer population [196]. However, limitations include suboptimal sperm quality even before cancer treatment, especially in testicular cancer, and the need to delay cancer therapy until several samples could be banked to ensure adequate and viable sperm [165]. Although the success of future fertility has yet to be established, pretreatment cryopreservation of testicular stem cell tissue can be considered for prepubertal boys who do not yet produce spermatozoa [158].

Sexual dysfunction among adolescent and young adult cancer survivors is an area of increasing research. A study of male and female CCS found that survivors treated for cancer as adolescents reported more delays in achieving sexual milestones compared with both survivors treated at a younger age and the age-matched general population [197]. Sexual dysfunction can result from psychosocial challenges of the survivor's cancer experience, including mood disorders, fatigue, altered body image, social isolation, and delayed psychosexual development [158]. Surgery and irradiation to the spine and pelvic region may also result in physiologic sexual dysfunction, defined as the inability to complete intercourse in males. In females, irradiation or scarring from graft-versus-host disease following transplantation may cause vaginal scarring and result in dyspareunia and vaginal dryness [159]. Estrogen deficiency can also cause vaginal atrophy and higher vaginal pH, which can result in infections, incontinence, and sexual dysfunction [159].

Additionally, young adult and adolescent cancer survivors should be counseled on reproductive health, sexually transmitted infections, and contraceptive methods. Young adults may assume that they have decreased fertility following cancer

treatment and may not follow safe sexual practices [198]. In one study involving 1041 nongynecologic cancer female survivors between 18 and 40 years of age, 21% of participants who had resumed menses reported unprotected intercourse in the prior month and were defined at risk of unintended pregnancy; this is a threefold higher risk of pregnancy than the general US population, according to the National Center for Health Statistics [199].

11.3.4 Hypoprolactinemia

Lactotroph cells are found in the anterior pituitary and produce prolactin (PRL), which is necessary for lactation. PRL deficiency can occur in adults with hypopituitarism and is related to the severity of hypopituitarism [200]. Impaired PRL secretion has been found in long-term survivors of childhood leukemia following cranial irradiation with a dose of 18–30 Gy [201]. In one study, six of the seven female survivors who became pregnant failed to lactate following childbirth. Patients with the lowest PRL levels (PRL $\leq$ 7 ng/l) also had significantly lower IGF-I levels. Other studies have shown that low prolactin levels have been independently associated with reduced levels of serum IGF-I in adults with severe GHD [202]. It has been theorized that GH may influence prolactin secretion, either by a direct effect or via IGF-I.

11.3.5 Adrenal Axis

Outside of the subsequent risk of iatrogenic adrenal insufficiency from glucocorticoid treatment, ACTH deficiency (central adrenal insufficiency) in CCS is relatively uncommon [127]. Acutely, ACTH deficiency can result following pituitary surgery or from direct tumor extension into the pituitary. Primary adrenal insufficiency is usually the result of direct tumor extension into the adrenal gland or secondary to the use of an adrenal-toxic drug such as ketoconazole or mitotane. Cranial irradiation, patients with a history of craniopharyngioma or medulloblastoma, or those receiving >18 Gy cranial irradiation have an increased incidence of ACTH deficiency [64, 203]. ACTH deficiency has also been found in survivors of head and neck rhabdomyosarcoma, likely secondary to radiation therapy [204].

A study evaluating the HPA axis of survivors of childhood ALL provided some evidence of HPA axis dysregulation; subjects had higher morning cortisol levels and increased cortisol suppression in response to oral dexamethasone compared to healthy age- and sex-matched controls [205]. Higher cortisol levels in survivors were also associated with more fatigue and poorer quality of life. HPA axis dysregulation was thought to be secondary to receiving chronic steroids as part of ALL treatment or secondary to the stress of illness [205].

11.3.6 Water Balance

11.3.6.1 Chronic SIADH

Outside of the immediate postoperative period, SIADH may develop years after treatment with cranial irradiation due to meningitis, vascular hemorrhage, or stroke. One must pay careful attention to changes in breakthrough urine output in patients previously diagnosed with DI who were treated with desmopressin and have risk factors for delayed onset SIADH. Careful history taking regarding fluid balance is essential in eliciting signs and symptoms of SIADH in at-risk patients. Laboratory findings consistent with SIADH include concentrated urinary output with low volume and increased osmolality, hyponatremia, and decreased serum osmolality.

Chronic SIADH is best managed by chronic oral fluid restriction. However, in very young children, fluid restriction may lead to calorie malnutrition. In this event, one may use demeclocycline therapy to induce a state of nephrogenic DI to allow for sufficient fluid intake, enhanced nutrition, and normal growth [206, 207]. Vaptans, a class of drugs that act as specific antagonists of the arginine vasopressin receptor 2 (AVPR2), have been approved by the US Food and Drug Administration (FDA) for treating euvolemic and hypervolemic hyponatremia in adult patients. There have been case reports of vaptan use in pediatric oncology patients with SIADH and hyponatremia to facilitate chemotherapy fluid administration and prevent worsening of the hyponatremia [208]. By inducing free water clearance, vaptan use enabled the administration of aggressive hydration as part of the management strategy to prevent complications from phosphate and other cell lysis products. However, data on

efficacy and safety of vaptans in the pediatric population to treat chronic SIADH are limited.

11.3.6.2 Chronic DI

Permanent DI commonly results from pituitary stalk surgery for resection of craniopharyngiomas and germinomas. In patients who have received cranial irradiation, DI may develop over a period of months to years after the completion of therapy. Clinically, DI may present with polydipsia in patients with an intact thirst mechanism, polyuria, dehydration, increased serum osmolality, and decreased urine osmolality. In young children, parents may report children drinking from unusual sources such as the toilet or bathtub. It is important to incorporate questions regarding new onset polyuria and polydipsia in the history taking process. Any suspicion for new onset DI should be investigated by measuring simultaneous serum electrolytes, serum osmolality, and urine osmolality. In the event that these laboratory findings are inconclusive, if clinical suspicion is high, formal water deprivation testing should be performed.

Treatment for central DI, regardless of the etiology, consists of replacing endogenous ADH secretion with exogenous synthetic hormone. Desmopressin therapy, in tablet or nasal spray form, can improve quality of life for patients with an intact thirst mechanism by creating periods of reduced urinary output during the day and overnight. For patients without an intact thirst mechanism, as is the case for many patients following a hypothalamic tumor, maintenance water replacement is given based on body surface area. During times of illness, insensible losses may increase due to fever, diarrhea, and tachypnea. To prevent dehydration and resulting hypernatremia, the amount of daily free water intake will need to be increased to account for these losses.

11.3.7 Obesity and Metabolic Syndrome

In the general population, obesity is a well-described risk factor for the development of comorbid conditions such as diabetes mellitus [209, 210], hypertension [211], dyslipidemia [212], and cardiovascular disease [213, 214]. Metabolic syndrome is characterized by central obesity, hypertension, dyslipidemia (elevated triglycerides, reduced HDL cholesterol), and insulin resistance (fasting hyperglycemia, hyperinsulinism, impaired glucose tolerance, and type 2 diabetes mellitus) [214–218]. Early diagnosis and intervention has been shown to reduce associated cardiovascular morbidity and mortality [218]. Several studies have suggested CCS, particularly ALL survivors, are at an increased risk of obesity and metabolic syndrome [24, 218–220]. Younger age, BMI at diagnosis, gender, race, and exposure to cranial irradiation and steroids are significantly associated with an increased risk [27, 221–226]. The CCSS has shown that cranial irradiation in doses of ≥20Gy is a primary risk factor for an increased prevalence of obesity, with the highest risk observed in survivors treated at age younger than 4 years [227]. In this population, cranial radiation exposure is associated with a greater rate of increasing BMI, particularly among females. CCS have been found to have a significantly lower total energy expenditure (TEE) with recommended levels of physical activity than estimated energy requirements [228]. Additionally, survivors who received cranial irradiation had a significantly lower TEE per kg body weight than survivors who did not receive CRT [224].

The increased risk of obesity may also be related to untreated GHD or decreased physical activity secondary to bone pain or decreased exercise tolerance [229]. Similar to the general population, there also may be some genetic predisposition to obesity among cancer survivors [230]. Non-Hispanic Black and Hispanic CCS are at an increased risk of diabetes even when adjusting for socioeconomic status and obesity [231]. Pathological eating behaviors such as frequent snacking, susceptibility to food stimuli, emotional eating, and eating at night have been found to occur more frequently in some groups of cancer survivors compared to obese, healthy controls [221, 222].

Additionally, patients, particularly those who received TBI to prepare for bone marrow transplantation, may develop features of metabolic syndrome in the absence of excess abdominal fat or without associated obesity [232, 233]. Metabolic syndrome has been reported in 12–39% of childhood ALL survivors, with greater risk in those treated with both chemotherapy and radiation [20, 234, 235]. In a cohort of 340 cancer survivors of hematological malignancies, brain tumors, sarcomas, and other pediatric

cancers, hypercholesterolemia was diagnosed in 20%, hypertriglyceridemia in 6%, and obesity in 8% after a median follow-up of 16.1 years [236]. Total body irradiation and GHD increased the risk of both hypercholesterolemia and hypertriglyceridemia. The risk of hypercholesterolemia was also higher in CCS who underwent autologous hematopoietic stem cell transplantation or platinum-based chemotherapy, whereas a previous diagnosis of brain tumor and exposure to anthracyclines significantly predicted obesity [236]. Additionally, a cohort of high-risk neuroblastoma patients treated with multimodal therapies, including chemotherapy, radiation, stem cell transplantation, and surgery, found that nearly half (11/24) of HgbA1c measured were in the prediabetes range of 5.7–6.4%. BMI and history of abdominal irradiation did not correlate with HgbA1c [237]. Those who received hematopoietic stem cell transplantation were also at increased risk of posttransplantation diabetes [238–240]. Patients with a history of stem cell transplantation and TBI have been found to have increased visceral fat distribution, increased total fat mass, and reduced lean mass which may be associated with increased insulin resistance.

Rapid and irretraceable weight gain that results from mechanical or functional disruption of the hypothalamic network is called hypothalamic obesity. Damage to the hypothalamus, which may occur in hypothalamic or pituitary tumors, can lead to low resting metabolic rates, autonomic imbalance, and reduced physical activity, in addition to endocrine deficits [241]. CRT-induced neuronal damage to the hypothalamus and pituitary may cause GHD and/or leptin insensitivity, which can lead to obesity and metabolic syndrome [27, 221, 222, 242, 243]. Mechanical damage from neurosurgery can also cause damage to the hypothalamus; prevalence of hypothalamic obesity has been reported in up to 55% of craniopharyngioma patients posttreatment [244–247]. In one study evaluating 261 patients with childhood-onset craniopharyngioma, history of hypothalamic involvement by the tumor was associated with severe weight gain during the first 8–12 years of follow-up (median BMI increase: +4.59 SD) compared to patients with no hypothalamic involvement (median increase: +1.20 SD) [248]. Quality of life in patients with hypothalamic involvement was impaired by obesity, physical fatigue, and reduced motivation. Some studies evaluating the use of stimulants, such as caffeine or dextroamphetamine, in children with hypothalamic obesity showed weight loss [249–251]. However, further trials are needed, and currently there are no approved options for the pharmacological treatment of hypothalamic obesity in children.

During routine annual follow-up care, all survivors should have height, weight, and blood pressure measured, BMI calculated, and percentiles noted. Short stature, due to GHD or spinal irradiation, or excess central obesity, secondary to abdominal or pelvic irradiation, may render BMI a less accurate measure of obesity [252]. Skinfold thicknesses, DXA scan for fat mass, or waist-to-height ratio may be used for better assessment of obesity. The COG-FTFU guidelines recommend measuring fasting blood glucose or HgbA1c and lipid profiles every 2 years, or more frequently if indicated based on patient evaluation, and counseling at every annual visit regarding proper nutrition, exercise, and obesity-related health risks [134]. Consider evaluation for other comorbid conditions including dyslipidemia, hypertension, and insulin resistance as needed. CCS may have increased screen time and decreased physical activity compared to healthy populations [253]. These are two areas on which specific lifestyle modifications could be focused. Lipid abnormalities should be managed according to the most current practice management guidelines for the given abnormality. Screening for other endocrine abnormalities that are associated with weight gain, such as hypothyroidism and GHD, should be performed.

Currently, the only FDA-approved treatments for type 2 diabetes mellitus in children are metformin [254] and insulin. Metformin is a biguanide that decreases hepatic glucose production and increases peripheral insulin sensitivity. Further, when used as monotherapy, metformin does not cause hypoglycemia. Some patients cannot tolerate the gastrointestinal side effects such as nausea and abdominal pain that occur in approximately one third of patients. Slowly titrating the dose upward over a period of 6–8 weeks and taking the medication with food help to alleviate these symptoms. Insulin is an option for individuals who are unable to tolerate or are poorly controlled on metformin. It is required for any patient who is ketotic. Usually given in combinations of short- and intermediate-acting formulations, insulin is an effective way to manage type 2 diabetes mellitus in this population.

11.3.8 Effects on Bone Strength

Low bone mineral density on DXA scan has been described in 24–50% of CCS [255–258]. Risk for osteopenia is dependent on numerous patient and treatment-specific factors, including cancer type [259], malnutrition/low BMI [260, 261], reduced muscle strength [262], chemotherapy [263], radiation exposure [261], hypogonadism [258], GHD [259], and glucocorticoid therapy [264]. There is some evidence that male cancer survivors have a higher risk of osteopenia [265]. During childhood and adolescence, skeletal development is characterized by sex- and maturation-specific increases in cortical dimensions and trabecular bone mineral density. This rapid accumulation of bone mass correlates with the rate of growth and requires the coordinated actions of growth factors and sex steroids in the setting of adequate biomechanical loading and nutrition. The COG-FTFU guidelines define low bone mineral density as a z-score more than 2.0 SD below the mean in survivors <20 years old or a *T*-score more than 1.0 SD below mean in survivors ≥20 years old [134]. Patients with exposure to chemotherapy (mainly methotrexate and glucocorticosteroids) or hematopoietic cell transplant and patients who have GHD, hypogonadism, delayed puberty, or hyperthyroidism should be screened with dual-emission X-ray absorptiometry (DXA) scan or quantitative computed tomography (qCT) at entry to a follow-up clinic and repeated as clinically indicated [134]. Low vitamin D levels, although common in the general population, may have a greater clinical significance in cancer survivors due to the increased risk of osteopenia and osteoporosis from multiple other factors. There is some evidence that the prevalence of vitamin D deficiency is higher among cancer survivors than healthy controls [266, 267]. Therefore, vitamin D level should be obtained annually and corrected with supplementation as needed.

Vertebral compression fractures have been reported to occur in children with newly diagnosed ALL. Although historically considered a rare manifestation, the Canadian Steroid-Associated Osteoporosis in the Pediatric Population research initiative showed that 16% of all patients with newly diagnosed ALL had evidence of vertebral compression fracture on radiographic screening [268]. Vertebral spine fractures can also be found in ALL survivors [269]. Numerous studies utilizing DXA have reported bone deficits and fractures in patients with childhood ALL at diagnosis and during treatment, particularly during the maintenance phase of chemotherapy [270, 271]. Additionally, a prospective study using qCT, which provides a three-dimensional measure of trabecular and cortical volumetric bone mineral density and geometry, found that the mean trabecular and cortical BMD z-scores were significantly lower in ALL participants within 2 years from treatment as compared to reference participants. Z-scores were not associated with participant demographics or ALL treatment characteristics. Although trabecular and cortical BMD improved significantly over the study interval, z-scores at the follow-up visit were lower than in the reference group [272]. Additionally, avascular necrosis (AVN) is a well-recognized complication of current therapy for childhood ALL, with a 3-year cumulative incidence of 9% in children treated for ALL [273]. Dexamethasone has been implicated as the main etiological factor. Additional risk is associated with adolescence and Caucasian race [274–276]. AVN is often multifocal, most commonly affecting weight-bearing joints and may cause a significant degree of pain resulting in immobility.

Elevated parathyroid hormone (PTH) levels can be secondary to vitamin D deficiency or hypocalcemia. In one study, hyperparathyroidism, defined as a PTH level >65 pg/mL, was detected in 15% ($n = 4$) of survivors of retinoblastoma. Vitamin D status negatively correlated with the levels of parathyroid hormone [267].

DXA is the preferred method for clinically assessing bone mineral content and areal bone mineral density (BMD). For pediatric and adolescent patients, the PA spine and total body less head measurements are the most accurate and reproducible skeletal sites to evaluate. However, confounding factors in this patient population, such as pubertal delay and GHD/short stature, make the interpretation of DXA results challenging. It is uncertain how much the decreased bone mineral density reported in the literature is due to either an underlying, untreated hormonal deficiency or bone toxicity from the cancer treatment itself. The development of normative data for the pediatric population correcting for height or pubertal development will improve utilization of DXA in these patients [277].

The increasing availability of magnetic resonance imaging (MRI) has enabled earlier

radiological diagnosis of AVN, prior to joint collapse when mild to moderate pain may be the only presenting symptom. The volume and extent of AVN measured on MRI has been shown to predict fracture and joint collapse [278]. Although MRI has a high sensitivity and specificity for diagnosis, no studies have been able to demonstrate the efficacy of using MRI as a screening tool. This is largely due to the lack of knowledge regarding natural history of clinically asymptomatic AVN [276].

It is important to keep in mind that the definitions of osteoporosis differ in the pediatric population compared to adults. The diagnosis of osteoporosis in children and adolescents should not be made on the basis of densitometric criteria alone. In fact, in children and adolescents, the diagnosis of osteoporosis requires the presence of both a clinically significant pathologic fracture and low bone mineral content or bone mineral density. A clinically significant fracture history is one or more of the following: long bone fracture of the lower extremities, vertebral compression fracture, or two or more long bone fractures of the upper extremities [279].

For adults, the European Society for Medical Oncology Clinical Practice Guidelines and Children's Oncology Group recommend a baseline fracture risk assessment, BMD measurement, and guidance on lifestyle modifications (i.e., increasing weight-bearing exercise, smoking cessation, etc.) and calcium and vitamin D supplementation [280]. Promoting an aerobic and resistance training program during cancer treatment has been shown to improve bone mineral density in small pilot studies [281]. In a randomized placebo-controlled trial in CCS from the St. Jude Children's Research Hospital, low-magnitude, high-frequency mechanical stimulation was found to improve mean whole-body BMD z-score by DXA scan compared to placebo [282]. Surveillance for other hormonal deficiencies (hypogonadism, hyperthyroidism, and GHD) in at-risk patients and initiation of replacement are also helpful. While it has been recognized that a majority of the skeletal deficits in this population are related to drug and radiation exposure during treatment, optimization of the hormonal milieu is beneficial.

Currently, the use of bisphosphonates is not routinely recommended in the pediatric population unless the criteria for osteoporosis are met and treatment is clinically indicated. Surgery, including joint replacement and core decompression, can provide symptomatic relief for AVN. However, there is minimal literature demonstrating the efficacy of core decompression in the treatment of AVN or the medical management of AVN using bisphosphonates in the pediatric population. The limited data available in cancer survivors suggest that intravenous pamidronate reduces pain and may delay the natural history of bony collapse but does not prevent late bone collapse and joint destruction [283]. Experience using oral alendronate resulted in gains in bone mineral density, improvement in motor function, and modest gains in health-related quality of life [284, 285].

Case Study

A.A. is a 14-year-old female who was diagnosed with a large cell medulloblastoma at 10 years of age. She underwent surgical resection, and her treatment was based on the Milan high-risk protocol with multi-alkylator chemotherapy, systemic methotrexate, and hyperfractionated accelerated radiotherapy (HART). After HART she received maintenance lomustine, an alkylating agent, and vincristine. A surveillance MRI 2 years post-resection showed a new contrasting small nodule along the resection cavity of the right cerebellum. She underwent repeat resection at age 12 years. At this time, she also underwent an autologous stem cell transplant with conditioning chemotherapy alkylating agents (carboplatin, temozolomide, and thiotepa).

She presented to endocrinology clinic at 13 years of age, following intracranial irradiation therapy with resultant radiation necrosis on brain MRI. Her anterior pituitary function was evaluated at this time. On review of her growth charts, A.A. had not gained any height since 12 years of age. Prior to her diagnosis of medulloblastoma, she had been growing along the 5th percentile, which was in line with her midparental growth potential. She was now well below the 5th percentile. She was Tanner stage 1 for both breast development and pubic hair on exam. Evaluation of her thyroid axis was significant for an inappropriately low TSH (1.65 uIU/mL (0.5–3.8) with a low free T4 (0.6 ng/dL (1–1.8). She was started on levothyroxine therapy, which normalized her T4. She also had prepubertal levels of LH and FSH and an undetectable

estradiol. Her IGF-I was 58 ng/mL (IGF-I z-score: −3.1). She was not started on GH therapy, as there were concerns for tumor recurrence at the time of diagnosis of GH deficiency. Her HPA axis was not evaluated at this time because A.A. presented on hydrocortisone therapy for radiation necrosis but was eventually weaned to hydrocortisone 5 mg AM and 2.5 mg PM (5.35 mg/m^2/day). Her family was taught to provide stress dosing with illness. She developed symptoms of fatigue and anorexia, and her hydrocortisone was increased to physiologic maintenance dosing with resolution of symptoms.

A.A. was non-weight bearing and used a wheel chair as a result of loss of balance from her cerebellar tumor. She developed multiple spine compression fractures and osteonecrosis of the humeral head. She was started on Ca++ 1000 mg daily and vitamin D 2000 IU daily.

11.4 Summary

Damage caused by chemotherapy and radiation therapy may not become clinically evident for many years after treatment. Endocrinopathies following cancer treatment vary widely by type of cancer, therapy, gender, and age. The COG-LTFU has issued specific guidelines for the cancer of CCS. Yet, general internists caring for adult CSS reported being "somewhat uncomfortable" on average caring for CSS and being generally unfamiliar with current guidelines for long-term follow-up of CSS [286]. It is important to educate both patients and providers regarding the need for long-term follow-up to ensure proper surveillance, diagnosis, and treatment of these late complications of therapy. Through the early identification and treatment of endocrine late effects, physicians have the ability to significantly improve patient quality of life and decrease morbidity and mortality.

Review Questions

1. A 10-year-old male who received cranial radiation for ALL at age 4 years has recently been started on GH. At this time, he has no additional anterior pituitary deficiencies. After 3 months of treatment, he experiences a rapid growth spurt, which translates to an annualized growth velocity of 9 cm/year. What aspects of his physical exam should be closely evaluated for an etiology of this growth spurt?
   A. Thyroid to monitor for a goiter
   B. Standard physical exam – his growth spurt is most likely secondary to his GH therapy
   C. Genital exam to assess for Tanner staging of pubic hair and testicular measurement
   D. Sitting height measurement to assess for disproportionate spinal growth
2. The most likely etiologies (from most to least likely) of growth failure in a childhood cancer survivor who received craniospinal radiation are:
   A. Hypothyroidism, ACTH deficiency, hypogonadotropic hypogonadism, and GHD
   B. GHD, ACTH deficiency, hypothyroidism, and hypogonadotropic hypogonadism
   C. GHD, hypothyroidism, hypogonadotropic hypogonadism, and ACTH deficiency
   D. Hypogonadotropic hypogonadism, ACTH deficiency, hypothyroidism, and GHD
3. Possible causes of obesity in the childhood cancer population are:
   A. Steroids
   B. Inactivity after the completion of cancer therapy
   C. Cranial radiation
   D. All of the above

Answers

1. (C) *Genital exam to assess for Tanner staging of pubic hair and testicular measurement.* Cranial irradiation, particularly to the hypothalamic region, has been associated with the development of central precocious puberty (CPP) at both lower doses for leukemia treatment (18–35 Gy) and higher doses for brain tumor treatment (>35 Gy). Rapid increase in growth velocity to pubertal rates can be mistaken as a response to GH therapy. A careful genital exam and laboratory evaluation with LH, FSH, and testosterone or estrogen should be done to assess for central precocious puberty. Such a robust response to GH may occur in child with true GHD *(B)* in

response to therapy. However, failure to identify CPP can cause premature closure of the epiphyseal plate and stature further below predicted height despite treatment with GH. Hyperthyroidism *(A)* may cause increase in height but is not a typical sequelae of cranial radiation. Hyperthyroidism may occur after radiation to the thyroid gland. Craniospinal radiation or vertebral fractures can disrupt spinal growth, causing scoliosis. This would decrease sitting height and would likely not manifest as an increase in growth velocity.

2. (C) *GHD, hypothyroidism, hypogonadotropic hypogonadism, and ACTH deficiency.* GH is the most vulnerable anterior pituitary hormone and is often the only anterior pituitary deficit to develop after cranial irradiation. Both secondary and primary hypothyroidism can occur after craniospinal radiation. Hypogonadotropic hypogonadism also can occur, but ACTH deficiency is the least common anterior pituitary defect after cranial radiation.
3. (D) *All of the above.* Steroids and inactivity both during and after treatment for ALL have been associated with increased weight gain and obesity. Cranial radiation may cause hypothalamic obesity with resultant rapid weight gain. During routine annual follow-up care, all survivors should have height, weight, and blood pressure measured, BMI calculated, and percentiles noted. The COG-FTFU guidelines recommend measuring fasting blood glucose or HgbA1c and lipid profiles every 2 years, or more frequently if indicated based on patient evaluation, and counseling at every annual visit regarding proper nutrition, exercise and obesity-related health risks.

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Endocrinologic Sequelae of Anorexia Nervosa and Obesity

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Key Points

- Restrictive eating disorders such as anorexia nervosa result in multiple endocrine alterations with accompanying relative growth hormone resistance and hypercortisolemia due to hyperactivity of the hypothalamic–pituitary–adrenal axis.
- Thyroid abnormalities represent a "euthyroid sick" profile and typically normalize with weight gain and psychological recovery, without the need for L-thyroxine replacement.
- Anorexia results in abnormalities in the reproductive axis in both boys and girls, and in girls, oligomenorrhea and, more commonly, amenorrhea can ensue.
- Early bone loss and/or lack of bone accrual represent serious complications in adolescent girls and boys with anorexia nervosa, with some of the skeletal losses representing irreversible changes.
- Obesity results in endocrine changes of the hypothalamic–pituitary–adrenal axis resulting in hypercortisolism and in the hypothalamic–pituitary axis resulting in altered growth hormone secretion.
- Adiposity accumulation and resultant obesity lead to multiple metabolic changes that increase the risk for future cardiovascular disease and type 2 diabetes.
- Mild thyroid abnormalities can be seen in obesity in adolescents and can be improved with weight loss.
- Obesity results in abnormalities in the reproductive axis in both boys and girls, including the common disorder, polycystic ovary syndrome (PCOS).
- Increased adiposity can impact bone health, resulting in increased fracture risk in some populations.

12.1 Anorexia Nervosa

12.1.1 Introduction

Anorexia nervosa (AN) is a severe psychiatric and medical condition once described as the "relentless pursuit of thinness" [1]. The Diagnostic and Statistical Manual of Mental Disorders, 5th edition (DSM-5) was published in 2013 and includes a significantly revised eating disorder section. By the new criteria, the lifetime prevalence for AN was 1.7% among over 2000 adolescents and young adults in a recent Dutch cohort study [2]. The disorder is most commonly seen among adolescent girls, with estimates that 19–30% of younger patients with AN are male, and the overall prevalence of this disorder among adolescent boys appears to be increasing [3, 4]. Testosterone deficiency and many of the endocrine alterations seen in girls are seen in adolescent boys with restrictive eating disorders [5, 6]. Eighty-five percent of patients with AN present between the ages of 13 and 20 years during a critical period for growth, pubertal development, and maximal bone accretion that culminates in peak bone mass. The disorder can result in a compromise in each of these important endocrinologic events, with lifelong sequelae. Reports over the past decade document an earlier age of onset of AN [7], and it is recognized that onset at a younger age is associated with poorer growth and bone health outcomes [8, 9].

Patients with AN also have a characteristic clinical picture of endocrine dysfunction, including amenorrhea, abnormal temperature regulation, elevated growth hormone (GH) levels, hypercortisolemia, and abnormal eating suggestive of hypothalamic or pituitary dysfunction. Therefore, endocrine function has been studied extensively in these patients. The multiple endocrine abnormalities appear to represent an adaptation to the starvation state.

The clinical features of AN by DSM-5 criteria are shown below. A major change in the transition to the new criteria is that amenorrhea is not included in the diagnosis of AN [10]. However, the absence of amenorrhea among the diagnostic criteria should not diminish bone health concerns in the young woman who manifests all other features. There can still be hormonal alterations at play that pose a risk to bone health despite ongoing menses.

DSM-5 Criteria for Anorexia Nervosa

1. Restriction of energy intake relative to requirements, leading to a significantly low body weight
2. Intense fear of gaining weight or of becoming fat or persistent behavior that interferes with weight gain, even though at a significantly low weight
3. Disturbance in the way in which one's body weight or shape is experienced, undue influence of body weight or shape on self-evaluation, or persistent lack of recognition of the seriousness of the current low body weight

12.1.2 Hypothalamic–Pituitary–Adrenal Axis in AN

Patients with AN exhibit hyperactivity of their hypothalamic–pituitary–adrenal (HPA) axis [11, 12]. These patients typically have elevated serum cortisol concentrations, accompanied by increased corticotropin-releasing hormone (CRH) secretion and normal circulating levels of adrenocorticotropic hormone (ACTH). The elevation in cortisol appears to be secondary to increased cortisol production, decreased clearance, or a combination of both factors [12]. Boyar and colleagues [13] were the first to report decreased cortisol metabolism in AN, subsequently confirmed by other groups. Walsh and colleagues [14] noted that when body size was taken into account (cortisol production/kg), cortisol secretion was significantly increased.

Overactivity of the HPA axis is largely secondary to increased CRH production but with circadian rhythmicity maintained. Adolescents with AN, as compared to healthy controls, have higher cortisol due to increased frequency of secretory bursts, suggesting increased cortisol secretion in this population [15]. Patients with AN may also exhibit inadequate suppression of cortisol after an overnight oral dexamethasone challenge [14, 16, 17]. Estour and colleagues [18] administered dexamethasone intravenously to 15 patients with AN and observed non-suppression in 93%. Results of those studies suggest that the hypercortisolism seen in AN is not suppressible by exogenous glucocorticoid. The abnormalities appear to improve after refeeding and weight gain. Gold and colleagues [11] found increased cortisol response to CRH, while Hotta and colleagues [19] showed a decreased response. Both groups interpret their findings as an indication that there is increased HPA axis activity due to increased CRH secretion in AN.

The adrenal androgens dehydroepiandrosterone (DHEA) and DHEA sulfate (DHEAS) have been reported to be abnormal in some, although not all, studies in young women with AN. In women with AN, DHEAS has been shown to be decreased [20, 21], increased [22], or unchanged [23, 24] as compared to healthy controls. Our group demonstrated that DHEAS inversely correlates with markers of bone resorption in adolescents and young women with this disease [25]. We also showed that a combination regimen of oral DHEA, estrogen, and progestin was found to be safe and effective for preserving BMD in young women with AN [26]. A follow-up study showed significant changes in measures of hip strength and cross-sectional geometry in the treated group compared to placebo that may translate to a lower fracture risk [25, 27].

12.1.3 Insulin, Leptin, and Adiponectin Abnormalities

Studies examining the question of insulin dynamics in AN have yielded contradictory results [28], demonstrating both insulin resistance and insulin deficiency in these patients. Low fasting glucose and insulin concentrations have been reported in AN [25], as have both normal [29] and increased insulin sensitivity [30]. Our group reported low baseline insulin levels as well as subnormal insulin rises after oral glucose in patients with AN compared to healthy, normal-weighted controls [31]. We concluded that adolescents with AN exhibit either an isolated resistance to glucose on a pancreatic level or compromised pancreatic

function after months of starvation, with a diminished ability to respond to high-glucose challenge.

Leptin, a peptide hormone secreted by adipose tissue, is altered by adaptations to a starvation state [32]. The subnormal plasma leptin concentrations seen in AN [33] likely reflect the decreased fat mass in these subjects. Subnormal leptin levels in patients with amenorrhea suggest that this hormone may serve as a metabolic signal to the reproductive axis [34–36]. Mantzoros and colleagues supported this theory through a study of eight women with hypothalamic amenorrhea, demonstrating that leptin administration improved reproductive, thyroid, and growth hormone axes and increased markers of bone formation [35]. However, of concern in studies of leptin replacement in these patients was a decrease in weight and fat mass that has been observed in two trials [37, 38].

Adiponectin, an adipokine produced by adipocytes, varies inversely with fat mass [36]. Low levels of adiponectin are associated with insulin resistance and hyperinsulinemia [38]. Adiponectin has been shown to be both elevated [39] and low [40] in adult women with AN. Similarly, adiponectin levels have been reported as normal [41] and elevated [42] in adolescents with AN, as compared to healthy controls and may explain in part the variability of BMD seen in these patients [41]. Alterations in adiponectin levels likely reflect low energy availability in patients with a decreased fat mass. Both adiponectin and leptin are adipokines. The fat-regulated hormones ghrelin and peptide YY appear to signal energy availability to the hypothalamus and may contribute to decreased gonadotropin pulsatility, hypothalamic amenorrhea, and ultimately a lower bone mass [43, 44].

12.1.4 Growth Hormone Abnormalities

Elevated serum growth hormone concentrations are found in at least one-half of emaciated anorexic patients [45, 46] and return to normal with weight gain [47]. Serum concentrations of insulin-like growth factor-I (IGF-I) are suppressed, indicating a state of acquired GH resistance, and levels normalize after nutritional therapy [48]. This GH resistance was not overcome in a trial of supraphysiologic recombinant human GH in women with AN, although the women exhibited an increase in lean body mass after treatment [49]. GH resistance has been attributed to consequences of starvation, but data are conflicting regarding the relative contributions of severity of weight loss and caloric deprivation.

Whereas increased basal levels of GH represent a reasonably consistent finding in emaciated patients with AN, GH responses to provocative tests have been less consistent [48]. Patients with AN exhibit impaired GH responses to L-dopa and apomorphine administration, and two classic studies showed that these findings persist even after nutritional rehabilitation [50, 51]. The GH response to arginine has been reported as normal in one study [52]. A paradoxical increase in GH secretion following a glucose load has also been reported [53].

12.1.5 Thyroid Hormone Abnormalities

Thyroid function tests are abnormal in many patients with AN and likely reflect an adaptive response to permit conservation of energy. Serum levels of T4 and T3 [23] in these patients are significantly lower than in normal individuals. In AN, as in starvation, peripheral deiodination of T4 is diverted from formation of active T3 to production of reverse T3 (rT3), an inactive metabolite [54]. Levels of T3 correlate linearly with body weight, expressed as a percentage of ideal [55], and normalize with weight gain [56]. Higher levels of rT3, the less active form of the hormone, may explain the occurrence of hypothyroid symptoms, such as fatigue, constipation, and hypothermia, which occur commonly in these patients despite normal to slightly subnormal T4 levels. Levels of thyroid-stimulating hormone (TSH) are within normal limits [56] and are not related to body weight in AN [56]. However, peak TSH response to thyroid-releasing hormone (TRH) stimulation appears to be delayed (e.g., to 120 min) [57] and may be augmented [55], suggestive of a hypothalamic defect.

12.1.6 Hypothalamic–Pituitary–Ovarian Axis Abnormalities

Amenorrhea is historically one of the cardinal features of AN and is due to hypogonadotropic hypogonadism. Studies of markedly underweight

patients with AN have shown low plasma gonadotropin levels in these patients [46]. A positive relationship between resting luteinizing hormone (LH) levels and body weight has been shown, and LH levels normalize with weight gain [58]. Studies of 24-h secretory patterns of gonadotropins demonstrate that significant weight loss induces a pattern of follicle-stimulating hormone (FSH) and LH secretion resembling that of prepubertal girls [59]. The pattern is characterized by either low LH levels throughout the day or decreased LH secretory episodes during waking hours. The LH response to gonadotropin-releasing hormone (GnRH) may also be significantly reduced in these patients. The response is correlated with body weight, so that patients with the greatest weight loss have the smallest rise in LH in response to GnRH [58].

Weight loss itself does not appear to explain the relationship between nutritional deprivation and disturbances in menstrual function, as amenorrhea precedes significant weight loss in half to two-thirds of patients [46] and may persist despite weight restoration [60]. Return of menstruation in patients with AN correlates with regaining weight, although not all patients recover menses [61]. A number of investigators have identified mean thresholds associated with reestablishment of menses in girls with AN based upon estimates of percentages of body fat using height and weight measurements [62], percentage of ideal body weight [63], and body mass index (BMI) [64]. However, it has been shown that return of menses does not show a simple relationship to weight or body fat [65], although the majority of patients resume menstruation when weight has returned to at least 90% of ideal [47]. These findings are in accord with the work of Frisch and colleagues [62] indicating that the onset and continuation of regular menstrual function in women are dependent on the maintenance of a minimal weight for height. This threshold has been proposed to represent a critical level of percentage body fat [62] and implies that body composition may be an important determinant of reproductive fitness in the human female. However, identifying clinical features that distinguish those who have restoration of menstruation from those who do not has been difficult and is not always explained by variations in body composition. In one recent study of women with prolonged amenorrhea despite weight restoration, Arimura and colleagues identified high baseline serum cortisol level as predictive of delayed restoration of menses [66]. Following weight restoration and resumption of menses, patients with AN appear to have normal fertility [61], although this has not been well-studied.

12.1.7 Prolactin

Fasting morning concentrations of prolactin are normal in adolescents and women with AN [56, 67], and there is no relationship between basal prolactin and body weight, estradiol, or gonadotropins [56]. Nighttime prolactin levels may be reduced, possibly secondary to dietary factors as nocturnal prolactin is reduced by a vegetarian diet in healthy, normal-weight subjects [68].

12.1.8 Vasopressin and Oxytocin

Partial diabetes insipidus has been reported in AN [67], as have abnormally high cerebrospinal fluid (CSF) arginine vasopressin (AVP) levels [69]. Patients with AN have also been shown to have decreased CSF oxytocin concentrations, along with reduced oxytocin responses to stimulation [70]. These findings appear to reverse with weight gain, suggesting that they may be secondary to malnutrition, abnormal fluid balance, or both [71].

12.1.9 Ghrelin and Peptide YY

Recent reports have demonstrated abnormalities in appetite regulatory peptides in women with AN, and ongoing research on these proteins is leading to a greater understanding of the underlying pathophysiology of AN. Ghrelin, an orexigenic hormone, has been shown to be elevated in adolescents and women with AN [72, 73]. Studies of peptide YY (PYY), an anorexigenic peptide released by the gut, have shown conflicting results, with both elevated [74] and normal levels [75] observed in women with AN.

12.1.10 Bone Loss and Abnormalities in Skeletal Dynamics

The amenorrhea that accompanies AN during adolescence and young adulthood appears to have permanent effects on bone density, since

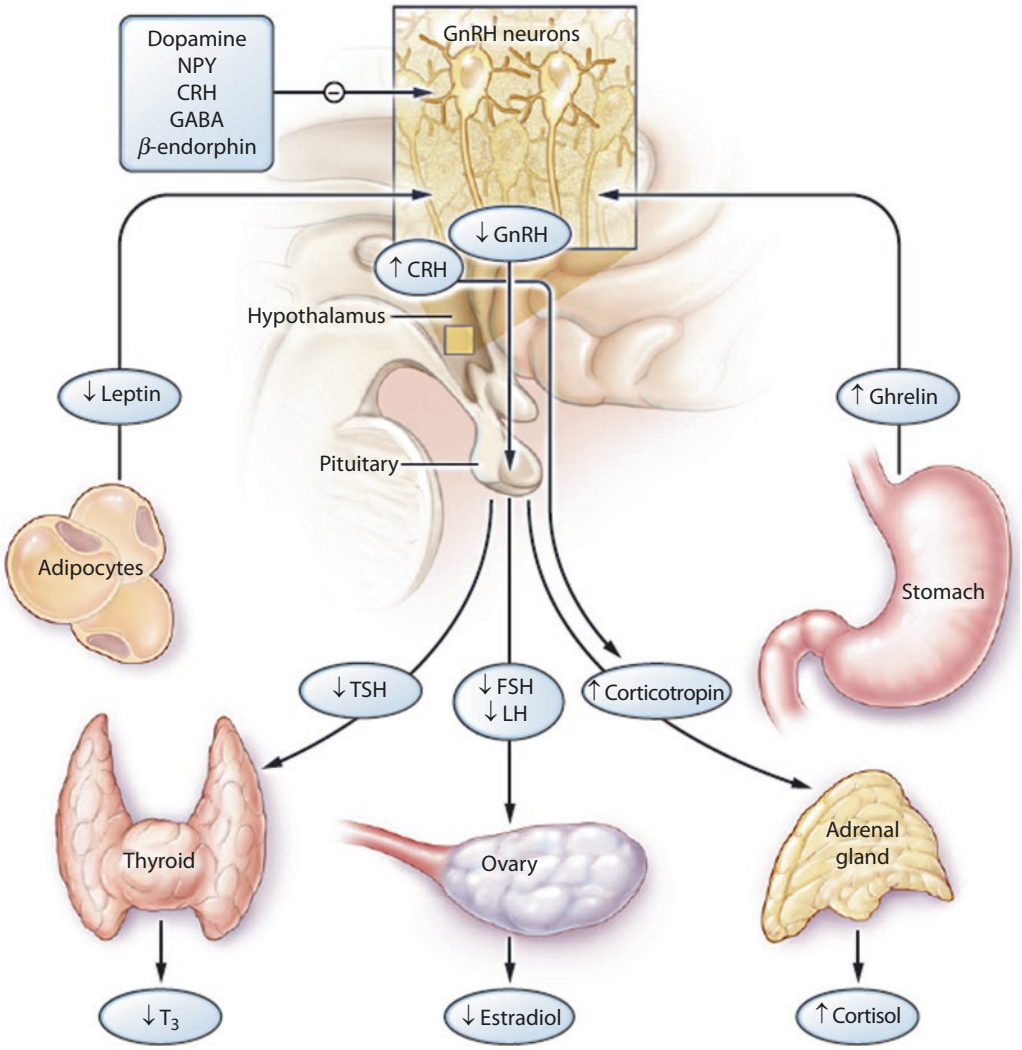

Fig. 12.1 Hormonal abnormalities in adolescent girls with AN and effects on multiple tissues (From: Gordon [44]. Copyright © 2010 Massachusetts Medical Society. Reprinted with permission from the Massachusetts Medical Society)

rapid bone accretion occurs during puberty [76–78]. A serious complication of AN includes profound deficits of both trabecular and cortical bone compartments [79] with spinal bone density reported to be greater than 2 SD below normal in 50% of young women with this disease. The bone loss is so severe that clinical fractures at multiple sites have been documented in women during late adolescence and young adulthood [79–81]. The multiple hormonal alterations which ultimately contribute to bone loss in AN, including increased bone resorption and decreased bone formation, are summarized in Fig. 12.1.

An uncoupling of bone formation and bone resorption is observed in women with AN [82], with multifactorial mechanisms contributing to the bone loss in AN. Although estrogen deficiency is characteristic, estrogen therapy alone does not result in significant increases in bone density. Klibanski and colleagues reported a positive effect of combined estrogen and progestin therapy on bone density only in young women who were <70% of ideal body weight [83]. Oral contraceptives containing both estrogen and progestin are generally not adequate to halt the bone loss seen in AN [84, 85]. There also appear to be direct effects of undernutrition on bone, as IGF-1 levels are subnormal and correlate with markers of bone formation [86, 87]. Misra and colleagues have studied short-term recombinant

human IGF-1 therapy in adolescents with AN for its potential effect on bone density and found that after 7–9 days of treatment, the girls had increases in surrogate markers of bone formation [88]. A randomized, controlled trial of combination therapy with oral contraceptive and IGF-1 in women with AN demonstrated modest gains in bone mineral density over 9 months [89]. A recent study of transdermal estrogen to adolescents with AN showed promising results after 18 months, with bone accrual rates almost matching those in the health control group [90].

Deficiencies in androgens, notably DHEA, have also been demonstrated in some studies [21, 91] which may be clinically significant as DHEA appears to have both anabolic and antiosteolytic effects on bone [87, 92]. As noted earlier, our group demonstrated that combined therapy with DHEA and a combined oral contraceptive pill preserves BMD and favorably alters hip bone cross-sectional geometry in young women with AN [27, 28]. Lastly, while some studies have demonstrated that bisphosphonate treatment reduces bone turnover and increases bone mineral density in adolescents and adults with AN [93, 94], a better understanding of the long-term risks of bisphosphonates is required before these drugs can be considered as part of routine care for use in this population. At the present time, these agents should be used with extreme caution and only under the guidance of a skeletal health expert [44].

A new area of investigation is the contribution of bone-fat interactions to skeletal remodeling. The bone marrow cavity contains significant fat tissue which may modulate bone remodeling [95]. Mesenchymal stem cells within bone marrow differentiate to become adipocytes or osteoblasts, driven by hormonal signals [6]. Adipocytes secrete cytokines and adipokines that may either stimulate or inhibit adjacent osteoblasts. Differentiated bone cells secrete factors that influence insulin sensitivity, and fat cells synthesize cytokines that regulate osteoblast differentiation; thus, the two pathways are closely interrelated. States of malnutrition, such as restrictive eating disorders, have been shown to increase marrow adiposity accompanying concurrent losses of subcutaneous adipose tissue, and there is an inverse association between marrow fat and areal BMD measures in adults with AN [96, 97]. In adolescents and young women with AN, increased marrow fat has been documented to be present in the peripheral skeleton [98], as well as lumbar spine [97].

12.1.11 Patient Evaluation

Patients in whom AN is suspected should undergo a careful patient and family history, physical examination, laboratory tests, and mental health and nutritional assessment. The patient history should focus on weight changes, self-perception of weight and desired weight, a history of bingeing and out-of-control cycles of eating and purging, and uses of laxatives, ipecac, and diet pills. Purging can include hyperexercising. Triggers for the weight loss should also be investigated, such as teasing at school or comments about weight that occurred either in the home or school setting. A careful history around the issues of growth, pubertal progression or delay, and menstrual history is critical as children and adolescents with AN may have delayed puberty and impaired growth and girls may have delayed menarche, amenorrhea, or oligomenorrhea. A family history should include information about eating disorders, obesity, thyroid disease, depression, alcoholism, substance abuse, or other evidence of mental illness.

A review of systems should include questions about abdominal pain, bloating, constipation, esophagitis associated with bulimia, hair loss or texture change associated with AN, cold intolerance, fatigue, weakness, fainting, substance use, and depression. The level of athletic participation and hours per day of physical exercise should be obtained. Special note should be made of previous stress fractures that may reflect an underlying low bone density for age. One should consider that it is often difficult to distinguish classic AN from the "female athlete triad" (recently retermed, "relative energy deficiency in sports" (RED-S)) [99] which includes osteoporosis, amenorrhea, and eating disorders [99]. The important underlying concept is that physically active adolescents and young women can develop an energy deficit that prevents normal hormonal secretion and dynamics that ultimately manifest as alterations in monthly menstrual periods. Adolescent girls with this triad are at increased risk for developing stress fractures not only because of skeletal deficits but also because of an altered pain threshold, including an inability to stop exercising and rest with the onset of pain.

A dietary history should include a 24-h recall of intake. The amounts may be inaccurate because teenagers with AN often overreport their intake. Triggers of bingeing such as stress are important to address. The calcium intake should be estimated by determining the number of servings of dairy products per day or the use of calcium supplements. This assessment is helpful in planning treatment interventions to assure adequate calcium and vitamin D intake because of the increased risk of osteoporosis in patients with AN. It is also important to ask about consumption of caffeine-containing beverages, as these may decrease a patient's appetite and increase heart rate at the time of medical evaluations. Documentation of soda consumption is also important as reports have suggested an association between consumption of these beverages and fractures in healthy adolescent girls [100–102].

The physical examination should include vital signs to assess bradycardia, hypotension, orthostasis, and hypothermia. The weight and height should be recorded in a gown, after urination, so that measurements are consistent between visits. Heights and weights should be plotted on age-appropriate growth charts to determine the patient's weight for height and body mass index. The urine-specific gravity should be measured since these patients often water load, and abnormalities of vasopressin (e.g., partial diabetes insipidus) have been reported [67]. During the skin examination, the clinician should assess for lanugo hair, dry skin, hypercarotenemia, hair changes, and calluses on the dorsum of the fingers (Russell's sign, indicative of bulimic behaviors). On the abdominal exam, the abdomen is typically scaphoid with palpable stool. Other findings include breast atrophy, hypoestrogenic vaginal mucosa, and cool and wasted extremities. The cardiac examination should include an assessment for bradycardia, arrhythmias, and mitral valve prolapse. From chronic vomiting, there may be dental caries or acid erosion of the anterior teeth and parotid hypertrophy.

In assessing the history and physical examination of an adolescent with suspected AN, the possibility of other diagnoses must be entertained: malignancy, central nervous system tumor, inflammatory bowel disease, celiac disease and other causes of malabsorption, diabetes mellitus, hypo- or hyperthyroidism, hypopituitarism, primary adrenal insufficiency, primary depression (with secondary anorexia), and human immunodeficiency virus (HIV), among others. The typical laboratory evaluation obtained at the initial visit includes complete blood count, differential, sedimentation rate, urinalysis, electrolytes, glucose, calcium, magnesium, phosphorus, blood urea nitrogen (BUN), creatinine, and thyroid function tests. If persistent or unexplained amenorrhea is present, serum levels of FSH, LH, and prolactin are obtained before initiation of hormonal replacement therapy. If a patient is sexually active, a urine pregnancy test is obtained. An electrocardiogram is also obtained if the patient is bradycardic or will be using a medication with cardiac side effects. CNS imaging should be considered in a patient with an early or unusual presentation of an eating disorder, growth failure, pubertal arrest, lab results consistent with central hypothyroidism, or neurologic signs and symptoms. Other tests, including endocrinologic assessments, may be considered depending on the patient's presentation.

12.1.12 Management

There are limited evidenced-based guidelines for the treatment of AN, and treatment guidelines often rely upon expert recommendations [103]. Clinical experience suggests that a multidisciplinary team approach can be helpful. A physician typically assumes the role as a manager of the team, performing vital sign and weight checks and coordinating the overall communication with the family. An endocrinologist can either assume the role of manager or help to address specific endocrinologic issues, such as the amenorrhea and bone loss commonly seen in these patients. A nutritionist works with the adolescent and family around meal planning and recommendations for caloric requirements and calcium intake. A psychotherapist provides individual and/or family therapy.

The indications for hospitalizations include unstable vital signs, hypotension, orthostasis, bradycardia, severe malnutrition (75% ideal body weight), dehydration, abnormal electrolytes, arrhythmias, acute food refusal, uncontrollable bingeing and purging, suicidality, and failure of outpatient therapy. Treatment options include medical hospitalization, psychiatric hospitalization, and day treatment psychiatric programs.

Osteoporosis is a significant health risk for adolescents with AN and often becomes a long-term follow-up issue for an endocrinologist. The bone loss and resulting low bone density are often irreversible and may be a source of both short- and long-term morbidity. The degree of bone loss has been associated with duration of both disease and amenorrhea. As short a period as 6 months of estrogen deficiency may have a negative impact on bone density. Other factors to consider include inadequate calcium and vitamin D intake, hypercortisolism, and adrenal androgen deficiency. The most important approach to the prevention and treatment of low bone mass in AN is the restoration of a normal body weight. Hotta and colleagues [64] found that BMI >16.4 ± 0.3 kg/m^2 was associated with an improvement in bone mineral density. Shomento and Kreipe [63] have found that a mean of >92 ± 7% of ideal body weight was associated with a return of menses. Our group showed that a higher percentage body fat by DXA was correlated with return of menses [104]. Golden and colleagues noted that even with restoration of normal body weight, persistent amenorrhea has been associated with low leptin levels [105]. Return of menses is an important milestone implying the provision of normal estrogen levels to all tissues, including the potential to improve bone mass. Hormonal therapies have been tested with mixed results. At this time, promising possibilities include a combination of estrogen, progestin, and DHEA replacement or gonadal steroids and IGF-I to limit BMD loss in AN. In young adolescents, transdermal estrogen replacement yielded near-normal bone accrual over a period of 18 months [90]. However, further studies are needed. For some patients who have reached their ideal weight but still have not had a return of menses, short-term (i.e., 4–6 months) estrogen monotherapy can be helpful to replete estrogen stores, particularly in those young women who show no withdrawal bleed in response to a progestin challenge (medroxyprogesterone acetate, 5–10 mg daily for 10 days) [44]. Provision of adequate calcium and vitamin D intake is important during the critical period for bone accretion. These patients should receive 1300 mg of elemental calcium and 600 international units of vitamin D daily [IOM], although appropriate supplementation doses are debated. Physical activity is associated with increased bone formation [106], and exercise regimens should be individually tailored to take into account hemodynamic stability, level of fitness, and extent of bone loss.

12.1.13 Conclusion

Patients with AN exhibit multiple endocrinologic abnormalities. Most of the abnormalities noted are an adaptive response to starvation and reverse with weight restoration. Bone loss with potential osteoporosis appears to be the only irreversible endocrinologic abnormality cited to date. Research is clearly needed to understand the multifactorial etiology of disordered eating in adolescents and to develop strategies to promote healthy eating patterns in young people. In addition, given that the bone loss seen is often irreversible, future research will hopefully elucidate mechanisms behind this complication and continue to provide guidance as to new treatment strategies for these young women.

Case Study

Patient and Diagnostic Evaluation

Janet is a 13-year-old girl who presents to the endocrinology clinic with the complaints of no menses for 3 months. She presents to clinic with her father who reports that she has always been thin but healthy. She started to run track last year and has become increasingly concerned about her weight. Since her annual health visit last year, she has lost 6 lb and has not grown. Her menarche was 6 months ago, at the age of 12 ½ years. She had a regular, 4-day menstrual period for 3 months and no subsequent menses. She denies purging (through hyperexercise or vomiting) and use of diet pills or laxatives. She denies depression, but her father notes that she does not seem to be as interested in interacting with her peers outside of school and her track practices and meets. Complaints include feeling cold and constipation. Otherwise, review of systems is negative.

Past medical history: She was born at term after an uncomplicated pregnancy; normal spontaneous vaginal delivery. No

chronic disease. She receives no medications and takes no supplements. She sustained a tibial stress fracture at age 12 at the end of track season.

Her primary care provider noted a BMI of 17 kg/m^2 at her 12 year annual physical and was somewhat concerned. BMI at this visit is now 15.8 kg/m^2. Heart rate is 51 and blood pressure 98/40. Her primary care provider obtained screening laboratory studies to start a diagnostic work-up.

The results were:

- Glucose 64 mg/dL, electrolytes normal
- AST: 64 unit/L
- ALT: 54 unit/L
- Cholesterol: 195 mg/dL
- LDL: 155 mg/dL
- Triglycerides: 195 mg/dL
- HDL: 42 mg/dL
- T4: 6.4 mcg/DL
- TSH: 0.9 mcIU/mL
- Total T3: 0.63 ng/mL
- Prolactin: 5.6 ng/mL
- DHEAS: 79 mcg/dL
- FSH: 1.0 mIU/mL
- Estradiol: undetectable

Janet's laboratory results are classic for a restrictive eating disorder. Reassuring is the fact that electrolytes are normal, as hypokalemia could reflect chronic purging (i.e., self-induced vomiting). Her glucose is low normal, transaminases are slightly elevated, and the lipid panel is altered (with elevated total cholesterol, LDL, and triglycerides). The low TSH and total T3 and low normal T4 represent the "euthyroid sick" pattern. The DHEAS is low for age. The constellation of results reflect an underlying adaptation to starvation. The appropriate next step would be for the pediatrician to refer the patient to a multidisciplinary program that would provide medical, nutritional, and psychological support for this young adolescent, ideally with a family-based component that would provide additional guidance and support for her family. While she is not hemodynamically unstable such that immediate hospitalization would be warranted, her borderline bradycardia and hypotension suggest that an immediate referral should be made. Long-term evaluation would include DXA screening given the history of stress fracture.

12.2 Obesity

12.2.1 Introduction and Background Information: Definition of Obesity and Prevalence in Adolescents

Obesity, defined by weight above the 95% for age and gender based on standardized growth curves (CDC, 2000; ▶ http://www.cdc.gov/growthcharts/clinical_charts.htm), is a growing public health crisis in the United States and worldwide. Over 30% of children and adolescents are overweight or obese, almost 17% are obese, and the prevalence does not appear to be plateauing in this age range [107]. The accumulation of adiposity during puberty contributes to the development of multiple metabolic and endocrinologic abnormalities. Adolescents are growing rapidly and progressing through pubertal changes and, therefore, teleologically are programed for efficiency in fuel utilization in preparation for the reproductive phase in life. Therefore, when adolescents gain excess weight and become obese, the physiologic insulin resistance and high levels of growth hormone, estrogens and androgens, lead to pathologic insulin resistance and resultant abnormalities in glycemia, lipids, liver function, musculoskeletal functioning, and reproductive function. In addition, obese adolescents demonstrate an endocrine profile that in some aspects is the polar opposite from AN but with some overlapping features including amenorrhea, altered growth hormone values, hypercortisolemia, and an altered appetite and metabolic rate due to alterations in central signaling pathways. In some cases, the abnormalities are the direct opposite of those present in the starvation state of AN, but obesity also represents a pathologic state of excess adiposity resulting in other compensatory mechanisms being activated.

12.2.2 Etiology

Childhood obesity is a multifactorial condition impacted by the perinatal environment, genetics and epigenetic changes, early childhood nutrition, levels of physical activity, and, in some cases, medical conditions and underlying syndromes. There have been some monogenic causes of obesity identified [108, 109], but even the most common of these the MCR4 (melanocortin 4 receptor) mutations accounts for less than 5% of obesity cases in most published series. Therefore, the genetic components are primarily polygenic, and there is increasing evidence that perinatal and early childhood factors may contribute to

epigenetic changes that impact the risk of obesity in later life. Obesity is caused by an imbalance in the energy intake and energy expenditure but is more complex than a simple scale imbalance. Once obesity is established, attempts to reduce energy intake to improve obesity are accompanied by a resultant reduction in energy expenditure, preventing the weight loss and increasing the need to further reduce energy intake. This has been validated through mathematical modeling algorithms [110]. Although obesity is defined by BMI, similar BMIs in different children can cause very different metabolic and endocrinologic effects. For example, adiposity that is deposited in the abdominal cavity, termed visceral adiposity, has a more metabolically unfavorable effect when compared to fat deposited under the skin or subcutaneous adiposity. Thus, individuals who tend to accumulate fat centrally with increased visceral fat, described as an apple phenotype vs. a pear phenotype to depict the characteristic shape of the fruit, are more metabolically and endocrinologically abnormal for the same BMI and overall percentage of body fat.

Fat tissue itself, once thought to be just a byproduct of weight gain, has been found to be metabolically active, secreting hormones to signal its presence or absence. This has an impact on metabolic rate and hunger and triggers many of the endocrinologic changes related to weight gain. The substances secreted from the adipocytes, called adipocytokines, have an impact throughout the body. Of note, there are also different types of fat. One type, brown fat, is actually a metabolically favorable fat tissue that is often present in young children and declines with age. In general, when we refer to adipose tissue that develops with weight gain, we are referring to white fat, as brown fat appears to be reduced in both obesity [111] and anorexia nervosa [112].

12.2.3 HPA Axis in Obesity

Cushing syndrome results in an overproduction of cortisol. Hypercortisolism results in central obesity, a full, round face, muscle atrophy and slowing of linear growth in children who have not yet reached final adult stature. In association with the central adiposity, patients can present with metabolic abnormalities including hyperglycemia, hyperlipidemia, and hypertension. Therefore, this syndrome can phenotypically overlap with obesity associated with metabolic abnormalities. In addition, in obesity, there is some degree of overproduction of cortisol termed pseudo-Cushing syndrome. The enzyme 11-beta hydroxysteroid dehydrogenase-1 is present in adipose tissue and converts cortisone to cortisol; thus, this may contribute to the increased cortisol present in obese individuals.

Several studies have been proposed to distinguish Cushing syndrome from pseudo-Cushing syndrome. The gold standard tests to diagnose Cushing syndrome are 24-h urinary collections for free cortisol, with at least two being elevated to consider further evaluation. The use of midnight salivary cortisol collections and/or dexamethasone suppression tests has also been well supported. However, if these values are elevated, then additional testing may be needed to distinguish between Cushing syndrome and pseudo-Cushing. Although mild elevations are most likely to be the result of pseudo-Cushing in an obese adolescent, it is important not to miss the diagnosis of Cushing syndrome, so in some cases further evaluation is warranted. Proposed tests to distinguish these two entities have included midnight serum and/or salivary cortisol and dexamethasone-CRH testing among others [113, 114]. The Endocrine Society practice guidelines suggest that first-line testing include 24-h urinary free cortisol, midnight salivary cortisol, and 1 mg overnight or 2 mg 48-h dexamethasone suppression tests. If abnormal, a referral to an endocrinologist is warranted to pursue additional testing which could include repeating the first-line tests or additional tests as outlined above.

12.2.4 Metabolic Syndrome

Obesity, the accumulation of adipocytes, creates an inflammatory condition that along with insulin resistance results in a series of features termed the metabolic syndrome. The metabolic syndrome was first defined by Reaven [115] as a list of criteria that all increase risk for cardiovascular disease in adults. This included elevated insulin, altered glucose tolerance, hypertension, low HDL, and elevated triglycerides. The World Health Organization [116], the International Diabetes Federation [117], the National Cholesterol Education Panel Adult Treatment Panel [118],

and the National Heart, Lung, and Blood Institute (NHLBI) [119] have all proposed definitions for the metabolic syndrome, and they have been adapted over time based on changes in criteria for altered glycemia. These features vary greatly among individuals and between different ethnic groups.

Multiple criteria have been proposed for the metabolic syndrome in the pediatric population, and thus there is a lack of consensus for the diagnosis of metabolic syndrome in adolescents. The International Diabetes Foundation [119] proposed criteria for children by age. The criteria for adolescents, ages 10–16 years, include:

- Obesity greater than or equal to the 90%; plus any two of the following:
  - Fasting glucose ≥100 mg/dL or known type 2 diabetes
  - Systolic BP ≥ 130 or diastolic blood pressure ≥ 85
  - Fasting triglycerides ≥150
  - HDL cholesterol <40 mg/dL

The criteria for over 16 years were consistent with the adult criteria and included a measure of waist circumference (>94 cm in men and >80 cm in women); as well as two of the criteria as outlined above with the HDL dichotomized by gender (HDL in men <40 mg/dL and in women <50 mg/dL).

Utilizing NHANES data from 1988 to 1994 and 1999 to 2000, de Ferranti et al. [120] defined metabolic syndrome as including:

- Three or more of:
  - ≥75% waist circumference for age and sex
  - ≥90% for systolic or diastolic blood pressure for age, sex, and height
  - Triglycerides ≥100 mg/dL
  - HDL < 45 in boys ages 15–19 years and <50 mg/dL in all others
  - Fasting glucose ≥110 mg/dL

Most criteria have subsequently lowered the glucose criteria consistent with the changing criteria for impaired fasting glucose to ≥ 100 mg/dL.

12.2.5 Type 2 Diabetes

Type 2 diabetes can be seen in adolescents and is almost always associated with obesity. This disease, when present in adolescents as a consequence of insulin resistance and inadequate insulin secretion, leads to hyperglycemia and increased risk for early progression to secondary complications. Type 2 diabetes in adolescents appears to be more rapidly progressive and results in earlier complications when compared to adult-onset type 2 diabetes. The American Diabetes Association [121] recommends testing children who are:

- Overweight (defined as having a BMI >85th percentile for age and sex, weight for height >85th percentile, or weight >120% of ideal for height)
- And have any two additional risk factors including:
  - Family history of type 2 diabetes in a first- or second-degree relative
  - Race/ethnicity of Native American, African-American, Latino, Asian American, or Pacific Islander
  - Signs of insulin resistance or conditions associated with insulin resistance such as acanthosis nigricans, hypertension, dyslipidemia, and PCOS
  - Maternal history of diabetes or gestational diabetes during the child's gestation

Testing is recommended to begin at age 10 years or at puberty if puberty occurs at a younger age and should be repeated every 3 years.

There are several methods for testing. Fasting plasma glucose can be used, but Hemoglobin A1c can also be used for screening and does not require fasting or timed testing. The criteria used to establish the diagnosis of diabetes mellitus include:

1. Symptoms of diabetes such as polyuria, polydipsia plus casual (random) plasma glucose concentration ≥200 mg/dL
2. Fasting plasma glucose ≥126 mg/dL
3. Plasma glucose ≥200 mg/dL at 2 h during a standard oral glucose tolerance test
4. HgbA1c ≥6.5%

In the absence of unequivocal hyperglycemia, confirmation of the abnormal test is required with a repeat blood sample. If two tests are discordant, the abnormal test should be repeated. The cutoff for diabetes diagnosis is the same in adults and children. Thus, some have questioned the validity of using the same criteria and whether all of the above tests are valid in the pediatric population. The recently published

guidelines from the American Academy of Pediatrics and the TODAY study group recommend the use of lifestyle modification, metformin and insulin, if needed for the treatment of type 2 diabetes in adolescents [122, 123]. There are many other classes of anti-hyperglycemic drugs approved for use in adults with type 2 diabetes. However, none have been extensively evaluated in children. See ▶ Chap. 32 on type 2 DM for more details.

12.2.6 Leptin

Leptin, discovered in 1994 and a product of the ob gene, is secreted by adipocytes, and provides a signal to the central nervous system that adipose tissue is present. Leptin is correlated with the degree of obesity. Leptin receptors are present in the central nervous system in the hypothalamic regions known to regular hunger and satiety but are also present on various organs. Low leptin levels stimulate hunger and also reduce energy expenditure with the goal of normalizing fat mass if underweight, as noted in anorexia nervosa. Therefore, elevated leptin levels in obesity should provide a protective mechanism against excessive adipose accumulation by reducing appetite and reducing adiposity. However, this does not appear to occur in obese individuals. Thus, there is some degree of leptin resistance that develops in obesity allowing for increased hunger in the setting of increased leptin.

Leptin is also thought to be a significant factor in signaling to the HPO axis. In states of underweight such as anorexia nervosa, the low levels of leptin serve to halt GnRH pulsatility and reduce the progression of puberty and ovulation to prevent reproductive capacity during times of physical stress. In the very rare patient diagnosed with true leptin deficiency, puberty is halted until leptin is replaced [124].

12.2.7 Adiponectin

Adiponectin is also an adipocytokine and it is increased in weight loss. Low levels of adiponectin are associated with more metabolic abnormalities including insulin resistance. It serves as a protective factor, suppressing some modulators of inflammation.

12.2.8 Growth Hormone

Obesity has an impact on the growth hormone axis, reducing basal and stimulated growth hormone levels [125, 126]. Growth hormone levels in the range used to diagnose growth hormone deficiency are present in obese adults and are associated with increased dysmetabolic profiles and metabolically unfavorable adipose tissue distribution, including increased visceral adiposity [127, 128]. In fact, recent studies have suggested that visceral adiposity may be more associated with lower growth hormone levels than overall weight or body mass index [129, 130].

Obesity induces a reduction in growth hormone secretion from the pituitary gland, but studies are mixed as to whether this results in a decrease, increase, or no change in total IGF-1 and/or free IGF-1 levels. This is an important practical point, as IGF-1 is often used to screen for growth hormone status, and the gold standard tests used to diagnose growth hormone deficiency in children, the growth hormone stimulation test, has been found to categorize significantly more children at a higher BMI with growth hormone deficiency when compared to lower BMI peers [131].

The mechanisms that induce a reduction in growth hormone secretion in obesity are likely multifactorial and interrelated. The reduction in growth hormone may occur due to hyperinsulinemia resulting in negative feedback on growth hormone production [132]. Other theories suggest that it is related to the hyperinsulinemia suppressing insulin-like growth factor-binding protein-1 (IGFBP-1), which, in term, modifies the free IGF-1 available and thus suppresses growth hormone secretion. However, studies are inconclusive when free IGF-1 is measured in the setting of obesity or acute overfeeding [133–135]. Some studies have suggested that free fatty acids are a primary factor in reduction of growth hormone levels in obesity. Free fatty acids are known to suppress growth hormone production and are increased in obesity [136]. In an experimental design, the use of a medication known to reduce free fatty acids resulted in improved growth hormone secretion in obese adults [137]. Free fatty acids may have a direct effect or act through somatostatin to decrease growth hormone in obesity [138]. Changes in ghrelin, a gut-derived peptide that stimulates hunger and binds to the growth hormone secretagogue receptor, may also

play a role in the impact of obesity on growth hormone [139].

Reduced growth hormone secretion can be reversed with weight loss [140]. The low levels of growth hormone may contribute to the worsening of the metabolic state in obesity, as low growth hormone levels are associated with increased visceral adiposity, which itself is associated with increased inflammation and worsening of metabolic risk factors [141]. In conditions that cause primary growth hormone deficiency, increased adiposity, particularly visceral adiposity, is a prominent feature.

As noted above in the discussion of the adrenal axis, 11-beta hydroxysteroid dehydrogenase is present in adipose tissue and converts cortisone to cortisol. Growth hormone plays a role in inhibiting this activity. Therefore, the reduction in growth hormone may also contribute to the increase in cortisol which then increases adiposity [142]. Therefore, the obesity induced reduction in growth hormone levels, like the increase in insulin levels may induce a cyclical reaction, promoting additional weight gain and increased adiposity, particularly visceral adiposity. Thus, it remains unclear whether the reduction in growth hormone secretion is a physiologic metabolically favorable development, inducing a reduction in glycemia, or an unfavorable development, increasing visceral adiposity through reduced lipolysis and increasing inflammatory mediators. Therefore, the use of supplementation of growth hormone or its stimulatory factors in obesity remains an area of active investigation.

12.2.9 Thyroid Hormone Abnormalities

A mildly elevated TSH is often present in obesity, causing clinical confusion as to whether the mild degree of compensated hypothyroidism is a causative factor in the development of obesity or whether it is a result of the weight gain. In general, only profound hypothyroidism causes rapid weight gain, and therefore, a mild TSH elevation is likely caused by the weight gain and not the cause of excessive weight gain. Another clinical clue is that hypothyroidism causes a reduction in linear growth, while obesity often promotes increased linear growth. Adipocytes have deiodinase and therefore participate in thyroid hormone metabolism, which may be a factor in these apparent abnormalities. In a recent study of obese and normal-weight children, the known mild elevation in TSH was demonstrated and noted to correlate with BMI, waist circumference, and lipid abnormalities [143]. Some investigators have speculated that the mild alterations in thyroid function studies are causative factors in the metabolic changes seen with obesity, but only associations have been demonstrated.

12.2.10 HPO/HPT Axis Including PCOS

Polycystic ovary syndrome (PCOS) is a group of clinical features noted in adolescent girls and women that is associated with obesity and insulin resistance, although it can also be present in lean adolescents and adults. The original consensus guidelines [144] included clinical hyperandrogenism (most commonly hirsutism and/or significant acne), hyperandrogenemia, and irregular menses. Associated features included insulin resistance. The diagnosis requires ruling out other causes of hyperandrogenism such as late-onset congenital adrenal hyperplasia and the rare occurrence of an adrenal tumor. Additional evaluation can include ultrasonography of the ovaries as classically PCOS is associated with an increase in ovarian size and in follicle number. However, the use of ultrasound in diagnosis has been questioned due to the frequency (up to 30%) of the PCOS ovarian morphology criteria reported in adolescent females without any other clinical or biochemical evidence of PCOS. Recent consensus statements have included new criteria for polycystic ovarian morphology based on the ever-evolving radiographic technologies [145], including increasing the threshold to 25 or greater follicles per ovary, from the previous threshold of 12. Furthermore, early hyperandrogenism and premature adrenarche, more commonly present in obese girls, have been linked in some populations to an increased risk of progression to PCOS in adolescence and adulthood [146].

Precocious puberty has been found to be more prevalent in obese girls compared to normal-weight girls. This had been controversial in earlier studies due to the criteria used to evaluate thelarche and whether the presence of early puberty might be confused with adipose deposition.

However, recent well-designed studies [147–149] have documented earlier pubertal progression of both thelarche and menarche in obese girls.

The impact of obesity on pubertal development in boys has been less well-studied. However, a recent large investigation of over 4000 boys demonstrated a correlation between age of pubertal onset by testicular size and BMI. Overweight and obese boys were found to begin puberty and progress through puberty at an earlier age [150]. Despite some evidence supporting earlier pubertal progression in boys, obesity has also been associated with reductions in testosterone level and increased risk of gynecomastia, both lipomastia and true breast development. This is thought to be related to the aromatization of androgens to estrogens by adipose tissue. Thus, some degree of hypogonadism may be present in some adolescent males with obesity.

The mechanisms for initiation of earlier pubertal progression in obese children remain an area of active investigation. Leptin is a hormone known to be permissive in pubertal initiation and when lacking causes lack of pubertal changes. Leptin is elevated in obesity. However, while leptin is known to be permissive for puberty, it has not been shown to be a causative factor. Additional mechanistic possibilities include hyperinsulinism leading to a suppression in sex hormone-binding globulin and thus increased bioavailability of sex steroids. The impact of androgens being aromatized to estrogens by adipose tissue has also been postulated to play an important role in early pubertal changes in girls and may have various impacts on the pubertal changes in boys.

12.2.11 Skeletal Impact of Obesity

As described in detail above, AN, a condition of extreme underweight and nutritional deficiency, results in a reduction in bone mineral density. However, the opposite extreme, elevated fat content in obesity also leads to changes in bone density and structure [151]. Despite measured increased bone mineral density, obese children may remain at increased risk for reduced bone strength [152] and fractures [153]. Increased adiposity may cause alterations at the level of the bone through inflammatory markers and mediators. Furthermore, as adipocytes and osteoblasts, the primary components of fat and bone are both derived from the progenitor mesenchymal stem cell; an alteration in one cell type may contribute to alterations in the other [154]. It is well-documented that 25-OH vitamin D levels are lower in obese individuals, which is thought to be attributable to both lack of dietary intake and increased distribution in adipose tissue. However, there does not seem to be a correlation between lower vitamin D levels and reduced bone mineral density or other markers of bone health in obese children [155].

Visceral adiposity, described above as the more metabolically active and detrimental depot of fat when compared to subcutaneous adiposity, seems to also be detrimental to bone health. Higher levels of visceral fat have been found to correlate with lower bone density in obese adolescent girls [156] and obese women [157].

Case Study

Patient and Diagnostic Evaluation

Josie is a 14-year-old girl who presents to the endocrinology clinic with the complaints of rapid weight gain and irregular menses. She and her mother report that she has always been "heavy" but that over the last 2 years, she has gained over 20 pounds per year, without any linear growth change. They have noticed darkening of the skin on her neck. She has had worsening facial acne and progressive hirsutism on the face and chest. Her weight has recently stabilized after reducing some of her daily intake of soda and processed snacks.

Past medical history: She was born at term after a pregnancy complicated by gestational diabetes. She has intermittent asthma but is not requiring daily medical therapy. She reached menarche at age 10 years but has never had regular cyclical menstrual cycling, with menses occurring about every 3–4 months. She is on no medications but has required oral steroid therapy in the past for acute asthma exacerbations.

Her primary care provider noted a BMI of 35 kg/m^2 at her 14 year annual physical and was concerned about her elevated BMI and rapid weight gain. Her primary care provider obtained screening laboratory studies to evaluate causative factors and to screen for potential comorbidities.

The results were:

- Fasting glucose 104 mg/dL
- Fasting insulin 28 uU/mL
- HgbA1C 6.2%
- AST 46 unit/L
- ALT 58 unit/L
- Cholesterol 195 mg/dL
- LDL 155 mg/dL
- Triglycerides 225 mg/dL
- HDL 38 mg/dL
- TSH (thyroid-stimulating hormone) 7.40 mcunit/mL
- Free testosterone 11.1 pg/mL
- DHEAS 400 mcg/dL

Therefore, she was noted to have impaired fasting glucose (>100 mg/dL), a prediabetes range HgbA1C (5.7–6.5%), and mildly elevated liver function studies, potentially consistent with nonalcoholic steatohepatitis. Her lipid panel demonstrated an elevated total cholesterol, LDL, and triglycerides and a low HDL. Her TSH was mildly elevated. Her free testosterone and DHEAS were also mildly elevated. The abnormalities were all consistent with obesity associated metabolic changes and the related syndrome of polycystic ovary syndrome. Specifically, she has insulin resistance as noted by her physical findings of acanthosis nigricans and elevated fasting insulin. She has impaired fasting glucose and a prediabetes range HgbA1C. This along with her history of being the result of a pregnancy complicated by gestational diabetes and family history increases her risk for progression to diabetes. She also has lipids and liver function studies consistent with a dysmetabolic state. Her clinical and biochemical hyperandrogenism along with her irregular menses are consistent with a clinical diagnosis of PCOS. All of the metabolic abnormalities would likely be improved with weight loss resulting in a reduction in inflammation and insulin resistance.

12.2.12 Management

First-line treatment for obesity and related metabolic factors is behavioral change supported by family-centered, multidisciplinary treatment in individual and/or group settings. The goal of behavioral change is to implement an improved diet, targeting reducing the glycemic index in foods, increasing fruits, vegetables, lean proteins and healthy fats while eliminating sugar containing beverages and significantly reducing processed carbohydrates. Physical activity with a goal of 60 min daily, including 30 min three times per week of moderate to vigorous activity, can improve the insulin resistance and inflammatory profile even without inducing weight loss. Changes in both diet and physical activity resulting in weight loss and improvement in metabolic status can improve all of the abnormalities described in the case above. Additional considerations for the treatment of nonalcoholic steatohepatitis (NASH) and PCOS are addressed elsewhere. The mild abnormalities noted in thyroid and growth hormone screening are the result of obesity and do not require hormonal supplementation.

Second-line therapies can include medications and in some cases bariatric surgery. The only FDA-approved weight loss medication in adolescents is orlistat (approved for ≥12 years), a lipase inhibitor. Orlistat, along with behavioral modification, can induce 19% of subjects to have a 5% or greater weight loss, with 9.5% of subjects losing more than 10% of body weight in clinical trials. The most common side effect was abdominal symptoms [158]. Other medications are currently under investigation. Bariatric surgery is also being performed in adolescents, and in recent published data, the results and side effects appear to be similar to those documented in adults when performed at experienced centers [159]. At 3 years, patients had an average weight reduction of 27%, and over 85% had more than a 10% weight loss. The large majority of patients had resolution of diabetes (95%) and hyperlipidemia (66%). The most common complications were micronutrient deficiencies, and 13% of patients needed an additional abdominal surgical procedure.

12.2.13 Summary

In summary, obesity is a common problem in childhood and results in multiple endocrinologic manifestations. These include changes in glycemia, lipids, and other cardiovascular risk factors. In addition, alterations in cortisol production, thyroid function studies, and growth hormone occur. Bone health and reproductive health are also impacted by adiposity. Primary treatment for the endocrine changes resulting from obesity includes treating the obesity with intensive lifestyle management.

Review Questions

1. Each of the following is a hormonal alteration in anorexia nervosa *except*:
   A. Hypercortisolism
   B. Hyperandrogenism
   C. Hypoestrogenism
   D. Low serum leptin
2. The following contributes to bone loss in girls with anorexia nervosa:
   A. Low serum insulin-like growth factor-I (IGF-I)
   B. Low serum cortisol
   C. Low serum ghrelin
   D. High serum leptin
3. Each of the following is a hormonal alteration that is known to occur in obesity *except*:
   A. Hypercortisolism
   B. Hyperandrogenism
   C. Low serum insulin
   D. High serum leptin
4. Which clinical finding would require further evaluation for Cushing syndrome in an obese adolescent in mid-puberty?
   A. BMI > 95% for age and gender
   B. An IGF-1 at the low end of the normal range
   C. A TSH at the high end of the normal range
   D. Slowing of linear growth

Answers

1. B
2. A
3. C
4. D

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III

Adrenal Disorders

Adrenal Insufficiency

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Key Points

- The adrenal glands produce glucocorticoids, mineralocorticoids, and androgens.
- Adrenal insufficiency may result in salt wasting, reduced ability to excrete free water, decreased vascular tone, hypoglycemia, inability to respond to stress, and/or sudden death.
- The most common causes of adrenal insufficiency include autoimmune disease and iatrogenic use of large doses of steroids to treat other disease processes.
- Treatment of adrenal insufficiency in children involves supplementation with synthetic steroids and mineralocorticoids at doses that must balance adequate treatment for a normal life with overtreatment that suppresses growth.

13.1 Introduction and Background Information

Disorders of adrenal function have long been known to cause clinically significant, often fatal, human disease. The initial description of anatomical abnormality of the adrenals in patients succumbing to a process manifested by progressive weakness, pallor, and overall physical decline was provided in 1849 by Thomas Addison [1]. While these initial cases may not have discerned the coincident sequelae of pernicious anemia and primary adrenal failure [2], Addison's continued efforts clarified the association between abnormal adrenals and systemic pathology [3]. The first experimental verification of the importance of the adrenals in animal systems was provided shortly thereafter by Brown-Sequard [4]. Despite recognition of the importance of the adrenal and adrenal hormones in human health and disease during the nineteenth century, the prognosis for patients diagnosed with primary adrenal insufficiency remained very poor until scientists and physicians developed the capacity to chemically synthesize and replace these hormones in the 1940s. The experience of Dunlop, who detailed the outcome of 86 individuals diagnosed with adrenal insufficiency over the period 1929–1958, is particularly instructive [5]. He found that the average life expectancy following diagnosis in 1929–1938, a time during which only salt supplementation and crude adrenal extracts were available, was approximately 1 year. With the ability to administer deoxycorticosterone, a mineralocorticoid, during the interval 1939–1948, the life expectancy for patients with primary adrenal insufficiency improved marginally to approximately 3 years following diagnosis. Not until the ability to specifically replace glucocorticoids in the 1950s did the prognosis for those individuals with primary adrenal failure considerably improve, such that the average life expectancy exceeded 10 years.

Primary adrenal insufficiency often combines defects in mineralocorticoid and glucocorticoid production. Glucocorticoid deficiency alone, however, can produce serious health risks as well. Patients treated with prolonged supraphysiological glucocorticoid doses for management of rheumatological disease were found to be at risk for sudden death during surgical stress if glucocorticoids had been recently terminated [6, 7]. Functions such as regulation of carbohydrate metabolism [8, 9], free water excretion [10–13], vascular tone [14], and the inflammatory response [15] have been ascribed to glucocorticoids. The truly essential aspect(s) of glucocorticoid action, however, remains uncertain.

Cortisol, the primary glucocorticoid in humans, exerts its effects by diffusion from the blood stream across cell membranes where it binds high-affinity glucocorticoid receptors (GRs) in the cytoplasm (Fig. 13.1). Two distinct gene products exhibit high-affinity glucocorticoid (GC) binding, the mineralocorticoid (type 1) receptor and the glucocorticoid (type 2) receptor. Similar to some other members of the nuclear hormone superfamily of receptors, in the non-ligand-bound state, GR exists in a cytoplasmic complex with heat shock proteins and immunophilins [16, 17]. These GR "chaperones" serve to mask the GR nuclear translocation sequence, abrogating modulation of gene transcription by preventing access of GR to glucocorticoid response elements (GREs) or heterodimer partners. When ligand is bound, GR undergoes a conformational change such that the heat shock proteins dissociate, the nuclear translocation sequence is exposed, and GR dimers enter the nucleus where specific genes are either activated or repressed. GR-mediated changes in gene transcription occur by many mechanisms, some only

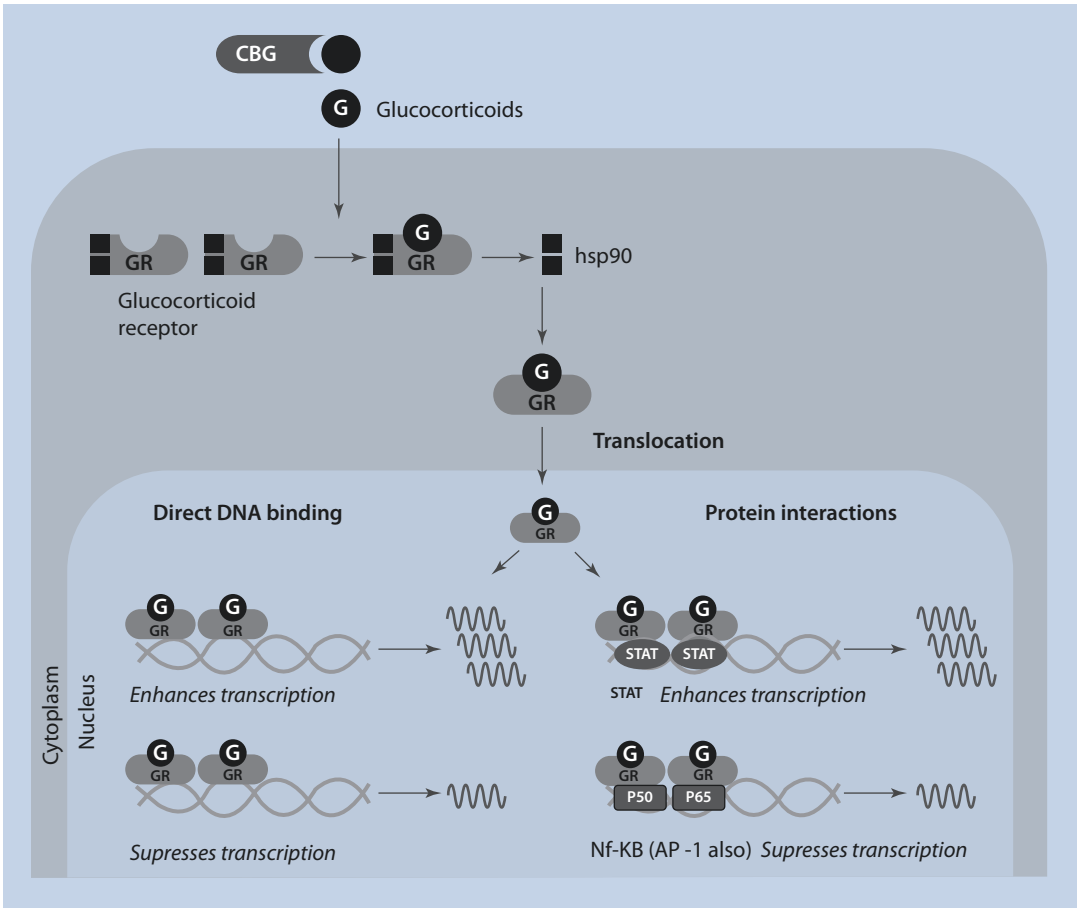

Fig. 13.1 Mechanism of glucocorticoid action. Glucocorticoids (G; small circles) circulate in the bloodstream primarily bound to corticosteroid-binding globulin (CBG). Glucocorticoid dissociates from CBG, diffuses across cellular membranes, and binds the cytosolic, heat shock protein (hsp)-complexed, glucocorticoid receptor (GR; ovals). Upon ligand binding, the GR undergoes a conformational change resulting in dissociation from its molecular chaperones, such as hsp90 (black squares), dimerization, and exposure of nuclear targeting sequences. The dimerized GR enters the nucleus (large circle) to alter transcription of chromatin-packaged genes by direct binding to glucocorticoid response elements or by protein–protein interactions with other transcription factor partners

beginning to be elucidated. One mechanism, for example, is that upon GR binding of GREs in nucleosome-packaged chromatin, coactivators (such as SRC-1/NcoA-1, TIF2/GRIP1, or p300/CBP) are recruited [18]. These GR–coactivator complexes are histone acetylases, serving to "open" DNA–histone complexes for more efficient transcription by the basal transcription machinery, in addition to exposing sites for other transcription activators to bind. Conversely, while not yet demonstrated for GR, other members of the nuclear hormone receptor superfamily can also recruit corepressors (such as SMRT and TIF1) with histone deacetylase activity which serve to "close" chromatin conformation and impede access of the basal transcription machinery [19, 20]. Alternatively, GR actions at composite GREs, consisting of a low-affinity GRE and a binding site for another type of transcription factor, can differentially modulate transcription depending upon the relative abundance of each monomeric component [21]. Finally, GR can modulate transcription of genes which do not contain GREs. For instance, GR has the capacity to directly interact with the p65 subunit of transcription factor nuclear factor κB (NFκB) to block NFκB-mediated gene induction [22, 23]. GR also induces transcription of a functional inhibitor of NFκB, IκBα, which then may serve to block NFκB-mediated gene activation [24, 25].

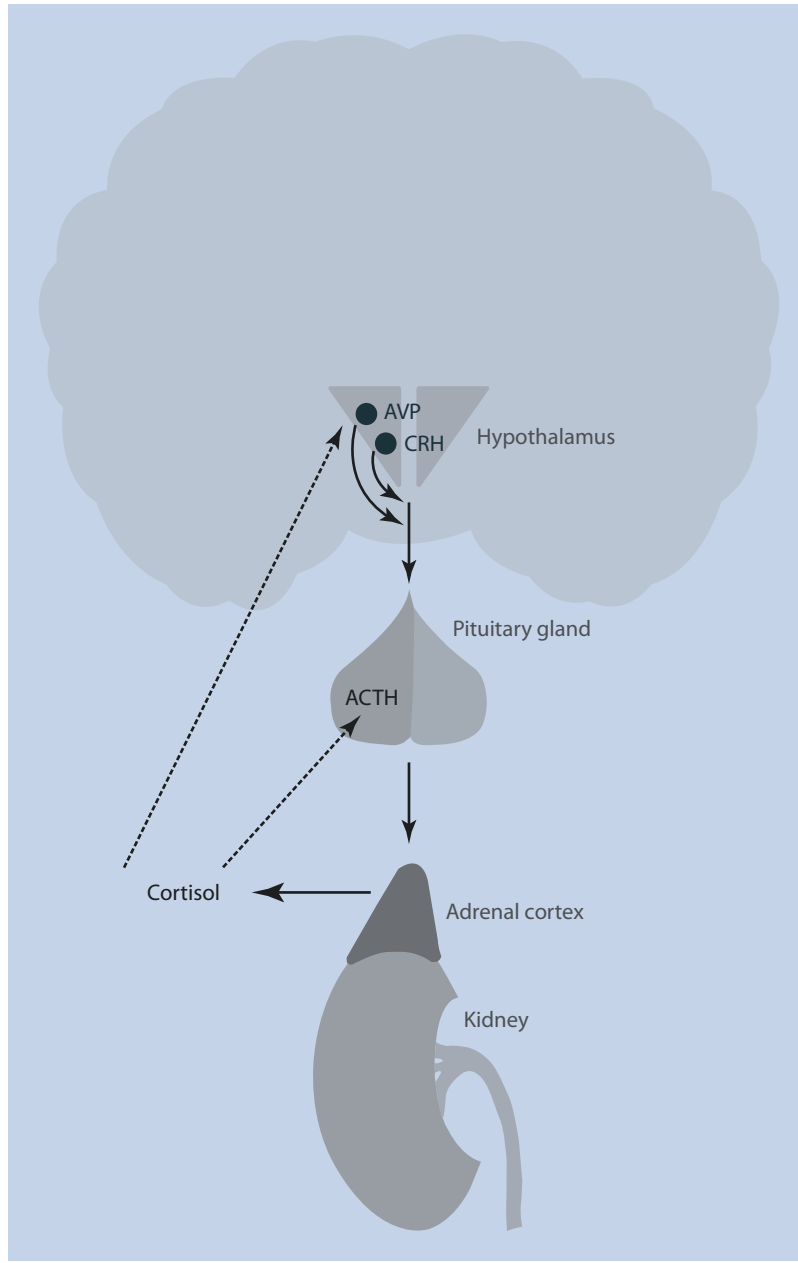

Fig. 13.2 Hypothalamic–pituitary–adrenal axis regulation. Stress, circadian stimuli, and glucocorticoid withdrawal stimulate cortical, hippocampal, and other higher neural centers to activate corticotropin-releasing hormone (CRH) and vasopressin (AVP) parvocellular neurons in the hypothalamic paraventricular nucleus (shaded triangles). These parvocellular neurons release CRH and AVP into the hypophysial portal circulation, augmenting release of ACTH from anterior pituitary corticotroph cells. ACTH directly stimulates adrenal cortisol release. Cortisol acts in a classical negative feedback manner (*dotted arrows*) to downregulate excessive release of hypothalamic and pituitary mediators

Considerable insight into the regulation of the hypothalamic–pituitary–adrenal (HPA) axis and the control of glucocorticoid release has been obtained through both human and animal studies (Fig. 13.2). Stress and circadian stimuli induce the release of hypothalamic neuropeptides, the most important of which are corticotropin-releasing hormone (CRH) and arginine vasopressin, into the hypophyseal portal circulation [26–29]. These neuropeptides then stimulate release of adrenocorticotropin (ACTH) from anterior pituitary corticotrophs.

ACTH released into the systemic circulation augments adrenocortical release of cortisol by acting upon specific G-protein-coupled receptors on steroidogenic cells of the zona fasciculata and zona reticularis (Fig. 13.3) [30, 31]. Cortisol then acts in a negative feedback manner at central nervous system and pituitary sites to decrease excessive release of hypothalamic neuropeptides

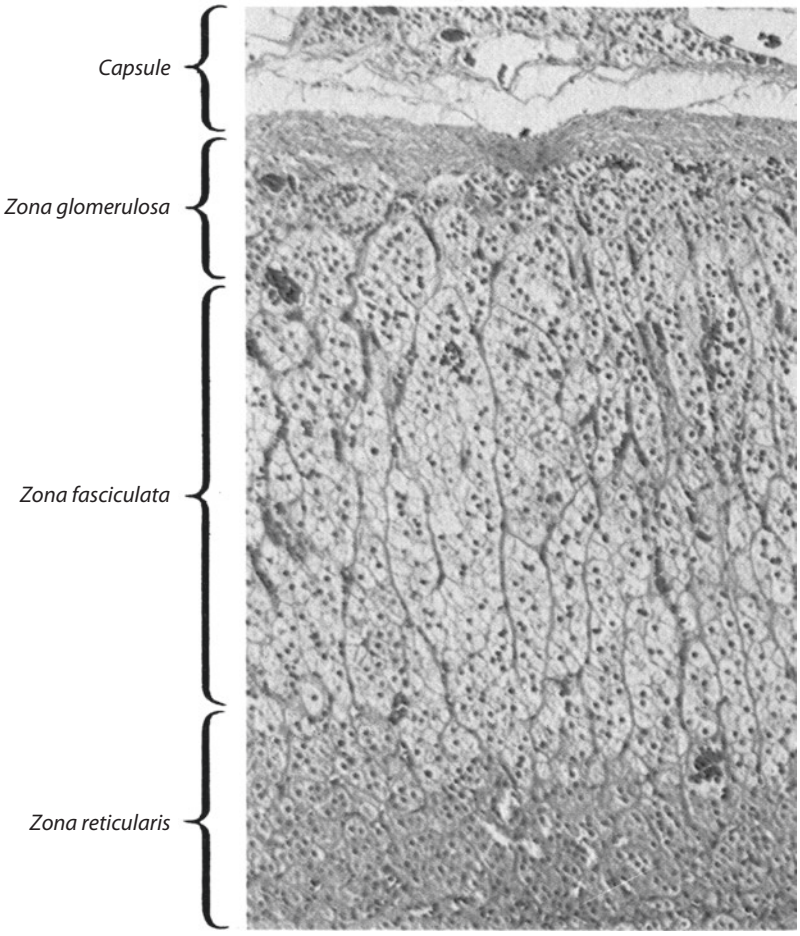

Fig. 13.3 Adrenal histology. Shown is a hematoxylin and eosin-stained section of a normal human adrenal. Relative sizes and positions of the zona glomerulosa, fasciculata, and reticularis are indicated (X40 magnification) (Reproduced with permission from Bethune [194] with copyright ©Pfizer Inc.)

and ACTH. Conversely, when insufficient glucocorticoid is present in the circulation, hypothalamic neuropeptide and ACTH release are augmented. In contrast, the control of mineralocorticoid (aldosterone) release by the zona glomerulosa of the adrenal is primarily determined by the renin–angiotensin system, with a smaller contribution from short-term changes in ACTH [32, 33]. Changes in vascular volume sensed by the renal juxtaglomerular apparatus result in increased secretion of renin, a proteolytic enzyme that cleaves angiotensinogen to angiotensin I. Angiotensin I is then activated through further cleavage by angiotensin-converting enzyme in the lung and other peripheral sites to angiotensin II. Angiotensin II and its metabolite angiotensin III demonstrate vasopressor and potent aldosterone secretory activity.

Adrenal insufficiency can result from impaired function at each level of the HPA axis. Direct involvement of the pathologic process at the level of the adrenal, or primary adrenal insufficiency, often causes both mineralocorticoid and glucocorticoid insufficiency by destruction of both glomerulosa and fasciculata/reticularis cells, respectively. Pituitary or hypothalamic defects result in secondary or tertiary adrenal insufficiency, respectively, manifest as isolated glucocorticoid insufficiency.

13.2 Etiology

13.2.1 Primary Adrenal Insufficiency

The most common cause of primary adrenal insufficiency, or Addison disease, is autoimmune adrenalitis (▶ Box 13.1). Antibodies that react to all three zones of the adrenal cortex can be found in 60–75% of patients with autoimmune adrenal insufficiency [34–37]. After the onset of adrenal insufficiency, the titers decrease and sometimes

Box 13.1 Causes of Adrenal Insufficiency

- Primary adrenal insufficiency
  - Autoimmune
    - Isolated adrenal insufficiency
    - Polyglandular autoimmune diseases I and II
  - Inborn errors of metabolism
    - Congenital adrenal hyperplasia
    - StAR deficiency
    - Smith–Lemli–Opitz syndrome
    - X-linked adrenoleukodystrophy
    - DAX-1 mutation (adrenal hypoplasia)
    - Familial glucocorticoid deficiency
    - Wolman's disease
    - SF-1 mutation
  - Drugs
    - Aminoglutethimide
    - Etomidate
    - Ketoconazole
    - Metyrapone
    - Suramin
    - Phenytoin
    - Barbiturates
    - Rifampicin
    - Mitotane
  - Adrenal hemorrhage
    - Birth trauma
    - Sepsis (Waterhouse–Friderichsen syndrome)
    - Shock
    - Coagulopathy
    - Ischemia
  - Infection
    - Tuberculosis
    - Amyloidosis
    - Hemochromatosis
    - Sarcoid
    - HIV/AIDS
    - Histoplasmosis
    - Blastomycosis
    - Cryptococcus.
    - Coccidioidiomycosis
- Secondary adrenal insufficiency
  - CNS lesions
    - Hypothalamic/pituitary/suprasellar tumors trauma/hemorrhage
    - Hemochromatosis
    - Sarcoidosis, tuberculosis, fungal infection
    - Empty sella syndrome
  - Cushing syndrome
  - Abnormalities in neuropeptides
    - POMC
    - CRH
  - Abnormalities in pituitary development
    - Septo-optic dysplasia
    - Hydrancephaly/anencephaly
    - Pituitary aplasia/hypoplasia
  - Iatrogenic (Supraphysiologic glucocorticoids)

completely disappear. Autoimmune adrenal insufficiency may occur as an isolated endocrinopathy or in association with other endocrinopathies [34, 36]. Addison disease in association with other endocrinopathies can be subdivided into two groups: polyglandular autoimmune disease types 1 and 2 [38]. Polyglandular autoimmune disease 3 is diagnosed when autoimmune thyroid disease is present with another autoimmune endocrinopathy without adrenal disease. The presence of adrenal antibodies in patients with other autoimmune diseases may precede the development of adrenal insufficiency by several years [39, 40].

Polyglandular autoimmune disease type 1 or APECED (autoimmune polyendocrinopathy candidiasis ectodermal dystrophy) is a rare autosomal recessive syndrome that usually presents in early childhood [41]. APECED is diagnosed when 2 of the following 3 diseases are present for at least 3 months: hypoparathyroidism, chronic mucocutaneous candidiasis, and Addison disease. Gonadal failure, enamel hypoplasia, and nail dystrophy are other common manifestations of this syndrome. Associated conditions include malabsorption syndromes, alopecia totalis or areata, pernicious anemia, autoimmune thyroid disease, chronic active hepatitis, vitiligo, type 1 diabetes mellitus, anterior hypophysitis, and diabetes insipidus [41–43]. The gene responsible for APECED [44] encodes the autoimmune regulator protein (AIRE), a zinc finger protein transcription factor involved in self-antigen presentation in the thymus [45]. Sequencing of the protein coding region of the AIRE gene is commercially available and detects more than 95% of mutations [46].

Polyglandular autoimmune disease type 2 is much more common than type 1 and usually presents in adulthood or late childhood. Polyglandular autoimmune disease 2 is diagnosed when adrenal failure and autoimmune thyroid disease or type 1 diabetes mellitus are present without hypoparathyroidism or candidiasis. Other diseases associated with this disorder include gonadal failure, celiac disease, autoimmune gastritis with vitamin B12 deficiency, vitiligo, diabetes insipidus, alopecia, pernicious anemia, myasthenia gravis, immune thrombocytopenia purpura, Sjogren's syndrome, and rheumatoid arthritis [34, 42, 43]. Much less is known about the etiology of polyglandular autoimmune type 2 other than an association with high risk class II HLA alleles [45].

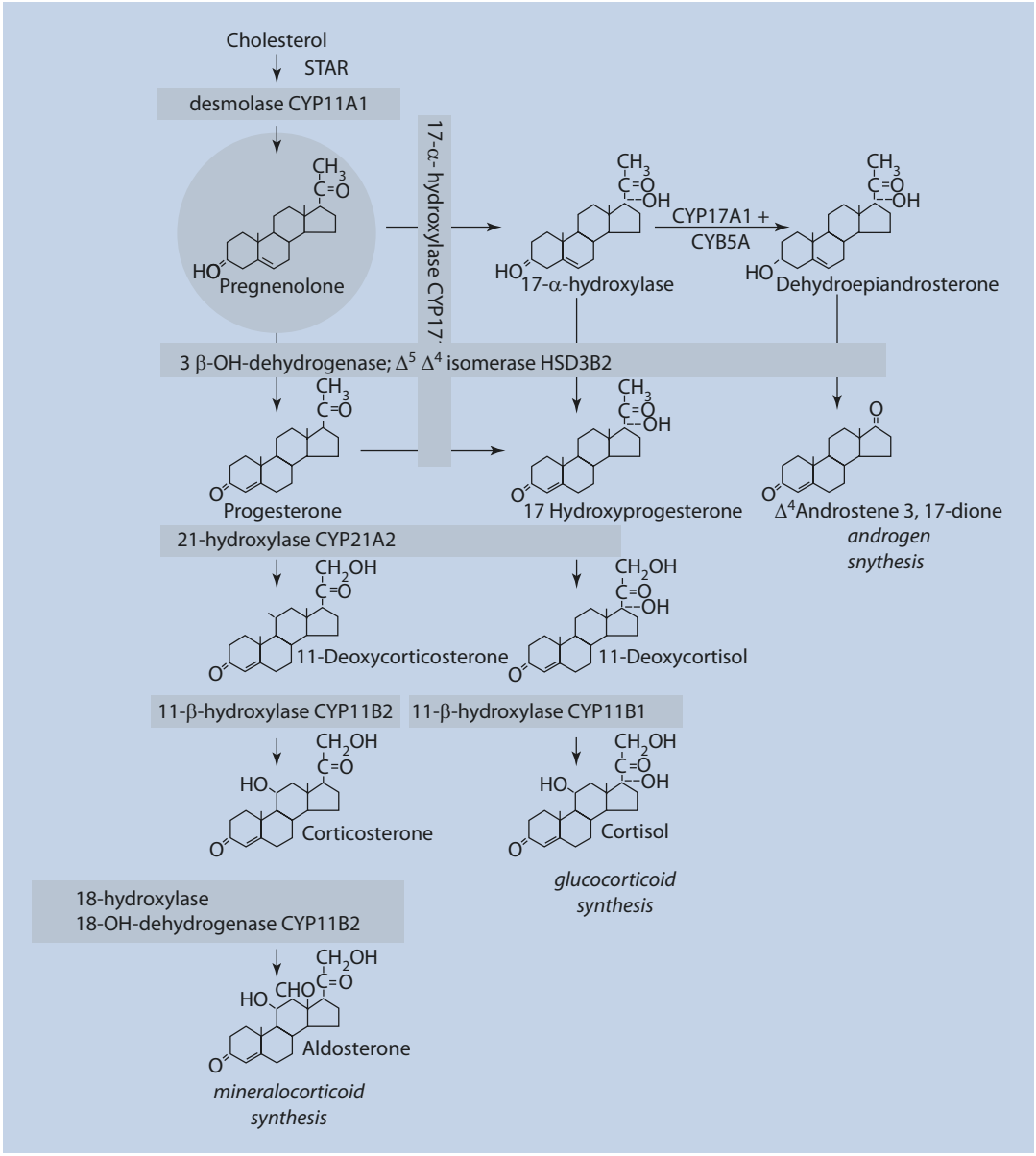

Fig. 13.4 Cortisol biosynthetic pathway. The enzymatic steps leading to mineralocorticoid, glucocorticoid, and adrenal androgen production are shown. Enzyme names and human gene nomenclature for each step are provided (Modified from Bethune [194] copyright ©Pfizer Inc.)

Inborn errors of steroid metabolism provide another common cause of adrenal insufficiency. Congenital adrenal hyperplasia (CAH) is an inborn error of steroid metabolism resulting from defects in enzymes involved in the biosynthesis of cortisol from cholesterol (Fig.13.4). Patients with congenital adrenal hyperplasia are cortisol deficient. Depending on the nature of enzyme deficiency, they may also be aldosterone deficient and require mineralocorticoid replacement and salt supplementation. The most common enzyme defects, 21-hydroxylase, 11β-hydroxylase, or 3β-hydroxysteroid dehydrogenase, lead to increased levels of the adrenal androgens androstenedione and/or DHEA [47]. These increases in adrenal androgens cause virilization of females, one of the primary clinical symptoms of congenital adrenal hyperplasia. Virilization of female infants

occurs at 8–10 weeks of gestation due to transient expression of the 3β-hydroxysteroid dehydrogenase enzyme, whose activity is maximal at 8–9 weeks gestation, in the fetal adrenal gland. This activity disappears by 14 weeks and reappears at 23 weeks gestation. Nearer to term, placental aromatases convert fetal androgens to estrogens [48]. Males with 21-hydroxylase or 11β-hydroxylase deficiency do not manifest genital ambiguity, while those with 3β hydroxysteroid dehydrogenase deficiency demonstrate undervirilization since testosterone production is diminished.

Congenital lipoid adrenal hyperplasia (StAR, or steroidogenic acute regulatory protein deficiency) is a rare autosomal recessive condition that results in deficiency of all adrenal and gonadal steroid hormones [49]. Males with this condition usually have female external genitalia. The defective gene is on chromosome 8 and encodes the StAR protein. The StAR protein mediates cholesterol transport from the outer to inner mitochondrial membrane [50].

Smith–Lemli–Opitz syndrome results from a deficiency of 7-dehydrocholesterol C-7 reductase. Individuals with this syndrome have low cholesterol and high 7-dehydrocholesterol [51] which may result in adrenal insufficiency and 46, XY gonadal dysgenesis [52]. Associated symptoms of this disorder include moderate-to-severe mental retardation, failure to thrive, altered muscle tone, microcephaly, dysmorphic facies, genitourinary anomalies, and limb anomalies [53].

X-linked adrenoleukodystrophy (X-ALD) is a sex-linked, recessively inherited defect in a peroxisomal membrane protein, the adrenoleukodystrophy protein (ALDP), which belongs to the ATP-binding cassette superfamily of transmembrane transporters [54, 55]. Defective ALDP function results in accumulation of very long-chain fatty acids (VLCFA), demyelination in cerebral white matter and destruction of the adrenal cortex [56]. Approximately 25% of patients with X-ALD develop adrenal insufficiency. Any male who presents with primary adrenal insufficiency should be screened for X-ALD by measuring serum VLCFA levels.

Genes affecting adrenal development in addition to those encoding steroid metabolic enzymes have also been found to cause congenital adrenal failure. X-linked adrenal hypoplasia congenita (AHC) with hypogonadotropic hypogonadism is a rare X-linked recessive disorder due to a deletion or mutation of the AHC (or DAX-1 (dosage-sensitive sex reversal-adrenal hypoplasia congenita gene on the X-chromosome-1) gene. Patients with this disorder have severe glucocorticoid, mineralocorticoid, and androgen deficiency [57–59]. In this disorder, the adrenal cortex resembles the fetal adrenal with large vacuolated cells. The miniature form of adrenal hypoplasia is a sporadic form associated with pituitary hypoplasia. More recently, heterozygous mutation of the autosomal steroidogenic factor – 1 (SF-1) gene has been found to result in adrenal failure and 46, XY sex reversal in humans [60]. Homozygous SF-1 deficiency has not been found in humans, though completely SF-1-deficient mice demonstrate agenesis of the adrenal cortex, testes, and ovaries [61].

Familial glucocorticoid deficiency is a rare autosomal recessive disorder. Patients with this disorder present in childhood with hyperpigmentation, muscle weakness, hypoglycemia, and seizures because of low cortisol and elevated ACTH levels. Initial families had been shown to have a defect in the ACTH receptor, but other families were thought to have a post-receptor defect [62–64]. Patients that also have achalasia and alacrima are classified as having Allgrove or Triple-A syndrome. More recent genetic analyses have found that in addition to mutations in *MC2R* which encodes the ACTH receptor, familial glucocorticoid deficiency can result from mutations in the melanocortin 2 receptor accessory protein (*MRAP*) [65] or steroidogenic acute regulatory protein (*STAR*) [66].

Wolman disease, a rare autosomal recessive disease that results from complete deficiency of lysosomal esterase, is usually fatal in the first year of life [67]. Features of this disease include mild mental retardation, hepatosplenomegaly, vomiting, diarrhea, growth failure, and adrenal calcifications. Calcifications that delineate the outline of both adrenals are pathognomonic for this disease as well as the less severe form of this disease, cholesterol ester storage disease [68].

Birth trauma may cause adrenal hemorrhage and should be considered in a newborn presenting with signs and symptoms of adrenal insufficiency. Adrenal hemorrhage has also been reported as sequelae of sepsis, traumatic shock, coagulopathies, or ischemic disorders. Adrenal hemorrhage in association with fulminant septicemia caused by *Neisseria meningitidis* is known as the Waterhouse–Friderichsen syndrome.

Infiltrative disease of the adrenal due to tuberculosis had been a frequent cause of adrenal failure when Addison first described adrenal insufficiency. Today, tuberculosis is a rare cause of adrenal insufficiency, especially in children. Amyloidosis, hemochromatosis, and sarcoidosis have all been reported to cause primary adrenal insufficiency by invasion of the adrenal gland.

Patients with acquired immunodeficiency syndrome (AIDS) or who are human immunodeficiency virus (HIV) positive may acquire adrenal insufficiency. In patients with HIV, cytomegalovirus infection can cause necrotizing adrenalitis. Infection with *Mycobacterium avium-intracellulare* or cryptococcus or involvement of the adrenal gland by Kaposi sarcoma also is a significant cause of primary adrenal insufficiency in HIV-positive patients [69]. In addition, most patients with AIDS have decreased adrenal reserves as measured by prolonged ACTH stimulation [70].

Fungal disease has also been shown to cause primary adrenal insufficiency. Disseminated infection with histoplasmosis or blastomycosis may invade and destroy the adrenal glands [71, 72]. Cryptococcus and coccidioidomycosis are rarer causes of adrenal insufficiency [73, 74]. Several drugs have been associated with the induction of adrenal insufficiency. Aminoglutethimide [75], etomidate [76], ketoconazole [77], metyrapone [78], and suramin [79] are drugs that may cause adrenal insufficiency by inhibiting cortisol synthesis. In most patients, an increase in ACTH will override the enzyme block, but in patients with limited reserve, adrenal insufficiency may ensue. Drugs that accelerate metabolism of cortisol and synthetic steroids such as phenytoin [80, 81], barbiturates, and rifampicin [80] also may cause adrenal insufficiency in patients with limited reserve. Mitotane accelerates the metabolism of halogenated synthetic steroids (dexamethasone and fludrocortisone) and may precipitate an adrenal crisis in patients taking both drugs [82].

13.2.2 Secondary and Tertiary Adrenal Insufficiency

With the widespread use of supraphysiological doses of glucocorticoids for the treatment of atopic, autoimmune, inflammatory, and neoplastic diseases, iatrogenic suppression of corticotroph ACTH release with secondary adrenocortical atrophy is a frequent, often unrecognized precipitant of adrenal insufficiency. Prolonged (greater than 7–10 days) supraphysiological glucocorticoid replacement places children and adults at risk for consequences of secondary/tertiary adrenal insufficiency [83]. Similarly, sustained, excessive glucocorticoid production in Cushing syndrome suppresses normal corticotroph responses. The duration of recovery of corticotroph function from iatrogenic adrenal suppression once pharmacological administration of glucocorticoids is discontinued, or Cushing syndrome after tumor resection, is quite variable, with evidence of suppression of the HPA axis evident in some patients for more than 1 year [84].

Several acquired and congenital lesions of the hypothalamus and pituitary are also causes of secondary/tertiary adrenal insufficiency (▶ Box 13.1). Disruption of corticotroph function commonly occurs by hypothalamic and pituitary tumors, or as a result of treatment of these tumors. In children, the most common tumors include craniopharyngiomas, dysgerminomas, and pituitary adenomas. Trauma to the hypothalamus, pituitary, or hypophysial portal circulation from significant head injury, cerebrovascular accident, Sheehan syndrome, or hydrocephalus/increased intracranial pressure provides additional etiologies of central adrenal insufficiency. Infiltrative diseases of the hypothalamus and pituitary such as autoimmune hypophysitis, sarcoidosis, tuberculosis, leukemia, and fungal infections also may result in adrenal insufficiency, often in the context of panhypopituitarism. Abnormalities in the development of the hypothalamus and pituitary associated with adrenal insufficiency include septo-optic dysplasia (de Morsier syndrome) [85, 86], hydrancephaly/anencephaly, and pituitary hypoplasia/aplasia. Secondary adrenal insufficiency together with diabetes insipidus is particularly ominous in these patients, as sudden death during childhood has been found [87].

Several groups have evaluated the frequency of central adrenal insufficiency in children and adults with Prader–Willi syndrome (PWS), a genetic disorder characterized by early hypotonia, failure to thrive, developmental delay, and hypogonadism, that later progresses to obesity due to hyperphagia and other behavioral problems. It has been widely appreciated that individuals with PWS are at increased risk for sudden and often

unexplained death. Stevenson et al. [88] reported small adrenal size in three children subject to postmortem analysis of the ten individuals in the series, and a later study demonstrated evidence of central hypoadrenalism in 60% of PWS subjects undergoing a single-dose metyrapone test [89]. This high frequency of central adrenal insufficiency, though, has not been found in two larger, recent studies of children and adults with PWS that employed low-dose cosyntropin, high-dose cosyntropin, or insulin tolerance testing [90, 91]. Clinically significant central adrenal insufficiency is likely rare in individuals with PWS.

Least commonly, inherited abnormalities of neuropeptides involved in HPA axis regulation have recently been reported. Deficiency of proopiomelanocortin results in adrenal insufficiency, pigmentary abnormalities, and obesity [92, 93]. One kindred with suspected CRH deficiency and Arnold-Chiari type 1 malformation has been described [94]. While the mutation in this kindred is linked to the CRH locus, a specific mutation in CRH gene has not yet been defined.

13.3 Clinical Presentation

13.3.1 Primary Adrenal Failure

Primary adrenal failure may present as acute, rapidly progressive deterioration or an insidious, chronic process. In infants with congenital adrenal hyperplasia, the first suspicion of adrenal insufficiency may be imparted by the observation of ambiguous genitalia in the delivery room. In Caucasian infants, physical examination may additionally reveal hyperpigmentation of the labioscrotal folds, areola, and buccal mucosa due to excessive proopiomelanocortin synthesis and processing to ACTH and MSH. If of a salt-wasting variety, untreated CAH commonly causes hyponatremia, hyperkalemia, acidosis, and shock at 7–14 days of age. Infants with the rarer adrenal hypoplasia congenita do not manifest genital ambiguity, but may present with a similar adrenal crisis [95].

Children and adults with primary adrenal insufficiency demonstrate a similar spectrum of signs and symptoms, whether of a gradual or sudden onset. The most common symptoms such as weakness, fatigability, anorexia, vomiting, constipation or diarrhea, and depression are nonspecific and do not immediately implicate adrenal insufficiency [37]. While salt craving is highly suggestive of adrenal insufficiency, this symptom may not be elicited at presentation. Weight loss is another very common finding with adrenal insufficiency, though again does not strongly indicate the diagnosis. More specific signs such as hyperpigmentation of skin folds, gingiva, and non-sun-exposed areas, hyponatremia with hyperkalemia, hypoglycemia, and hypotension often point toward the correct diagnosis. Hypercalcemia is sometimes found at presentation due to volume depletion and associated increased intravascular protein concentration [96]. In children with polyglandular autoimmune syndrome type 1, mucocutaneous candidiasis and hypoparathyroidism usually precede the appearance of adrenal insufficiency [41]. In X-linked adrenoleukodystrophy, neurological manifestations may either precede or follow the evolution of adrenal insufficiency [97, 98].

13.3.2 Secondary Adrenal Failure

The findings of secondary adrenal failure in large part recapitulate the consequences of isolated glucocorticoid deficiency in primary adrenal insufficiency, such as weakness, fatigability, and an increased tendency toward hypoglycemia. Of note, salt wasting does not occur because the renin–angiotensin–aldosterone system remains intact. Because glucocorticoids are required for appropriate renal free water clearance [11], secondary adrenal insufficiency is associated with hyponatremia without hyperkalemia or volume depletion. Additionally, since ACTH production and secretion are the primary defects in secondary disease, skin hyperpigmentation does not occur unless as a manifestation of recently treated Cushing disease. Signs and symptoms of a central nervous system tumor, such as headaches, vomiting, or visual disturbances, should be sought. Infants with congenital central nervous system malformations, physical evidence for possible hypopituitarism such as midline facial defects or microphallus, or optic nerve atrophy should be evaluated for adrenal deficiency. Individuals at risk for iatrogenic adrenal insufficiency will often appear Cushingoid on examination, with round facies, thinned skin, striae, and a buffalo hump due to prior glucocorticoid therapy.

13.4 Diagnostic Evaluation

13.4.1 Baseline Hormone Measurements

To verify suspected primary adrenal insufficiency in the patient presenting with classic signs and symptoms of Addison disease, often little more is necessary than measurement of plasma ACTH, cortisol, renin activity, and aldosterone. Elevated ACTH (>2-fold above the upper limit of the reference range) and plasma renin activity together with low plasma cortisol and/or aldosterone confirms primary adrenal failure [99]. In patients more than 6 months of age, the age at which the circadian pattern of glucocorticoid production has been established [100], not presenting in fulminant adrenal crisis, morning (approximately 8 a.m.) ACTH, and cortisol levels, along with electrolytes, plasma renin activity, and aldosterone, may establish the diagnosis. A fasting morning cortisol of less than 5 mcg/dl is indicative of adrenal insufficiency, while concentrations exceeding 20 mcg/dl make adrenal failure quite unlikely [99, 101]. Those with early adrenal failure, or secondary disease, will often require additional provocative testing as described below. Adrenal autoantibodies can be used to further establish the etiology of adrenal insufficiency as autoimmune [35, 36]. All males diagnosed with primary adrenal failure without evidence of other autoimmune pathology should have plasma very long-chain fatty acids measured to exclude X-linked adrenoleukodystrophy.

Screening for CAH caused by 21-hydroxylase deficiency on the newborn screen is universally required by law in all 50 of the United States [102]. In newborns with ambiguous genitalia or a salt-wasting crisis where CAH is being considered, random measurement of cortisol precursors and precursor by-products usually confirms or excludes the diagnosis. For a virilized female, where 21-hydroxylase, 11β-hydroxylase, or 3β-hydroxysteroid dehydrogenase deficiencies are possible, a typical hormone profile consists of measuring 17-hydroxypregnenolone, 17-hydroxyprogesterone, dehydroepiandrosterone, androstenedione, testosterone, cortisol, plasma renin activity, and aldosterone. Males with undervirilization should be evaluated for 3β-hydroxysteroid dehydrogenase, 17-hydroxylase, or StAR deficiency, as well as non-adrenal etiologies of genital ambiguity, while normally virilized males with salt wasting should be evaluated for 21-hydroxylase deficiency and aldosterone biosynthetic defects. In 11β-hydroxylase deficiency, hypertension, hypokalemia, and a suppressed plasma renin are found in normally virilized males, or virilized females. Since salt wasting due to CAH does not typically occur within the first 3 days after birth, and adrenal hormone levels change dramatically in normal infants within this period [103–105], random adrenal hormone measurements should be obtained on day of life 2–3. Additionally, the normal range of adrenal hormones differs for term and preterm infants and should be accounted for in interpretation of results [106–108]. Late-onset forms of CAH and proximal lesions in the cortisol biosynthetic pathway such as StAR deficiency or adrenal hypoplasia often require cosyntropin stimulation testing, as described below, with cortisol precursors measured in addition to cortisol.

13.4.2 Cosyntropin Stimulation Test

Direct stimulation of adrenal cortisol release by administration of cosyntropin (1–24 ACTH; Cortrosyn) is the most commonly used diagnostic tool in evaluation of adrenal function [101, 109]. In the standard cosyntropin test (250 mcg cosyntropin in adults and children ≥2 years of age, 125 mcg for children <2 y, and 15 mcg/kg for infants), baseline ACTH and cortisol samples are obtained, and then cosyntropin is administered intravenously [99]. If mineralocorticoid deficiency is also suspected, plasma renin activity, aldosterone, and electrolytes should be obtained with the baseline laboratories. Thirty and/or 60 min following cosyntropin administration, a second plasma sample is obtained for cortisol determination. Plasma cortisol concentration greater than or equal to 18 mcg/dl, along with a normal baseline ACTH level, rules out primary adrenal insufficiency. In addition, a normal response to this standard stimulation test also rules out long-standing, severe secondary adrenal insufficiency. To evaluate recent-onset secondary adrenal insufficiency or milder forms of secondary adrenal insufficiency, a more sensitive stimulation test employing a lower dose of cosyntropin has been devised [110–113]. In this case, 1 mcg, or 0.5 mcg/M^2 of body surface area, of

cosyntropin is administered intravenously, with cortisol measured at baseline and 20–60 min after administration. Plasma cortisol concentration above 18 mcg/dl is considered a normal response. False-positive tests employing these criteria may be relatively frequent, however, and should be considered to prevent overdiagnosis of adrenal insufficiency [114]. The 1 mcg cosyntropin test is not recommended for diagnosis of suspected primary adrenal insufficiency [99].

13.4.3 Insulin-Induced Hypoglycemia

The response to hypoglycemia (blood glucose <40 mg/dl) requires the integrity of the entire HPA axis. After an overnight fast, 0.10–0.15 U/kg of regular insulin is administered intravenously. Blood glucose and cortisol are measured prior to and then 15, 30, 45, 60, 75, 90, and 120 min following insulin injection. Patients will experience some degree of discomfort during the hypoglycemic phase of this test due to neuroglycopenia and the consequences of catecholamine release, such as tachycardia, diaphoresis, anxiety, and tremulousness. A plasma cortisol of above 20 mcg/dl is considered a normal response [101, 115]. This test should be avoided in patients with a history of seizures or significant cardiovascular disease, and dextrose for intravenous rescue should be immediately accessible in the event of sustained severe hypoglycemia or a seizure.

13.4.4 Corticotropin-Releasing Hormone Stimulation Test

To directly assess corticotroph function, ovine corticotropin-releasing hormone can be administered intravenously at a dose of 1 mcg/kg or 100 mcg followed by measurement of plasma ACTH and cortisol levels over the next 2 h [116–118]. Flushing occurs in some patients after administration. Peripheral CRH administration provides a less robust stimulus for ACTH release than hypoglycemia, and the normal range has been less well established. However, studies directly comparing the responses to CRH and insulin-induced hypoglycemia have demonstrated good concordance in defining adrenal status. In normal subjects, plasma ACTH peaks rapidly (15–30 min) following administration and remains at an elevated level. Cortisol peaks slightly later, at 30–60 min following injection, and also persists at an elevated level for 2 h. In patients with hypothalamic lesions, an exaggerated ACTH response is often obtained with an even longer prolongation in the duration of elevation. In contrast, patients with pituitary lesions do not respond to CRH administration with increases in either ACTH or cortisol.

13.4.5 Glucagon Stimulation Test

The glucagon stimulation test provides an alternative to insulin-induced hypoglycemia in evaluating central adrenal insufficiency as it requires endogenous ACTH secretion to cause cortisol release [119, 120]. While glucagon doses of 0.03 mg/kg have been routinely used as a provocative test for growth hormone assessment, studies evaluating adrenal function have employed somewhat higher doses (0.1 mg/kg IM; maximum 1.0 mg in children; in adult studies 1.0 mg if <90 kg and 1.5 mg if >90 kg) [121–123]. After an overnight fast, plasma is obtained at baseline, then 30, 60, 90, 120, 150, and 180 min following injection. A normal response is achieved if peak cortisol exceeds 20 mcg/dl.

13.4.6 Metyrapone Test

Metyrapone inhibits the activity of the enzyme 11-beta-hydroxylase, blocking the conversion of 11-deoxycortisol to cortisol. Thus, cortisol is unable to provide negative feedback at central nervous system and pituitary sites increasing ACTH secretion. The increased plasma ACTH concentration stimulates increased production of 11-deoxycortisol and its urinary metabolites. Two general forms of the metyrapone test have been standardized: an overnight, single-dose test [124] and a multiple-dose form [125]. Because of convenience, the single-dose test is the more commonly performed. For the single-dose test, 30 mg/kg to a maximum of 3.0 g is given at midnight with a snack to decrease the nausea associated with metyrapone ingestion. Cortisol, 11-deoxycortisol, and ACTH are measured at 8 am following the dose. A normal response is the increase in plasma 11-deoxycortisol to more

the 7 mcg/dl [126]. Cortisol levels above 5 mcg/dl imply inadequate suppression of enzyme activity such that low 11-deoxycortisol levels cannot be taken as an index of inadequate hypothalamic or pituitary function.

13.4.7 Radiological Tests

In general, imaging studies should be utilized after the diagnosis of adrenal insufficiency is established by biochemical methods. The obvious exception to this rule is the patient presenting with symptoms suggesting an intracranial mass lesion. The resolution of magnetic resonance imaging of the hypothalamus and pituitary in general exceeds that of computed tomography [127] and is the initial imaging study of choice for evaluation of documented central adrenal insufficiency. If a mass is found, computed tomography may be performed to establish whether the tumor has calcifications characteristic of a craniopharyngioma.

In patients with primary adrenal insufficiency with positive adrenal autoantibodies or elevated VLCFA, establishing autoimmune adrenalitis or X-ALD as the etiology, respectively, adrenal imaging is not required. If these entities are not established as the diagnosis, CT or MRI of the adrenal should be performed [128–131]. Observation of calcifications in an older child is suggestive of tuberculosis or other granulomatous disease, while in an infant, the diagnosis of Wolman disease should also be entertained. In a limited number of cases, CT-assisted needle biopsy for pathological diagnosis may be required.

13.4.8 Diagnosing Adrenal Insufficiency in Critical Illness

During critical illness, activation of the HPA axis is essential for survival. Individuals with known primary or central adrenal insufficiency require additional steroids during critical illness. Over the last decade, there have been many studies published on adrenal function in the intensive care setting in patients not previously diagnosed with adrenal insufficiency. Patients in the ICU with unrecognized adrenal insufficiency may experience treatment-resistant hypotension, prolonged ventilation, and increased mortality [132]. However, how to diagnose patients in the ICU with adrenal insufficiency is highly controversial. Intensivists often diagnose relative adrenal insufficiency if the rise in cortisol 30 min or 60 min after administering 0.5–250 mcg of cosyntropin intravenously is less than 9 mcg/dl [133–137]. Still others use a baseline cortisol of less than 7 mcg/dl [135, 138, 139] or a stimulated cortisol of <18 mcg/dl [135, 140] in response to low-dose cosyntropin as evidence of adrenal insufficiency [137]. However, there are many factors that affect our ability to diagnose adrenal insufficiency in the setting of critical illness. During critical illness inflammatory cytokines such as IL-1, Il-6, and TNF-α, the autonomic nervous system and factors in the innate immunity response such as Toll-like receptors and macrophage inhibitory factor (MIF) have either been shown or postulated to play a significant role in the HPA axis response to stress [141]. Alterations in these confounding factors as well as polymorphisms in individual genes likely contribute to variations in the normal HPA axis response to each stressor. Second, it has recently been recognized that the HPA axis response to acute critical illness differs from its response to prolonged critical illness. Third, in critical illness, transcortin levels may decrease depending on the exact illness. Since more than 90% of cortisol is bound to transcortin and albumin, this change may affect the validity of measuring total cortisol, especially when albumin levels are also low. Ideally, free cortisol levels would be measured to determine HPA axis status, but these assays are not easily available. Fourth, the most readily available cortisol assay used is the immunoassay, which exhibits nonuniformity that may increase in the setting of critical illness. Lastly, what constitutes adrenal insufficiency varies between studies. Thus, adrenal insufficiency in the setting of a critical illness is difficult to diagnose, but should be considered and the patient treated appropriately when warranted by the clinical scenario.

13.4.9 Diagnosing Adrenal Insufficiency in Neonates

It has been increasingly recognized that critically ill neonates may have relative adrenal insufficiency [140]. Steroidogenic activity has been detected in fetal adrenal glands by 50–52 days postconception with a peak in cortisol production at 8–9 weeks gestation and undetectable levels by 14 weeks

gestation. Due to lack of 3β-hydroxysteroid dehydrogenase activity between 14 and 23 weeks and suppression from maternal cortisol in early gestation, very little fetal cortisol is produced between 14 and 30 weeks gestation [48]. Later in gestation, the placenta begins to express 11β-hydroxysteroid dehydrogenase 2, which inactivates maternal cortisol, allowing the fetal adrenal to take over cortisol production. In the infant born preterm, this immaturity of the HPA axis may lead to relative adrenal insufficiency during stress. Several studies have demonstrated that preterm infants with hypotension have lower baseline and stimulated cortisol [142–145]. Critically ill term neonates may also be at risk for relative adrenal insufficiency for the same reasons. However, they may also be at risk because of hormonal shifts during the transition from fetal to extrauterine life. As the placenta matures, it produces more and more CRH that drives the fetal adrenal. Unlike hypothalamic CRH, placental CRH is stimulated by cortisol. At birth, the placental CRH is suddenly withdrawn. During the transition, the hypothalamus and pituitary may be transiently suppressed, leading to relative adrenal insufficiency during critical illness. Further studies in neonates are necessary before glucocorticoid treatment of critically ill neonates can be recommended as standard of care. However, glucocorticoid therapy should be considered in neonates both preterm and term who are hemodynamically unstable. If glucocorticoids are used, a cortisol level should be drawn prior to initiating therapy. If treatment is initiated, the length of treatment should be minimized as much as possible.

13.5 Outcomes and Possible Complications

Children with adrenal insufficiency in general lead normal lives. However, glucocorticoid deficiency places them at increased risk for usual illnesses becoming life-threatening. If appropriate stress steroid coverage is not given during an illness, these children have the potential of dying. Therefore, for both the child and the entire family, reinforcement of stress steroid administration during illness is essential. If oral intake of medications and salt is not possible due to gastrointestinal disease or mental status changes, further instruction for emergency assistance is important. All children with adrenal insufficiency should be provided with a medical alert bracelet stating their diagnosis to facilitate urgent therapy if required. Other factors affecting quality of life are determined by the etiology of adrenal failure.

13.5.1 Polyglandular Autoimmune Syndromes

Children with adrenal insufficiency in the context of one of the polyglandular autoimmune syndromes must contend with other endocrinopathies, or the anticipation of developing other endocrinopathies. Often, complicated, multidrug therapeutic regimens develop with significant financial and emotional cost to the families. The development of type 1 diabetes mellitus, especially, places added demands on daily life. Additionally, enamel hypoplasia, nail dystrophy, vitiligo, and alopecia may be considered disfiguring by the patients with these disorders.

13.5.2 Central Adrenal Insufficiency

Secondary or tertiary adrenal insufficiency may be associated with insufficiency of other pituitary hormones. Similar to patients with polyglandular autoimmune syndromes, multi-hormone deficiency states are common and require frequent medication administration and dosage adjustment. Long-term issues with decreased fertility and excessive weight gain due to hypothalamic damage pose the most challenging concerns.

13.5.3 Congenital Adrenal Hyperplasia

Determining the etiology of ambiguous genitalia is an endocrine emergency. Sex assignment of a newborn has obvious long-term implications, with optimization of adult sexual and emotional function being the primary goal. The timing for sex assignment and reconstructive surgery remains controversial [102]. Members of the Intersex Society of North America (ISNA) propose that no reconstructive genital surgery should be performed on children before they are old enough to consent. In general though, 46, XX patients with CAH, if treated adequately, may be

fertile as adult females. Therefore, the recommendation that 46, XX patients with CAH be raised as females, with prompt reconstructive surgery if needed, had been the standard of care. If a female infant is severely virilized (Prader score >3), it is suggested in Endocrine Society Clinical Practice Guidelines that clitoral and perineal surgery during infancy by an experienced team be considered [102]. Further, for infants with a low vaginal confluence, it is recommended that a complete repair be done during infancy. However, more long-term outcome studies are necessary to make firm recommendations for surgical repair. Management of all forms of genital ambiguity benefits from a multidisciplinary approach with input from experienced endocrinologists, surgeons, geneticists, and psychologists, allowing formulation of a consistent long-term plan for the child and family.

13.5.4 Adrenoleukodystrophy

The phenotype of X-ALD is variable with at least seven clinical subtypes: childhood cerebral ALD, adolescent ALD, adult cerebral ALD, adrenomyeloneuropathy, Addison disease only, and asymptomatic and heterozygote women. All of these subtypes can be present within the same family [98]. In 6–8% of cases, Addison disease is the only manifestation of ALD. Any male sibling of an affected patient has a 50% chance of also being affected. Therefore, all male siblings should be screened and, if positive, evaluated for adrenal insufficiency. Any female sibling of a patient has a 50% chance of being a carrier. Screening should be offered to any female siblings to evaluate the risk of disease to their children. The major psychosocial consideration in this disease is the anticipation of progressive neurological deterioration, with limited interventions of proven efficacy.

13.6 Treatment

13.6.1 Primary Adrenal Failure

13.6.1.1 Chronic Replacement

Since the bioavailability of oral steroids is poor and varies from individual to individual, the recommended dose for replacement hydrocortisone therapy is a starting guide [146, 147]. For adults, consensus guidelines suggest 15–25 mg of hydrocortisone in 2–3 divided doses per day, with the highest dose given in the morning upon awakening and a lower dose later in the day. For children, the consensus guidelines recommend a total daily dose of 8 mg/M^2 body surface area of hydrocortisone in 3–4 divided doses (▶ Box 13.2) [99]. It has been shown that twice-daily hydrocortisone produces a nonphysiological low cortisol level 2–4 h prior to the next dose [148, 149].

Box 13.2 Management of Adrenal Insufficiency

- Management of adrenal crisis
- Obtain blood for:
  - Electrolytes
  - Cortisol
  - ACTH
- Intravenous fluid administration
- 500 cc/M^2 of D5NS over first 30–60 min to restore cardiovascular stability
- Correct sodium at a maximal rate of 0.5 mEq/L to prevent central pontine myelinolysis.
- Stress dose steroid
- Intravenous 50 mg/M^2 hydrocortisone, or if a new presentation, use 1 mg/M^2 of dexamethasone until ACTH stimulation test is done
- Continue 50–100 mg/M^2/day hydrocortisone divided q 6 h (or 1–2.5 mg/M^2/day dexamethasone) until stable for 24 h
- If a new presentation, perform ACTH stimulation test
- Frequent assessment of electrolytes, blood glucose, and vital signs
- Chronic replacement
- Glucocorticoid replacement
- 8 mg/M^2/day of hydrocortisone divided TID-QID
- Monitor clinical symptoms and morning plasma ACTH (Addison disease) or adrenal androgens/cortisol precursors (congenital adrenal hyperplasia)
- Mineralocorticoid replacement
- Florinef 0.5–2.0 mg QD
- Infants need 1–4 g NaCl added to their formula divided QID
- Monitor blood pressure, plasma renin activity, and electrolytes
- Treatment of minor febrile illness or stress
- Increase steroid dose to 30–100 mg/M^2/day until 24 h after symptoms resolve.
- Do not alter Florinef dose
- If unable to tolerate oral intake, administer 30–100 mg/M^2 of hydrocortisone acetate or 1–2.5 mg/M^2 of dexamethasone IM
- Obtain medical alert bracelet

Therefore, younger children, who are more prone to hypoglycemia when cortisol levels are low, or children with CAH, where efficient suppression of adrenal androgens is required, should receive hydrocortisone divided three to four times per day. Although steroids other than hydrocortisone may be used, hydrocortisone is preferred in children since it has less growth suppressive effects than synthetic steroids [102, 150–152]. Hydrocortisone tablets and liquid suspension are not equivalent. Hydrocortisone drug is unevenly distributed in the suspension and is not recommended for use in children [102, 153].

Patients with primary adrenal insufficiency often do not produce adequate aldosterone. Although hydrocortisone has some mineralocorticoid activity, physiologic doses do not usually provide enough mineralocorticoid activity to prevent salt wasting in children with primary adrenal insufficiency. Thus, children with mineralocorticoid deficiency are also treated with 0.05–0.2 mg/day of fludrocortisone. Since the aldosterone secretion rate after the first week of life does not increase from infancy to adulthood, mineralocorticoid doses do not vary significantly with body size [154]. Because infant formulas are low in salt, infants up to 1 year of age should be treated with 1–4 g per day of NaCl supplementation [155]. Older children and adults usually have enough salt in their diet without additional salt supplementation to maintain normal electrolytes with the use of fludrocortisone.

It is important that treatment adequacy be monitored on a regular basis. Growth velocity, weight gain, blood pressure, serum electrolytes, and plasma renin activity are the most useful tests every 3–6 months. Hyperpigmentation in non-sun-exposed areas is also an important sign that indicates inadequate therapy. Some physicians also like to monitor ACTH levels and to maintain these in the high normal to mildly elevated range. Special considerations for children with CAH are discussed below.

13.6.1.2 Stress Replacement

In normal individuals, plasma ACTH and cortisol levels increase in response to surgery, trauma, and critical illness. Many researchers have measured plasma or urinary-free cortisol in healthy adults undergoing surgery or in acutely ill individuals and have found that the daily secretion rate of cortisol is proportional to the degree of stress [156–161]. Estimates of the daily cortisol secretory rate in adults after surgery range from 60 to 167 mg/24 h. Based on repeated cortisol measurements, it has been estimated that adults undergoing minor surgery secrete 50 mg/day of cortisol [162, 163] and 75–150 mg/day after major surgery [160]. One comprehensive review of the literature [164] recommends that adults receive 25 mg for minor stress, 50–75 mg for minor surgery, and 100–150 mg hydrocortisone per day for major surgery for 1–3 days.

Based on data from adults, children should receive double or triple their usual dose of hydrocortisone with the onset of fever, gastrointestinal, or other significant illness and continued for 24 h after symptoms resolve [47, 99, 165, 166] (▶ Box 13.2). If the child has emesis within 1 h of the dose, it should be repeated, and if emesis occurs again, an intramuscular injection of 50 mg/M^2/day hydrocortisone or its equivalent should be given. The night prior to surgery, children should be given triple their normal dose of hydrocortisone. On the day of surgery, on-call to the operating room prior to anesthesia administration, they should be given an intravenous dose of 50 mg/M^2 of hydrocortisone and then continued on 50–100 mg/M^2/d divided every 6 h for the next 24–48 h postoperatively [99]. There is no need to give extra fludrocortisone when the dose of hydrocortisone is at least 50 mg/M^2; however, salt intake must be maintained to prevent electrolyte imbalance.

During a suspected adrenal crisis, electrolytes, cortisol, and ACTH levels should be drawn and treatment begun before lab values are available. Normal saline with 5% dextrose at a volume of 500 cc/M^2 or 20 mL/kg should be infused over the first hour. Initially 50–100 mg/M^2 of hydrocortisone can be given intravenously and then 50–100 mg/M^2/day divided every 6 h. In order to confirm a suspected diagnosis of adrenal insufficiency, equivalent doses of dexamethasone (1–2.5 mg/M^2/day) should be given instead of hydrocortisone [99]. Dexamethasone does not cross-react in standard cortisol assays and allows cosyntropin stimulation testing to be performed shortly after the initiation of therapy. Once a cosyntropin stimulation test has been performed, children should be changed to the less growth suppressive hydrocortisone. After re-expansion of vascular volume with normal saline to restore cardiovascular stability, hyponatremia should be corrected at a maximal rate of 0.5 mEq/L/h to minimize the risk for central pontine myelinolysis.

Additionally, a glucose infusion should be continued during rehydration to avoid hypoglycemia.

13.6.2 Central Adrenal Failure

ACTH or CRH deficiency is treated in much the same as primary adrenal insufficiency. The major difference is that while these patients do not require mineralocorticoid replacement, they do require evaluation for deficiency of other pituitary hormones. In addition, when first diagnosed, a head MRI, with special attention to views of the pituitary and hypothalamus, should be performed to look for a tumor or other anomalies.

13.6.3 Special Considerations for Virilizing Forms of CAH

13.6.3.1 Standard Therapy

Treatment of all forms of classical CAH consists of replacement and, when indicated, stress doses of cortisol. Treatment of virilizing forms of CAH requires more than replacement doses of hydrocortisone to prevent further virilization and rapid fusion of the growth plates. The dose required varies from individual to individual but averages 10–20 mg/M^2/day divided three times per day [47, 102, 167, 168]. When the dose exceeds 20 mg/M^2/day in infants and 15–17 mg/M^2/day in pubertal patients, there is evidence that final adult height is compromised [102, 169–172]. There has been controversy as to whether it is better to give a larger dose of hydrocortisone in the morning to mimic the normal diurnal rise in cortisol, or to give a larger dose in the evening to suppress the diurnal rise of ACTH. Recent data demonstrate that there is no difference in disease control or well-being of the patient [173]. Stress dosing (triple usual dose) is recommended for febrile illness (>38.5 °C), gastroenteritis with dehydration, surgery with general anesthesia, or major trauma. However, it is not recommended for mental and emotional stress, minor trauma, or exercise [102, 174].

Patients with CAH, with or without salt wasting, may benefit from mineralocorticoid therapy. In the salt-losing forms of CAH with elevation in plasma renin activity, the addition of fludrocortisone at 0.05–0.2 mg per day is required. In patients with mildly elevated plasma renin activity without overt salt wasting, the addition of fludrocortisone often helps to suppress excess adrenal androgen production. Depending on the degree of enzyme deficiency, children with CAH may also have aldosterone deficiency or increased levels of antagonists of aldosterone action [175, 176]. Aldosterone deficiency causes hyperkalemia, hyponatremia, and volume depletion. Hyponatremia and volume depletion lead to increased renin and angiotensin II. Angiotensin II not only stimulates aldosterone secretion directly at the level of the adrenal cortex; it also stimulates ACTH secretion [177–179]. Therefore, by suppressing plasma renin activity with the use of fludrocortisone, patients may require a lower dose of glucocorticoid. It is recommended that all neonates and infants with classical CAH regardless of salt-wasting status should be given fludrocortisone and salt supplementation [102]. Infants with CAH require approximately 1–4 grams per day of salt added to their formula or as supplementation to breast feeding [155].

Follow-up evaluation should occur every 2–4 months to monitor electrolytes, plasma renin activity, growth velocity, blood pressure, 17-hydroxyprogesterone, and androstenedione. Suppression of 17-hydroxyprogesterone and androstenedione into the normal range may compromise growth [180, 181]. In any patient where normal levels of these precursors suppress growth, steroid doses should be reduced to maintain levels in the slightly elevated range [102]. In patients with elevated blood pressure and/or suppressed plasma renin activity, a reduction in the fludrocortisones dose should be considered. A bone age should be monitored yearly to ensure that skeletal maturation is not advancing faster than the chronological age.

13.6.4 Newer Therapies

13.6.4.1 CAH

A major shortcoming in the therapy of CAH is compromised final adult height [182–184]. Inadequate suppression of 17-hydroxyprogesterone leads to relative advancement of bone age and ultimately short stature. Too much glucocorticoid also results in suppression of growth. Bilateral adrenalectomy reduces the risk of virilization and allows the use of lower doses of steroids. However, this treatment is controversial because of the added risk of surgery, possible increased risk of

adrenal crisis, and possible loss of other adrenal hormones (epinephrine, DHEA). Ideal therapy for CAH would be more physiological replacement of cortisol.

Current approved therapies for treatment of adrenal insufficiency do not mimic the normal circadian rhythm of endogenous cortisol. In addition, giving hydrocortisone three times per day is inconvenient and may result in missed doses. Researchers and pharmaceutical companies have been trying to improve treatment of patients with adrenal insufficiency. There are currently several orphan drugs that are approved by the FDA and/or EMA for use to treat adrenal insufficiency in adults and one to use in children [185–187].

Plenadren or Duocort is a once-daily hydrocortisone modified-release tablet (OD). It is designed to be taken once a day in the morning. It has an outer coating that supplies a high concentration of hydrocortisone in the morning and an extended-release core for continuous release throughout the day. It comes in 5 mg and 20 mg doses. The immediate-release form of the drug gives physiological cortisol levels within 20 min of ingestion. The extended-release form of the drug decreases the exposure to steroids over a 24-h period compared to thrice-daily hydrocortisone. The safety profile is similar to thrice-daily hydrocortisone. During times of mild-to-moderate stress, the drug should be given every 8 h. In a study of 64 patients, the OD hydrocortisone demonstrated decreased body weight, blood pressure compared to the subjects on thrice-daily immediate-release hydrocortisone. In the patients with concomitant diabetes mellitus, there was an improvement in HbA1c after 12 weeks of treatment with the OD hydrocortisone. In addition, QoL improved in subjects at 12 weeks of therapy on the OD hydrocortisone [185].

Chronocort is another hydrocortisone modified-release tablet (MR-HC). The MR-HC is a bilayer tablet consisting of an insoluble coating that covers all but one face of the pill. The unprotected face has a layer which slowly erodes to release the sustained release hydrocortisone resulting in a delayed and extended release of hydrocortisone. When given at 10 PM once daily, the peak cortisol level occurs at 6 AM the next day. In a small study of adults with congenital adrenal hyperplasia, twice-daily MR-HC, given at 7 AM and 11 PM, resulted in a peak cortisol at 7 AM and reduced androgens [186, 187].

Infacort is a hydrocortisone preparation composed of granules with taste masking for infants and neonates. The inert core is surrounded by the hydrocortisone which is coated with a binding layer and covered with a taste-masking layer. The granules are placed in a capsule, with the dose of hydrocortisone modified by altering the fill weight of the capsules. Infacort is administered by emptying the capsule onto a teaspoon, placing the granules on the back of the infant's tongue followed by administration of water. A study in adults demonstrated that Infacort was well-tolerated, neutral taste, and easy to administer and produced serum cortisol levels equivalent to conventional hydrocortisone [188].

Verucerfont is a corticotropin-releasing factor 1 receptor antagonist. Theoretically, this drug would decrease ACTH release, allowing patients with CAH to be treated with lower doses of hydrocortisone. Verucerfont was granted orphan drug status by the FDA in early 2015. However, clinical trials of this drug for use to treat CAH were put on hold due to new preclinical findings [189].

Other potential treatment for virilizing CAH is to use a drug that inhibits a key androgenic enzyme, thereby allowing one to use physiological (rather than supraphysiological) doses of steroids to treat a patient with virilizing CAH. Abiraterone acetate is an oral prodrug for abiraterone, which inhibits the 17 alpha-hydroxylase/17, 20 lyase (P450c17, CYP17A1) enzyme. This drug has been used to successfully reduce androgens in men with prostate cancer and was recently shown to reduce androgens in women with 21-hydroxylase deficiency CAH in a Phase 1 dose escalation trial. However, since this drug also inhibits gonadal steroids, it cannot be used during pregnancy or during puberty [190].

13.6.4.2 X-Linked Adrenoleukodystrophy

Conventional therapy consists of replacement and stress doses of cortisol, if indicated. Restriction of VLCFA intake and supplementation with glycerol trioleate and glycerol trierucate (Lorenzo's oil) has very little benefit [191]. In animal studies, 4-phenylbutyrate promotes the expression of a peroxisomal protein that corrects the metabolism of VLCFA and prevents the accumulation in the brain and adrenal glands [56]. Bone marrow transplantation has shown some success when performed before significant cognitive changes have occurred [192, 193]. However, bone marrow transplantation does not reverse damage that has already occurred.

Case Study

A 16-year-old male was referred to the emergency department (ED) by his primary care provider for hyponatremia, weight loss, weakness, and recurrent vomiting.

The patient reported progressive weakness, fatigue, weight loss, and nausea over the past 6–8 weeks. He was previously a star track athlete but resigned due fatigue and weakness. He was unable to carry his book bag or climb the stairs at school. He reported daily nausea and periumbilical abdominal pain with emesis for 2 weeks prior to presentation. He endorsed intense cravings for salt, sought pickles, and potato chips. During the prior 2 months, he had been evaluated at the ED twice for similar symptoms. He reported that his sodium was low both times, but was not addressed. During one visit he was given antibiotics for a "muscle infection" because there were signs of "muscle breakdown" in his blood. At the other ED visit, he was diagnosed with asthma because he found it exhaustive to breathe. He endorsed that he felt much better during his 5-day course of prednisone and worsened again after completion of treatment.

At the third ED visit, his exam was notable for tachycardia, postural hypotension, delayed capillary refill, diffuse hyperpigmentation, and significant increased pigmentation on hand creases and scrotum. He had mild, diffuse abdominal pain and decreased muscle strength. BMI was at the 40th percentile. Laboratory tests showed hyponatremia (127 mEq/L) and mildly elevated blood urea nitrogen with normal serum potassium, creatinine, TSH, and free thyroxine, along with very low random cortisol concentration (less than 0.8 mcg/dl). He was diagnosed with primary adrenal insufficiency, given an intravenous (IV) fluid bolus and IV hydrocortisone. He was continued on 50 mg/m^2/day of hydrocortisone for 1 week and then changed to 9 mg/m^2/day of hydrocortisone divided three times per day along with fludrocortisone, 0.1 mg per day. Laboratory values that came back later were consistent with autoimmune adrenal insufficiency. Specifically, he had an elevated ACTH (1380 pg/ml; (normal: 7.2–63.3)) and plasma renin activity (43.36 ng/ml/h; (normal: 0.25–5.82)), normal very long-chain fatty acids (VLCFA), and positive 21-hydroxylase antibodies. He had normal liver enzymes, negative celiac antibodies, and negative thyroid antibodies but does have elevated glutamic acid decarboxylase-65 antibodies.

The patient and his family members were educated on the pathophysiology of adrenal insufficiency, importance of adherence with medications, indication for use of stress dose of hydrocortisone, and use of intramuscular emergency hydrocortisone injection. They were also educated about the symptoms of diabetes. The patient has been gaining weight, has rejoined his track team, and no longer craves salt.

This case illustrates the fact that since primary adrenal insufficiency is rare, with a prevalence of 100–140 per million in the developed countries, that is often overlooked. The most common cause of primary adrenal insufficiency is due to adrenal autoimmunity. However, in males, VLCFA levels should be sent to ensure that the patient does not have X-linked adrenoleukodystrophy as the cause of adrenal failure. Since autoimmune adrenal disease may be part of a polyglandular autoimmune syndrome, these patients should be regularly screened for other autoimmune diseases such as thyroid disease, celiac disease, type 1 diabetes, and vitamin B12 deficiency.

13.7 Summary

In summary, adrenal insufficiency is a rare disorder. It is vital, however, to consider it in the diagnostic differential of an appropriate patient, as left untreated it is often a fatal disease. Symptoms of adrenal insufficiency may include hypotension, hyponatremia, hypoglycemia, abdominal pain/vomiting, and/or hyperpigmentation. Treatment includes replacement steroids plus or minus mineralocorticoids and salt supplements in infants. During times of stress, patients with adrenal insufficiency need extra steroids. In males with primary adrenal insufficiency, it is critical to screen for X-linked adrenoleukodystrophy. Any patient with primary adrenal insufficiency of autoimmune etiology should be screened for other autoimmune diseases. Growth and development needs to be closely monitored in these patients to ensure proper dosing of medication.

Review Questions

1. The Endocrine Society recommends the use of the standard dose (250 mcg in adults) intravenous corticotropin stimulation test as the preferred diagnostic test for primary adrenal insufficiency.
   A. True
   B. False
2. Dexamethasone is the preferred glucocorticoid for treatment of primary adrenal insufficiency since it only needs

to be taken once a day thus improving adherence.
   A. True
   B. False
3. Symptoms of primary adrenal insufficiency may include which of the following:
   A. Hyponatremia
   B. Hypoglycemia
   C. Hyperpigmentation
   D. Salt craving
   E. All of the above

Answers

1. (A) *True.* In the standard corticotropin test, baseline ACTH and cortisol samples are obtained, and then corticotropin (250 mcg in adults and children ≥2 years, 15 mcg/kg in infants, and 125 mcg for children <2 years) is administered intravenously. Thirty and/or 60 min following corticotropin administration, plasma samples are obtained for cortisol determination. Peak plasma cortisol concentration less than 18 mcg/dl (500 nm/L) at all times indicates adrenal insufficiency.
2. (B) *False.* Dexamethasone is not the preferred drug for treatment of adrenal insufficiency due to difficulties in dose adjustment leading to iatrogenic Cushing disease. Hydrocortisone at a total daily dose of 8 mg/M^2 body surface area in 3–4 divided doses for children and 15–25 mg per day divided two to three times per day in adults is the preferred medication.
3. (E) *All of the above.* Since patients with untreated primary adrenal insufficiency lack adequate negative feedback to the pituitary, ACTH levels are elevated. Since ACTH is cleaved from the precursor polypeptide, POMC, which also contains the melanocortin-stimulating hormone, these patients will develop hyperpigmentation in non-sun-exposed areas. The degree of hyperpigmentation may be subtle, so should not be the only criteria used to consider this diagnosis. Other symptoms may include hyponatremia (due to decreased free water clearance), hypoglycemia, fatigue, weight loss, muscle weakness, and abdominal pain. If there is mineralocorticoid deficiency, these patients will also crave salt.

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Congenital Adrenal Hyperplasia

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Key Points

- 21-Hydroxylase deficiency accounts for 95% of all cases of CAH.
- Newborn screening has allowed for early diagnosis and treatment of CAH.
- Glucocorticoid therapy remains the mainstay of CAH treatment.
- Prenatal diagnosis and treatment are still considered experimental and not routinely recommended.

14.1 Introduction

Congenital adrenal hyperplasia (CAH) is a family of autosomal recessive disorders in which there is a deficiency of one of the enzymes necessary for cortisol synthesis [1] (see Fig. 14.1). Abnormalities in each of the enzymatic activities required for cortisol synthesis have been described. As a result of the disordered enzymatic step, there is decreased cortisol synthesis, increased ACTH via a negative feedback system, overproduction of the hormones prior to the enzymatic step or not requiring the deficient enzyme, and deficiency of the hormones distal to the disordered enzymatic step. Since several of the enzymatic steps are required for sex hormone synthesis by the gonad, a disordered enzymatic step in the gonad resulting in gonadal steroid hormone deficiency may also be present [2].

The symptoms of the disorder depend upon which hormones are overproduced and which are deficient. As a result, CAH may present with a spectrum of symptoms: virilization of an affected female infant, subsequent signs of androgen excess

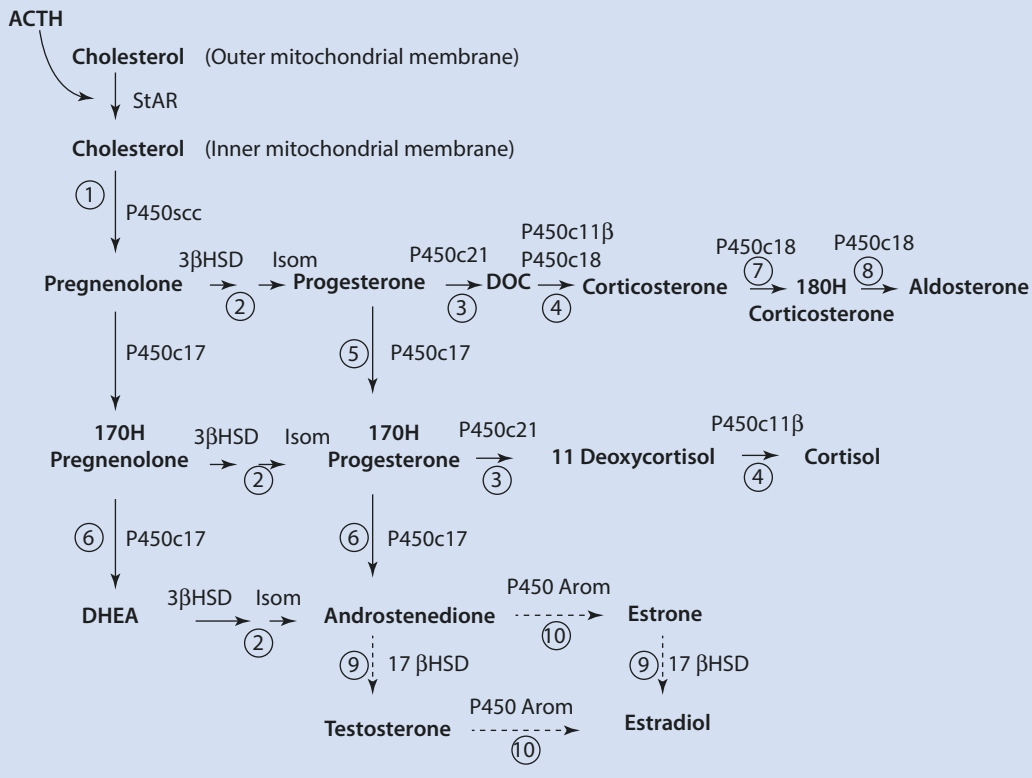

Fig. 14.1 Simplified scheme of adrenal steroidogenesis. Two reactions (*dotted arrows*) occur primarily in gonads, not in adrenal gland. Chemical names for enzymes shown above or to the right of arrows; circled numbers refer to traditional names, (1) 20,22-desmolase, (2) 3β(beta)-hydroxysteroid dehydrogenase/isomerase, (3) 21-hydroxylase, (4) 11β(beta)-hydroxylase, (5) 17α(alpha)-hydroxylase, (6) 17,20-lyase, (7) 18-hydroxylase, (8) 18-oxidase, (9) 17β(beta)-hydroxysteroid dehydrogenase, (10) aromatase; StAR, steroidogenic acute regulatory protein; DOC, 11-deoxycorticosterone (for P450 oxidoreducatse deficiency, combined deficiency of 3 and 5)

Table 14.1 Enzymes and genes in congenital adrenal hyperplasia

Enzymatic activity	Enzyme	Cellular location	Gene	Chromosomal location
Cholesterol desmolase (side-chain cleavage)	P450scc (CYP11A1)	Mitochondrion	*CYP11A1*	15q23–24
3β(Beta)-hydroxysteroid dehydrogenase	3β(Beta)HSD (3β(Beta)HSDII)	Endoplasmic reticulum	*HSD3B2*	1p13.1
17α(Alpha)-hydroxylase/17,20 lyase	P450c17 (CYP17)	Endoplasmic reticulum	*CYP17*	10q24.3
21α(Alpha)-hydroxylase	P450c21 (CYP21A2)	Endoplasmic reticulum	*CYP21A2*	6p21.3
11β(Beta)-hydroxylase	P450c11 (CYP11B1)	Mitochondrion	*CYP11B1*	8q21–22
Combined 21α(alpha)-hydroxylase and 17α(alpha)-hydroxylase/17,20 lyase (P450 oxidoreductase)	P450 oxidoreductase	Microsome	*POR* (P450 oxidoreductase)	7q11.2
Aldosterone synthase (corticosterone 18-methylcorticosterone oxidase/lyase)	P450c18 (CYP11B2)	Mitochondrion	*CYP11B2*	8q21–22

Data from Ref. [1]

in both males and females, incomplete virilization of the male, signs of sex hormone deficiency at puberty in both males and females, salt-wasting crisis secondary to aldosterone deficiency, and hormonal hypertension secondary to increased deoxycorticosterone (DOC), a mineralocorticoid [1–6]. The enzymes of adrenal steroidogenesis, their cellular location, the genes encoding the enzymes, and their chromosomal locations are presented in Table 14.1. The clinical presentations of this family of disorders are presented in Table 14.2. This chapter presents an overview of all of the enzymatic deficiencies resulting in CAH with the most extensive review of 21-hydroxylase deficiency which is the most common, first described, and most intensively studied of the enzymatic disorders.

14.2 Lipoid Adrenal Hyperplasia

Lipoid adrenal hyperplasia is due to a deficiency of cholesterol desmolase activity. It is one of the most severe types of CAH [7]. There is a deficiency in all of the adrenal hormones as a result: glucocorticoids (cortisol), mineralocorticoids (aldosterone), and sex steroids (Fig. 14.1). In addition, since this enzymatic activity is necessary for sex hormone synthesis in the gonad, there is also a deficiency of gonadal steroids. Affected infants usually present early in life with salt-wasting crisis manifested by cardiovascular collapse, hyponatremia, and hyperkalemia. Males have phenotypically female external genitalia. Females exhibit no genital abnormalities. Increased pigmentation secondary to increased MSH from POMC cleavage may be of such a degree as to produce "bronzing" in the newborn. Occasionally, infants have been reported to present with salt-wasting crisis beyond the newborn period. Because of the gonadal sex steroid deficiency, males are unable to produce gonadal steroids at the time of puberty. Affected females may have sufficient gonadal function remaining at puberty to begin feminization and progress to menarche; this is likely due to the fact that there is some degree of steroidogenesis that is independent of StAR [8]. However, gonadal failure ultimately ensues likely from progressive lipid deposition in ovarian follicular cells (Table 14.2). Laboratory evaluation in patients with lipoid adrenal hyperplasia reveals low

Table 14.2 Clinical and hormonal data

Enzymatic deficiency	Signs and symptoms	Laboratory findings	Therapeutic measures
Lipoid CAH (cholesterol desmolase deficiency)	Salt-wasting crisis Male DSD Incomplete female puberty	Low levels of all steroid hormones, with decreased/absent response to ACTH Decreased/absent response to HCG in males ↑↑ ACTH ↑↑ PRA Hypergonadotropic hypogonadism	Glucocorticoid and mineralocorticoid administration Sodium chloride supplementation Gonadectomy of male DSD Sex hormone replacement consonant with sex of rearing
3β(Beta)-HSD deficiency	Classic form: Salt-wasting crisis Male and female DSD Precocious pubarche Disordered puberty	↑↑ baseline and ACTH-stimulated Δ(delta)5 steroids (pregnenolone, 17-hydroxypregnenolone, DHEA and their urinary metabolites) ↑↑ Δ(delta)5/Δ(delta)4 serum and urinary steroids ↑↑ ACTH ↑↑ PRA Suppression of elevated adrenal steroids after glucocorticoid administration	Glucocorticoid and mineralocorticoid administration Sodium chloride supplementation Surgical correction of genitalia and sex hormone replacement as necessary consonant with sex of rearing
3β(Beta)-HSD deficiency	Nonclassic form: Precocious pubarche Disordered puberty Menstrual irregularity Hirsutism Acne Infertility	↑ baseline and ACTH-stimulated Δ(delta)5 steroids (pregnenolone, 17-hydroxypregnenolone, DHEA and their urinary metabolites) ↑ Δ(delta)5/Δ(delta)4 serum and urinary steroids ↑ ACTH ↑ PRA Suppression of elevated adrenal steroids after glucocorticoid administration	Glucocorticoid administration
21-Hydroxylase deficiency	Classic form: Salt-wasting crisis Female DSD Postnatal virilization	↑↑ baseline and ACTH-stimulated 17-hydroxyprogesterone and pregnanetriol ↑↑ serum androgens and urinary metabolites ↑↑ ACTH ↑↑ PRA Suppression of elevated adrenal steroids after glucocorticoid administration	Glucocorticoid and mineralocorticoid administration Sodium chloride supplementation Vaginoplasty and clitoral recession or clitoroplasty with preservation of neurovascular bundle in female DSD
21-Hydroxylase deficiency	Nonclassic form: Precocious pubarche Disordered puberty Menstrual irregularity Hirsutism Acne Infertility	↑ baseline and ACTH-stimulated 17-hydroxyprogesterone and pregnanetriol ↑ serum androgens and urinary metabolites ↑ ACTH ↑ PRA Suppression of elevated adrenal steroids after glucocorticoid administration	Glucocorticoid administration

Table 14.2 (continued)

Enzymatic deficiency	Signs and symptoms	Laboratory findings	Therapeutic measures
11β(Beta)-hydroxylase deficiency	Classic form: Female DSD Postnatal virilization in males and females Hypertension	↑↑ baseline and ACTH-stimulated compound S, DOC, and their urinary metabolites ↑↑ serum androgens and their urinary metabolites ↑↑ ACTH ↓ PRA Hypokalemia Suppression of elevated steroids after glucocorticoid administration	Glucocorticoid administration Vaginoplasty and clitoral recession or clitoroplasty with preservation of neurovascular bundle in female DSD
17α(Alpha)-hydroxylase/17,20 lyase deficiency	Male DSD Sexual infantilism Hypertension	↑↑ DOC, 18-OH DOC, corticosterone, 18-hydroxycorticosterone Low 17α(alpha)-hydroxylated steroids and poor response to ACTH Poor response to HCG in male DSD ↓ PRA ↑ ACTH Hypokalemia Suppression of elevated adrenal steroids after glucocorticoid administration	Glucocorticoid administration Surgical correction of genitalia and sex hormone replacement in male DSD consonant with sex of rearing Sex hormone replacement in females
Combined deficiency of 21α(alpha)-hydroxylase and 17α(alpha)-hydroxylase/17,20 lyase deficiency (P450 oxidoreductase deficiency)	Females: Prenatal virilization of external genitalia Primary amenorrhea Males: Feminized or undermasculinized external genitalia Maternal: Gestational virilization	Variable combination of 21 and 17α(alpha)-hydroxylase/17,20 lyase deficiency (e.g., ↑↑ ACTH, ↑↑ 17-hydroxyprogesterone, ↑↑ progesterone, normal or ↓↓ androgens or ↓ or normal cortisol)	Glucocorticoid administration Sex hormone replacement as needed

Data from Ref. [184]
DSD disorder of sex development

levels of all steroid hormones with no response to ACTH or human chorionic gonadotropin (HCG) administration. ACTH and plasma renin activity (PRA) are very elevated. Imaging studies of the adrenal gland will reveal marked enlargement of the adrenals secondary to the accumulation of lipoid droplets. Females with this disorder have normal internal and external genitalia.

Males as noted above are phenotypically female, which affirms the absence of testosterone production between 6 and 12 weeks of gestation [9]. Males incorrectly diagnosed as females with adrenal insufficiency have been noted subsequently to have inguinal gonads, which has led to the correct diagnosis.

Lipoid adrenal hyperplasia is an autosomal recessively inherited disorder that is found in most ethnic groups but most frequently in patients of Japanese, Korean, and Palestinian Arab descent [7, 10, 11]. In this disorder, the gene coding for P450 SCC, a mitochondrial enzyme, has been normal in almost all cases studied (Table 14.1). Congenital lipoid adrenal hyperplasia is most often due to mutations in the

gene for steroidogenic acute regulatory protein (*StAR*). The *StAR* gene is located on chromosome 8p11.2 and is expressed in adrenals and gonads. *StAR* is a mitochondrial protein that enables the movement of cholesterol from the outer to the inner mitochondrial membrane. Lipoid adrenal hyperplasia is one of only two forms of CAH, which is not caused by a mutation in a gene coding for a steroidogenic enzyme [7, 10, 12–20], the second one being P450 oxidoreductase deficiency [21].

Certain *StAR* mutations are more commonly identified in certain ethnic groups. The majority of Japanese and Korean patients (65–70% and over 90%, respectively) carry the Q258X mutation, while R182L is found in Palestinian Arabs, R182H in eastern Saudi Arabians, and L260P in the Swiss [8, 10, 11]. A founder *StAR* mutation c.201_202delCT has been described in four Palestinian families as perhaps the most common mutation resulting in lipoid adrenal hyperplasia in this particular population [12]. Saenger et al. first described diagnosing congenital lipoid adrenal hyperplasia prenatally by measuring maternal estriol and amniotic fluid steroid levels [13]. It is now possible to diagnose congenital lipoid adrenal hyperplasia prenatally using targeted molecular genetic analysis for the c.201_202delCT mutation [10]. Other novel mutations in *StAR* are still being described [14, 22–25].

The prevalence of testicular neoplasia is currently unknown in this patient population; however, gonadectomy in affected 46,XY patients is recommended before puberty to prevent malignant progression. Of great importance, however, is the recent discovery that gonads of affected 46,XY patients have shown neoplastic changes as early as age one; thus early gonadectomy at the time of diagnosis may be necessary in these patients [12]. A nonclassic form of the disorder has also been described in which patients have been found to have partial loss-of-function mutations in *StAR*, which appears to result in a phenotype of late-onset adrenal insufficiency with minimally disordered sexual development [15]. Most patients with nonclassic lipoid CAH carry the StAR mutation R188C [8]. While patients with lipoid CAH and those with P450scc mutations have almost identical clinical and hormonal findings, P450scc deficiency should not be considered a form of lipoid CAH [8].

14.3 3β(Beta)-Hydroxysteroid (3β(Beta)-HSD)/Δ(Delta)4,5-Isomerase Deficiency

3β(Beta)-HSD/Δ(delta)4,5-isomerase deficiency is also a rare form of CAH occurring in fewer than 5% of patients. As can be seen in Fig. 14.1, 3β(beta)-HSD/Δ(delta)4,5-isomerase is necessary for the conversion of pregnenolone to progesterone, 17-hydroxypregnenolone to 17-hydroxyprogesterone, and dehydroepiandrosterone (DHEA) to Δ(delta)4-androstenedione. The decreased ability to convert these Δ(delta)5 steroids to Δ(delta)4 steroids results in diminished synthesis of cortisol, aldosterone, and Δ(delta)4-androstenedione. In the testes, it results in a decreased ability to form testosterone. The deficiency of cortisol results in increased ACTH with overproduction of Δ(delta)5 steroids, including DHEA. The increased level of DHEA is sufficient to result in some degree of virilization of the external genitalia in females, although the virilization is not as marked as in the other forms of virilizing CAH such as 21-hydroxylase deficiency and 11-hydroxylase deficiency. Female infants with 3β(beta)-HSD/Δ(delta)4,5-isomerase deficiency may have clitoromegaly and partial fusion of labial folds. Males with this disorder manifest a deficiency of prenatal testosterone and are born with varying degrees of ambiguity of the external genitalia ranging from hypospadias to more significant degrees of incomplete virilization with partial fusion of scrotal folds. Most infants with 3β(beta)-HSD/Δ(delta)4,5-isomerase deficiency have aldosterone deficiency and may present in the newborn period with salt-wasting crisis. Postnatally there is continued excessive DHEA secretion with growth acceleration and the early onset of pubic and/or axillary hair. Symptoms of ongoing excessive adrenal androgens include hirsutism, acne, menstrual irregularity or amenorrhea, and infertility. Increased pigmentation of skin creases occurs secondary to increased MSH from POMC cleavage.

Laboratory evaluation reveals elevation of the Δ(delta)5 steroids, specifically the diagnostic hormone 17-hydroxypregnenolone and DHEA, with a further rise following ACTH stimulation to levels of 10,000–60,000 ng/dL and 3000–12,000 ng/dL, respectively. The ratios of Δ(delta)5 to Δ(delta)4 steroids (17-hydroxypregnenolone/17-hydroxyprogesterone and DHEA/Δ(delta)4-androstenedione)

post ACTH stimulation have been reported to reach 18–25 and 18–30, respectively. Males with this disorder have been reported to undergo normal male puberty. However, this occurs with marked elevation of the Δ(delta)5 steroids sufficient to produce adequate levels of testosterone. ACTH levels are increased and in those with aldosterone deficiency, PRA is markedly elevated as well. Glucocorticoid administration results in a decrease in ACTH followed by a decrease in the overproduced adrenal androgens (Table 14.2).

The 3β(beta)-HSD enzyme, located in the endoplasmic reticulum, mediates both 3β(beta)-HSD and isomerase activities (Table 14.1). In humans, there are two 3β(beta)-HSD isoenzymes, designated types I and II, which are encoded by the *HSD3B1* and *HSD3B2* genes. 3β(Beta)-HSD/Δ(delta)4,5-isomerase deficiency is due to a mutation in the *HSD3B2* gene, located on chromosome 1, and expressed in adrenal and gonadal tissue [26]. A number of mutations in this gene have been described, and while rare, it occurs with greater frequency among Old Order Amish of North America due to a founder effect [27]. Mutations in the *HSD3B2* gene result in a number of molecular defects, which are associated with the different phenotypic manifestations of 3β(beta)-HSD deficiency [26, 28].

In the past, signs of mild androgen excess in children and adults (precocious pubarche, acne, hirsutism, menstrual problems) have been attributed to a nonclassic form of 3β(beta)-HSD deficiency in which a less severe enzymatic deficiency results in lesser elevations of the Δ(delta)5 steroids and Δ(delta)5/Δ(delta)4 ratios. Although mutations in the *HSD3B2* gene have been described in premature pubarche, a number of children and adults thought to have nonclassic 3β(beta)-HSD deficiency have been demonstrated to have normal *HSD3B2* genes, bringing into question the diagnosis and suggesting that hormonal criteria remain to be established for the diagnosis of the nonclassic or mild form of the disease [26, 28–34].

14.4 17-Hydroxylase/17,20-Lyase Deficiency

17-Hydroxylase/17,20-lyase deficiency is another relatively rare form of CAH with a reported incidence of approximately 1:50,000 births [35–37]. In this disorder, there is a deficiency of 17-hydroxylation by which pregnenolone and progesterone are converted to 17-hydroxypregnenolone and 17-hydroxyprogesterone, as well as deficiency in the 17,20-lyase reaction resulting in the conversion of 17-hydroxypregnenolone and 17-hydroxyprogesterone to DHEA and Δ(delta)4-androstenedione, respectively (Fig. 14.1). Similar to other forms of CAH, the deficiency in cortisol results in increased ACTH. Overproduction of DOC, a mineralocorticoid, produces hypertension and hypokalemia that may be the presenting symptoms. Because this enzymatic deficiency is present also in the gonad, there is a deficiency of sex steroids as well so that affected males are incompletely virilized; they may be phenotypically female or ambiguous. These males are unable to undergo normal male puberty due to testosterone deficiency. Affected females have normal female external genitalia and may present with failure of sexual development at adolescence (Table 14.2). The majority of patients are infertile [38]. Rarely, patients have been described with isolated 17,20-lyase deficiency in which there is sex hormone deficiency without mineralocorticoid excess [39–42].

17-Hydroxylase/17,20-lyase deficiency is diagnosed by the presence of low levels of all 17-hydroxylated steroids with a poor response to ACTH and HCG administration. Levels of DOC (10–40×), 18-OH DOC (30–60×), corticosterone (B) (30–100×), and 18-OHB (10×) are markedly elevated, and PRA and aldosterone are suppressed. Glucocorticoid administration results in suppression of the overproduced hormones. As DOC is suppressed, there is resolution of the volume expansion and PRA increases, thus stimulating aldosterone secretion.

P450c17, found in the endoplasmic reticulum, is responsible for catalyzing steroid 17-hydroxylation and 17,20-lyase reactions. The *CYP17* gene is located on chromosome 10q24–25 and is expressed both in the adrenal cortex and in the gonads (Table 14.1). Almost 100 different genetic mutations of the *CYP17* gene have been documented with wide variation in the clinical and biochemical presentations (e.g., 10–15% patients are normotensive at diagnosis [36], and novel mutations in the *CYP17* gene are still being described [38, 42–46]. In Brazil, due to two founder mutations, the disease is more common [38]. There are also case reports of patients whose clinical and biochemical profiles support

the diagnosis of 17-hydroxylase/17,20-lyase deficiency, but no pathologic mutations in the *CYP17* gene have been found, suggesting that the defect may be in other areas of the gene as yet not detected by molecular analysis or in posttranslational processes [26]. The molecular basis for isolated 17,20-lyase deficiency has been elucidated [39–41].

14.5 21-Hydroxylase Deficiency

21-Hydroxylase deficiency is the most common form of CAH, affecting approximately 95% of individuals with CAH. It occurs with a worldwide frequency of approximately 1:15,000 newborns with increased frequency among certain ethnic groups [47]. In this disorder, there is impaired ability to 21-hydroxylate progesterone and 17-hydroxyprogesterone to DOC and 11-deoxycortisol (S), respectively (Fig. 14.1). As a result, there is decreased cortisol secretion, increased ACTH, adrenal hyperplasia, and overproduction of steroids prior to 21-hydroxylation. 17-Hydroxyprogesterone is most elevated and is the diagnostic hormone in this disorder. There is overproduction of the adrenal androgens, especially Δ(delta)4-androstenedione, and by peripheral conversion testosterone, which results in virilization, the hallmark of this disorder.

In addition, approximately 2/3 of these patients will have aldosterone deficiency and present with salt-wasting crisis in the newborn period, most often between 1 week and 1 month of age. Salt-wasting can manifest later in infancy and occasionally beyond the time of infancy, often in the setting of an intercurrent illness. Because this disorder begins in utero, the female fetus is exposed to excessive adrenal androgens resulting in virilization of the external genitalia ranging from clitoromegaly, with or without mild degrees of labial fusion, to marked virilization of the external genitalia such that the female infant appears to be a male infant with hypospadias (occasionally with the appearance of a penile urethra) and undescended testes. There is a urogenital sinus with one outflow track to the perineum. As with all forms of CAH, a female infant will have normal ovaries, fallopian tubes, uterus, and proximal vagina.

Postnatally, there is continued virilization with progressive clitoromegaly and penile enlargement, rapid growth, and premature development of pubic and/or axillary hair. Additionally, signs of androgen excess secondary to late or inadequate treatment include acne, delayed menarche or primary amenorrhea, menstrual irregularity, hirsutism, and infertility. Although rapid growth and tall stature are present in early childhood, bone age advancement is typically greater than height advancement, resulting in short final height in late or poorly treated patients. True precocious puberty may occur with bone age advancement to 10 years or older contributing to short final height. Increased MSH from POMC cleavage results in hyperpigmentation of skin creases, nipples, and genitalia. Unilateral testicular enlargement may occur secondary to stimulation of adrenal rest tissue and result in the formation of adrenal rest tumors (Table 14.2).

A milder nonclassic form of 21-hydroxylase deficiency is well recognized. The prevalence in the general Caucasian population is approximately 0.1–0.2%, but it may occur in up to 1–2% among certain inbred populations such as Ashkenazi Jews [48]. Salt-wasting is absent in the nonclassic disorder and female genitalia are normal at birth. Signs of androgen excess may appear in childhood. Premature pubarche, acne, hirsutism, menstrual irregularity, and infertility may be presenting symptoms. Males with this disorder may also present with unilateral testicular enlargement similar to males with the classical disorder (Table 14.2).

The diagnostic hormone in 21-hydroxylase deficiency is 17-hydroxyprogesterone. Levels in the classic form are markedly elevated throughout the day in the range of 10,000–100,000 ng/dL and rise to levels of 25,000–100,000 ng/dL or greater following ACTH stimulation. Δ(Delta)4-androstenedione levels are also elevated and may be in the range of 250 ng/dL to greater than 1000 ng/dL. Testosterone levels are elevated to a variable degree and range from early male pubertal levels to levels in the adult male range (350–1000 ng/dL). While these are no longer commonly checked, the 24-h urinary excretion of pregnanetriol and 17-ketosteroids, the metabolic products of 17-hydroxyprogesterone and androgens, respectively, is also elevated. The elevated serum and urinary hormones promptly decrease following glucocorticoid administration. ACTH levels are also increased throughout the day in classic 21-hydroxylase deficiency. PRA and PRA/

aldosterone are increased in overt or subtle salt-wasting. Salt-wasting crisis presents with hyponatremia, hyperkalemia, acidosis, and azotemia. Hypoglycemia may also be present. Cortisol levels may be decreased or in the normal range but usually do not increase with ACTH, indicating that the adrenal gland has maximally compensated for the enzymatic deficiency.

Laboratory findings are less marked in the nonclassic form. 17-Hydroxyprogesterone may be only mildly elevated, particularly if drawn in late morning or afternoon, paralleling the diurnal pattern of ACTH. An early morning baseline level of <200 ng/dL can safely rule out the nonclassic form [49]. Following ACTH administration, 17-hydroxyprogesterone may rise to levels of 2000–10,000 ng/dL, although some older patients have shown stimulated values between 1000 ng/dL and 1500 ng/dL [50]. Serum androgens are also less elevated compared to the classic form. Glucocorticoid administration results in a prompt decrease in the elevated hormones (Table 14.2).

21-Hydroxylation is mediated by P450c21, found in the endoplasmic reticulum (Table 14.1). The gene for P450c21 was initially mapped to within the HLA complex on the short arm of chromosome 6 between the genes for HLA-B and DR by HLA studies of families with classic CAH. In these studies, it was demonstrated that within a family, all affected siblings were HLA-B identical and different from their unaffected siblings. Family members sharing one HLA-B antigen with the affected index case were predicted to be heterozygote carriers of the *CAH* gene, and family members sharing no HLA-B antigen with the affected index case were predicted to be homozygous normal. Subsequently, molecular genetic analysis demonstrated that there are two highly homologous human *P450c21* genes—one active (*CYP21A2*) and one inactive (*CYP21AP*). The two genes are located in tandem with two highly homologous genes for the fourth component of complement (*C4A*, *C4B*). A number of other genes of known and unknown function are also located in this cluster.

The genetic mutations in patients with 21-hydroxylase deficiency have been extensively studied. More than 200 *CYP21A2* mutations have been described to date with 90% of cases resulting from 10 common *CYP21A2* mutations [51]. *CYP21A2* mutations can be grouped into three categories depending on level of enzymatic activity: Group A mutations (often deletions or nonsense mutations) in which enzyme activity is completely absent, Group B mutations (missense) which retain only 1–2% of normal enzyme activity, and Group C mutations in which 20–60% of enzyme activity is preserved [51]. The severity of the disease is determined by the less severely affected allele. Most patients (65–75%) are compound heterozygotes, having a different mutation on each allele. Approximately 75% of mutations are recombinations between the inactive *CYP21AP* and the active *CYP21A2* gene, resulting in microconversions. A valine-to-leucine substitution in codon 281 is a frequently found point mutation in nonclassic 21-hydroxylase deficiency and is highly associated with HLA-B14DR1 [1–6]. The severity of the disease is determined by the less severely affected allele.

The classic form of the disorder results from the combination of two severe deficiency genes, while the nonclassic form of the disease results from a combination of a severe *CYP21A2* deficiency gene (found in the classic form of the disease) and a mild *CYP21A2* deficiency gene or a combination of two mild deficiency genes. Molecular genetic diagnosis of patients with CAH can be difficult given the complexity of gene duplications, deletions, and rearrangements within chromosome 6. Although false positives are still possible, multiplex ligation-dependent probe amplification (MLPA) analysis has been more widely used for detection of abnormalities in the CYP21A2 gene [52].

14.6 11β(Beta)-Hydroxylase Deficiency

11β(Beta)-hydroxylase deficiency is the second most common cause of CAH and accounts for approximately 5–8% of reported cases. It occurs in approximately 1:100,000 births in a diverse Caucasian population but is more common in Jews of North African origin. In this disorder, the enzymatic deficiency results in a block in 11-hydroxylation of 11-deoxycortisol (compound S) to cortisol and 11-deoxycorticosterone (DOC) to corticosterone (B). Impaired cortisol production results in increased ACTH and adrenal hyperplasia, as well as overproduction of 11-deoxycortisol and DOC. As in 21-hydroxylase deficiency, there is shunting into the androgen pathway with

overproduction of adrenal androgens, especially Δ(delta)4-androstenedione, and by peripheral conversion, testosterone (Fig. 14.1).

Prenatal virilization of the female fetus and postnatal virilization of affected males and females are similar to 21-hydroxylase deficiency. The excessive DOC secretion results in sodium and water retention with plasma volume expansion. Hypertension and hypokalemia may ensue.

The diagnosis of 11β(beta)-hydroxylase deficiency is based upon marked elevation of serum 11-deoxycortisol (1400–4300 ng/dL) and DOC (183–2050 ng/dL). Increased excretion of their metabolites, tetrahydro-11-deoxycortisol (THS) and tetrahydro-11-deoxycorticosterone (THDOC), in a 24-h urine can confirm the diagnosis. Serum Δ(delta)4-androstenedione and testosterone, as well as urinary ketosteroids, are also elevated. PRA and aldosterone are suppressed secondary to the volume expansion mediated by the excessive DOC; hypokalemia may also be present. Glucocorticoid therapy results in suppression of the excessive S, DOC, and androgens. As DOC is suppressed, there is remission of the volume expansion, PRA and aldosterone rise, and hypokalemia reverses. Nonclassic 11-hydroxylase deficiency has also been reported presenting in later childhood, adolescence, or adulthood with signs of androgen excess (premature pubarche, acne, hirsutism, menstrual irregularity, and infertility). These patients may also exhibit short stature and higher than normal blood pressure [53] (Table 14.2).

P450c11β(Beta), a mitochondrial enzyme, is coded for by *CYP11B1*. It mediates 11β(beta)-hydroxylation in the zona fasciculata, leading to cortisol synthesis. P450c18, also located in the mitochondria and coded for by *CYP11B2*, mediates 11β(beta)-hydroxylase, 18-hydroxylase, and 18-oxidase activities in the zona glomerulosa, leading to aldosterone synthesis. These genes lie on chromosome 8q21–22, about 40 kb from the highly homologous aldosterone synthase gene (*CYP11B2*) [54] (Table 14.1). CAH due to 11β(beta)-hydroxylase deficiency results from mutations in the *CYP11B1* gene. More than 50 *CYP11B1*-inactivating mutations have been described in patients, most with classic 11β(beta)-hydroxylase deficiency, and novel mutations continue to be described [55–63]. Almost all Moroccan Jewish patients with this disorder have a point mutation in codon 448 in *CYP11B1*, resulting in an arginine-histidine substitution, although recently several novel mutations leading to 11-hydroxylase deficiency have been described in this population [55, 64–67].

14.7 P450 Oxidoreductase Deficiency

P450 oxidoreductase deficiency is a rare autosomal recessive disorder of steroidogenesis with a wide spectrum of clinical phenotypes and is unique in that it also affects non-endocrine systems. Biochemically, this form of CAH appears to be a combined form of 21-hydroxylase deficiency and 17-hydroxylase deficiency. In 1985, a case report described a 46,XY patient with genital ambiguity and abnormal serum and urinary steroids that suggested partial deficiencies in steroid 17α-hydroxylase, 17,20-lyase, and 21-hydroxylase, although the gene responsible for the disordered steroidogenesis was obscure at the time [68]. In 2004, the first reports describing mutations in the P450 oxidoreductase gene were published [69–72]. Since those initial reports, at least 130 additional patients with P450 oxidoreductase deficiency have been described [73]. P450 oxidoreductase (*POR*), located on chromosome 7, is an essential electron donor for all microsomal P450 enzymes, including three of the enzymes involved in steroidogenesis, P450c17 (17α(alpha)-hydroxylase/17,20-lyase), P450c21 (21-hydroxylase), and P450aro (aromatase) [21]. *POR* knockout mice are embryonically lethal, most likely from extrahepatic *POR* deficiency; however, mutations in *POR* result in the variable clinical spectrum seen. Numerous *POR* mutations have been described to date, the majority of which are missense and frameshift mutations, although nonsense and splice site mutations and deletions have been reported [21, 74, 75]. The most common mutation in people of European descent is A287P, while R457H is often found in Japan. Analysis of different *POR* mutants and their enzymatic capabilities allows for correlations between genotype and phenotype. Currently, an assay that employs genetically modified yeast and bacteria expressing human P450c17 and *POR* mutants provides excellent correlation [21, 74].

Many, although not all, of the patients also have skeletal abnormalities associated with the Antley-Bixler syndrome (ABS). ABS is a skeletal malformation syndrome that can consist of craniosynostosis, midface hypoplasia, radiohumeral and/or radioulnar synostosis, femoral bowing, fractures, and other skeletal deformities [76]. Approximately 50% of patients with ABS exhibit genital abnormalities [77, 78]. Thus, the current understanding is that ABS is really two distinct genetic disorders. In those patients with normal genitalia, the condition is due to gain-of-function mutations in the gene for fibroblast growth factor receptor 2 (FGFR2), while recessive *POR* mutations are responsible for those patients with ABS, genital anomalies, and/or abnormal steroid profiles [79]. While the basis for the skeletal abnormalities in POR deficiency is still being elucidated, research has suggested that *POR* mutations affect the CYP26B1-mediated degradation of retinoic acid, which is instrumental for osteoblast maturation into osteocytes [80].

With P450 oxidoreductase deficiency, patients typically have normal to increased ACTH with increased17-hydroxyprogesterone, progesterone, and DOC, and decreased cortisol and androgens postnatally, although steroid profiles can vary since *POR* deficiency affects multiple enzymes [21] (Table 14.2). 46,XY males are typically undervirilized because of the decreased 17,20-lyase activity, while 46,XX females are frequently virilized at birth, although the virilization does not advance postnatally. Aromatization of fetal androgens is impaired, which may lead to virilization of the mother and low urinary estriol levels during pregnancy. A role for the "backdoor pathway" to fetal androgen production, in which 21-carbon steroid precursors are ultimately converted to dihydrotestosterone via 5α(alpha)-reduction, has been proposed [21, 81, 82].

Because of the wide variability in phenotype and steroid hormone profile, the diagnosis and treatment of P450 oxidoreductase deficiency are not straightforward. Currently, gas chromatography-mass spectrometry (GC/MS) of urine steroids followed by confirmatory genetic testing is the preferred method of diagnosis [73]. Long-term outcomes for severe P450 oxidoreductase deficiency are unknown. The most critical issue concerns the potential for cortisol deficiency, which, if undetected, may lead to adrenal crisis and death. Aldosterone deficiency with salt-wasting has not yet been reported; it is possible in theory given the 21-hydroxylase deficiency. Orthopedic management is essential for those patients with ABS [21]. Pubertal events in these patients are not fully understood, although there are reports of pubertal failure, lack of breast development and pubic hair, and bilaterally enlarged ovarian cysts in a handful of patients [75, 82]. Drug metabolism may be abnormal in these patients and prescribing medications that are metabolized by P450 enzymes must be done with caution because the majority of drugs are metabolized by hepatic P450 enzymes and *POR* mutants affect drug metabolism in vitro [21, 73, 82]. Further study into the genetics and clinical outcomes of these patients is ongoing.

14.8 Therapy, Monitoring, and Outcome

The principle of therapy for CAH is to replace the hormones that are deficient and to decrease the hormones that are overproduced. Glucocorticoids have been the mainstay of treatment for over 50 years. Proper treatment with glucocorticoids prevents adrenal crisis and virilization, allowing for normal growth and development. As noted previously, administration of glucocorticoids reduces ACTH overproduction, reverses adrenal hyperplasia, and reduces the levels of hormones that are overproduced: androgens in the virilizing disorders (21-hydroxylase, 11-hydroxylase, 3β(beta)-HSD/Δ(delta)4,5-isomerase deficiencies) and DOC in the hypertensive disorders (11-hydroxylase, 17-hydroxylase). In the salt-wasting disorders (lipoid adrenal hyperplasia, 3β(beta)-HSD/Δ(delta)4,5-isomerase, 21-hydroxylase), mineralocorticoid and sodium supplementation are provided. In disorders with sex steroid deficiency (lipoid adrenal hyperplasia, 17-hydroxylase, 3β(beta)-HSD/Δ(delta)4,5-isomerase), sex hormone replacement consonant with the sex of assignment is necessary. Surgical correction of ambiguous genitalia may also be necessary.

The objective of therapy is to achieve normal growth and pubertal development, normal sexual function, and normal reproductive function in those disorders with potential fertility. Therapy must be individualized according to the clinical course and hormonal levels.

14.8.1 Glucocorticoids

Hydrocortisone is most commonly used in childhood. Because of the short half-life, three daily divided doses are generally recommended. The standard dose of hydrocortisone is usually in the range of 8–15 mg/m^2/day; however, at the time of diagnosis, in order to lower considerably elevated adrenal hormone levels, it may be advisable to exceed recommended glucocorticoid doses, as long as the dose is rapidly tapered when steroid levels reach target ranges. Hormone levels must be reassessed frequently, particularly in infants [83]. Lower doses can often be used in the nonclassical disorders if treatment is required. Whether the dose should be equally divided or a higher dose given in the morning or in the evening is controversial; some authors advocate avoidance of a higher evening dose so as not to interfere with sleep and to better mimic the physiologic profile of cortisol secretion [84, 85]. Hydrocortisone suspension and hydrocortisone tablets are not bioequivalent. The oral suspension may not provide adequate control in children, given the non-predictable distribution of the drug in liquid. With severe 21-hydroxylase deficiency, there is an inability to mount a sufficient cortisol response during times of stress, and as such, glucocorticoid doses must be increased during such episodes [83]. For patients with the nonclassic disorder, stress dose steroids are required for those on daily treatment due to iatrogenic suppression of adrenal function. Additionally, about 1/3 of nonclassic patients may not mount a sufficient cortisol response to stimulation testing, and as such, glucocorticoid therapy may be indicated during periods of stress [86]. Equivalent doses of longer-acting steroids, such as prednisone or dexamethasone, may be used in the older adolescent/young adult, allowing for less frequent dosing. The longer-acting steroids are used less frequently in children because of concerns regarding side-effect profile and risk for growth suppression, although there are reports of successful treatment with the more potent steroids in childhood [1–6, 87]. When the growth-suppressive effects of the longer-acting steroids were evaluated, prednisolone was about 15-fold more potent than hydrocortisone, while dexamethasone was approximately 70–80-fold more potent [87, 88]. More recently, modified-release hydrocortisone preparations have shown promise with regard to more closely mimicking physiologic cortisol release and deserve further study [85].

14.8.2 Mineralocorticoids

In the presence of aldosterone deficiency, fludrocortisone, a synthetic mineralocorticoid, is administered. The dose is usually between 0.1 mg and 0.3 mg daily. Current recommendations are that all infants with salt-wasting 21-hydroxylase deficiency require mineralocorticoid therapy, as well as glucocorticoid treatment and sodium chloride supplementation [53]. The dose of sodium chloride supplementation needed is typically in the range of 1–3 g daily. As sodium chloride in the diet increases, it may be possible to decrease and ultimately discontinue sodium supplementation. Similarly, there may be a decreasing dose requirement for fludrocortisone. It is important to monitor blood pressure in infants and children being treated with mineralocorticoids. Hypertension has been documented in children on higher doses of fludrocortisone supplementation who had suppressed plasma renin activity [89].

14.8.3 Sex Steroids

In those conditions with sex steroid deficiency (lipoid adrenal hyperplasia, 17-hydroxylase, 3β(beta)-HSD/Δ(delta)4,5-isomerase), sex hormone replacement therapy to induce or maintain normal secondary sexual characteristics may often be required. Therapy is begun at an age appropriate for puberty and the achievement of a satisfactory final height. Estrogen therapy to induce breast development is often begun with conjugated estrogens. A progestational agent is added to induce menses in the genetic female, and therapy is often subsequently changed to an oral contraceptive agent. Testosterone enanthate is used to induce male pubertal changes. The newer transdermal testosterone preparations may also be used. Menstrual irregularity or amenorrhea may occur in females with the virilizing disorders. Oral contraceptives may also be used in these patients.

14.8.4 Genitalia Surgery

In females with virilizing forms of CAH, surgical correction of the genitalia may be necessary depending on the degree of virilization. If significant clitoromegaly is present but not marked, clitoral recession may be possible. The clitoris is freed and repositioned beneath the pubis with preservation of the glans, corporal components, and all neural and vascular elements. If there is marked clitoromegaly, the clitoris is typically reduced with partial excision of the corporal bodies and preservation of the neurovascular bundle. Current consensus guidelines suggest that for severely virilized females, clitoral and perineal reconstruction should be considered during infancy in centers where this surgery is frequently performed [83]. Later revision may still be necessary.

In conditions with gonadal sex hormone deficiency resulting in incomplete virilization of the external genitalia in the genetic male, surgical correction to conform with the sex of rearing is often necessary. Males with lipoid adrenal hyperplasia have phenotypically normal female genitalia and traditionally have been raised as females. A gonadectomy is performed to avoid the risk of gonadal malignancy. The degree of genital ambiguity in males with 3β(beta)-HSD/Δ(delta)4,5-isomerase or 17-hydroxylase deficiencies is variable and ranges from phenotypically female to male with hypospadias. In those given a female sex assignment, gonadectomy and surgery to create normal appearing female external genitalia are performed. In incompletely virilized males given a male sex assignment, corrective surgery may include repair of hypospadias, orchiopexy, and phalloplasty.

Our present practices in regard to genital surgery in infants with intersex problems are currently undergoing intensive reexamination and reevaluation. Some patient groups and professionals have suggested that surgery should not be performed until the child can participate in the decision. The need for better education of parents, more attention to psychosocial issues, and better communication between all of the involved professionals, parents, and patients is clearly demonstrated in reports of problematic psychosocial outcomes for some intersex patients. Many groups are exploring methods to improve outcomes, but there is at the present time no clear consensus on how this can best be achieved [90–93]. Most experts do agree however that identifying centers of excellence, which offer a multidisciplinary approach, is crucial [94].

14.8.5 Experimental Therapies

The goal of many new treatment approaches is to normalize growth and development in children with CAH. Precocious puberty may occur in late diagnosed or poorly controlled children whose bone ages are advanced to 10 years or more. Luteinizing hormone-releasing hormone (LHRH) agonists have been used to delay puberty, retard bone age advancement, and prolong the time available for continued growth. The dose of Lupron Depot-Ped, commonly used in the USA, is similar to that used in non-CAH children with precocious puberty [95, 96].

The combination of an antiandrogen (to block androgen effect) and an aromatase inhibitor (to block conversion of androgen to estrogen) has been reported [97–99].

The final short stature of many adults with CAH patients may be due in part to periods of excess cortisol treatment or inadequate suppression of androgens or both. There are reports of growth hormone treatment, with or without LHRH agonists, in children with CAH. Initial data had suggested an improvement in predicted adult height, but long-term results and final heights were not available at the time [100, 101]. While more recent publications have demonstrated improvement in final or near final height in CAH patients treated with growth hormone [102, 103], the current consensus statement recommends that further studies be undertaken to examine this issue [83, 104, 105]. Adrenalectomy has been reported in children with 21-hydroxylase deficiency and 11-hydroxlyase deficiency who could not be well controlled medically [106–109]. Bilateral adrenalectomy for CAH remains controversial. Studies of these new treatment regimens are required to determine if they result in better final outcomes.

14.8.6 Monitoring

Monitoring treatment can be difficult in CAH. Therapy is evaluated by clinical course and appropriate hormone levels. Normal gains

in height and weight, normal onset and progress of puberty, absence of signs of androgen excess in virilizing disorders (rapid growth, acne, hirsutism, phallic enlargement), and normotension in the hypertensive disorders and in patients on mineralocorticoid and/or salt replacement are goals of therapy. Glucocorticoid excess can result in growth suppression, excess weight gain, and Cushingoid appearance. Undertreatment resulting in inadequate androgen suppression can lead to rapid growth and bone age advancement and carries the risk of adrenal crisis. Inadequate sodium repletion may result in poor growth and worsening of hormonal control. Hormonal monitoring includes measurement of adrenal androgens in the virilizing disorders, PRA in the salt-losing disorders, and PRA and DOC in the hypertensive disorders. 17-Hydroxyprogesterone, testosterone, and Δ(delta)4-androstenedione are currently the best measures of adequate glucocorticoid treatment in 21-hydroxylase deficiency with limitations [83].

Measurement of the precursor hormones, such as 17-hydroxyprogesterone in 21-hydroxylase deficiency, compound S in 11-hydroxylase deficiency, and 17-hydroxypregnenolone in 3β(beta)-HSD/Δ(delta)4,5-isomerase deficiency, should also be performed (■ Table 14.2).

Normal levels of 17-hydroxyprogesterone and the other steroids should not be the treatment goal, but instead may be an indication of overtreatment. The aim of therapy is to keep the precursor hormones in a range sufficiently low to maintain adrenal androgens in the normal range in the virilizing disorders. PRA should be in the high normal range in the salt-wasting disorders. PRA and DOC should be in the normal range in the hypertensive disorders.

The optimal time and relationship to dose for the hormonal measurements are not established, but hormonal measurements should be consistently timed. Early morning blood work before the morning glucocorticoid dose and random blood work drawn while on the usual therapeutic regimen are often utilized. Measurement of 24-h excretion of urinary metabolites can provide valuable information about the androgen status of CAH patients, and more recent data may provide reference points for treatment monitoring [1–6, 83, 110].

14.8.7 Outcome

The outcome of treatment for CAH due to 21-hydroxylase deficiency has been the most extensively reported. Although normal final height and normal pubertal development, sexual function, and fertility have been reported, there have been frequent reports of short stature, disordered puberty, menstrual irregularity, infertility, inadequate vaginal reconstruction, and lack of sexual function. Gender dysphoria has been described for some patients with CAH, although at significantly lower rates compared to other patients with DSD [1–6, 91, 93, 111–125]. Decreased bone mineral density (BMD) in adult women with CAH has been reported [126]. Increases in BMD have been reported with cautious reduction in HC dosing [127]. Further studies are needed to determine the best glucocorticoid regimens with respect to optimizing bone health.

The hope is that earlier diagnosis by newborn screening, the development of improved methods to monitor these patients, improved surgical techniques, and new therapies will result in better outcomes.

The increased awareness of psychosocial issues and the need for extensive psychological support for patients and families, as well as the current reexamination and discussion of issues relating to genital surgery, should contribute to the development of more successful therapies and better outcomes.

14.9 Prenatal Diagnosis and Treatment of CAH

14.9.1 Prenatal Diagnosis

There have been numerous reports on the prenatal diagnosis and treatment of CAH due to 21-hydroxylase deficiency, although the 2010 CAH guidelines continue to regard prenatal therapy as experimental [83]. Initially, the prenatal diagnosis of CAH due to 21-hydroxylase deficiency was based upon elevated levels of 17-hydroxyprogesterone and Δ(delta)4-androstenedione (and testosterone in females) in amniotic fluid of an at-risk pregnancy. The demonstration of genetic linkage between CAH

due to 21-hydroxylase deficiency and HLA made possible the prenatal prediction of the disorder by HLA genotyping of cultured amniotic fluid cells and cultured chorionic villous cells. A fetus HLA identical to the affected index case would be predicted to be affected. The fetus that has one HLA haplotype in common with the index case would be predicted to be a heterozygous carrier, and the fetus in which both HLA haplotypes are different from the index case would be predicted to be homozygous normal. Molecular genetic analysis of DNA extracted from chorionic villous cells or amniocytes has largely replaced hormonal evaluation and HLA genotyping for prenatal diagnosis of CAH due to 21-hydroxylase deficiency; however, these methods are invasive, and the earliest they can be performed is late in the first trimester. Causative mutations can now be identified in 95% of chromosomes by *CYP21A2* gene analysis. At the present time, polymerase chain reaction (PCR)-based technique of either allele-specific oligonucleotide hybridization or allele-specific PCR for the mutation(s) detected in the index case can be performed.

If prenatal treatment is being considered, it must be instituted early in the first trimester before karyotyping and *CYP21A2* genotyping has been determined [128]. As such, seven out of eight fetuses in at-risk pregnancies receive unneeded treatment until the genetic diagnosis is made [129]. Fetal sex determination (SRY test) has shown reliability and is sensitive from approximately 5 weeks of gestation; thus, it may provide a way to avoid prenatal dexamethasone treatment in CAH males [130]. A newer noninvasive technique for prenatal diagnosis very early in the first trimester utilizing cell-free fetal DNA (ffDNA) from maternal plasma has been developed [131, 132]. Early work has shown promise in determining fetal CAH status, but cell-free fetal DNA is not yet the established standard of care [133, 134]. De novo mutations, found in patients with CAH but not in parents, are found in 1% of disease-causing *CYP21A2* mutations [1–6, 135, 136].

Prenatal diagnosis of 11β(beta)-hydroxylase deficiency has been made utilizing measurement of amniotic fluid 11-deoxycortisol and THS and DNA analysis of chorionic villus cells [137, 138]. Lipoid adrenal hyperplasia has also been diagnosed prenatally using ultrasonography, amniotic fluid hormone levels, and maternal plasma and urinary hormone measurements [139, 140]. Theoretically, all forms of CAH can now be diagnosed prenatally by DNA analysis of chorionic villus cells.

14.9.2 Prenatal Treatment

As per the current consensus guidelines on CAH, prenatal treatment is regarded as experimental, and no specific treatment protocols can be recommended [83]. The first report of successful prenatal treatment of CAH due to 21-hydroxylase deficiency to prevent virilization of a female fetus was in 1984. In an at-risk pregnancy, dexamethasone 0.5 mg twice daily was administered to the mother from 5 weeks of fetal age. The fetus was identified as an affected female by karyotyping and HLA genotyping of amniotic cells; dexamethasone was continued to term. The infant had normal genitalia at birth and was confirmed to have CAH. In a second pregnancy in this report, administration of hydrocortisone to the mother resulted in an affected female with minimally virilized genitalia [141].

Since this initial report, there have been numerous at-risk pregnancies in which prenatal treatment was instituted, although long-term outcome data are limited. Dexamethasone, in doses as low as 0.5 mg to as high as 1.5 mg/day, has been administered in two to three divided doses. Dexamethasone is used since it is not inactivated by placental 11β(beta)-hydroxysteroid dehydrogenase type 2 [142].

In the largest series, among 532 pregnancies assessed for carrying a fetus with CAH, 281 underwent prenatal treatment. Of the female fetuses who were exposed to dexamethasone before age 9 weeks in utero, 11 out of 25 CAH-affected females had normal genitalia by report and 11 had minimal virilization [143]. Variability in maternal metabolic clearance and placental metabolism may contribute to the variability of results in addition to inadequate dosing and interruption or delay in treatment.

Dexamethasone is a category B drug (safety in pregnancy not established). Thus, prenatal treatment of CAH with dexamethasone is still considered an off-label use in the USA and European Union. Spontaneous abortion, late pregnancy fetal demise, intrauterine growth retardation, reduction in birth weight, liver steatosis, hydrocephalus, agenesis of the corpus callosum, and

hypospadias with unilateral cryptorchidism occasionally have occurred in short-term-treated unaffected pregnancies or long-term-treated affected pregnancies. These events have not been considered to be related to the treatment. In long-term follow-up of most infants treated prenatally until mid-gestation or throughout the pregnancy, development seems to be normal, and growth has been consistent with the family pattern and the other affected siblings. Rare adverse events include failure to thrive, and psychomotor and psychosocial delays in development have been observed but cannot be definitively ascribed to the prenatal therapy [135, 136, 144–147].

Concerns have been raised for abnormal cognitive and behavioral development due to prenatal treatment with dexamethasone, but long-term follow-up data are limited with conflicting findings. An early pilot study raised concern for increased shyness and inhibition in treated children, but other follow-up studies have not demonstrated differences on standard motor, cognitive, and social development scales [148–150]. Hirvikoski and colleagues have reported that early dexamethasone treatment in CAH-unaffected children may negatively affect cognitive functioning and most recently published data showing that the effects of dexamethasone treatment may disproportionately affect females [151, 152]. New and colleagues did not find similar cognitive effects in those children exposed to short-term treatment, but did report slower mental processing in CAH-affected females exposed to long-term prenatal dexamethasone [153].

Successful prenatal treatment has also been reported in 11β(beta)-hydroxylase deficiency [138]. If prenatal therapy is pursued, it should only be instituted at centers that have IRB-approved protocols and where it is possible to collect outcome data on a large number of patients, so that the risks and benefits of this treatment can be further defined [83].

14.9.3 Maternal Complications of Prenatal Treatment

There have been a number of reports of maternal adverse effects related to prenatal dexamethasone treatment. The frequency of adverse effects has varied from approximately 1/3 to 100% in mothers treated until delivery. The most common problem reported has been marked weight gain, found in ¼ to 100% of mothers in various reports. Side effects reported include edema, irritability, nervousness, mood swings, hypertension, glucose intolerance, epigastric pain, gastroenteritis, Cushingoid facial features, increased facial hair growth, and severe striae with permanent scarring. Studies of possible long-term maternal adverse effects have not been reported [145, 147, 154].

The maternal effects have prompted decreasing the dose or discontinuing treatment. Noncompliance and unsatisfactory genital outcome may have resulted. Symptoms of glucocorticoid deficiency following tapering or discontinuing treatment have rarely been observed [144].

Maternal anxiety about short- and long-term side effects of prenatal dexamethasone treatment on the fetus and child and on the mother has been documented [155]. In one report, 30 of 44 dexamethasone-treated women indicated that they would decline prenatal treatment for a subsequent pregnancy [147].

14.9.4 Further Recommendations

Prenatal treatment for CAH due to 21-hydroxylase deficiency appears to be effective in ameliorating the virilization of the affected female fetus. However, at present, the short- and long-term complications to the fetus and mother are not fully defined. Researchers in Sweden have halted recruitment of pregnant women in a prospective prenatal treatment study due to concerns for adverse fetal outcomes [156]. Some authors have argued that prenatal treatment is unwarranted and unethical due to the potential adverse consequences, both known and unknown, for what is primarily a cosmetic outcome [156–158]. Therefore, parents seeking genetic counseling should be fully informed of the presently unknown long-term side effects on treated mothers and prenatally treated children, the known possible maternal side effects, and the variable genital outcome, and treatment only offered as part of IRB-approved research protocols [159]. In the presence of maternal medical or mental conditions that may be worsened by dexamethasone treatment, such as hypertension, overt gestational diabetes, or toxemia, treatment should not

be undertaken or undertaken only with extreme caution [144].

14.10 Newborn Screening for CAH

The development in 1977 of the methodology to measure 17-hydroxyprogesterone in a heel stick capillary blood specimen on filter paper made possible newborn screening for CAH due to 21-hydroxylase deficiency [160]. Shortly thereafter, a pilot newborn screening program was developed in Alaska [161]. Screening programs have been developed worldwide in various countries. All 50 states within the USA and over 40 other countries screen for CAH.

First-tier screens for CAH use immunoassays to measure 17-hydroxyprogesterone in a filter paper blood spot sample obtained by the heel prick technique concurrently with samples collected for newborn screening of other disorders. Data on more than 17 million neonates screened is available. The disorder occurs in 1 of 21,000 newborns in Japan; 1 of 10,000–16,000 in Europe and North America; 1 in 5000 in La Reunion, France; and 1 in 300 Yupik Eskimos of Alaska. About 75% of affected infants have the salt-losing form, and 25% have the simple virilizing form of the disorder. The nonclassic form is not reliably detected by newborn screening, and its frequency remains to be established.

Almost all of the screening programs use a single-sample screening test, although a number of programs perform a second test on the initial sample in the presence of a borderline level on the initial screen, and a few programs utilize two-sample screenings. The current consensus guidelines recommend a two-tier protocol in which a positive result on the first-tier screen (immunoassay) is further evaluated by a second-tier screen. Accurate measurement of serum 17-hydroxyprogesterone requires an assay with high specificity with an extraction step because of the many cross-reacting steroids present. To improve sensitivity, the cutoff levels of 17-hydroxyprogesterone are set low enough so that approximately 1% of all tests will be reported as positive. Nonetheless, only approximately 1 in every 100 neonates with a positive screening test will have CAH due to the overall low prevalence of the disorder [83]. The majority of false-positive results have occurred in low birth weight, sick, stressed, and premature infants since 17-hydroxyprogesterone levels are generally higher in these populations. Separate normative reference values based on birth weight or gestational age have been developed, which have minimized the false-positive rates among this population of newborns. The false-negative rate for screening is actually quite low [160–175]. However, in those infants who test positive based on immunoassay, a second-tier screening test is necessary. Liquid chromatography followed by tandem mass spectrometry (LC–MS/MS) as a second-tier screen has been shown to improve the positive predictive value of CAH screening in Minnesota from 0.8% to 7.6% during a 3-year follow-up period [176]. LC–MS/MS may become the method of choice for confirming positive results [51, 177–183].

Case Study

A 6-year 4-month-old female is referred by her pediatrician for evaluation of body odor and pubic hair. Her parents report that the body odor has been present for the past 6 months requiring deodorant use. The pubic hair has also been present for the past 6 months, and parents report that they have noticed an increase in the amount of hair over time. They have also noticed a few pimples on her face over the past several months. While she has always been tall compared to her peers, they feel that recently she has had a "growth spurt." Other than body odor and pubic hair, she is asymptomatic. Her pediatrician obtained a bone age prior to making the referral, which was advanced at 9 years. On review of old growth records, you see that her height has increased over the past year from the 75th to the 90th percentile. On physical exam, she has a few small pimples on her face. She has axillary wetness. She has Tanner 1 stage breasts and Tanner 3 stage pubic hair. There is no evidence of clitoromegaly. An early morning 17-hydroxyprogesterone level is 284 ng/dL. Molecular genetic testing confirms the diagnosis of nonclassic CAH.

14.11 Conclusion

Our understanding of the pathophysiology of the disorders of adrenal steroidogenesis, which result in CAH, expanded markedly in the second half of the twentieth century. The clinical spectrum of these disorders and their biochemical basis; the cellular locations, function, and abnormalities of the affected enzymes; and the genes encoding these enzymes and the molecular mutations resulting in CAH have been elucidated. Newborn screening has allowed for early diagnosis and treatment with the goal of preventing significant morbidity and mortality. Prenatal diagnosis and treatment are now possible but still considered experimental and not routinely recommended. Despite 50 years of treatment, however, the optimal therapy eludes us, and efforts must continue in the twenty-first century to develop better treatment protocols to achieve more successful outcomes for these disorders.

Review Questions

1. Hypertension may be present in which type(s) of CAH:
   A. 21-Hydroxylase deficiency
   B. 11β(Beta)-hydroxylase deficiency
   C. 17-Hydroxylase/17,20-lyase deficiency
   D. Both A and B
   E. Both B and C
2. Hyperpigmentation may occur in CAH patients due to:
   A. Increased MSH from POMC cleavage
   B. Direct effects of ACTH on melanocytes
   C. Overproduction of adrenal androgens
3. 17-Hydroxypregnenolone is the diagnostic hormone in which type of CAH:
   A. P450 oxidoreductase deficiency
   B. 11β(Beta)-hydroxylase deficiency
   C. 3β(Beta)-HSD/Δ(delta)4,5-isomerase deficiency
   D. 21-Hydroxylase deficiency

Answers

1. (E) Excessive 11-deoxycorticosterone (DOC) secretion in both 11β(beta)-hydroxylase deficiency and 17-hydroxylase/17,20-lyase deficiency results in sodium and water retention with plasma volume expansion. Hypertension occurs as a result and hypokalemia may also be present.
2. (A) The lack of cortisol production in CAH results in increased production of ACTH by the pituitary gland. ACTH is derived from a precursor molecule called pro-opiomelanocortin (POMC). POMC is also a precursor for melanocyte-stimulating hormone (MSH). MSH stimulates melanocytes and can cause skin darkening. In secondary and tertiary forms of adrenal insufficiency, skin darkening does not occur since ACTH is not overproduced.
3. (C) 3β(Beta)-HSD/Δ(delta)4,5-isomerase is necessary for the conversion of pregnenolone to progesterone, 17-hydroxypregnenolone to 17-hydroxyprogesterone, and dehydroepiandrosterone (DHEA) to Δ(delta)4-androstenedione. Laboratory evaluation reveals elevation of the Δ(delta)5 steroids, specifically the diagnostic hormone 17-hydroxypregnenolone and DHEA.

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Cushing Syndrome in Childhood

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Key Points

- Cushing syndrome is rare in children; however, without early detection, it can cause considerable morbidity and potential death; patients should be referred to multidisciplinary centers of excellence with experience in endocrinology and neurosurgery.
- The first-line treatment for pituitary adenomas that cause Cushing disease is surgical removal via transsphenoidal surgery.
- Some children with cyclical symptoms of Cushing syndrome may in fact have bilateral adrenocortical hyperplasia; our understanding of the pathophysiology of this condition has grown immensely in recent years.
- While exceedingly rare in children, adrenocortical cancer must be considered as part of the differential diagnosis in any patient with Cushing syndrome and low corticotropin.
- Cushing syndrome in children may be related in part to distinct germline or somatic mutations, each with important potential prognostic implications for the patient and their family members.

15.1 Introduction and Background Information

In 1932, Harvey Cushing described a series of clinical findings including central adiposity, skin striae, hypertrichosis, and hypertension in 12 patients with pituitary basophil adenomas. It emerged later that what we call today "Cushing syndrome" could also result from tumors of the adrenal cortex. It was not until 1962 that the first case of ectopic Cushing syndrome was described. Over the last 50 years, significant advances in the understanding of the pathophysiology and treatment of Cushing syndrome have been made.

Cushing syndrome is a multisystem disorder resulting from the body's prolonged exposure to excess glucocorticoids. It is characterized by truncal obesity, growth deceleration, characteristic skin changes, muscle weakness, and hypertension [1, 2]. Most cases of Cushing syndrome in childhood result from the exogenous administration of glucocorticoids (iatrogenic Cushing syndrome). Only endogenous Cushing syndrome is discussed in this chapter.

15.1.1 Normal Hypothalamic–Pituitary–Adrenal Axis

Corticotropin-releasing hormone (CRH) is synthesized in the hypothalamus and carried to the anterior pituitary in the portal system. CRH stimulates corticotropin (ACTH) release from the anterior pituitary, which in turn stimulates the adrenal cortex to secrete cortisol (hypothalamic–pituitary–adrenal or HPA axis) [3, 4]. Cortisol inhibits the synthesis and secretion of both CRH and ACTH in a negative feedback regulation system (Fig. 15.1a). In Cushing syndrome, the HPA axis has lost its ability for self-regulation, due to excessive secretion of either ACTH or cortisol and the loss of the negative feedback function (Fig. 15.1b). Diagnostic tests, on the other hand, take advantage of the tight regulation of the HPA axis in the normal state and its disturbance in Cushing syndrome to guide therapy toward the primary cause of this disorder.

15.2 Etiology

Cushing syndrome is a rare entity, especially in children [1]. The overall incidence of Cushing syndrome is approximately 2–5 new cases per million people per year. Up to 10% of the new cases each year occur in children. Pediatric Cushing disease is found to have equal distribution between males and females, unlike adults where females predominate [5].

The most common cause of Cushing syndrome in children is exogenous or iatrogenic Cushing syndrome. This is the result of chronic administration of glucocorticoids or ACTH. Glucocorticoids are being used more frequently for the treatment of many non-endocrine diseases including pulmonary, autoimmune, dermatologic, hematologic, and neoplastic disorders. In addition, ACTH is being used for the treatment of certain seizure disorders. While this chapter focuses on endogenous Cushing syndrome, we can learn about the effects of prolonged glucocorticoid exposure in children from our experience with these unique patients.

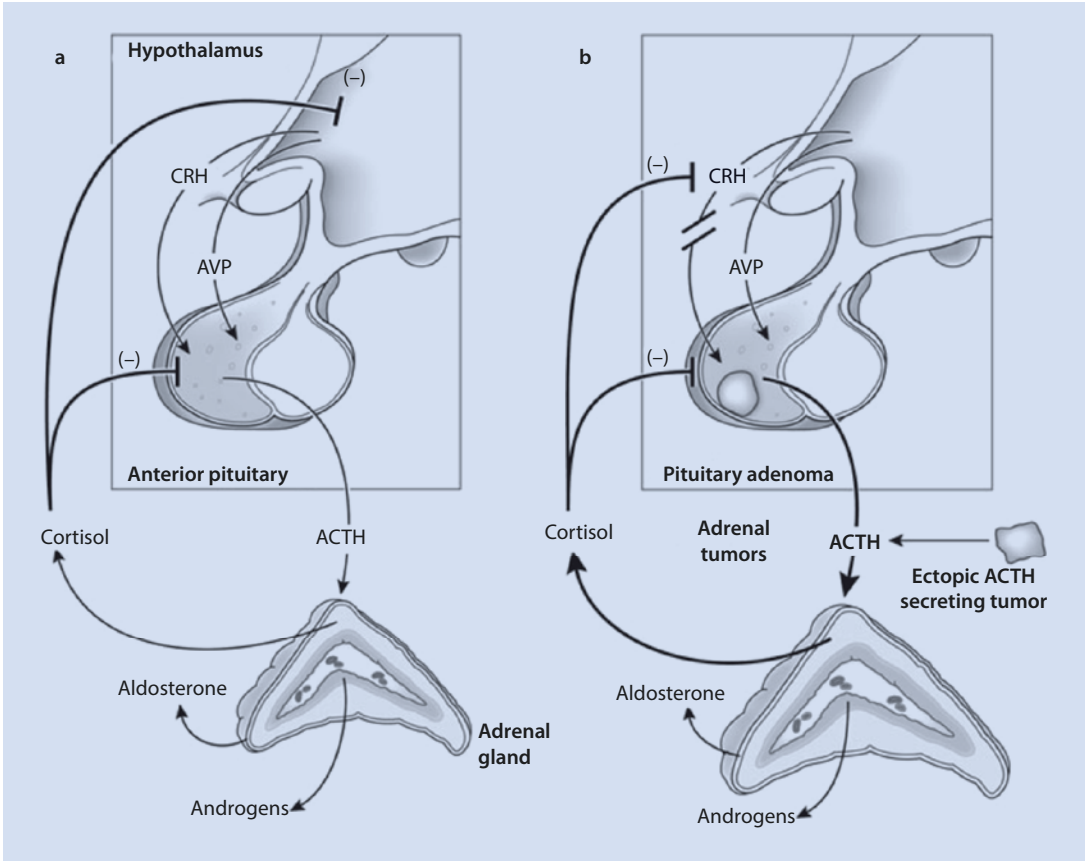

Fig. 15.1 **a** (*Left panel*) Physiologic regulation of cortisol secretion (*abbreviations*: CRH corticotropin-releasing hormone, AVP arginine vasopressin, ACTH adrenocorticotropin). **b** (*Right panel*) Causes of Cushing syndrome; adrenal neoplasms include PPNAD and other hypeprlasias, benign tumors, and adrenocortical carcinomas. *Straight arrows* represent stimulation

The most common cause of endogenous Cushing syndrome in children is ACTH overproduction from the pituitary; this is called Cushing disease. It is usually caused by an ACTH-secreting pituitary microadenoma and, rarely, a macroadenoma (i.e., greater than 1 cm). ACTH secretion in this disease maintains some of the feedback of the HPA axis. Cushing disease accounts for approximately 75% of all cases of Cushing syndrome in children over 7 years. In children under 7 years, Cushing disease is less frequent; adrenal causes of Cushing syndrome (adenoma, carcinoma, or bilateral hyperplasia) are the most common causes of the condition in infants and young toddlers. Ectopic ACTH production occurs rarely in young children; it also accounts for <1% of the cases of Cushing syndrome in adolescents. Sources of ectopic ACTH include small cell carcinoma of the lung, carcinoid tumors in the bronchus, pancreas or thymus, neuroblastomas, medullary carcinomas of the thyroid, pheochromocytomas, and other neuroendocrine tumors, especially those of the pancreas, and gut carcinoids.

Rarely, ACTH overproduction by the pituitary may be the result of CRH oversecretion by an ectopic CRH source [6]. Its significance lies in the fact that diagnostic tests that are usually used for the exclusion of ectopic sources of Cushing syndrome have frequently misleading results in the case of CRH-induced ACTH oversecretion.

Autonomous secretion of cortisol from the adrenal glands, or ACTH-independent Cushing syndrome, accounts for approximately 15% of all the cases of Cushing syndrome in childhood [7]. Although adrenocortical tumors are rare in older children, in younger children they are more

frequent. In prepubertal children, adrenocortical lesions are the most frequent cause of Cushing syndrome.

Adrenocortical neoplasms account for 0.6% of all childhood tumors; Cushing syndrome is a manifestation of approximately one-third of all adrenal tumors [3, 8]. In young children, unilateral (single) adrenal tumors presenting with Cushing syndrome are often malignant (more than 70%). The majority of patients present under age 5, contributing thus to the first peak of the known bimodal distribution of adrenal cancer across the life span. As in adults, there is a female to male predominance. The tumors usually occur unilaterally; however, in 2–10% of patients, they occur bilaterally. Germline *TP53* mutations have been found in over 70% of cases of adrenocortical tumors in children regardless of whether they have other signs of Li–Fraumeni syndrome or not, as many *TP53* mutations may only cause adrenocortical adenomas or cancer [9].

More recently, bilateral nodular adrenal disease has been appreciated as more frequent than previously thought cause of Cushing syndrome in childhood [7, 8]. Primary pigmented adrenocortical nodular disease (PPNAD) is a genetic disorder with the majority of cases associated with Carney complex, a syndrome of multiple endocrine gland abnormalities in addition to characteristic skin freckling and connective tissue tumors. The adrenal glands in PPNAD are most commonly normal or even small in size with multiple pigmented nodules surrounded usually (but not always) by an atrophic cortex. Children and adolescents with PPNAD frequently have periodic, cyclical, or otherwise atypical Cushing syndrome [10].

Massive macronodular adrenal hyperplasia (MMAD), also known as primary macronodular adrenocortical hyperplasia (PMAH), is another rare bilateral disease, which leads to Cushing syndrome [8]. The adrenal glands are massively enlarged with multiple, huge nodules that are typical, yellow-to-brown cortisol-producing adenomas. In many patients with MMAD, the cause is due to dominantly inherited inactivating mutations of *ARMC5*, a putative tumor-suppressor gene [11].

Adrenal adenomas or, more frequently, bilateral macronodular adrenal hyperplasia can also be seen in McCune–Albright syndrome (MAS) and Beckwith–Wiedemann syndrome [12, 13]. In Cushing syndrome associated with MAS, there is a somatic mutation of the *GNAS1* gene leading to constitutive activation of the Gsα protein and continuous, non-ACTH-dependent activation of steroidogenesis by the adrenal cortex. Cushing syndrome in MAS is rare and usually presents in the infantile period (before 6 months of age); interestingly, a few children with MAS have had spontaneous resolution of their Cushing syndrome [14].

15.2.1 Molecular Genetics

Figure 15.2 summarizes the genetic and molecular mechanisms implicated in Cushing syndrome, subdivided into those associated with Cushing disease, ectopic ACTH-secreting tumors, and adrenal Cushing syndrome [15].

15.2.2 Adrenocortical Hyperplasias

Aberrant cAMP signaling has been linked to genetic forms of cortisol excess [16]. For example, MMAD may be associated with *GNAS1* mutations as seen in MAS or in some sporadic adrenal tumors as well as associated with *ARMC5* mutations [11, 17, 18]. In addition, most micronodular forms of bilateral adrenocortical hyperplasia (BAH) are associated with defects of the cAMP-dependent protein kinase (PKA) pathways; for example, germline inactivating mutations of the *PRKAR1A* gene cause most cases of PPNAD [19]. Several forms of micronodular BAH are not associated with inactivating mutations of the *PRKAR1A* gene but may occur due to mutations in the *PDE11A* or *PDE8B* genes [10, 20, 21]. In adults with isolated cortisol-secreting adenomas, somatic activating mutations of the catalytic subunit of the PKA enzyme have been found to be the underlying genetic cause of 42% of these sporadic tumors [22]. In children, de novo or inherited genetic rearrangements in the chromosome 19p13 locus result in copy number gains encompassing the *PRKACA* gene that lead to increased PKA activity and adrenocortical hyperplasia and Cushing syndrome [23].

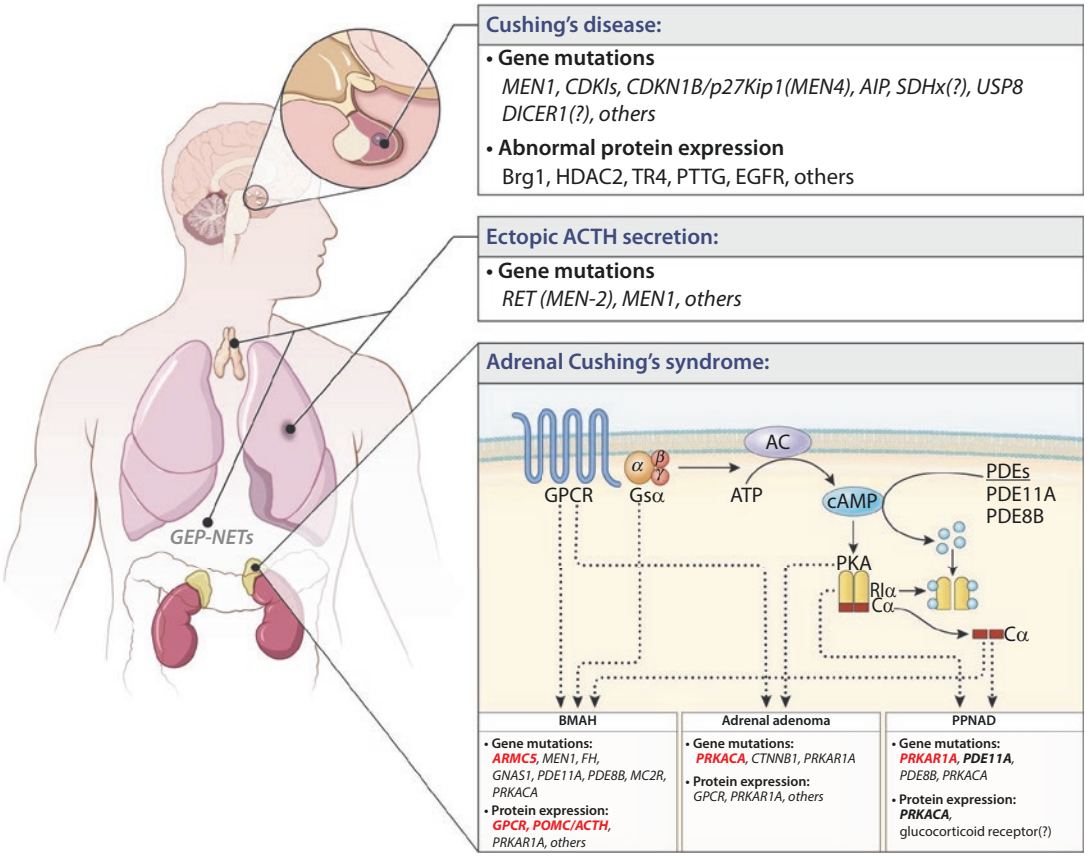

Fig. 15.2 Summary of genetic and molecular mechanisms implicated in Cushing syndrome. Almost all have been identified in the last 15 years. The various genetic mutations or abnormal protein expression believed to play a role in the pathophysiology are indicated. Highlighted in red are frequent and confirmed genetic defects, whereas other characterized mechanisms are highlighted in bold. The remaining are less frequent genetic defects, and some are shown with a question mark because they are not yet confirmed. ACTH adrenocorticotrophic hormone, AC adenylate cyclase, ATP adenosine triphosphate, cAMP cyclic adenosine monophosphate, EGFR epidermal growth factor receptor, GEP-NETs gastroenteropancreatic neuroendocrine tumors, GPCR G protein-coupled receptor, PDE phosphodiesterase, PPNAD primary pigmented nodular adrenocortical disease

15.2.3 Pituitary Corticotropinomas

Among functional pituitary tumors in early childhood, ACTH-producing adenomas are probably the most common although they are still considerably rare. Childhood corticotropinomas only rarely occur in the familial setting and then most commonly in the context of multiple endocrine neoplasia type 1 (MEN 1) and rarely due to *AIP* mutations [24]. Recently, somatic mutations in the gene coding for the ubiquitin-specific protease 8 (USP8), a protein that modulates turnover of the EGF receptor, have been found in adult and pediatric corticotropinomas [25, 26]. This may ultimately lead to targeted therapeutic intervention with EGFR inhibitors in a subset of individuals with Cushing syndrome.

15.3 Clinical Presentation

In most children, the onset of Cushing syndrome is rather insidious [1, 3, 4, 27]. Lack of height gain concomitant with persistent weight gain is the most common presentation of Cushing syndrome

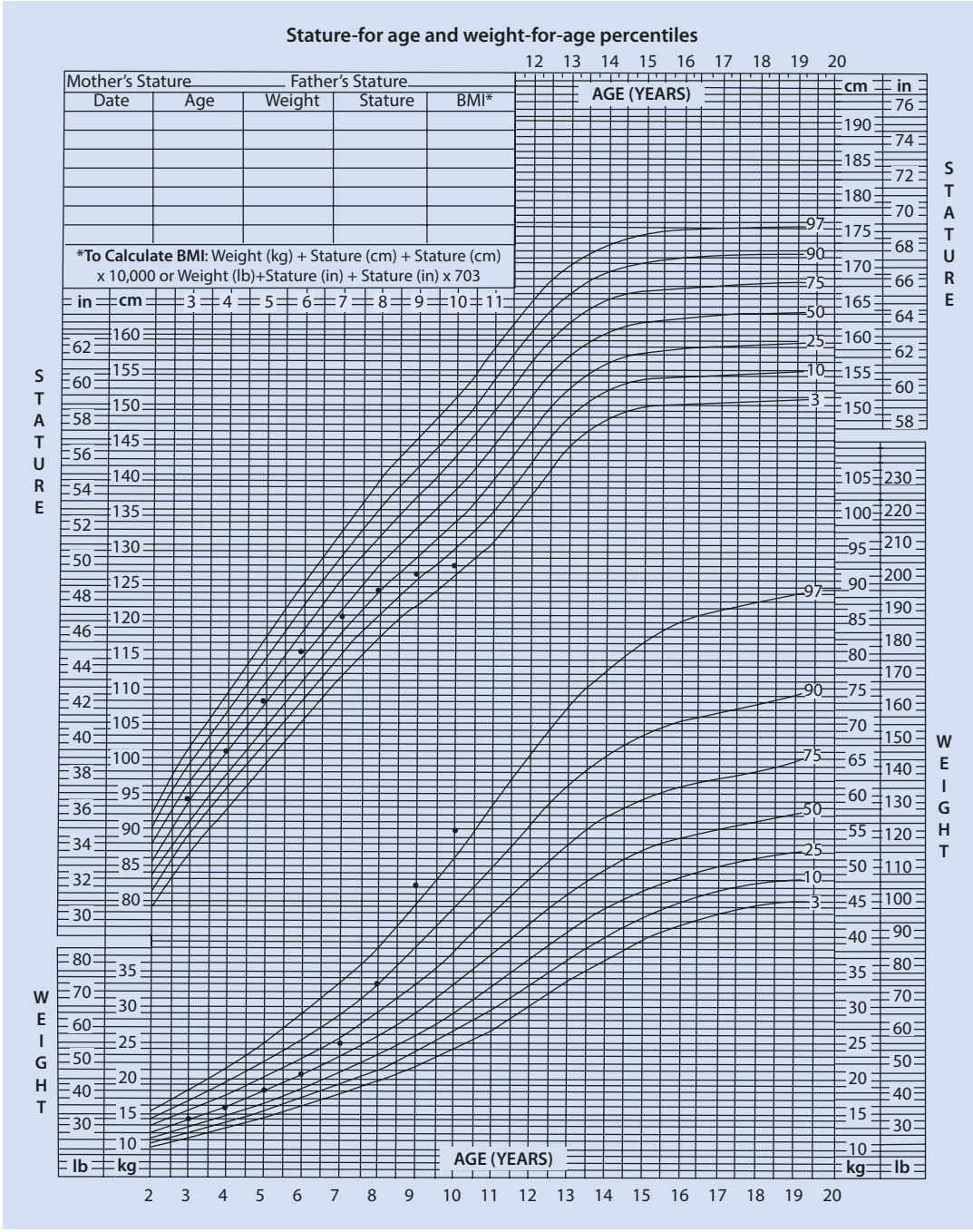

Fig. 15.3 Growth chart of a child with Cushing syndrome demonstrating growth rate deceleration with concomitant weight gain

in childhood, as illustrated in a typical growth chart for a child with Cushing syndrome is shown in Fig. 15.3.

Other common problems reported in children (Table 15.1) include facial plethora, headaches, hypertension, hirsutism, amenorrhea, and delayed sexual development [2, 28]. Pubertal children may present with virilization. Skin manifestations, including acne, purple striae, fungal infections, bruising, and acanthosis nigricans,

Table 15.1 Clinical presentation of CS in pediatric patients

Symptoms/signs	Frequency (%)
Weight gain	90
Growth retardation	83
Menstrual irregularities	81
Hirsutism	81
Obesity (body mass index >85 percentile)	73
Violaceous skin striae	63
Acne	52
Hypertension	51
Fatigue–weakness	45
Precocious puberty	41
Bruising	27
Mental changes	18
"Delayed or inappropriate" bone age	14
Hyperpigmentation	13
Muscle weakness	13
Acanthosis nigricans	10
Accelerated bone age	10
Sleep disturbances	7
Pubertal delay	7
Hypercalcemia	6
Alkalosis	6
Hypokalemia	2
Slipped femoral capital epiphysis	2

National Institutes of Health series—data from Ref. [1]

are also common [1] (Fig. 15.4). Glucose intolerance and diabetes, fractures, and kidney stones are also associated presenting symptoms [29–31]. In comparison to adult patients with Cushing syndrome, symptoms that are less commonly seen in children include sleep disruption, muscular weakness, and problems with memory. Morbidity and mortality are increased in Cushing syndrome; early diagnosis is associated with improved outcomes [32–34].

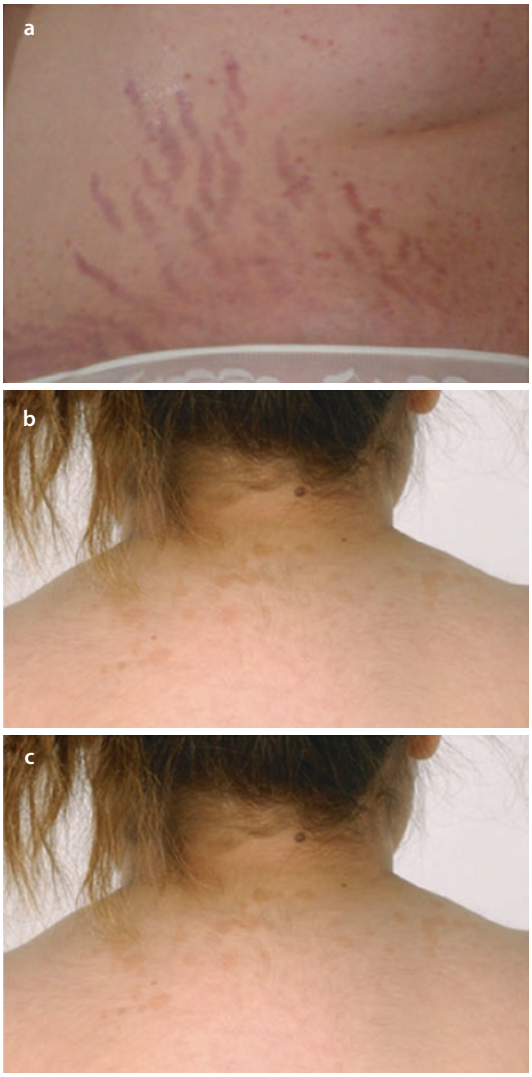

Fig. 15.4 Striae caused by endogenous Cushing syndrome in an 18-year-old girl **a**, acanthosis nigricans and ringworm (*tinea corporis*) lesions in a 9-year-old **b**, and hypertrichosis in a girl **c**; both patients had long-standing Cushing disease

15.4 Diagnostic Evaluation

The appropriate therapeutic interventions in Cushing syndrome depend on accurate diagnosis and classification of the disease. The medical history and clinical evaluation, including review of growth data, are important to make the initial diagnosis of Cushing syndrome. Upon suspicion of Cushing syndrome, laboratory and imaging confirmations are necessary. An algorithm of the diagnostic process is presented in Fig. 15.5.

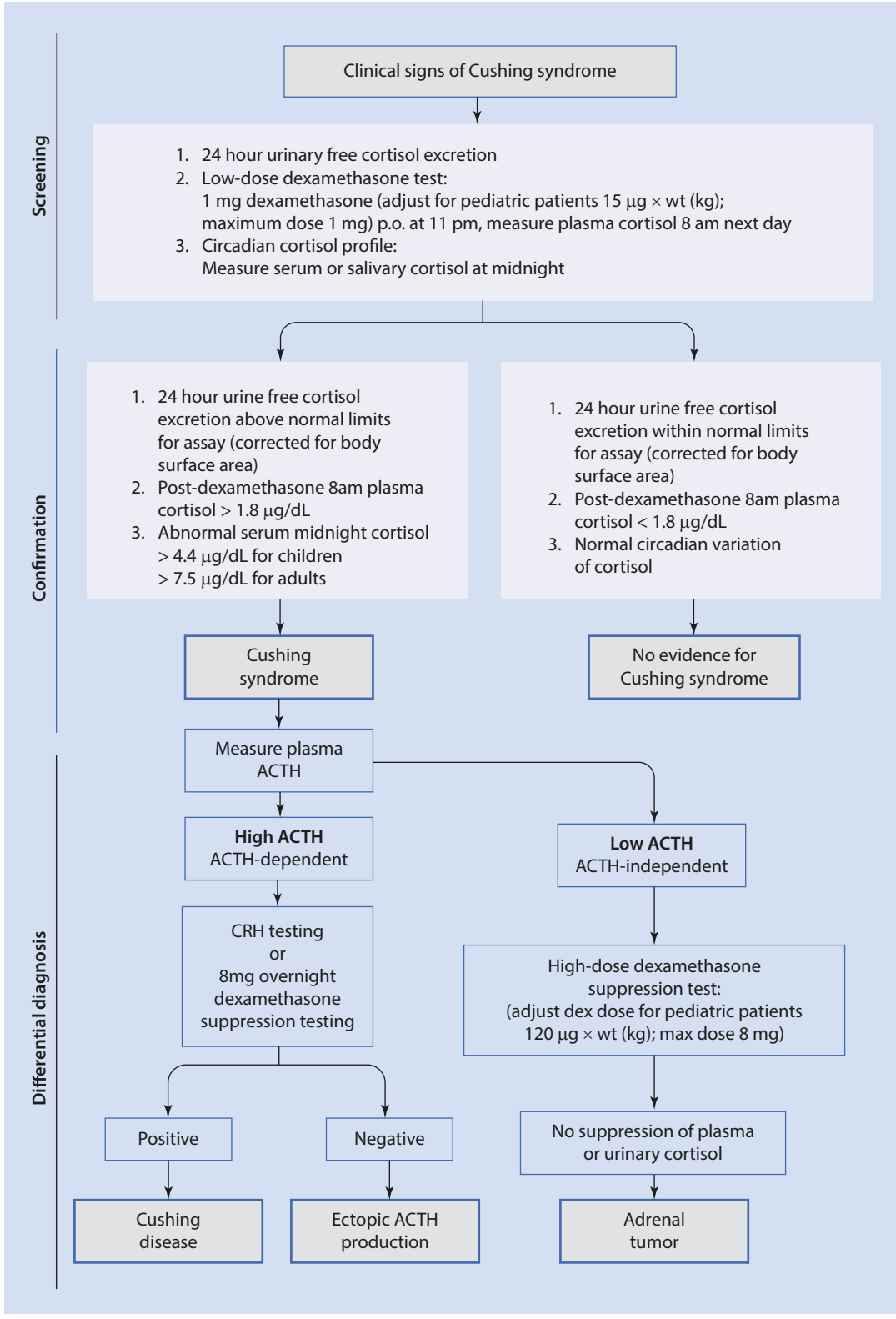

Fig. 15.5 Suggested diagnostic algorithm for the workup of suspected Cushing syndrome or hypercortisolemia

The first step in the diagnosis of Cushing syndrome is to document hypercortisolism [35, 36], which is typically done in the outpatient setting. Because of the circadian nature of cortisol and ACTH, isolated cortisol and ACTH measurements are not of great value in diagnosis. One excellent screening test for hypercortisolism is a 24-h urinary free cortisol (UFC) excretion (corrected for body surface area). However it is often difficult to obtain a 24-h urine collection reliably in the outpatient setting, particularly in the pediatric population. Falsely high UFC may be obtained because of physical and emotional stress, chronic and severe obesity, pregnancy, chronic exercise, depression, poor diabetes control, alcoholism, anorexia, narcotic withdrawal, anxiety, malnutrition, and high water intake. These conditions may cause sufficiently high UFCs to cause what is known as pseudo-Cushing syndrome. On the other hand, falsely low UFC may be obtained with inadequate collection.

Another baseline test for the establishment of the diagnosis of Cushing syndrome is a low-dose dexamethasone suppression test. This test involves giving 1 mg of dexamethasone at 11 pm (corrected per weight in kg as 15 μg/kg) and measuring a serum cortisol level the following morning at 8 am. If the serum cortisol level is greater than 1.8 μg/dL, further evaluation is necessary [37]. This test has a low percentage of false normal suppression; however, the percentage of false positives is higher (approximately 15–20%). Salivary cortisol is a noninvasive test that provides an index of serum-free cortisol concentration and may be useful for screening of diurnal cortisol patterns in children in an outpatient setting. However, a number of factors may introduce error with the collection of specimens (e.g., collection device, activity, food intake, storage), and there are wide variations in types of assays for salivary cortisol. The LC-MS/MS analysis provides greater sensitivity and specificity than RIA and is now available in routine clinical chemistry laboratories [38]. It should be noted that the 1-mg overnight and salivary cortisol tests, like the 24-h UFCs, do not distinguish between hypercortisolism from Cushing syndrome and other hypercortisolemic states.

If the response to the 1-mg dexamethasone overnight suppression test and the 24-h UFC are both normal, a diagnosis of Cushing syndrome may be excluded with the following caveat: 5–10% of patients may have intermittent or periodic cortisol hypersecretion and may not manifest abnormal results to either test. If periodic or intermittent Cushing syndrome is suspected, continuous follow-up of the patients is recommended, including monitoring of growth and 24-h UFC.

If one of the test results is suggestive of Cushing syndrome or if there is any question about the diagnosis, then tests that distinguish between pseudo-Cushing syndrome states and Cushing syndrome may be obtained. One such test is the combined dexamethasone-CRH test [39]. In this test the patient is treated with low-dose dexamethasone (0.5 mg adjusted for weight for children <70 kg) every 6 h for eight doses prior to the administration of CRH (ovine CRH—oCRH) the following morning. ACTH and cortisol levels are measured at baseline (−15, −5, and 0 min) and 15 min after the administration of oCRH (plasma dexamethasone level is measured once at baseline). The patient with pseudo-Cushing syndrome will exhibit low or undetectable basal plasma cortisol and ACTH and have a diminished or no response to oCRH stimulation. Patients with Cushing syndrome will have higher basal cortisol and ACTH levels and will also have a greater peak value with oCRH stimulation. A cortisol level of greater than 1.4 μg/dL (38 nmol/L) 15 min after oCRH administration supports a diagnosis of Cushing syndrome, and further evaluation is indicated. However, severe obesity (BMI >2 standard deviations) confounds the interpretation of the dexamethasone-CRH test; the criteria of peak cortisol of 2.2 ug/dl have been found to have higher sensitivity and specificity for diagnosing Cushing syndrome in children with elevated BMI [40]. Confirmed height gain is a simple way to help distinguish children with pseudo-Cushing from those with Cushing syndrome [41].

15.4.1 Differential Diagnosis to Distinguish the Etiology of Endogenous Cushing Syndrome

Once the diagnosis of Cushing syndrome is confirmed, there are several tests to distinguish ACTH-dependent disease from the ACTH-independent syndrome. A spot morning plasma ACTH may be measured; a cutoff value of 29 pg/mL (with the newer, high-sensitivity ACTH assays) in children with confirmed Cushing syndrome has a sensitivity of 70% in identifying

children with an ACTH-dependent form of the syndrome [36]. It is important to consider the variability in plasma ACTH levels and the instability of the molecule after the sample's collection.

The standard high-dose dexamethasone suppression test (HDDST) is used to differentiate Cushing disease from ectopic ACTH secretion and adrenal causes of Cushing syndrome. The HDDST test has been modified to giving a high dose of dexamethasone (120 μg/kg, maximum dose 8 mg) at 11 pm and measuring the plasma cortisol level the following morning. In children, 20% cortisol suppression from baseline had a sensitivity and specificity of 97.5% and 100%, respectively, with the HDDST for differentiating patients with Cushing disease from those with adrenal tumors [36].

Indications for obtaining the classic Liddle's test, a low-dose dexamethasone (30 μg/kg/dose; maximum 0.5 mg/dose) every 6 h for eight doses, followed by high dose (120 μg/kg/dose; maximum 2 mg/dose) every 6 h for eight doses (instead of the modified overnight HDDST), include non-suppression of serum cortisol levels during the HDDST and/or negative imaging studies and/or suspected adrenal disease. UFC and 17-hydroxysteroid (17OHS) excretion are measured at baseline and after dexamethasone administration during Liddle's test. Approximately 85% of patients with Cushing disease will have suppression of serum cortisol, UFC, and 17OHS values, whereas <10% of patients with ectopic ACTH secretion will have any suppression. UFC values should suppress to 90% of baseline value, and 17OHS excretion should suppress to <50% of baseline value. This test has been shown to be useful mostly in patients who have suspected micronodular adrenal disease; in this case it is used with the aim to identify a "paradoxical" stimulation of cortisol secretion, which is found in patients with PPNAD and other forms of BAH, but not in other forms of primary adrenocortical lesions [42].

Following confirmation of elevated 24-h UFC (three collections), a single midnight cortisol value of >4.4 μg/dL followed by an HDDST (>20% suppression of morning serum cortisol) is the most rapid and accurate way for confirmation and diagnostic differentiation, respectively, of hypercortisolemia due to a pituitary or adrenal tumor [36]. However, for accuracy, diurnal testing requires an inpatient stay, and this may limit its use as a routine screening test [36].

An oCRH stimulation test may also be obtained for the differentiation of Cushing disease from ectopic ACTH secretion [43] and/or adrenal lesions. In this test, 85% of patients with Cushing disease respond to oCRH with increased plasma ACTH and cortisol production. Ninety-five percent of patients with ectopic ACTH production do not respond to administration of oCRH. The criterion for diagnosis of Cushing disease is a mean increase of 20% above baseline for cortisol values at 30 min and 45 min and an increase in the mean corticotropin concentrations of at least 35% over basal value at 15 min and 30 min after CRH administration. When the oCRH and high-dose dexamethasone (Liddle's or overnight) tests are used together, diagnostic accuracy improves to 98%. The oCRH test should not be used in patients with atypical forms of Cushing syndrome, because individuals with normal pituitary function respond to oCRH like patients with Cushing disease; interpretation of oCRH testing in the differential diagnosis of Cushing syndrome is only possible when the normal corticotrophs are suppressed by consistently elevated cortisol levels.

Another important tool in the localization and characterization of Cushing syndrome is diagnostic imaging. The most important initial imaging when Cushing disease is suspected is pituitary magnetic resonance imaging (MRI). The MRI should be done in thin sections with high resolution and always with contrast (gadolinium). The latter is important since only macroadenomas will be detectable without contrast; after contrast, an otherwise normal-looking pituitary MRI might show a hypoenhancing lesion, usually a microadenoma. More than 90% of ACTH-producing tumors are hypoenhancing, whereas only about 5% are hyperenhancing after contrast infusion. However, even with the use of contrast material, pituitary MRI may detect only up to approximately 50% of ACTH-producing pituitary tumors. Post-contrast spoiled gradient-recalled MRI (SPGR-MRI) is superior to spin echo MRI (SE-MRI) in the detection of a microadenoma in children and adolescents with Cushing disease [44] (Fig. 15.6).

Computed tomography (CT) (more preferable than MRI) of the adrenal glands is useful in the distinction between Cushing disease and adrenal causes of Cushing syndrome, mainly unilateral adrenal tumors. The distinction is harder in the presence of micronodular forms of BAH (such asPPNAD) or the rare case of bilateral adrenal carcinoma. Most patients with Cushing disease

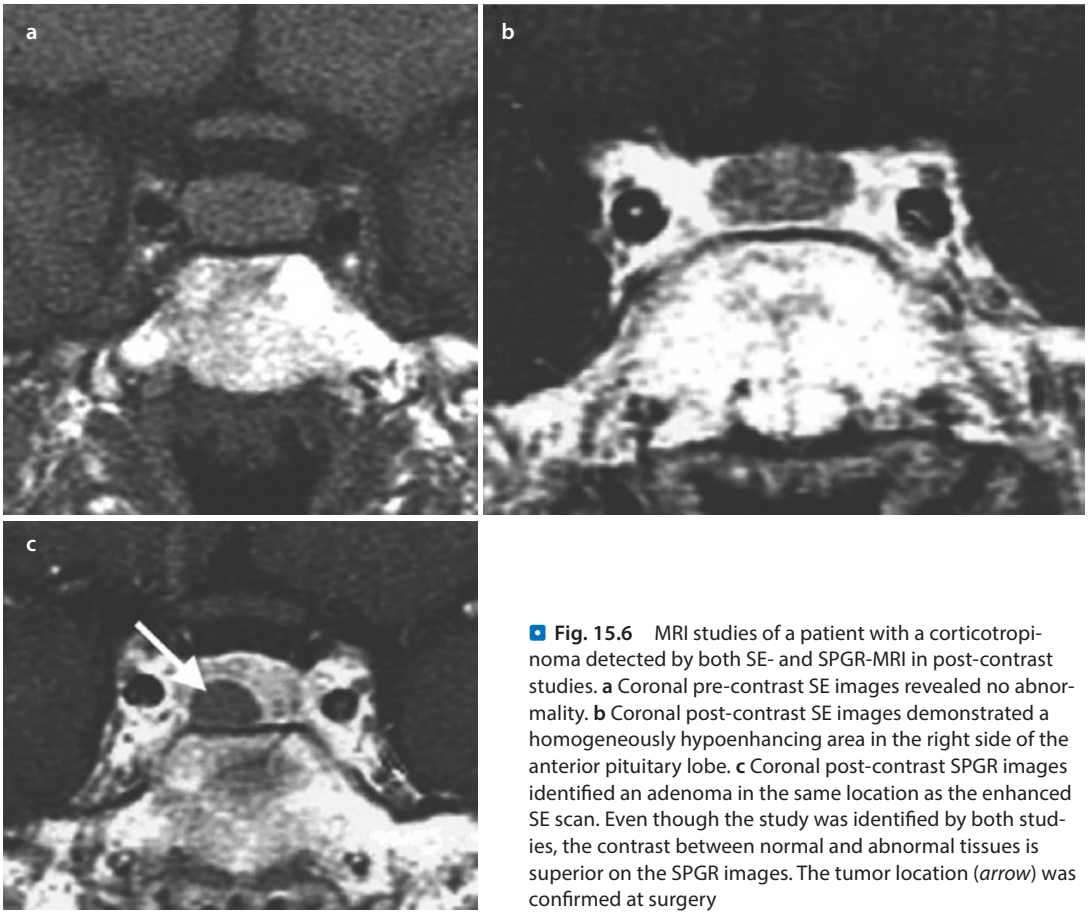

Fig. 15.6 MRI studies of a patient with a corticotropinoma detected by both SE- and SPGR-MRI in post-contrast studies. **a** Coronal pre-contrast SE images revealed no abnormality. **b** Coronal post-contrast SE images demonstrated a homogeneously hypoenhancing area in the right side of the anterior pituitary lobe. **c** Coronal post-contrast SPGR images identified an adenoma in the same location as the enhanced SE scan. Even though the study was identified by both studies, the contrast between normal and abnormal tissues is superior on the SPGR images. The tumor location (*arrow*) was confirmed at surgery

have ACTH-driven bilateral hyperplasia, and both adrenal glands will appear enlarged and nodular on CT or MRI. Most adrenocortical carcinomas are unilateral and quite large by the time they are detected. Adrenocortical adenomas are usually small, <5 cm in diameter, and like most carcinomas, they involve one adrenal gland. MMAD presents with massive enlargement of both adrenal glands, whereas PPNAD and other forms of micronodular disease are more difficult to diagnose radiologically because they are usually associated with normal or small-sized adrenal glands, despite the histologic presence of hyperplasia [45].

Ultrasound may also be used to image the adrenal glands, but its sensitivity and accuracy is much less than CT or MRI. A CT or MRI scan of the neck, chest, abdomen, and pelvis may be used for the detection of an ectopic source of ACTH production. Labeled octreotide scanning, positron-emission tomography (PET), and venous sampling may also help in the localization of an ectopic ACTH source, as well as newer modalities such as 18F–fluorodopa PET (F-DOPA-PET) [46].

Since up to 50% of pituitary ACTH-secreting tumors and many of ectopic ACTH-producing tumors may not be detected on routine imaging and often laboratory diagnosis is not completely clear, catheterization studies must be used to confirm the source of ACTH secretion in ACTH-dependent Cushing syndrome. Bilateral inferior petrosal sinus sampling (IPSS) has been used for the localization of a pituitary microadenoma [47]; however, IPSS is a poor predictor of the site of a microadenoma in children [48]. In brief, sampling from each inferior petrosal sinus is taken for measurement of ACTH concentration simultaneously with peripheral venous sampling. ACTH is measured at baseline and at 3 min, 5 min, and 10 min after oCRH administration. Patients with ectopic ACTH secretion have no gradient between either

sinus (central) and the peripheral sample. On the other hand, patients with an ACTH-secreting pituitary adenoma have at least a 2-to-1 central-to-peripheral gradient at baseline or 3-to-1 after stimulation with oCRH. IPSS is an excellent test for the differential diagnosis between ACTH-dependent forms of Cushing syndrome with a diagnostic accuracy that approximates 100%, as long as it is performed in an experienced clinical center. IPSS, however, may not lead to the correct diagnosis, if it is obtained when the patient is not sufficiently hypercortisolemic or if venous drainage of the pituitary gland does not follow the expected, normal anatomy or with an ectopic CRH-producing tumor.

15.5 Treatment

The treatment of choice for almost all patients with an ACTH-secreting pituitary adenoma (Cushing disease) is transsphenoidal surgery (TSS). In most specialized centers with experienced neurosurgeons, the success rate of the first TSS is close to or even higher than 90% [34, 49]. Treatment failures are most commonly the result of a macroadenoma or a small tumor invading the cavernous sinus. The success rate of repeat TSS is lower, closer to 60%. Recurrence of Cushing disease after an initial TSS is approximately 10–20% in pediatric patients, likely secondary to progression of remaining adenoma.

Postoperative complications include transient diabetes insipidus (DI) and, occasionally, syndrome of inappropriate antidiuretic hormone secretion (SIADH), central hypothyroidism, growth hormone deficiency, hypogonadism, bleeding, infection (meningitis), and pituitary apoplexy. The mortality rate is extremely low, at <1%. Permanent pituitary dysfunction (partial or panhypopituitarism) and DI are rare, but they are more likely after repeat TSS or larger adenomas.

Pituitary irradiation is considered an appropriate treatment in patients with Cushing disease following a failed TSS. Up to 80% of patients will have remission after irradiation of the pituitary gland. Hypopituitarism is the most common side effect and is more frequent when surgery precedes the radiotherapy. The recommended dosage is 4500/5000 cGy total, usually given over a period of 5–6 weeks. Stereotactic radiotherapy (proton beam therapy or gamma knife) offers the benefit of shorter time required for the procedure and the potential for lower rates of side effects. Radiation requires approximately 2 years for control of ACTH levels to be achieved and is associated with the development of other pituitary hormone deficiencies over time [50].

The treatment of choice for benign adrenal tumors is surgical resection, usually via laparoscopic adrenalectomy. After unilateral adrenalectomy, glucocorticoid replacement is required until the hypothalamic–pituitary–adrenal axis recovers from suppression. Treatment guidelines for children with adrenal cancer are lacking as it is a rare disease; however, a protocol was developed in 2006 to investigate the role of surgery and chemotherapy including etoposide, cisplatin, mitotane, and doxorubicin for adrenal cancer in pediatrics [9].

Bilateral total adrenalectomy is usually the treatment of choice in bilateral micro- or macronodular adrenal disease, such as PPNAD and MMAD. Lifelong replacement with mineralocorticoids and glucocorticoids is required. Bilateral adrenalectomy may be considered as a treatment for those patients with Cushing disease, who have either failed transsphenoidal surgery or radiotherapy, or in patients with ectopic ACTH-dependent Cushing syndrome, when the tumor has not been localized. A recent retrospective review including pediatric as well as adult patients concluded that early bilateral adrenalectomy in patients with uncontrolled Cushing syndrome improved adverse events [51]. Nelson syndrome, which includes increased pigmentation, elevated ACTH levels, and a growing pituitary ACTH-producing pituitary tumor, may develop in up to 24% of adults with Cushing disease who are treated with bilateral adrenalectomy, while exact rates in children are not known [52, 53].

Pharmacotherapy is an option in the case of failure of surgery for Cushing disease or in ectopic ACTH secretion where the source cannot be identified. Currently three types of pharmacologic therapies exist, including those directed at the pituitary gland, the adrenal gland, and the glucocorticoid receptor. Dopamine and somatostatin agonists, including cabergoline and pasireotide, have shown success in up to 30% of adults with Cushing disease; however, experience in children is lacking [54]. Adrenal enzyme inhibitors, including mitotane, metyrapone, and

ketoconazole, may be used to control hypercortisolism. Careful monitoring of liver functions and cortisol level is required when using these medications as they are associated with hepatotoxicity, gastrointestinal side effects, and adrenal insufficiency [55]. The glucocorticoid receptor antagonist mifepristone has been studied in adults with Cushing syndrome, yet side effects include hypokalemia, adrenal insufficiency, and endometrial thickening [56]. While medical therapy for Cushing syndrome may be effective in some individuals, it is important to point out that none of these therapies are approved for use in children. As our understanding of the genetic etiologies of Cushing syndrome grows, targeted therapies may become standard of care in the future.

In ectopic ACTH production, if the source of ACTH secretion can be identified then the treatment of choice is surgical resection of the tumor. If surgical resection is impossible or if the source of ACTH cannot be identified then pharmacotherapy is indicated as previously discussed. If the tumor cannot be located then repeat searches for the tumor should be performed at least yearly. Bilateral adrenalectomy should be performed in the case of failure of pharmacotherapy or failure to locate the tumor after many years.

15.5.1 Glucocorticoid Replacement

After the completion of successful TSS in Cushing disease or excision of an autonomously functioning adrenal adenoma, there will be a period of adrenal insufficiency, while the hypothalamic–pituitary–adrenal axis is recovering [57]. During this period, glucocorticoids should be replaced at the suggested physiologic replacement dose (12–15 mg/m^2/day bid or tid). The patient should be followed every few months, and the adrenocortical function should be periodically assessed with a 1-h ACTH test (normal response is a cortisol level over 18 μg/dL at 30 min or 60 min after ACTH stimulation).

After bilateral adrenalectomy, patients require lifetime replacement with both glucocorticoids (as above) and mineralocorticoids (fludrocortisone 0.1–0.3 mg daily). These patients also need stress doses of glucocorticoids immediately postoperatively; they are weaned to physiologic replacement relatively quickly. In addition, stress dosing for acute illness, trauma, or surgical procedures is required for both temporary and permanent adrenal insufficiency.

15.6 Outcomes and Possible Complications

Cushing syndrome has the potential for long-term adverse health outcomes in children. The prolonged exposures of the body to high levels of glucocorticoids, combined with the morbidity from the surgical or radiation treatment itself, are associated with complications. Chronic hypercortisolemia may lead to poor growth, obesity, insulin resistance, dyslipidemia, hypertension, hypercoagulability, osteopenia, and psychiatric disorders. Cushing syndrome in children is associated with an increased risk of venous thromboembolism and elevated procoagulants and antifibrinolytics. The adverse effects of abdominal adiposity insulin resistance and hypertension and cardiovascular dysfunction may persist even after cure [2, 29, 58, 59]. Pubertal development and linear growth are affected by childhood Cushing syndrome; however, studies have shown conflicting outcomes with regard to final height and catch-up growth [60–62]. Children who develop Cushing syndrome during puberty or who have had a prolonged course of disease prior to intervention are at a higher risk of short stature; early diagnosis and appropriate treatment with growth hormone are important to consider in certain clinical scenarios.

Cushing syndrome has been associated with multiple psychiatric and psychological disturbances, most commonly emotional lability, depression, and/or anxiety. Other abnormalities have included mania, panic disorder, suicidal ideation, schizophrenia, obsessive–compulsive symptomatology, psychosis, impaired self-esteem, and distorted body image. Significant psychopathology can even remain after remission of hypercortisolism and even after recovery of the hypothalamic–pituitary–adrenal axis. Up to 70% of patients will have significant improvements in the psychiatric symptoms gradually after the correction of the hypercortisolism.

We recently reported that children with Cushing syndrome may experience a decline in cognitive and school performance 1 year after surgical cure, without any associated psychopa-

thology [63]. Our recent study of health-related quality of life reported that active Cushing syndrome, particularly in younger children, was associated with low physical and psychosocial scores and that despite improvement from pre- to 1-year post-cure, residual impairment remained in physical function and role-emotional impact score. Although most self-reported CS symptoms showed improvement, forgetfulness, unclear thinking, and decreased attention span did not improve after cure of CS [64]. Importantly, we recently described mental health disorders, including suicidal ideation, in a subgroup of children and adolescents after remission of CS [65].

Case Study

A 10-year-girl presents with the growth chart shown in Fig. 15.3 and the physical appearance in Fig. 15.7. Her parents report that she is tired more easily and has been eating more than usual. She has been complaining of headaches off and on for 1 year. On physical examination, she has a blood pressure of 135/80 mm Hg, heart rate of 110 beats/min, and respiratory rate of 20 breaths/min. She is overweight, with posterior cervical fat pad and abdominal adiposity. She also has facial acne, a rounded face with bright red cheeks, lipomastia (Tanner stage 1), pubic hair (Tanner stage 3), and a normal clitoris. The rest of her examination findings are normal. Her pediatrician had ordered a complete blood cell count, which was normal, and thyroid function tests, which revealed a TSH concentration of 0.45 mIU/L (reference range 0.35–5.0 mIU/L) and a free T4 concentration of 0.9 ng/dL (reference range 0.8–1.7 ng/dL).

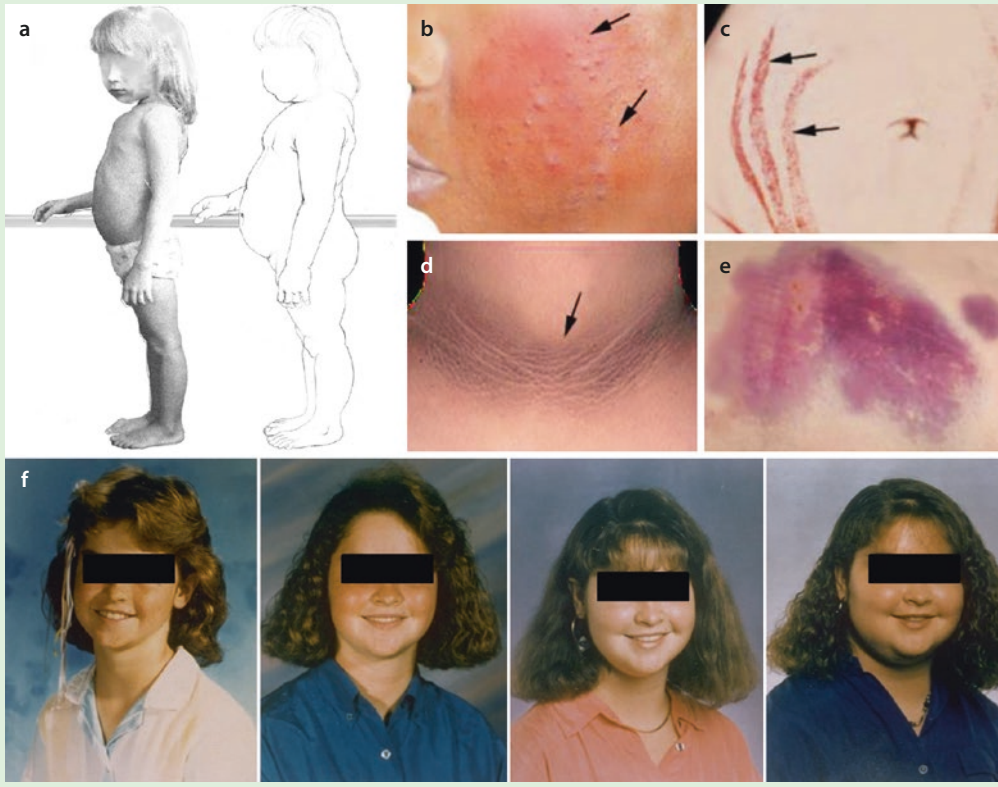

Fig. 15.7 **a** Gradual progression to the classic appearance of Cushing syndrome in a young child. **b** Facial plethora with acne (*arrows*) in a patient with Cushing syndrome. **c** Striae with bleeding (*arrows*). **d** Acanthosis nigricans (*arrows*) in a patient with Cushing syndrome and severe insulin resistance and glucose intolerance. **e** Skin bruising is frequent in older patients with Cushing syndrome but absent in toddlers and young children. **f** The gradual facial changes of a pediatric patient with Cushing syndrome over 4 years

15.7 Summary

Cushing syndrome can be caused by tumors that produce ACTH in the pituitary gland, in which case it is termed Cushing Disease, alternatively, the source of corticotropin may be outside of the pituitary itself (known as ectopic Cushing syndrome). Adrenocortical lesions, including adrenocortical cancer, adrenocortical adenomas, and bilateral adrenocortical hyperplasias, also cause Cushing syndrome. We now know that Cushing syndrome in children is often related to germline or somatic mutations in specific genes – each of which may have implications for the prognosis of the patients and for their families. Early recognition of Cushing syndrome is imperative in children; late diagnosis is associated with significant morbidity and mortality. Individuals with suspected Cushing syndrome should be referred to specialized clinical centers with experienced endocrinologists, neurosurgeons, and endocrine surgeons. Individualized medicine that takes into account mutation status in the context of Cushing syndrome is at the frontier of treatment for Cushing syndrome in children.

Review Questions

1. Which one of the following is the best next step in the patient's care (presented in the case study above)?
   A. No additional testing now; in 3 to 6 months, measure height and weight and assess thyroid function again.
   B. Perform a pituitary MRI immediately.
   C. Order growth hormone (GH) stimulation testing.
   D. Instruct the patient and her parents to collect a 24-h urine specimen for measurement of urinary free cortisol.
   E. Immediately prescribe levothyroxine replacement therapy.
2. A 14-year-old boy presents with a 1-year history of weight gain, easy bruising, stretch marks, and increased tiredness. Of note, his father was diagnosed with Cushing syndrome when he was in his teens. On physical examination:
   The patient is obese with a height of 150 cm and a weight of 75 kg (BMI = 33 kg/m2). He ishypertensive with a blood pressure of 147/104 mm Hg.

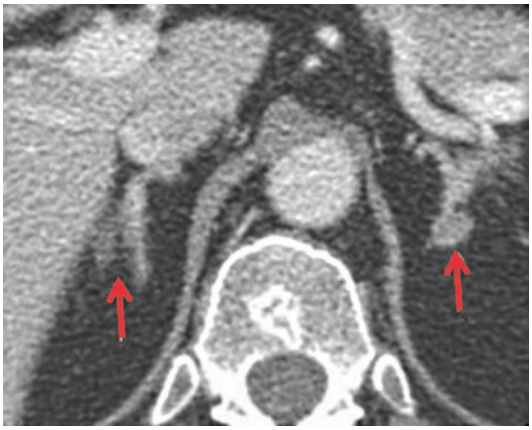

Fig. 15.8 Classic "beads-on-a-string" appearance of adrenal glands in a patient with primary pigmented nodular adrenocortical disease

   He has a plethoric round face with acne, gynecomastia, and multiple striae on his abdomen and upper thighs.
   Laboratory test results:
   ACTH (8 AM) = <5 pg/mL (reference range 10–60 pg/mL)
   Midnight serum cortisol = 16 μg/dL (reference range < 5 μg/dL)
   24-h urinary free cortisol = 320 μg/24 h (reference range 10–55 μg/24 h)
   Adrenal CT is shown in Fig. 15.8
   Which one of the following is the most likely diagnosis, putting together the CT findings, family history, and the biochemical profile of this patient?
   A. Adrenal cancer
   B. Congenital adrenal hyperplasia
   C. Primary pigmented nodular adrenocortical disease
   D. Cushing disease
   E. Exogenous steroid use
3. A 6-year-old boy presents with a 1-year history of acne and pubic hair growth. Review of his growth chart indicates that he has crossed percentiles from the 50% to the 75% in 1 year. On physical examination, stretched penile length is 10 cm and pubic hair is tanner 3. The testis is prepubertal at 2 cc bilaterally, no masses palpated. Bone age is read as 9 years of age. Lab tests show:
   17-Hydroxyprogesterone 120 ng/dL (reference range < 90 ng/dL)

17-Hydroxypregenelone 200 ng/dL (reference range 10–186 ng/dL)
DHEA-S 650 ug/dL (reference range 13–115 ug/dL)
Total testosterone 280 ng/dL (reference range < 10 ng/dL)
What is the most likely diagnosis?

A. Anabolic steroid exposure
B. 21-Hydroxylase deficiency
C. Germ cell tumor
D. Adrenal adenoma or carcinoma
E. 11-beta-hydroxylase deficiency

4. A 14-year-old female is referred to her pediatrician for irregular menses and concern for facial hair that she has started to bleach. She is started on oral contraceptives and returns for a follow-up visit 6 months later with complaints of further weight gain, and the development of flesh colored stretch marks on her thighs. She is doing well in school and enjoys participating in band and theater. At this point her pediatrician orders a 4 pm cortisol level that is resulted at 24 mcg/dL (reference range 5–25 mcg/dL). She is referred to you for evaluation for suspected Cushing syndrome.
What is the etiology of the high-normal cortisol?

A. The patient has Cushing syndrome.
B. The patient is depressed.
C. The cortisol at 4 PM is a very reliable time to measure cortisol; thus, this level warrants pituitary MRI.
D. The level of blood cortisol may be related to her oral contraceptive treatment, and a more reliable test would be urinary free cortisol.

Answers

1. (D) The patient's presentation and growth chart are typical of Cushing syndrome with increase in weight coupled with concurrent slowing of growth. In addition, hypertension, headaches, facial plethora, and acne are all characteristic signs of Cushing syndrome. The inappropriately advanced pubic hair without breast development is another clue to the diagnosis as excess adrenal androgens are produced in the context of pituitary tumors producing ACTH (or, more rarely, from an adrenocortical tumor producing both androgens and cortisol). Central, partially compensated hypothyroidism seen in this patient is not unusual at presentation of patients with Cushing syndrome. It is generally mild hypothyroidism and rarely requires treatment, and thyroid functions normalize after cure of Cushing syndrome. After excluding exogenous steroids as the cause of the patient's symptoms, the best next step is to confirm endogenous hypercortisolemia, via the collection of 24-h urine for the determination of urinary free cortisol. Generally, two to three consecutive 24-h collections are needed, because over- or under-collection in the course of a single 24-h collection often leads to false-positive or false-negative results, respectively. Once hypercortisolism is identified by urinary free cortisol, the next task is to confirm it by measuring midnight cortisol levels. In all causes of CS, the normal diurnal variation of ACTH and cortisol is lost, and cortisol levels in blood or saliva are high at midnight. To identify the cause of CS, ACTH must be measured next, followed by overnight dexamethasone testing. The most common cause of CS at this patient's age would be a pituitary adenoma. However, it would be inappropriate to perform MRI before confirming the diagnosis biochemically and documenting ACTH levels that indicate an ACTH-dependent cause of CS. Pituitary MRI, an expensive test that often requires sedation in young children, is frequently normal in patients with ACTH-producing microadenomas. Alternatively, a false-positive MRI finding (indicating an "incidentaloma") can misguide the investigation if imaging is obtained inappropriately early in the process of finding the cause of CS. It would also be inappropriate to wait and reassess and retest thyroid function in 3–6 months or assess for short stature by ordering GH testing.
2. (C) The case history is suggestive of familial Cushing syndrome. As the ACTH level is undetectable, the laboratory data point to adrenal hypersecretion of cortisol that is independent from the action of the pituitary. Adrenal CT is consistent with the classic appearance of PPNAD.

3. (D) This 6-year-old boy has rapidly progressive virilization with advanced bone age, elevated testosterone, and DHEA-S. This child most likely has an adrenal adenoma or carcinoma (D) In the case of congenital adrenal hyperplasia, steroid precursors would be elevated. While a testicular tumor is possible, the marked DHEA-S elevation points toward an adrenal source. Anabolic steroid exposure would be highly unlikely.
4. (D) Estrogen therapy is associated with an increase in corticosteroid-binding globulin, leading to increase in total cortisol levels, while free cortisol levels remain unchanged. In order to avoid this pitfall in diagnosis, urinary free cortisol is a more reliable measure of cortisol levels in an individual taking oral contraceptives. Depression may lead to a pseudo-Cushing state; however, we have no reason from the history to suspect depression. Thin flesh colored stretch marks are commonly found in obesity, to be distinguished from the wide purple colored striae in Cushing syndrome.

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Mineralocorticoid Disorders and Endocrine Hypertension

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Key Points

- Disorders of mineralocorticoid action should be considered in patients with abnormal serum levels of sodium and potassium.
- Mineralocorticoid deficiency or resistance to aldosterone should be considered in a patient with hyponatremia or hyperkalemia.
- Disorders of excess mineralocorticoid action should be considered in children suspected of having secondary hypertension. The absence of hypokalemia should not be used as evidence to exclude such disorders.

16.1 Introduction

Mineralocorticoid production in the adrenal cortex functions within the renin-angiotensin-aldosterone (RAA) system in the control of intravascular volume. The RAA system acts in concert with antidiuretic hormone (ADH) secretion from the posterior pituitary to maintain normal blood pressure and normal plasma osmolality, sodium, and potassium concentrations. This control of intravascular volume is necessary to maintain a normal blood pressure, while plasma sodium and potassium concentrations are controlled due to the role of sodium and potassium in the electrochemical gradients necessary for cell signaling. As discussed in ▶ Chap. 20, ADH is predominantly responsible for controlling plasma osmolality through regulation of free water excretion in the kidney, although, with significant volume loss, the body sacrifices osmolality to maintain extracellular volume. ADH deficiency, as in diabetes insipidus, can result in hypernatremic/hyperosmolar dehydration, while overaction of ADH, as in the syndrome of inappropriate antidiuretic hormone (SIADH), causes hyponatremia/hyposmolality. The RAA system, through regulation of sodium and potassium handling in the kidney, regulates intravascular volume and the serum potassium level. Derangements of this system can lead to hypertension and hypokalemia when overactive or to dehydration, hyponatremia, and hyperkalemia when underactive. The most common cause of deficiency of the RAA system is mineralocorticoid deficiency in the setting of primary adrenal insufficiency. This is discussed in ▶ Chap. 13. This chapter will discuss more specific disorders leading to an underactive RAA system, as well as the disorders within this system that lead to hypertension from an overactive RAA system.

Over 99% of the sodium and water that are filtered through the glomera of the kidney are subsequently reabsorbed. Approximately 90% of this occurs prior to the distal tubules. It is in the distal convoluted tubule, however, that aldosterone mediates the regulated reabsorption of sodium and secretion of potassium to maintain extracellular volume and potassium levels. Electrolyte handling in the distal convoluted tubule (DCT) is mediate by the epithelial sodium channel (ENaC), the sodium-potassium ATPase (Na^+/K^+ ATPase), and the renal outer medullary potassium channel (ROMK) (◘ Fig. 16.1). The Na^+/K^+ ATPase on the basolateral membrane of the principal cells in the DCT moves sodium from inside the cells to the interstitium. This drives the reabsorption of sodium from the lumen (urine) through apically expressed ENaC. As sodium is moved from the lumen to the interstitium, this ultimately drives water reabsorption. In addition, the rise in intracellular potassium due to the action of the Na^+/K^+ ATPase drives potassium secretion into the urine through ROMK.

In the kidney, the mineralocorticoid receptor (MR, systematic name NR3C2) is expressed in the cells of the DCT, mediating this regulation of sodium, potassium, and water handling of the DCT by the RAA system. Aldosterone is the primary ligand activating MR signaling in the DCT. It is produced exclusively in the zona glomerulosa of the adrenal cortex due to the specific expression of aldosterone synthase (*CYP11B2*) in these cells [1]. Aldosterone synthase contains enzymatic activity for 11β-hydroxylation, 18-hydroxylation, and 18-methyloxidation and thus metabolizes 11-deoxycorticosterone through corticosterone and 18-OH-corticosterone to produce aldosterone. (Note that 11β-hydroxylase, the gene product of the *CYP11B1* gene, is responsible for the conversion of 11-deoxycortisol to cortisol in the zona fasciculata; the 11β-hydroxylase conversion of deoxycorticosterone (DOC) to corticosterone in the zona glomerulosa is mediated by aldosterone synthase.) The aldosterone precursors 11-deoxycortisol and corticosterone can also bind

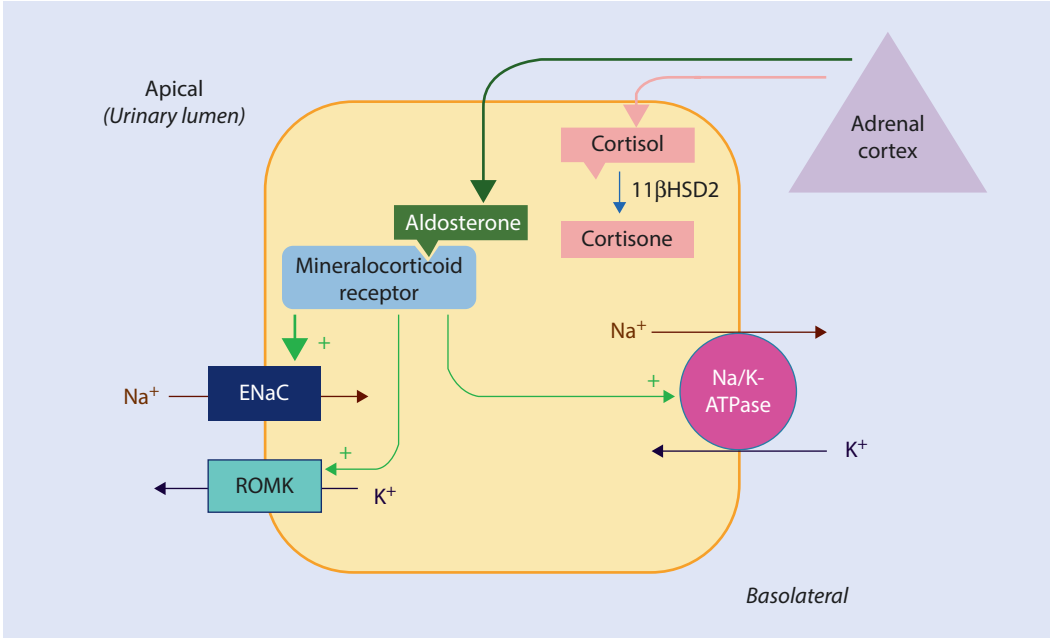

Fig. 16.1 Mineralocorticoid regulation of electrolyte and water balance in the distal nephron. Cortisol that enters the cell is converted by 11β-HSD2 into cortisone, which does not activate the MR. Thus, under normal circumstances, only aldosterone activates the MR. Activation of the MR increases the expression of ENaC on the apical membrane. The rapid effect of aldosterone is to increase the amount of ENaC expressed at the apical membrane by promoting translocation of intracellularly sequestered ENaC. MR activation also increases the production of ENaC, ROMK, and the Na+/K + -ATPase. The increased expression of these transporters and channels increases the reabsorption of sodium from the urinary lumen into the interstitial space and increases potassium excretion into the urine. The increase in sodium reabsorption subsequently drives an increase in water reabsorption

to the MR, albeit with low affinity. Therefore, at the usual physiologic circulating concentrations, these compounds do not activate MR signaling.

Cortisol binds to the MR with equal affinity to that of aldosterone [2] and circulates in levels 100 to 1000 times higher than of aldosterone. Therefore, if there were not a mechanism to keep cortisol from activating the MR, aldosterone would not be able to regulate the activity within the DCT; MR signaling would always be "on." However, the cells of the DCT express the enzyme 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2), which prevents usual circulating concentrations of cortisol from activating the MR (Fig. 16.1) [3]. This enzyme catalyzes the conversion of cortisol to the inactive steroid cortisone. In addition, conversion of cortisol to cortisone by 11β-HSD2 generates NADH, and this increase in NADH prevents cortisol-occupied MR from initiating downstream signals [4].

As a steroid hormone, aldosterone enters the cells of the DCT by passive diffusion and binds to the cytoplasmic MR. The MR is a member of the nuclear hormone receptor superfamily, acting as a transcription factor to regulate gene expression. In the DCT, MR activation increases the expression of ENaC, the Na^+/K^+ ATPase, and ROMK (Fig. 16.1) [5]. Even before there is an increase in the expression of these proteins; however, aldosterone stimulates an increase in the localization of ENaC to the apical membrane [5]. Because ENaC is rate-limiting for sodium reabsorption, it is primarily this increase in ENaC at the apical membrane that causes the increases in sodium reabsorption. As is true of other steroid hormone receptors, the MR has rapid, non-genomic actions, including the activation of multiple kinase cascades [6]. Indeed, it is these rapid, non-genomic actions that result in the earliest increase in ENaC expression at the apical membrane. The movement of ENaC to the plasma membrane is mediated in large part through the serine/threonine-protein kinase 1 (SGK1); the non-genomic actions of the MR can phosphorylate and activate SGK1, while the genomic actions of the MR increase SGK1 expression (before

increasing ENaC expression). The final effect of aldosterone action on the cells of the DCT is an increase in sodium and water reabsorption from the urine and an increase in potassium secretion into the urine. Aldosterone also stimulates hydrogen ion section into the urine through both direct and indirect mechanisms.

Aldosterone production is stimulated by the renin-angiotensin system and by hyperkalemia. The juxtaglomerular cells (JG cells) in the afferent arteriole in the kidney secrete renin into the systemic circulation in response to a fall in the renal perfusion pressure. This will occur with a decrease in the systemic blood pressure, with a change to an upright posture, and with a decrease in the serum sodium concentration. Renin is also secreted in response to an increase in the sympathetic nervous system input to the kidney. Renin catalyzes the cleavage of angiotensinogen (produced constitutively in the liver) into angiotensin I. Angiotensin-converting enzyme (ACE), produced constitutively in the lung, then cleaves angiotensin I into angiotensin II. Angiotensin II binds to the AT1 receptor in the adrenal cortex, stimulating aldosterone production. An elevated serum potassium level directly stimulates aldosterone production in the adrenal cortex, independent of the RAA system. ACTH also can stimulate aldosterone production. However, since the RAA system is the primary regulator of aldosterone production, ACTH deficiency does not result in mineralocorticoid deficiency.

Disorders of mineralocorticoid action include disorders of insufficient action and disorders of excess action. These are listed below. The consequences of impaired or excess mineralocorticoid action can be predicted from the known actions of aldosterone. Disorders of impaired mineralocorticoid action typically result in hyponatremia, dehydration, and hyperkalemia. These patients also typically have a metabolic acidosis from impaired acid secretion. Primary adrenal insufficiency, with impaired production of all adrenal steroid hormones, is discussed in ▶ Chap. 13. Certain forms of congenital adrenal hyperplasia (CAH) result in mineralocorticoid deficiency, as discussed in ▶ Chap. 14. Disorders of excess mineralocorticoid action typically result in hypertension with hypokalemia and can have a metabolic alkalosis. The hypertensive forms of CAH are discussed in ▶ Chap. 14.

Disorders of Mineralocorticoid Action

- **Impaired Mineralocorticoid Action**
  - *Decreased aldosterone production*
    - Primary adrenal insufficiency
    - Aldosterone synthase deficiency
    - Congenital adrenal hyperplasia (CAH)
      - 21α-Hydroxylase deficiency
      - 3β-Hydroxysteroid dehydrogenase deficiency
      - StAR deficiency
  - *Decreased aldosterone action*
    - Pseudohypoaldosteronism (PHA)
      - Renal PHA1
      - Generalized PHA1
      - Secondary PHA
- **Excess Mineralocorticoid Action**
  - *Increased aldosterone production*
    - Primary aldosteronism
      - Conn syndrome
      - Bilateral idiopathic hyperplasia
    - Renal artery stenosis
    - Glucocorticoid remediable aldosteronism (GRA)
  - *Non-aldosterone mediated*
    - Apparent mineralocorticoid excess (AME)
    - Glycyrrhetinic acid-mediated
    - Congenital adrenal hyperplasia (CAH)
      - 11β-Hydroxylase deficiency
      - 17-Hydroxylase deficiency

16.2 Aldosterone Synthase Deficiency

Mutations of the *CYP11B2* gene, the gene for the aldosterone synthase enzyme, result in isolated aldosterone deficiency, with normal glucocorticoid production. This is a rare, autosomal recessive disorder [7]. Two variants of aldosterone synthase deficiency have been described, referred to as aldosterone synthase deficiency type I and type II. These were previously referred to as type I and II corticosterone methyl oxidase deficiency (CMO I and CMO II deficiency.) Type I and type II aldosterone synthase deficiencies are differentiated by differences in the levels of aldosterone and 18-hydroxycorticosterone, reflecting differences in completeness of the enzymatic block and perhaps on the differential impact of the mutation on the three enzymatic reactions carried out by the enzyme. However, the same mutation in *CYP11B2* can cause both variants, indicating that there must be other mechanisms resulting in these differences in clinical phenotype. It is worth noting that

deoxycorticosterone (DOC) and corticosterone, both weak mineralocorticoids, can be produced in patients with aldosterone synthase deficiency. DOC is produced because it is proximate in the steroid pathway to aldosterone synthase; corticosterone may be produced either through retained 11β-hydroxylation activity of the mutant aldosterone synthase gene or through the action of the 11β-hydroxylase enzyme. However, unlike in the 11β-hydroxylase deficiency form of CAH where DOC levels are markedly elevated (driven by the high ACTH levels), DOC and corticosterone levels in aldosterone synthase deficiency are insufficient to prevent salt loss in infants and young children.

16.2.1 Clinical Presentation

Individuals usually present with a salt-wasting crisis in the first days to weeks of life. They will typically have a history of vomiting and will present with evidence of dehydration on physical examination. Electrolytes typically demonstrate hyponatremia and hyperkalemia. This presentation is very similar to infants with salt-wasting forms of CAH. However, in aldosterone synthase deficiency, there is no ambiguity of the genitalia, as there can be with CAH. Less severe forms may present later in childhood with failure to thrive or growth retardation.

16.2.2 Diagnostic Evaluation

The diagnosis of mineralocorticoid deficiency is made based on a low serum aldosterone level with a high plasma renin level. CAH is excluded by normal serum levels of cortisol and the relevant adrenal steroid levels. In type I aldosterone synthase deficiency, the aldosterone level will be undetectable or low, while in type II aldosterone synthase deficiency, the level is low but detectable. Tetrahydroaldosterone is a metabolite of aldosterone, and the urinary tetrahydroaldosterone level is low in both forms of aldosterone synthase deficiency. The characteristic difference between type I and type II aldosterone synthase deficiency is the level of 18-hydroxycorticosterone: low in the type I form and markedly elevated in the type II form.

16.2.3 Treatment and Outcomes

Treatment with 9α-fludrocortisone corrects the mineralocorticoid deficiency in these infants. Some infants may also require salt supplementation. In most patients the disorder becomes less severe over time so that by 3–4 years of age they often remain asymptomatic even without treatment with 9α-fludrocortisone. However, at least some of these patients continue to have orthostatic hypotension and remain at risk of electrolyte abnormalities at times of stress from dehydration or sodium depletion.

16.3 Pseudohypoaldosteronism

Pseudohypoaldosteronism (PHA) refers to a clinical presentation that suggests hypoaldosteronism (dehydration, hyponatremia, hyperkalemia, and metabolic acidosis) but with an elevated aldosterone level that indicates that the cause is aldosterone resistance, rather than aldosterone deficiency. PHA1 is discussed below. PHA2 is now also known as Gordon syndrome, and it is not a true aldosterone resistance disorder. This disorder does have hyperkalemia and metabolic acidosis from impaired renal excretion in common with aldosterone resistance. But in contrast with true aldosterone resistance, patients with PHA2/Gordon syndrome have hypertension, rather than salt loss and hyponatremia. PHA3 refers to transient and secondary forms of aldosterone resistance as described below.

16.3.1 Pseudohypoaldosteronism Type 1 (PHA1)

There are two distinct forms of PHA1 that differ in clinical features as well as inheritance pattern and are caused by mutations in unique genes [5]. The renal form of PHA1 has autosomal dominant inheritance and is caused by inactivating mutations in the *NR3C2* gene that encodes the MR. The generalized or systemic form of PHA1 has autosomal recessive inheritance and is due to inactivating mutations in one of the genes encoding subunits of the ENaC channel.

16.3.2 Renal PHA1

16.3.2.1 Clinical Presentation

Renal PHA1 is caused by heterozygous mutations in the *NR3C2* gene that result in impaired MR function [8]. As for other forms of deficient mineralocorticoid action, infants with renal PHA1 classically present with vomiting, failure to thrive, and dehydration, most often presenting between 2 weeks and 6 months of age. However, there is a wide spectrum of clinical findings in patients with *NR3C2* mutations, including in patients with the same mutation within a given family [5, 9]. Thus, patients can present with the classical salt-wasting disease in infancy; some individuals can be asymptomatic with normal electrolytes but with laboratory evidence of mineralocorticoid resistance (elevated renin and aldosterone levels); and some individuals can be completely unaffected.

16.3.2.2 Diagnostic Evaluation

Laboratory findings in patients with renal PHA1 are those expected in mineralocorticoid resistance: a high plasma renin level and an elevated aldosterone level. The infants present with hyponatremia and hyperkalemia, although the hyperkalemia is generally mild. While metabolic acidosis may be seen, it is often not present. Genetic testing will demonstrate a heterozygous mutation in the *NR3C2* gene.

16.3.2.3 Treatment and Outcomes

Treatment of renal PHA1 is with sodium supplementation. The usual doses of sodium required are between 3 and 20 mEq/kg/day. The amount of sodium supplement required is determined by adjusting the dose to achieve normal serum potassium and plasma renin levels. Interestingly, in spite of this disease being caused by a germ line mutation, there is amelioration of the disease with age, such that sodium supplements can be discontinued by around 18 to 24 months of age. This likely is due to a combination of things, including the increase in renal MR expression during infancy, as well as the increase in sodium intake with the introduction of solid food in the diet. With this clinical resolution, the plasma renin level is normal without sodium supplementation. The aldosterone level may continue to be elevated, evidence of ongoing mineralocorticoid resistance, but in other individuals the aldosterone level will normalize.

16.3.3 Systemic PHA1

16.3.3.1 Clinical Presentation

Systemic PHA1 is caused by defective function of ENaC [10]. The ENAC channel is composed of three subunits, ENaCα, ENACβ, and ENaCγ, produced by the *SCNN1A*, *SCNN1B*, and *SCNN1G* genes. ENaC has important functions beyond the kidney, and patients with systemic PHA1 have evidence of salt loss from the distal colon and from salivary and sweat glands, in addition to the salt loss from the kidney [5]. In addition, the ENaC mutations impair salt and water handling in the respiratory tract. Infants with systemic PHA1 typically present as neonates with vomiting, failure to thrive, and severe dehydration, just as do infants with renal PHA1. The salt wasting in systemic PHA1 is more severe, however, so these infants typically present earlier than those with renal PHA1, often in the first weeks of life. In addition, because of the extrarenal effects of ENaC mutations, these patients also have significant respiratory symptoms. They can have persistent clear rhinorrhea and congestion, chronic cough, tachypnea, and wheezing. They often have recurrent lower respiratory tract infections. The impairment in ENaC function in skin can result in cutaneous lesions that resemble miliaria rubra.

16.3.3.2 Diagnostic Evaluation

Laboratory testing demonstrates hyponatremia, hyperkalemia, metabolic acidosis, and high renin and aldosterone levels. The hyperkalemia can be severe and can present a significant mortality risk. The sodium concentration in sweat is high, which can distinguish this form of PHA1 from the renal form, where the sweat sodium concentration is normal. Genetic testing will demonstrate biallelic mutation of one of the three genes encoding the ENaC channel subunits.

16.3.3.3 Treatment and Outcomes

As with the renal form of PHA1, the primary treatment of the systemic form of PHA1 is with sodium supplements. The sodium supplementation required in the systemic form is higher than that required in the renal form, consistent with the generally more sever salt wasting in the systemic form. Doses of sodium are typically between 20 and 50 mEq/kg/day. Tube feedings are sometimes needed to achieve this high degree of salt intake. At presentation, treatment with ion exchange

resins such as sodium polystyrene sulfonate may be necessary to correct the hyperkalemia, and some infants may require ongoing treatment with these resins. Occasionally, even more aggressive treatment of life-threatening hyperkalemia present at diagnosis is needed, such as treatment with beta-agonists or glucose and insulin infusions.

Unlike renal PHA1 patients, systemic PHA patients cannot stop their salt supplementation as they get older. While there may be some amelioration with increasing age, systemic PHA1 requires ongoing, lifelong treatment with sodium supplementation. The lung disease of PHA1 can be clinically very similar to that of cystic fibrosis (CF). However, despite frequent respiratory exacerbations, as well as having colonization with *Pseudomonas aeruginosa,* PHA1 patients do not have the destructive lung disease that occurs in CF patients. Nonetheless, these patients do require appropriate symptomatic treatment for the pulmonary exacerbations.

16.3.4 Secondary Pseudohypoaldosteronism (PHA3)

A transient form of pseudohypoaldosteronism can develop from either infection or obstruction of the urinary tract [11]. This secondary form of PHA is referred to as either secondary PHA1 or as PHA3, and it resolves with correction of the underlying abnormality. It has also been described in children treated with calcineurin inhibitors such as tacrolimus and cyclosporine [12].

16.3.4.1 Clinical Presentation

The typical presentation for PHA3 is similar to that of infants with other forms of PHA: an infant with vomiting and dehydration and failure to thrive. When there is a urinary tract infection, these infants present with fever. In infants where there is no underlying renal abnormality and the cause is from an acute urinary tract infection, there will not be failure to thrive. Most commonly these infants are less than 3–6 months of age [11].

16.3.4.2 Diagnostic Evaluation

As with genetic forms of PHA, laboratory testing will demonstrate hyponatremia, hyperkalemia, metabolic acidosis, and high renin and aldosterone levels. In older children, the PHA due to a urinary tract infection may cause hyponatremia with normokalemia. Because urinary tract obstruction or infection can cause PHA, all infants presenting with laboratory results that suggests PHA should have testing to investigate for a urinary tract infection and ultrasound imaging to explore for evidence of urinary tract abnormalities.

16.3.4.3 Treatment and Outcomes

Acutely, these infants are treated with fluid replacement, which typically results in normalization of electrolytes within 24–48 h. With obstructive uropathy, the salt loss may transiently worsen in the immediate postoperative period. However, with treatment of the underlying cause, there will be resolution of the salt loss unless there has been permanent renal damage. In most cases the resolution occurs within a few days, although in some cases the salt loss may last a few weeks.

16.4 Excess Mineralocorticoid Action

16.4.1 Increased Aldosterone Production

As expected from the actions of aldosterone, mineralocorticoid excess causes an excess retention of sodium and excretion of potassium. Thus, these patients develop hypertension and hypokalemia. They also typically develop a metabolic alkalosis. In adults, mineralocorticoid excess is the underlying cause in up to about 10% of patients with hypertension [13]. While hypertension is more often caused by an underlying disorder in children compared to adults, endocrine causes are uncommon, with primary renal or renovascular diseases much more likely. However, along with pheochromocytoma, Cushing syndrome, hypertensive forms of CAH, and hyperthyroidism, primary aldosteronism is a cause of hypertension in children, albeit an exceedingly rare cause.

16.5 Primary Aldosteronism

Primary aldosteronism is caused by either Conn syndrome, an aldosterone-secreting tumor of the adrenal cortex, or by idiopathic, bilateral hyperplasia of the adrenal glomerulosa. Rare, familial forms

of bilateral hyperplasia are the result of a germ line mutation of the *KCNJ5* and *CACNA1D* genes, and children within these pedigrees present at a young age with the hypertension and hypokalemia expected of aldosteronism [14]. The children with CACNA1D gene mutations have also had neuromuscular disorders [15]. Somatic mutations are often found within Conn syndrome tumors, most often of *KCNJ5* and less commonly of *CACNA1D*, *ATP1A1*, or *ATP2B3* [14]. Rarely, patients with MEN1 can develop Conn syndrome [16].

Secondary hypertension, including aldosteronism, should be investigated as a possible cause of hypertension in prepubertal children with any degree of hypertension and in a pubertal child with severe hypertension (blood pressure more than 5 mmHG above the 99th percentile). In addition, aldosteronism should be suspected in the hypertensive child with hypokalemia or one with a family history of early onset hypertension or cerebrovascular hemorrhage at a young age, which suggests the possibility of glucocorticoid remediable aldosteronism (GRA). Finally, secondary hypertension, including aldosteronism, may be considered in the thin adolescent with any degree of hypertension. The evaluation for aldosteronism begins with measuring plasma aldosterone level and plasma renin activity.

16.5.1 Clinical Presentation

Disorders of aldosterone excess are most often identified in the evaluation of the child with asymptomatic hypertension. It might be expected in a younger child with more severe hypertension. If the hypertension is severe, the child may have a history of headaches. The symptoms of hypokalemia can include muscle weakness and cramping, as well as polydipsia and polyuria from the renal concentrating defect caused by hypokalemia.

16.5.2 Diagnostic Evaluation

The laboratory finding that would suggest aldosteronism in a child with hypertension is hypokalemia and metabolic alkalosis. However, in adults, many of these patients will present with a normal serum potassium level, particularly those with low salt intake in their diet. While there are insufficient data in children to determine the frequency of hypokalemia in children with aldosteronism, it may be more common than in adults. Normokalemia is more common in adults with bilateral hyperplasia (~80%), while only 50% of adults with Conn syndrome are normokalemic [17]. In children, aldosteronism caused by bilateral hyperplasia is more likely to be caused by a somatic mutation (such as KCNJ5) than in adults (where an idiopathic cause is most common), and these genetic causes may be less likely to have normokalemia. Nonetheless, a substantial proportion of children with aldosteronism will not have hypokalemia.

The diagnosis of aldosteronism is supported by an elevated aldosterone level in the presence of a suppressed plasma renin activity (PRA), resulting in a high plasma aldosterone concentration (PA) to PRA ratio. Note that in a hypertensive patient, a normal PA concentration is inappropriately elevated. In adults, most patients with aldosteronism will have a PA/PRA ratio (measure in ng/dl and ng/mL per hour, respectively) of over 20–40 [13]. Confirmation of the diagnosis of aldosteronism can be made using one of four testing procedures: oral sodium loading, saline infusion, fludrocortisone suppression, and captopril challenge [13]. Because of the rarity of aldosteronism in children, the utility of the confirmation testing and specific cutoffs for the PA/PRA ratio have not been determined for children.

Once aldosteronism is identified, testing should be done to identify those children with GRA (see below). If GRA is not the cause, imaging is utilized to determine if the cause is a unilateral adrenal tumor (Conn syndrome) or bilateral hyperplasia as seen in the germ line *KNCJ5* and *CACNA1D* mutations. If Conn syndrome is not identified on imaging, genetic testing looking for mutations of *KNCJ5* is indicated.

16.5.3 Treatment and Outcomes

The treatment of aldosteronism from Conn syndrome is surgical excision of the tumor, which is curative. For patients with aldosteronism from bilateral adrenal hyperplasia, treatment with mineralocorticoid antagonists, either spironolactone or eplerenone, is the initial therapy, although some may require treatment with additional antihypertensive medications. For pubertal patients, eplerenone may be preferable to avoid

the antiandrogenic effects of spironolactone. Patients with germ line *KNCJ5* mutations causing massive adrenal hyperplasia have required bilateral adrenalectomies to control the hypertension [18], although more mild forms have also been described [19].

Patients with hypertension due to aldosteronism of any cause have a higher risk of renal and cardiac damage than those with equivalent degrees of hypertension from other causes [20, 21]. While MR expression in the cells of the DCT of the kidney is the primary mechanism for sodium, potassium, and extracellular volume regulation by the RAA system, MR is also expressed in cells outside the kidney. This extrarenal MR expression likely contributes to some of the complications arising from chronic mineralocorticoid excess, including cardiac fibrosis.

16.6 Renal Artery Stenosis

In a child with hypertension, a high PA concentration in the absence of a suppressed PRA, with a PA/PRA that is not elevated, suggests a diagnosis of renal artery stenosis (RAS). RAS leads to a decrease in the renal perfusion pressure sensed by that kidney, resulting in an increase in renin secreted by the JG cells of that kidney. This renal artery stenosis can be unilateral or bilateral. Once the patient's blood pressure is elevated, the renin level returns to normal. Initially the renin from the contralateral (non-affected) kidney is suppressed, and ultimately the renin from the affected kidney also declines as the hypertension overcomes the stenosis. Therefore, an elevated renin level, which is preferably measured as a plasma renin activity (PRA), may not be found in patients with renal artery stenosis.

16.6.1 Diagnostic Evaluation

The normal response to hypertension is suppression of renin secretion. Thus, a PRA that is not suppressed suggests inappropriate production of renin as the cause of the hypertension. As noted above, PRA is typically in the normal range in patients with RAS. An elevated PRA in a hypertensive child would suggest the very rare situation of a renin-secreting juxtaglomerular cell tumor [22]. As in aldosteronism, PA levels are not suppressed in RAS, but in contrast to the elevated PA/PRA ratio in aldosteronism, this ratio is normal in RAS. Hypokalemia may be found in patients with RAS, but the degree of hypokalemia tends to be mild or absent. In about 50% of patients an abdominal bruit is present.

16.7 Glucocorticoid Remediable Aldosteronism (GRA)

Secretion of steroid hormones is coupled to production. The main rate-limiting step in steroid synthesis is at the level of transport of cholesterol from the outer to the inner mitochondrial membrane through the action of the steroidogenic acute regulatory protein (StAR). However, the subsequent determination of which steroid is produced is controlled through the level of expression of enzymes specific to the separate steroid synthesis pathways. Production of cortisol is dependent on the level of expression of 11β-hydroxylase. The 11β-hydroxylase gene is expressed at high levels in the zona fasciculata, and this expression is driven in part through the action of upstream regulatory elements, including those that are responsive to ACTH stimulatory pathways. Aldosterone production in the zona glomerulosa is dependent on the high level of expression of the gene for aldosterone synthase. Its expression is increased through potassium- and angiotensin II-responsive elements upstream of the gene.

GRA occurs through an unequal crossover event during sister chromatid exchange in meiosis between the 11β-hydroxylase gene and the aldosterone synthase gene (Fig. 16.2) [23]. This occurs due to their close proximity to each other on chromosome 8, as well as to the high degree of sequence homology of the coding regions of these genes. This results in the translocation of the ACTH-responsive regulatory elements of the 11β-hydroxylase gene to a position upstream of the coding sequence of the aldosterone synthase gene. The effect of this is the ACTH-dependent production of high levels of aldosterone in the zona glomerulosa. These individuals do not have any impairment of glucocorticoid production: they have two normal 11β-hydroxylase genes (as well as two normal aldosterone synthase genes) in addition to the chimeric gene.

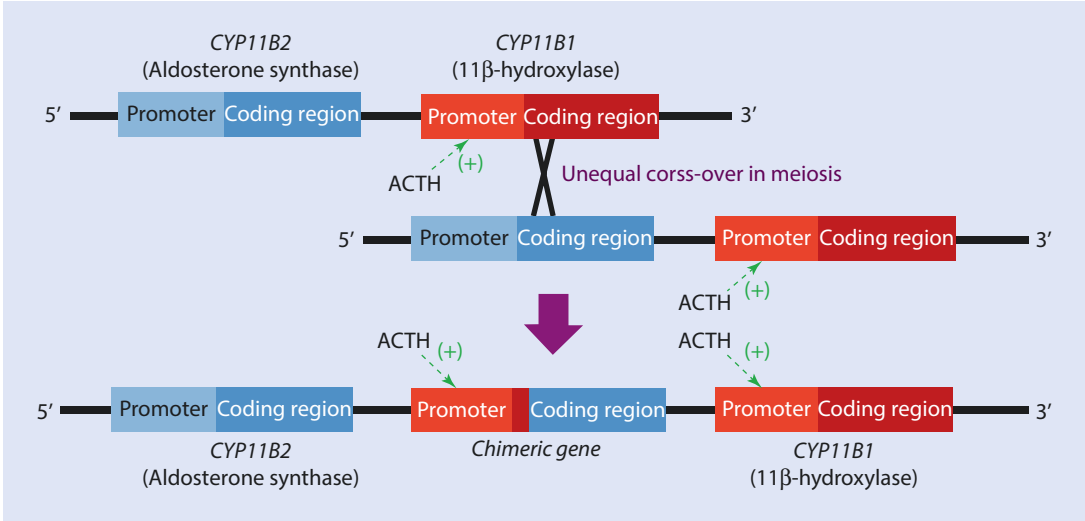

Fig. 16.2 Chimeric gene formation in GRA. In GRA, an unequal crossover event in meiosis results in the fusion of the 5′ sequences of CYP11B1 (the gene for 11β-hydroxylase) with 3′ sequence of CYP11B1 (the gene for aldosterone synthase). The chimeric gene contains the CYP11B1 promoter, the very N-terminal coding sequence of 11β-hydroxylase, and C-terminal aldosterone synthase coding sequence. This results in the ACTH-dependent expression of high levels of a chimeric protein with aldosterone synthase activity

16.7.1 Clinical Presentation

GRA is inherited in an autosomal dominant manner. It typically presents with hypertension in the first two decades of life, with one report finding that 80% of children with the chimeric gene were hypertensive by 13 years of age [24]. As with other forms of aldosteronism, the patients may or may not have hypokalemia. GRA is associated with an increased risk of early cerebral hemorrhage, although these events typically occur in young adulthood [25].

16.7.2 Diagnostic Evaluation

Investigation for GRA starts with determining if there is biochemical evidence for aldosteronism as discussed above. Any child who is found to have aldosteronism should undergo genetic testing for GRA. While dexamethasone suppression of aldosterone and very high serum and urine levels of 18-hydroxycortisol and 18-oxocortisol levels are characteristic of GRA, these tests lack specificity. Therefore, testing for the chimeric gene should be performed to evaluate for GRA. Because of the autosomal dominant inheritance, first-degree relatives of individuals with GRA should also undergo genetic testing.

16.7.3 Treatment and Outcomes

Aldosterone production in GRA is ACTH-dependent. Therefore, aldosterone levels can be lowered by treating the patient with glucocorticoid. Longer-acting synthetic glucocorticoids such as dexamethasone and prednisone are preferred and should be given at bedtime. The starting dose for adults is 0.125–0.25 mg of dexamethasone or 2.5–5 mg of prednisone. These translate to surface area-adjusted doses for children of approximately 0.1 mg/m^2 of dexamethasone and 2 mg/m^2 of prednisone. If low doses of glucocorticoid are not fully effective in controlling the hypertension, a mineralocorticoid antagonist (spironolactone or eplerenone) can be added. Eplerenone may be preferable to avoid the antiandrogenic effects of spironolactone in pubertal children. The use of mineralocorticoid antagonist to minimize the glucocorticoid dose may be particularly important to avoid any growth-suppressing effects from excessive glucocorticoid doses. As with other forms of aldosteronism, potassium-wasting diuretics should only be used with caution (and are probably best avoided) as they are likely to induce hypokalemia.

Because of the high rate of cerebrovascular events in patients with GRA, screening with appropriate imaging is recommended, starting at puberty.

16.8 Apparent Mineralocorticoid Excess (AME)

Apparent mineralocorticoid excess (AME) is caused by biallelic inactivating mutations of the 11β-HSD2 gene [4]. It is inherited in an autosomal recessive manner. As expected, the loss of 11β-HSD2 function in the kidney results in continuous activation of the MR in these cells by cortisol, resulting in hypertension and potassium loss.

16.8.1 Clinical Presentation

There can be a range of presentations from mild to severe [26]. In the severe phenotype, the infant is born with low birth weight and demonstrates failure to thrive in early childhood, with severe hypertension developing in early childhood. These children typically present with hypercalciuria and nephrocalcinosis [27]. They may also have polyuria due to the nephrogenic diabetes insipidus that is a consequence of the hypokalemia. Milder phenotypes can present without electrolyte changes, with the only manifestation being hypertension that develops later in childhood or even in adulthood.

16.8.2 Diagnostic Evaluation

As for other forms of aldosteronism, hypokalemia and metabolic alkalosis suggest the diagnosis but may not be present in all patients. However, in contrast to patients with aldosteronism, the aldosterone level in this disorder is low. The diagnosis is made by demonstrating an increase in the ratio of 11-hydroxysteroids to 11-ketosteroids in the urine: in a 24-h urine sample, there is an increase in cortisol/cortisone and an increase in (tetrahydrocortisol + allotetrahydrocortisol)/tetrahydrocortisone. The normal ratio of urinary cortisone/cortisol is less than 0.5. The normal ratio of (tetrahydrocortisol + allotetrahydrocortisol)/tetrahydrocortisone is approximately 1; in AME this is increased to approximately 6–60 [28].

16.8.3 Treatment and Outcomes

Treatment for AME can include some combination of mineralocorticoid receptor antagonism, ENaC inhibition, potassium supplementation, and sodium restriction. In mild forms, a high potassium diet or potassium supplementation along with a low dose of either spironolactone or eplerenone may be sufficient. In more severe forms, more aggressive therapy is needed, adding sodium restriction along with amiloride or triamterene to block ENaC. Thiazide diuretics may be beneficial in decreasing urinary calcium excretion, as these patients typically have hypercalciuria and may develop nephrocalcinosis. In some patients there may be a role of a long-acting glucocorticoid such as dexamethasone to suppress cortisol levels.

16.9 Glycyrrhetinic Acid-Mediated

Chronic ingestion of licorice can result in hypertension and laboratory findings similar to those of AME: hypokalemia and metabolic alkalosis with a low plasma aldosterone level [29]. This occurs because licorice contains glycyrrhetinic acid, which is an effective inhibitor of 11β-HSD. Resolution of the hypertension and metabolic abnormalities occurs with cessation of licorice ingestion.

16.10 Congenital Adrenal Hyperplasia

Two forms of congenital adrenal hyperplasia (CAH) result in hypertension: due to 11β-hydroxylase deficiency and 17-hydroxylase deficiency. In the case of 11β-hydroxylase deficiency, the 46,XX baby will present with ambiguous genitalia, while the 46,XY baby will present with early signs of virilization before puberty. Overproduction of deoxycorticosterone causes hypertension, hypokalemia, and metabolic alkalosis, although these may not be present at birth. While deoxycorticosterone has relatively low affinity for the MR, it is produced in markedly high levels in this form of CAH, sufficient to cause MR activation. CAH due to 17-hydroxylase deficiency may present with ambiguous genitalia in the 46,XY infant due to the impaired testosterone production. In 46,XX infants, or in forms with complete loss of enzyme action, both the 46,XX child and the 46,XY child will present as girls with a lack of development of secondary sexual characteristics at puberty. These children also

develop hypertension, hypokalemia, and metabolic alkalosis, in this case due to overproduction of both deoxycorticosterone and corticosterone. Like deoxycorticosterone, corticosterone has weak mineralocorticoid activity. In 17-hydroxylase deficiency, deoxycorticosterone and corticosterone are produced at levels high enough to activate the MR. See ► Chap. 14 for more details about these and other forms of CAH.

16.11 Summary

Isolated disorders of mineralocorticoid action are rare in children. However, when present, they can cause significant morbidity and in severe cases can be life-threatening. Disorders that result in deficient mineralocorticoid action are typically congenital and present within the first months of life. The exception would be milder forms of the congenital disorders or secondary pseudohypoaldosteronism that can be acquired due to renal disease. These mineralocorticoid-deficient states typically present with vomiting and dehydration or with failure to thrive if more indolent. The classical electrolyte disturbance found is hyponatremia and hyperkalemia. Overactivity of mineralocorticoid action leads to hypertension and should be considered in the workup of secondary causes of hypertension in children. Hypokalemia in a patient with hypertension is an important clue to excess mineralocorticoid activity, but hypokalemia is not always present in these disorders, and therefore aldosteronism should not be excluded on the basis of a normal serum potassium.

Review Questions

1. Why does cortisol generally not activate the mineralocorticoid receptor?
   - A. Cortisol has a low affinity for the mineralocorticoid receptor.
   - B. Cortisol circulates at a much lower level than aldosterone, and therefore the mineralocorticoid receptor is generally occupied by aldosterone binding.
   - C. Cortisol binding to the mineralocorticoid receptor does not recruit the appropriate coactivators to allow active signaling.
   - D. Cortisol is inactivated by 11β-HSD2 in the cells of the distal nephron.
2. A female infant presents at 4 days of age with a history of vomiting and lethargy. On exam she is severely dehydrated. Laboratory studies reveal serum sodium of 120 mEq/L, potassium of 9 mEq/L, and bicarbonate 8 mEq/L. The infant is resuscitated and discharged on sodium chloride supplements. Over the first year, she had multiple hospital admissions for respiratory distress with fever, cough, and tachypnea, with wheezing and crackles on exam. What is the most likely inheritance pattern for this disorder, and what is expected for the natural history of this disease?

	Inheritance pattern	Natural history
A	Autosomal recessive	Persistent symptoms throughout life
B	Autosomal dominant	Persistent symptoms throughout life
C	Autosomal recessive	Resolution in the first years of life
D	Autosomal dominant	Resolution in the first years of life

3. An 18-month-old child presents with fever and vomiting for the past 2 days. The past history is unremarkable with normal growth and development. On exam the child has evidence of dehydration with tachycardia, decrease skin turgor, and decreased capillary refill time. Serum electrolytes show a sodium level of 126 mEq/L and a serum potassium of 5.6 mEq/L. Which of the following is the most likely diagnosis?
   - A. Secondary pseudohypoaldosteronism
   - B. Homozygous mutation of ENaC
   - C. Congenital adrenal hyperplasia
   - D. Homozygous mutation of the mineralocorticoid receptor
   - E. Glucocorticoid remediable aldosteronism

Answers

1. D
2. A
3. A

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Thyroid Disorders

Congenital Hypothyroidism

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Key Points

- Congenital hypothyroidism is a devastating yet preventable cause of mental retardation.
- Newborn screening for congenital hypothyroidism is crucial for prevention of long-term consequences.
- Interpretation of laboratory results requires an understanding of newborn screening methodologies and timing of data collection.
- Optimal treatment of congenital hypothyroidism requires timely confirmation of diagnosis and adequate medication administration.

17.1 Introduction and Background Information

17.1.1 Newborn Screening

Most infants born with CH have no appreciable physical stigmata of the disease; therefore, screening to detect which newborns are at risk for thyroid disease is paramount. The introduction of NBS in the 1970s has decreased the incidence of untreated CH although prevalence rates have increased [1]. Prevalence of CH ranges from 1:1415 to 1:4000 depending on region and ethnicity [2]. Prior to screening, only one-third of hypothyroid infants was clinically detected before 3 months of age, leading children to suffer from severe mental retardation language, learning, and coordination delays and severe mental retardation. With the introduction of screening, age of detection has steadily declined. In the early phases of NBS, the target was to identify and initiate treatment by 2 months of life. Currently, with rapid specimen transport of newborn specimens collected on average day of life 2, and advances in technology which allow rapid thyroxine (T4) and thyrotropin (TSH) analysis, many screening programs detect and initiate treatment within 1–2 weeks of age, allowing more normal developmental outcomes [3]. NBS can be implemented in several ways, some more sensitive than others. In general, appropriate interpretation of TSH and/or T4 values, be it total T4 or free T4 (fT4), is required to appropriately diagnose CH.

17.1.2 Primary T4, Secondary TSH NBS Method

A common method for screening newborns for CH consists of collecting blood spots on filter paper for the whole blood total T4. Subsequently TSH screening occurs only on a select group, based on low T4 values, often the lowest 3rd to 10th percentiles [4]. Using this methodology, the specific numeric cutoff may vary from week to week. The benefits of primary T4 screening are its sensitivity for detection of newborns with hypothalamic-pituitary hypothyroidism. Known as central hypothyroidism, delayed detection of this form of hypothyroidism also results in developmental deficits. In this form of pituitary dysfunction, TSH is not elevated, often normal; therefore, primary TSH screening would not detect this abnormality. Even more profound is the high prevalence of coexistent pituitary hormone deficiencies such as adrenocorticotropic (ACTH) and growth hormone deficiency. Isolated central hypothyroidism is rare (incidence 1:65,000); therefore if central hypothyroidism is detected, consequences such as hypoglycemia and adrenal crisis can be averted [5]. Primary T4 assays offer a significant benefit in detecting a rare but high-risk thyroid disease.

Unfortunately, primary T4 NBS is nonspecific in other high-risk populations such as low and very low birth weight infants (V/LBW). Prematurity also lowers efficacy of the primary T4 assay. Low T4 concentrations in V/LBW and premature infants increase the number of TSH assays by 10% [6]. Low total T4 concentrations may result from deficient protein binding of thyroid hormones, the primary cause of transient neonatal hypothyroidism in premature infants and in infants with T4-binding globulin (TBG) deficiency. Transient hypothyroidism also occurs as a result of maternal TSH receptor blocking antibodies, exposure to antithyroid medications during pregnancy, and iodine deficiency or excess [4]. T4 screening in these infants leads to unnecessary testing and possible treatment in children who require no intervention.

17.1.3 Primary TSH with and without T4 NBS Method

Used in most developed countries, the primary TSH screening is overall a better screening test than the T4/TSH screening [7]. TSH is a suitable indicator of iodine deficiency [8] and a sensitive marker of pituitary triiodothyronine (T3) concentrations. Intraneural concentration of T3 (derived from intracellular conversion of T4 to T3) is essential for neurogenesis, neuronal migration, neuronal and glial cell differentiation, as well as myelination in the developing fetus [9]. Given that pituitary T3 levels cannot be measured, TSH is the most sensitive surrogate.

Despite its specificity, a primary TSH assay may miss the delayed TSH elevation seen in premature infants, V/LBW babies, children with congenital heart disease, or those who are acutely ill. Infants in this high-risk category often undergo interventions that affect interpretation of screening, especially interventions including hyperalimentation, transfusions, and treatments with thyroid interfering medications such as iodine, glucocorticoids, neuromodulators (i.e., dopamine), or antibiotics [10]. Numerous medications, alimentation, and states of illness affect the results and significance of primary TSH screening (Table 17.1). Interferences with thyroid function lead to transient neonatal hypothyroidism and sick euthyroid states in critically ill neonates [11]. Sick euthyroid, also known as non-thyroidal illness, is a functional state in which derangements in thyroid function occur as a result of illness and not from actual thyroid disease. Several anomalies in thyroid function can occur in various stages of illness, including higher TSH values. These higher TSH values can mislead clinicians to diagnose thyroid disease in euthyroid patients. In a TSH/T4 program, T4 assays increase by 6% given the higher number of TSH elevations [12].

Also problematic is the hospital management systems' implementation of early discharge. Infants discharged from the hospital before 48 h of life often undergo screening on day of life 1, leading to a high rate of false positives in those infants who have a physiologic postnatal rise in TSH [12]. After birth, as a physiological response to temperature change, TSH concentration increases in order to stimulate neonatal rise in T4 and T3. TSH values peak between 70 mIU/L and to 100 mIU/L within 1 h of life. Thyrotropin

Table 17.1 Medications that alter thyroid function testing

	Laboratory results			
	↓TSH	↑TSH	↑(f)T4	↓(f)T4
Medications	Amphetamines Bromocriptine Dopamine GCC Octreotide	Amiodarone Iodinated contrast media Metoclopramide	Amiodarone Iodinated contrast media IV furosemide (↑fT4) IV heparin (↑↑↑fT4) Methadone (↑T4) NSAIDS Salicylates (↑fT4)	$Al(OH)_3$ Ca^+ carbonate/citrate/acetate Carbamazepine Cimetidine $FeSO_4$ GCC IV furosemide (↓T4) Phenobarbital Phenytoin Phosphate Binders PPI Salicylates (↓T4) β-blockers

↓ deceased, ↑ increased, *TSH* thyroid-stimulating hormone (thyrotropin), *fT4* free thyroxine, *T4* total thyroxine, *IV* intravenous, *Al(OH)*$_3$ aluminum hydroxide, *β* beta, *Ca*$^+$ calcium, *FeSO4* ferrous sulfate, *GCC* glucocorticoids (dexamethasone, hydrocortisone), *PPI* proton pump inhibitors (omeprazole, lansoprazole), *NSAIDS* nonsteroidal anti-inflammatory drugs

values progressively decline over the following 48 h of life, while T4 and T3 values normalize by 3–5 days [13]. As noted in discussion of primary T4 assays, TSH surge is blunted in the preterm and V/LBW infant. In term infants, early hospital discharge has increased the ratio of false-positive TSH elevations from 3:1 to 5:1. Recommendations suggest that NBS be performed at 48 h of age; however the optimal time to obtain a primary TSH NBS is around 3–5 days of life [4].

17.1.4 Dual TSH and T4 NBS Method

Recent data show that primary TSH screening is more cost-effective in reducing false-positive and false-negative cases for mass screening despite the dual TSH and T4 approach theoretically being the ideal method of screening [14]. Dual TSH and T4 screening is expensive and not practical in most countries. As with any screening, the value of identifying an infant with CH must be balanced against the cost of screening normal newborns. NBS is expensive and not weighed in the costs are the psychosocial burdens created by screening for non-disease [15]. Given recall rates of 0.05%, over 1000 children will undergo further diagnostic testing with negative results for each child with true-positive results. Even more costly is children without true hypothyroidism who undergo therapeutic intervention. Not limited to monetary disadvantages are the psychosocial burdens associated with non-disease, including, but not limited to, parental anxiety, aversion of child to repeat intravenous catheters, and risks of therapeutic side effects.

17.1.5 Confirmatory Results

Essential in the efficacy of NBS is confirmation of true disease if screening is abnormal. Confirmation consists of serum analysis of thyroid function tests (TSH and T4 or fT4) using ultrasensitive assays. Diagnosis of CH should not be based on screening tests alone, although treatment may be initiated based on absolute results (Fig. 17.1). Clinicians should be aware of assay

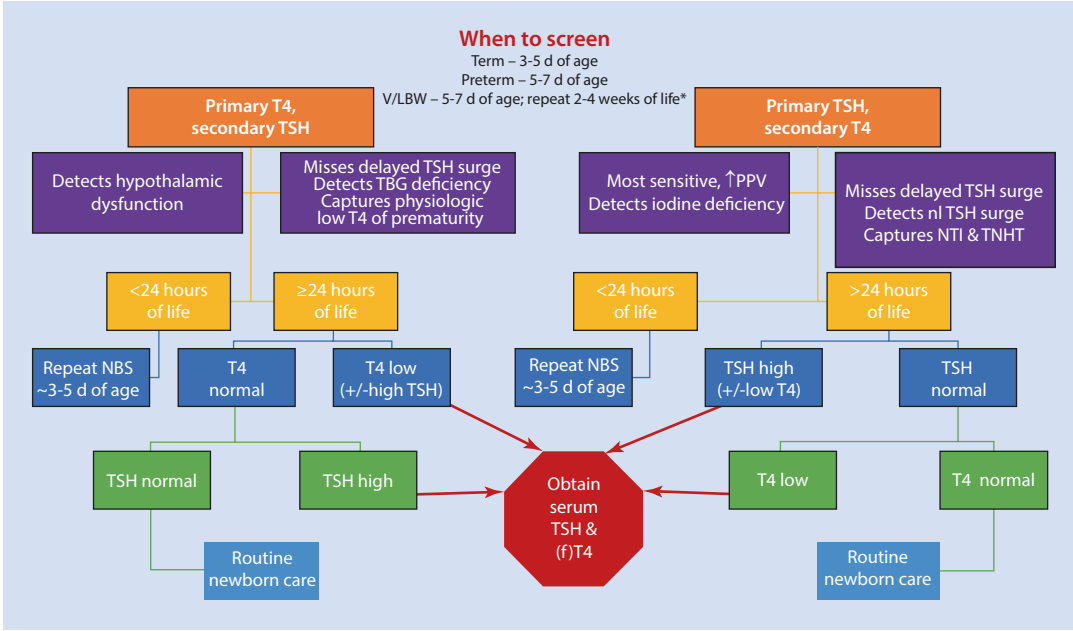

Fig. 17.1 Newborn screening of congenital hypothyroidism: practical guidelines. Practical guidelines for the follow up of abnormal thyroid screening in the newborn. *A strategy of second screening should be considered for the following conditions: preterm neonates; low-birth weight (LBW) and very low birth weight (VLBW) neonates; ill and preterm newborns admitted to neonatal intensive care units (NICU); specimen collection within the first 24 h of life; and multiple births (particularly same-sex twins). D Days, PPV Positive predictive value, NI Normal, NTI Non-thyroidal illness, TNHT Transient neonatal hypothyroidism, T4 Thyroxine, TSH Thyroid-stimulating hormone, fT4 Free thyroxine, V/LBW Very low and low birth weight

methodology available within their local laboratory. If ultrasensitive assay techniques are not available, reference ranges should be assessed with caution, especially if pediatric reference ranges are not available.

NBS test results must be communicated rapidly back to the physician or hospital identified on the screening filter paper card. The responsibility for transmission of these results rests with the authority or agency that performed the test. In general, when an abnormal screening result is found, the responsible physician is notified immediately so that he or she can arrange for follow-up testing. Screening test results should be entered into the patient's record. If the informed physician is no longer caring for or cannot locate the infant, he or she should notify the NBS laboratory immediately. In such situations, the local health department is often helpful in locating these infants to ensure that they are not lost to follow-up [12].

17.2 Etiology

The most common cause of CH is thyroid dysgenesis (■ Table 17.2). Anatomical defects in thyroid gland morphology include agenesis, hypoplasia, and ectopy. Displacement of the thyroid gland outside of its usual location explains two-thirds of permanent CH cases worldwide. Initially thought to be sporadic, Castanet et al. discovered a 15-fold increase in the prevalence of infants with thyroid

■ **Table 17.2** Etiology, genotype, and phenotype of congenital hypothyroidism [26, 27]

	Thyroid dysgenesis	Thyroid dyshormonogenesis	Thyroid receptor mutations
	Defects in thyroid anatomy	Defects in thyroid hormone production	Defects in thyroid action
Causes			
	Ectopy Aplasia Hypoplasia Hemiagenesis	Sodium-iodide symporter defect (NIS) Thyroid peroxidase defects Hydrogen peroxide generation defects Pendrin defect Thyroglobulin defect Iodotyrosine deiodinase defect	TSH receptor (TSHR) defect Thyroid receptor β(TRβ) mutation
Mutations			
	TTF-1, TTF-2, PAX8	NIS, DUOX2, DUOXA2, SLC26A4, DEHAL1, SECISBP2	TSHR, TRβ
Testing			
	Aplasia TSH >100[a] Hypoplasia TSH >20 Hemiagenesis TSH >6	TSH >20[a] T4 <8[b]	Variable depending on degree of resistance
Imaging			
Ultrasound	Aplasia = absent Hypoplasia = small or normal Hemiagenesis = small	Large gland	Variable depending on degree of resistance
Scintigraphy	Aplasia no uptake Hypoplasia ↓uptake Ectopia ↓uptake	↑uptake in eutopic location	Variable depending on degree of resistance

↓ decreased, ↑ increased, *TSH* thyroid-stimulating hormone (thyrotropin), *T4* total thyroxine, *TSHR* TSH receptor, *TRβ* thyroid receptor βeta
[a]mIU/L
[b]mcg/dL

dysgenesis with an affected first-degree relative [16]. Therefore, diagnosis of CH should take into account family history of thyroid dysfunction. There is also a direct linear relationship between TSH values and amount of thyroid tissue present. Most infants with severe CH (TSH >100 mIU/L) have agenesis which can be easily confirmed by absence of thyroid tissue on thyroid ultrasonography. A serum thyroglobulin concentration below the lower limit of normal also strongly correlates with absent thyroid gland.

Genetic predisposition to thyroid dysgenesis may in part explain the observed higher incidence of CH among certain ethnic groups. Mutations in developmental genes, which play important roles in embryogenesis and developmental cell migrations, contribute to genetic predisposition to thyroid dysgenesis. Mutations in genes (such as TTF-1, TTF-2, and PAX8) have been associated with developmental anomalies including thyroid dysgenesis and likely account for the higher incidence of CH in Hispanic patients [3].

Aside from anatomical defects in the thyroid gland, inborn errors of thyroid hormone synthesis account for up to 15% of cases of CH (Table 17.2). Errors in thyroid hormone synthesis can occur at any step along the path of thyroid hormone production (Fig. 17.2). Dyshormonogenesis results from insufficient intake of iodine or from a number of mostly autosomal recessive mutations. These mutations include but are not limited to defects in:

1. *Sodium-iodide symporter (NIS).* NIS is responsible for actively transporting iodine into thyroid follicular cells.
2. *Pendrin (Pendred gene SLC26A4, also termed PDS).* PDS is a sodium-independent chloride/iodide transporter found on the apical membrane of thyroid follicular cells. Its role is to transport iodide from the cytoplasm into the follicular lumen. PDS is also found in the inner ear; hence, the association with sensorineural hearing loss noted in Pendred syndrome.
3. *Dual oxidase 2 (DUOX) and dual oxidase maturation factor 2 (DUOXA2).* Within follicular cells, DUOX2 proteins are essential for thyroid hormone biosynthesis. The presence

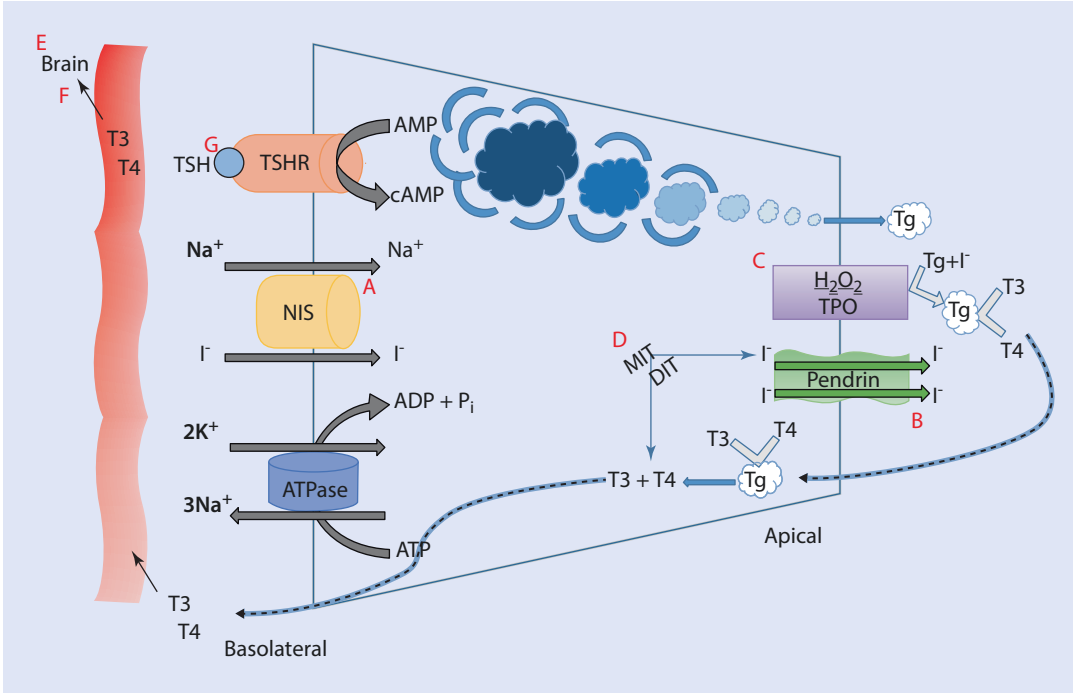

Fig. 17.2 Thyroid hormone synthesis and defects in thyroid hormone production. Schematic of thyroid follicular cell and location of defects associated with congenital hypothyroidism. (A) Sodium-iodide symporter (NIS) (B) Pendrin (Pendred gene SLC26A4, also termed PDS) (C) Dual oxidase 2 (DUOX) and dual oxidase maturation factor 2 (DUOXA2) (D) Iodotyrosine dehalogenase (DEHAL1) (E) Monocarboxylate transporter 8 (MCT8) (F) Selenocysteine insertion sequence-binding protein 2 (SECISBP2) (G) Thyroid-stimulating hormone (TSH) and TSH receptor (TSHR) (Adapted from Ref. [28])

of hydrogen peroxide (H2O2) is required for organification of iodine. Duox2 proteins, glycosylated and bound at the apical membrane, catalyze formation of H2O2. DUOXA2 is required for DUOX2 maturation and activation [17].

4. *Iodotyrosine dehalogenase (DEHAL1).* Homozygous mutations of DEHAL1 prevent recycling of iodide at the apical membrane of the follicular cell. Iodotyrosine accounts for almost 70% of iodine in thyroglobulin and hence is the rate-limiting step in T4 and T3 production [18].
5. *Monocarboxylate transporter 8 (MCT8).* Allan-Herndon-Dudley syndrome results from a mutation of the thyroid hormone transporter MCT8 (also referred to as SLC16A2). Located on the X chromosome, the MCT8 gene is responsible for transport of T3 across the blood-brain barrier, resulting in uptake of T3 into neuronal cells [19]. This X-linked hypothyroidism is associated with mental retardation and several neurologic problems.
6. *Selenocysteine insertion sequence-binding protein 2 (SECISBP2).* Deiodinases are selenoenzymes responsible for conversion of T4 to the biologically active form, T3. Deiodinases possess enzymatic activity, requiring selenocysteine for its action. SECISBP2 aids in the recognition of selenocysteine binding. As a cell membrane transporter element, SECISBP2 regulates intracellular thyroid metabolism by regulating the influx and efflux of T4 and T3 within the neuron [20].

Defects in thyroid hormone metabolism are exquisitely rare with only a handful of case reports. Prior to such a diagnosis, defects in thyroid hormone action such as resistance to thyroid hormone (RTH) should be considered (Fig. 17.2G). RTH is essentially impaired responsiveness of peripheral and central tissue to thyroid hormone. However, defects in thyroid hormone action including RTH are rarely diagnosed in the newborn period, given propensity for normal TSH values and elevated T4 values. The thyrotropin receptor (TSHR) is also involved in thyroid organogenesis. Thus, mutations in the TSHR gene can cause CH as well.

17.3 Clinical Presentation

Except in geographic regions of iodine deficiency, the diagnosis of CH is rarely made on physical examination. In industrialized countries with high emphasis on prenatal care, signs and symptoms of CH are rarely apparent. Unfortunately, it is within this newborn period when missing clinical features of hypothyroidism lend to irreversible brain damage.

Symptoms and signs suggestive of CH include the following, with additional signs evident as infants become older:

0 days of life
- Cardiac malformations
- Delay in skeletal maturation (absence of femoral and tibial epiphyses)
- Hypothermia
- Jaundice
- Lethargy/decreased activity
- Macroglossia
- Macrosomia
- Mottled and dry skin
- Open and wide posterior fontanelle (>2 cm)
- Poor feeding
- Umbilical hernia
- Wide anterior fontanelle

Early infancy (0–2 months of life)
- Constipation
- Decreased muscle tone
- Failure to thrive
- Hoarse cry
- Linear growth deceleration
- Poor feeding
- Prolonged jaundice

Late infancy (>2 months of life)
- Delayed tooth eruption
- Growth failure
- Pseudomuscular hypertrophy
- Psychomotor retardation

Infants with CH are often described as "good babies" as they sleep well and cry infrequently. As with any newborn, a complete history, including maternal thyroid status and family history, should be obtained, and careful physical examination must be performed.

17.4 Diagnostic Evaluation

The most significant confounder in the confirmation of CH is its transient form. The most common form of transient hypothyroidism worldwide is iodine deficiency. Iodine deficiency must be considered in high-risk populations such as those from underdeveloped countries, infants of mothers whose diets are limited in meats (vegans) or iodinated salts (sea salt consumers), and in infants whose condition limits gastric intake of nutrients (total parenteral nutrition-dependence). Even in infants with transient neonatal hypothyroidism, treatment is recommended if certain diagnostic criteria are met (Fig. 17.3).

The most common cause of transient neonatal hypothyroidism in developed countries is prematurity (gestational age <37 weeks). Temporary thyroid dysfunction also occurs in infants who are born 10% below expected body weight (i.e., V/LBW), ill neonates admitted to the neonatal intensive care unit, neonates who are discharged within the first 24 h of life, and cohorts of multiple births, including monozygotic twins. In these special populations, a repeat NBS specimen should be collected at about 2 weeks of age or 2 weeks after the first screening test was carried out [7].

To confirm the diagnosis of permanent CH, the European Society for Pediatric Endocrinology (ESPE) recommends performing imaging studies

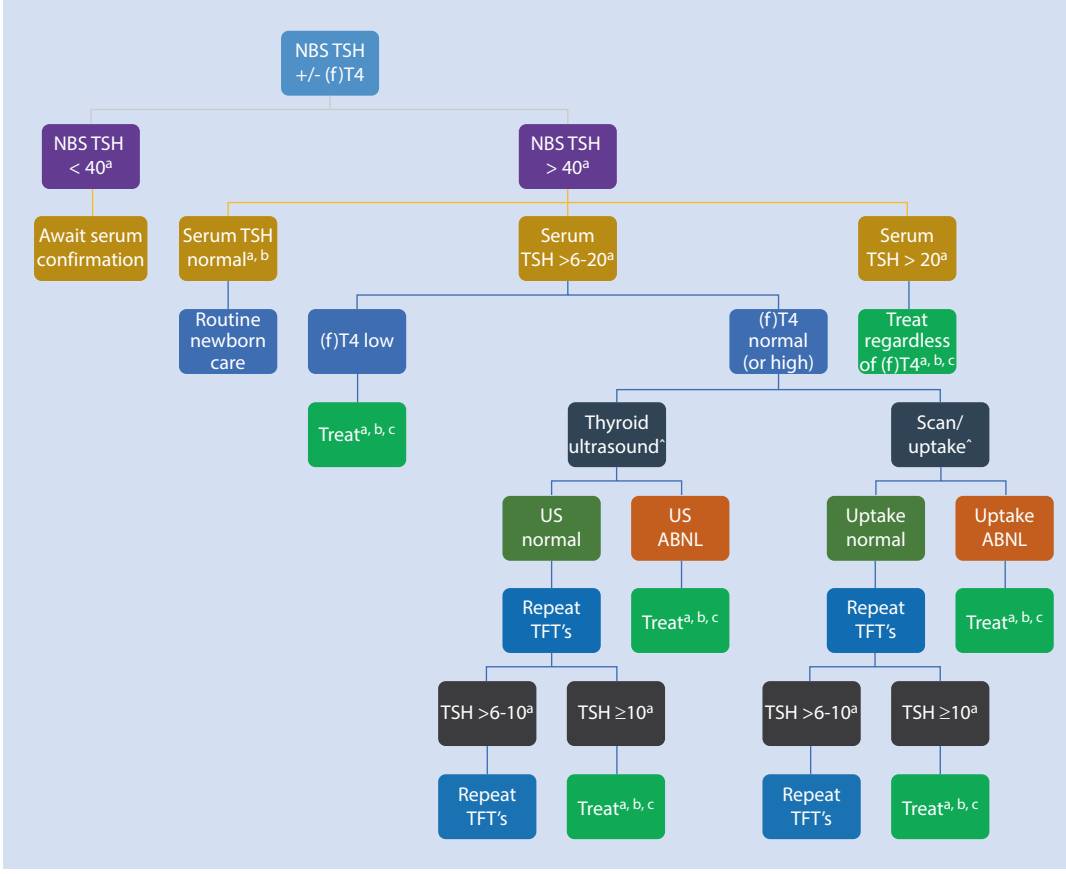

Fig. 17.3 Congenital hypothyroid treatment algorithm. Practical guidelines for the management of abnormal thyroid screening in the newborn. Perform serum confirmation by 24–48 h of abnormal NBS. Serum confirmation need not delay thyroid hormone therapy in infants strongly suspected to have CH. [a]mIU/L. [b]Normal serum TSH but low (f)T4 should prompt further diagnostic workup to exclude central hypothyroidism. [c]Initial dose: Levothyroxine 10–15 ug/kg/day. ^Imaging should never delay treatment. US (ultrasound) and/or scan/uptake (^{123}I scintigraphy or technetium Tc 99 m pertechnetate) may be performed to determine etiology of CH. If persistent TSH elevations >21–28 days of life, consider starting thyroid hormone therapy with goal to retest at 3 years of age, OR retest TFT's ~ 6 weeks of life. NBS Newborn screen, TFT thyroid function tests [i.e., TSH and (f)T4]

to determine its specific etiology [7]. Diagnostic imaging may be helpful in cases in which CH diagnosis is not clear. Treatment may be altered based on results, especially if clinical evaluation is also consistent with CH. The option to initiate, hold, and potentially stop treatment may be based on imaging results as well. When the cause of abnormal thyroid studies cannot be determined as either transient or permanent, both scintigraphy and ultrasound can be considered in neonates with abnormal TSH values (Fig. 17.3). Of course, the time interval to imaging should not delay treatment in any circumstance in which there is a high degree of suspicion for thyroid disease. Even in circumstances in which differentiation between transient and permanent CH is blurred, imaging can always be deferred until the child reaches the age of 3 years. At 3 years, neuronal migration and myelination are fairly advanced; thus risk of neurocognitive dysfunction as a result of thyroid hormone withdrawal at age 3 years is lower than what would have been expected in the first 3 years of life [9].

In the era of genetic testing, several mutations have been discovered which account for some cases of CH. Genetic studies for mutations in thyroid hormone synthesis can confirm inborn errors of thyroid hormone biosynthesis [21]. Despite the importance of confirming CH in permanent cases, the costs of genetic testing do not yet outweigh the benefits. The exceptions include families in which multiple siblings are affected. In these cases, genetic counseling is suggested to ensure families are aware of potential risks in future pregnancies.

17.5 Treatment

Once diagnosis of CH is confirmed, levothyroxine replacement is recommended as the treatment of choice. There is no place in pediatric thyroid disease for formularies including T3 treatment or desiccated animal product. Treatment with levothyroxine should be started as soon as possible. Goal for initiation of treatment is within the first 2 weeks of life. However, given that some abnormal screenings require repeated measurements or further diagnostic testing, treatment should begin once testing confirms disease (Fig. 17.3). Initial treatment is with levothyroxine 10–15 mg/kg/d; however, several factors interfere with thyroid hormone absorption. Severity of disease also affects choice of replacement dose. More severe disease, such as in athyreosis, indicates use of doses at the upper end of the treatment scale (15 mg/kg/d), while the same dose in infants affected by defects in thyroid hormone synthesis may lead to overtreatment. Goal of all therapy is to normalize thyroid hormone function [7].

Based on TSH concentration, the dosage of levothyroxine is adjusted. Use of serum fT4 can be adjunctive in ensuring an appropriate clinical response. In the first 3 years, serum total T4 and/or fT4 values should be in the upper half of the reference range, and serum TSH levels should be between 0.5 mIU/L and 2.0 mIU/L [12]. Relative pituitary resistance may delay normalization of serum TSH, resulting in a normal or increased serum T4 concentration with an inappropriately high TSH level. In these cases, the dose should be titrated based on the TSH value, after first ruling out nonadherence to treatment.

Normalizing thyroid function may be challenging in the first year of life. Despite tightened Federal Drug Administration regulations, levothyroxine preparations can vary by up to 12% in the amount of active hormone, even within brand [22]. Guidelines recommend brand name levothyroxine; however, cost can be prohibitive for most families whose formularies require generic prescriptions [7]. Children initiated on generic formulations should remain on generic, and those started on brand name formulations should remain on brand. If there is any change in thyroid hormone manufacturer, follow-up laboratory testing should be ordered to ensure euthyroidism.

Other factors affecting thyroid hormone dosing include absorption in the gastrointestinal tract, ranging from 70% to 80%, hence the pharmaceutical company's recommendations for consuming levothyroxine on an empty stomach [23]. Practically, however, infants are in a perpetual postprandial state. Adhering to specific preprandial timing of medication administration has been associated with lower medication adherence [24]. Therefore, medication can be given with or without food as long as there remains consistency with each dose. Dosing can be adjusted based on the most realistic mode of consumption.

Several other factors can also interfere with thyroid hormone absorption, some to such a degree that the medication and interfering substance should be separated by at least 3–4 h. These

products include those listed below as "BASIC" acronym:

- B = Bioacid-binding resins (i.e., cholestyramine)
- A = Aluminum-containing antacids (i.e., Rolaids®)
- S = Sucralfate/soy
- I = Iron
- C = Calcium/caffeine

Rapid normalization of thyroid hormone levels (within the first 2 weeks after treatment initiation) and maintenance of relatively higher fT4 concentrations during the first year of life lead to better intellectual outcomes. However, overtreatment has also been associated with lower intelligence quotients in children with CH [25].

Thyroid function testing should be performed every 2–3 months for the first year of life, 3–4 months in the 2nd and 3rd years of life, and at least twice yearly thereafter. Perform thyroid function testing approximately 4 weeks after dose changes or changes in brand or administration route. If clinical symptoms of thyroid disease develop, repeat testing is indicated even if normal previously.

17.6 Case Studies

Case Study #1

A 9-day-old former 36-week twin B born to 25-year-old gravida 4, para 3 mother is admitted to NICU for poor oral intake. NBS obtained on postnatal day 2 revealed TSH 302 (normal, <34 mIU/L) and T4 5.9 mcg/dL (normal, 7.6–16 mcg/dL). Serum confirmation was significant for TSH 435 mIU/L (normal, 0.5–5 mIU/L). He was started on levothyroxine 50 mcg, followed by 37.5 mcg on day 2, then 25 mcg daily. At 23 days of life, he was discharged from the NICU on 25 mcg of daily levothyroxine replacement. He subsequently missed his first outpatient endocrinology appointment; however, repeat labs obtained by his pediatrician revealed elevated TSH 100 mIU/L and low fT4 0.4 ng/dL (normal, 0.8–2 ng/dL). Levothyroxine was increased to 37.5 mcg. At his first ambulatory endocrinology visit at 3 months of age, elevated TSH 488 mIU/L persisted, and exam revealed dry skin and macroglossia. Thus, levothyroxine was increased further to 50 mcg daily. Thyroid studies did not normalize until 12–14 months of age after home health and child protective services involvement. At 14 months of age, he exhibited normal growth and development with laboratory results confirming euthyroid state. He did not return for any clinical care until age 8. At 8 years of age, he exhibited symptoms including fatigue, sluggish movements, cold sensitivity, dry skin, hoarse voice, joint stiffness, and difficulty arousing from sleep. He lacked toilet-training skills and was unable to speak in full sentences. Growth parameters showed severe abnormalities with weight 13.7 kg [−6.1 standard deviations (SD)], height 83.5 cm (−8.7 SD), and BMI 19.7 kg/m2 (93rd percentile).

This case clearly demonstrates that NBS works to diagnose severe CH but is not enough to prevent the debilitating effects of thyroid hormone deficiency. CH patients require daily administration of medication, frequent clinical examination, dosage adjustments, laboratory assessments, and evaluation for potential barriers to care.

Case Study #2

A 2-month-old male presents for the evaluation of abnormal thyroid studies. He was a full term baby born by Cesarean section delivery, after in vitro fertilization. His NBS returned abnormal with the following results: TSH 35.2 mIU/L (normal, <34 mIU/L) and T4 14.4 (normal, >8 ng/dL). Repeated serum thyroid studies at 1 week of life revealed TSH 9.8 (normal, 1.0–5.6) and T4 9.4 mcg/dL (normal, 8.1–16.5). Repeat thyroid function testing 2 weeks later revealed TSH of 9.8 (normal, 1.0–5.6) and fT4 1.4 (normal, 1–2 ng/dL). Due to consistent TSH elevation beyond 1 month of age, he was started on levothyroxine 25 mcg daily. He was adherent to therapy and follow-up, remaining on 25 mcg of levothyroxine until 2.8 years of age when parents agreed to trial off medication. At that time, he was receiving less than 10 mcg/kg/day of thyroid hormone replacement. The following is a summary of thyroid function testing once levothyroxine therapy was discontinued.

	Normal range	Day 0	+1-month	+2 months	+3 months
TSH	0.6–4.0 mIU/L	3.2	6.8 (A)	5.9 (A)	4.4 (A)
T4 free by direct dialysis	1.0–2.8 ng/dL	2.0	1.3	1.5	1.4

One month after discontinuing thyroid hormone replacement, mother called with concerns about behavior change including refusing to take naps and irritability. This was thought to be a viral illness, so TSH elevation was attributed to non-thyroidal illness. Two months after trial off medication, patient was noted to be back to normal behavior but constipated and more tired than usual. Since TSH was normalizing, labs were repeated a third time. Patient continued to have worsening constipation, fatigue, and reported weight gain. Ultrasonography of thyroid gland revealed absence of the left thyroid lobe and isthmus. Right thyroid lobe was normal but ectopic thymic tissue was noted between the right common carotid artery and internal jugular vein. Thyroid hormone was restarted, after which constipation, fatigue, and excess weight gain resolved.

This case illustrates how thyroid imaging can aid in differentiating permanent versus transient CH.

17.7 Summary

Neurocognitive development and growth are dependent on sufficient serum thyroid hormone concentration in fetal and early child development. Iodine is essential for thyroid hormone production, and deficiency (hypothyroidism) can affect maternal, fetal, and postnatal thyroid function. Hypothyroidism remains the leading cause worldwide of treatable mental retardation. At birth, there may be few signs or symptoms of hypothyroidism, making NBS for thyroid function a critical step in detecting CH. Interpretation of NBS in various clinical settings may alter diagnosis and treatment. Once identified early and treated appropriately. CH becomes a treatable condition with normal or near-normal developmental outcomes [3].

Review Questions

1. Given the physiologic TSH surge of the newborn, when is the optimal timing for CH screening?
2. If NBS TSH values exceed 100 mIU/L, serum laboratory testing is not required to confirm the diagnosis. TRUE or FALSE?
3. A primary T4 assay is performed in a 36-week EGA (estimated gestational age) infant at 2 days of life. Further testing returns a normal serum TSH. No further workup is required. TRUE or FALSE?
4. A 7-day-old presents with abnormal newborn screening and serum confirmatory screening suggestive of CH. The most likely abnormality to be described on scintigraphy would be?
5. An infant is started on 15 mcg/kg/d of levothyroxine replacement. Four weeks later, TSH is <0.01 mIU/L, and fT4 is elevated at 3 ng/dL. What is the most likely cause of biochemical hyperthyroidism?

Answers

1. See Fig. 17.1. Ideally timed, NBS should occur at approximately 3–5 days of life in term infants and 1 week of life in preterm or V/LBW infants. Realistically, given modern discharge criteria, 48 h of life is acceptable with rescreening in at risk populations.
2. FALSE. NBS programs screen for high-risk disease and attempt to capture all infants with disease. As such, infants without disease are also captured lending to high false-positive rates. This is quite prevalent in vulnerable populations such as preterm infants. Screening should always be followed by serum confirmation. It is imperative that confirmatory venous results be obtained within 1–2 days of

NBS. However, results of serum confirmation need not delay treatment.

3. FALSE. A primary T4 assay is adventitious in detecting hypothalamic-pituitary hypothyroidism. A normal TSH with concurrent low (f)T4 should be further investigated to rule out central hypothyroidism.
4. Technetium 99 m scan showing uptake in ectopic location, i.e., ectopic gland. Dysgenesis is the most common cause of congenital hypothyroidism, occurring in 85% of cases. Ectopia accounts for almost 70% of dysgenic CH, followed by athyreosis and hypoplasia.
5. Overtreatment. Although higher doses of levothyroxine lead to better overall developmental outcomes, higher doses have also been associated with decrements in cognitive outcomes. Goal of all treatment is to ensure normalization of thyroid function.

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Autoimmune Thyroid Disease

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Key Points

- The most common cause of goiter in the pediatric population is autoimmune (Hashimoto's) thyroiditis.
- Typical symptoms of thyroid dysfunction may be present, and growth and puberty may be deranged.
- There may be a prolonged period of euthyroidism until overt hypothyroidism develops, and therefore, the decision to treat children with subclinical hypothyroidism remains controversial.
- Graves' disease is due to TSH receptor antibodies that stimulate the thyroid gland and result in the production of excess thyroid hormone.
- Initial treatment consists of the use of antithyroid drug (methimazole); however, serious side effects of methimazole must be monitored and include agranulocytosis, hepatitis, arthritis, and Stevens-Johnson syndrome.
- More prolonged use of antithyroid medications may be required to achieve remission in children relative to adults.
- If remission does not occur or an adverse reaction develops, then permanent therapy with either radioactive iodine or surgical thyroidectomy can be considered.
- Neonatal Graves' disease has a high morbidity and mortality rate, thus prompt diagnosis and treatment are necessary.
- Most cases of neonatal Graves' disease will spontaneously remit after 3–12 weeks as the maternal TSH receptor antibodies clear the infant's circulation.

18

18.1 Introduction

Autoimmune thyroid disease affects approximately 2% of the female population and 0.2% of the male population [1]. Although its prevalence peaks in adulthood, it is the most common etiology of acquired thyroid dysfunction in pediatrics [2, 3]. This chapter presents a summary of autoimmune thyroid disease, discussing chronic autoimmune thyroiditis and Graves' disease, with an emphasis on their clinical management. Normal levels of thyroid hormone are critical to neurodevelopment and growth, and by maintaining an appropriate index of suspicion, the clinician can often recognize thyroid dysfunction in its early stages.

18.2 Chronic Autoimmune Thyroiditis

The childhood prevalence of chronic autoimmune thyroiditis peaks in early to mid puberty, and a female preponderance of 2:1 has been reported [4]. Presentation is rare under the age of 3 years, but cases have been described even in infancy [5, 6].

18.2.1 Terminology and Definitions

In 1912, Hashimoto described four women with thyromegaly and the apparent transformation of thyroid into lymphoid tissue ("struma lymphomatosa"). These patients comprise the first report of Hashimoto's disease, which is now recognized as chronic autoimmune thyroiditis. Improvements in the measurement of circulating autoantibodies have obviated the need for biopsy in the diagnosis of autoimmune thyroid disease, and the nomenclature has been redefined in recent years (▶ Box 18.1) [7]. The term thyroiditis is defined as evidence of "intrathyroidal lymphocytic infiltration" with or without follicular damage. Two types of chronic autoimmune thyroiditis (also known as chronic lymphocytic thyroiditis) are causes of persistent hypothyroidism, Hashimoto's disease (goitrous form, type 2A) and atrophic thyroiditis (nongoitrous form, type 2B). Both are characterized by circulating thyroid autoantibodies and varying degrees of thyroid dysfunction, differing only by the presence or absence of goiter. The transient disorder of postpartum thyroiditis is believed to be a manifestation of chronic autoimmune thyroiditis (type 2C) [8]. The term chronic autoimmune thyroiditis does not include subacute (de Quervain's) thyroiditis.

Box 18.1 Classification of Autoimmune Thyroiditis

- Type 1 autoimmune thyroiditis (Hashimoto's disease type 1)
  - 1A Goitrous
  - 1B Nongoitrous
  - Status: Euthyroid with normal TSH
- Type 2 autoimmune thyroiditis (Hashimoto's disease type 2)
  - 2A Goitrous (classic Hashimoto's disease)
  - 2B Nongoitrous (primary myxedema, atrophic thyroiditis)
  - Status: Persistent hypothyroidism with increased TSH
  - 2C Transient aggravation of thyroiditis (e.g., postpartum thyroiditis)
  - Status: May start as transient, low radioactive iodine uptake (RAIU) thyrotoxicosis, followed by transient hypothyroidism
- Type 3 autoimmune thyroiditis (Graves' disease)
  - 3A Hyperthyroid Graves' disease
  - 3B Euthyroid Graves' disease
  - Status: Hyperthyroid or euthyroid with suppressed TSH. Stimulatory autoantibodies to the TSH receptor are present (autoantibodies to thyroglobulin (Tg) and TPO are also usually present)
  - 3C Hypothyroid Graves' disease
  - Status: Orbitopathy with hypothyroidism. Diagnostic levels of autoantibodies to the TSH receptor (blocking or stimulating) may be detected (autoantibodies to Tg and TPO are also usually present)

Data from Davies, *Thyroid* 1993 [7]

18.2.2 Pathophysiology

The activation of CD4 (helper) T lymphocytes specific for thyroid antigens is believed to be the first step in pathogenesis. Activated, self-reactive CD4 T cells recruit cytotoxic CD8 T cells and autoreactive B cells into the thyroid. The three main targets of thyroid antibodies are thyroglobulin (Tg), thyroid peroxidase (TPO), and the thyrotropin receptor (TSHR). Anti-TPO antibodies have been shown to inhibit the activity of thyroid peroxidase in vitro, but direct killing by CD8 T cells is believed to be the main mechanism of hypothyroidism in vivo [8]. Anti-TSH receptor antibodies (TRAbs) may contribute to hypothyroidism in a minority of adult patients with the atrophic form of chronic autoimmune thyroiditis, but this has not been proven in children [9–11].

Histologically, goitrous autoimmune thyroiditis is characterized by diffuse lymphocytic infiltration with occasional germinal centers. Thyroid follicles may be reduced in size and contain sparse colloid. Individual thyroid cells are often enlarged with oxyphilic cytoplasm (Hurthle or Askanazy cell). In contrast, the gland of atrophic autoimmune thyroiditis is small with lymphocytic infiltration and fibrous replacement of the parenchyma.

18.2.3 Clinical Presentation

The presentation of chronic autoimmune thyroiditis includes either hypothyroidism, goiter, or both. A goiter or firm thyroid is commonly the first physical sign of chronic autoimmune thyroiditis. Thyromegaly is typically diffuse with a "pebbly" or "seedy" surface that evolves into a firm and nodular consistency [12]. As the disease progresses, subclinical and then clinical hypothyroidism appears. Subclinical hypothyroidism is defined as a TSH concentration that is above the upper limit of the normal range, with a free T_4 that is within the normal range, whereas overt hypothyroidism is defined as an elevated TSH along with a low free T_4 level. Typically, the TSH in subclinical hypothyroidism is only mildly elevated, between 5 μU/mL and 10 μU/mL. In adults, the natural history of subclinical hypothyroidism is fairly well-described, with progression to overt hypothyroidism seen in up to 20% of cases. Symptoms of hypothyroidism may be subtle, even with marked biochemical derangement (▶ Box 18.2).

Box 18.2 Symptoms and Signs of Hypothyroidism

- Goiter
- Growth retardation
- Skeletal maturational delay
- Pubertal disorders (delay or pseudoprecocity)
- Slowed mentation (lethargy and impaired school performance)
- Fatigue
- Bradycardia (decreased cardiac output)
- Constipation
- Cold intolerance
- Hypothermia
- Fluid retention and weight gain (impaired renal free water clearance)
- Dry, sallow skin
- Delayed deep tendon reflexes

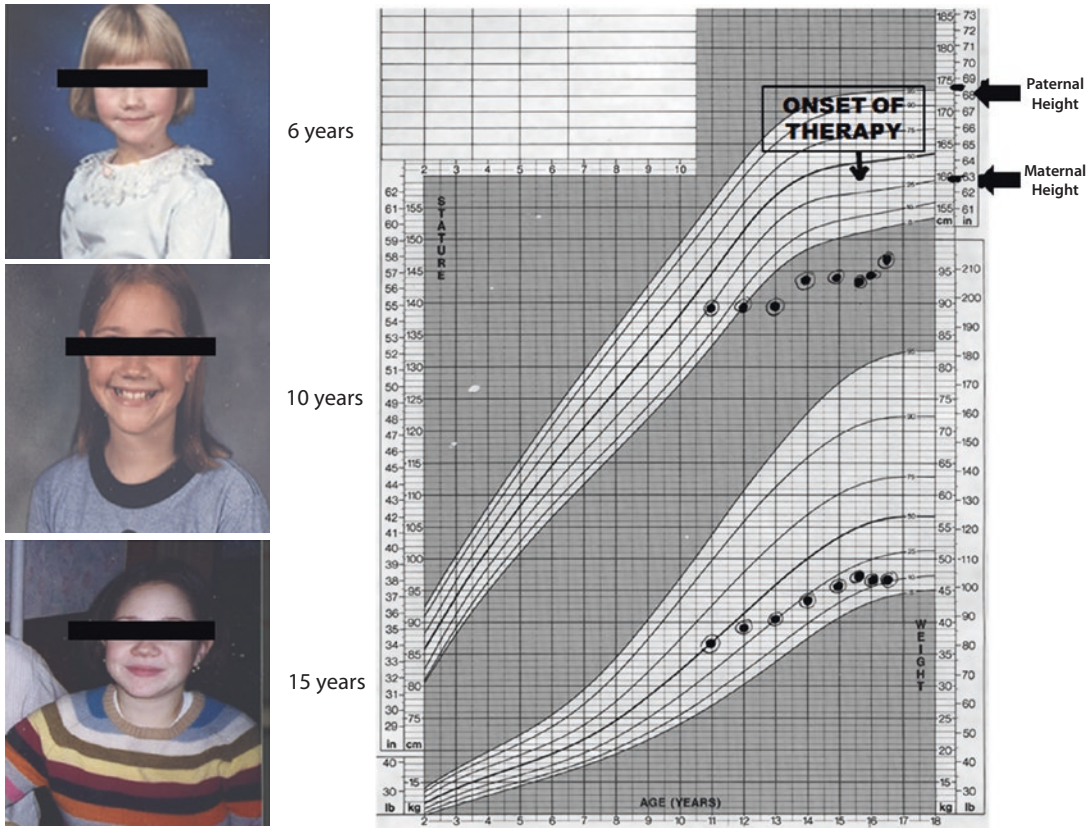

Fig. 18.1 A patient with chronic autoimmune thyroiditis. The growth failure of hypothyroidism characteristically affects height to a greater degree than weight. The initiation of thyroid hormone replacement is associated with an acute decrease in weight due to the mobilization of myxedematous fluid, followed by an acceleration in linear progression or "catch-up growth." (Chart and photographs are from the files of Rosalind Brown, MD, CM, FRCP(C), Boston Children's Hospital. Used with permission)

The initial history should include inquiries into energy level, sleep pattern, menses, cold intolerance, bowel patterns, and school performance. In addition to palpation of the thyroid, assessment of the extraocular movements, fluid status, and deep tendon reflexes are important components of the physical examination. Chronic autoimmune thyroiditis may be the initial presentation of an autoimmune polyglandular syndrome, and the possibility of coexisting autoimmune diseases such as type 1 diabetes, Addison's disease, and pernicious anemia must be addressed by the past medical history and the review of systems.

Growth and pubertal development may be deranged. Similar to other endocrine causes of growth failure, linear progression is compromised to a greater degree than weight gain, and the bone age is delayed (Fig. 18.1) [13, 14]. Hypothyroidism typically causes pubertal delay but may also induce a syndrome of pseudoprecocity manifested as testicular enlargement in boys and breast enlargement and vaginal bleeding in girls [15, 16]. This is known as Van Wyk-Grumbach syndrome and differs clinically from true precocity by the absence of accelerated bone maturation and linear growth (▶ Box 18.2).

18.2.4 Diagnosis

The serum TSH concentration is elevated in primary hypothyroidism, and its determination is an appropriate screen for thyroid dysfunction. If the differential diagnosis includes central

hypothyroidism or if the overall suspicion for hypothyroidism is high, a free T4 (or calculated free T4 index) should be included on the initial screen. In mild hypothyroidism, serum T3 can remain in the normal range due to the increased conversion of T4 to T3 by type 2 deiodinase and the preferential secretion of T3 by residual thyroid tissue under the influence of hyperthyrotropinemia [17, 18]. For these reasons, measurement of the serum T3 concentration is not a useful test in the diagnosis or monitoring of patients with primary hypothyroidism.

The presence of goiter or hyperthyrotropinemia should prompt the measurement of anti-TPO antibodies. Anti-TPO antibodies are the most sensitive screen for chronic autoimmune thyroiditis [19]. Little further benefit is gained by the additional measurement of antithyroglobulin antibodies, although they may be added if anti-TPO titers are negative. The typical patient with hypothyroidism secondary to chronic autoimmune thyroiditis will have an elevated TSH (over 10 μU/ml), a low free T4, and positive anti-TPO antibodies. In early stages of the disease, TSH may be normal, and anti-TPO antibodies may be positive with goiter (type 1A). Later, TSH elevation becomes modest (between 5 μU/ml and 10 μU/ml) with a normal free T4 (biochemical or subclinical hypothyroidism). Up to 90% of patients with hypothyroidism secondary to autoimmune thyroiditis are anti-TPO antibody positive. It should be noted that 10–15% of the general population are positive for anti-TPO antibodies and that low titers (less than 1/100 by agglutination methods or less than 100 IU/l by immunoassays) are less specific for autoimmune thyroid disease [1]. If anti-TPO antibodies are absent, less common etiologies of primary hypothyroidism such as transient hypothyroidism (post subacute thyroiditis), external irradiation, and consumptive hypothyroidism should be considered [20–22].

Subclinical hypothyroidism is defined as TSH elevation with normal concentrations of circulating thyroid hormones (T4 and T3). The log-linear relationship between serum TSH and free T4 explains how small reductions in serum free T4 lead to large deviations in TSH. The majority of these patients are asymptomatic, but individuals with the combined risk factors of hyperthyrotropinemia and positive thyroid antibodies (antithyroglobulin or anti-TPO) are at high risk for progression to overt hypothyroidism [23]. For this reason, it is our practice to recommend thyroid hormone replacement in all patients with TSH values greater than 10 μU/ml or with TSH values greater than 5 μU/ml in combination with goiter or thyroid autoantibodies [24, 25]. Given the critical importance of thyroid hormone in neurodevelopment, persistent hyperthyrotropinemia in infancy should be empirically treated and a trial with reduced therapy considered after the age of 2–3 years. Similarly, the presence of growth failure may lower the threshold to initiate replacement for persistent hyperthyrotropinemia. Euthyroid children with autoimmune thyroiditis (type 1A or type 1B) who are observed without treatment should be monitored carefully with TSH measurements every 6–12 months, as a significant fraction will progress to overt hypothyroidism [26].

18.2.5 Therapy

Levothyroxine (L-T4) is the replacement of choice. There are virtually no adverse reactions, and its long half-life of 5–7 days allows the convenience of daily administration. Of note, while both desiccated thyroid extracts and liothyronine (T3) are commercially available as replacement options, current consensus guidelines recommend against the routine use of these preparations due to the lack of long-term outcome data and their risk of iatrogenic hypertriiodothyronemia [27].

Although very rare, case reports have described the development of pseudotumor cerebri around the initiation of levothyroxine in a small number of school-age children [28]. Some authors advocate a graded approach to the initiation of levothyroxine [29]. Alternatively, a starting dose can be estimated based upon the patient's age and ideal body weight (Table 18.1) [4]. The medication's long half-life insures a gradual equilibration over the course of 5–6 weeks. Average daily requirements approximate 100 μg/m^2/day, but dosing will ultimately be individualized on the basis of biochemical monitoring [4]. TSH normalization is the goal of replacement. In growing children, we aim for a target range of 0.5–3 μU/ml

Table 18.1 Levothyroxine replacement doses

Recommended L-T4 treatment doses	
Age	**L-T4 dose (mcg/kg)**
0–3 months	10–15
3–6 months	8–10
6–12 months	6–8
1–3 years	4–6
3–10 years	3–4
10–15 years	2–4
>15 years	2–3
Adult	1.6

Data from LaFranchi, *Pediatric Annals* 1992 [4]

to reduce the risk of overt hyperthyrotropinemia as their thyroid hormone requirements increase with age. This will usually be associated with a free T4 in the upper half of the normal range. Thyroid function tests should be obtained 6 weeks after the initiation or adjustment of the levothyroxine dosage. Growth and sexual development should be followed systematically as in any pediatric patient. Once biochemical euthyroidism has been achieved, TSH can be monitored every 4–6 months in the growing child and yearly once final height has been attained.

A variety of conditions or drugs may alter levothyroxine requirements (Table 18.2). Thus, an unexplained increase in levothyroxine requirements should prompt a careful medication history and potential evaluation for enteral malabsorption. In theory, levothyroxine should be administered at least 30 min before eating or any medication known to impair its absorption. However, from a practical viewpoint, the most important goal is to establish a regular time for levothyroxine administration. Parents of children with chronic autoimmune thyroiditis should be advised that the hypothyroidism will likely be permanent, although exceptions have been reported [30, 31]. The monitoring of thyroid function is lifelong. A TSH should be checked if pregnancy is diagnosed, and the frequency of monitoring should be increased. Levothyroxine requirements increase by an average of 47% during gestation, and untreated maternal hypothyroidism may adversely affect the intellectual development of the fetus [32–34].

Table 18.2 Conditions that alter levothyroxine requirements

Increased levothyroxine requirements	
Pregnancy	
Gastrointestinal disease	Mucosal diseases of the small bowel (e.g., sprue)
	Helicobacter pylori-related gastritis
	Atrophic gastritis
Drugs which impair L-T4 absorption	Cholestyramine
	Sucralfate
	Aluminum hydroxide
	Calcium carbonate
	Ferrous sulfate
Drugs which may enhance CYP3A4 and thereby accelerate levothyroxine clearance	Rifampin
	Carbamazepine
	Phenytoin
	Estrogen (?)
	Sertraline (?)
Drugs which impair T4-to-T3 conversion	Amiodarone
Conditions which may block type 1 deiodinase	Selenium deficiency (due to dietary deficiencies as in PKU and cystic fibrosis)
	Cirrhosis

18.3 Graves' Disease

Robert Graves reported the clinical syndrome of goiter, palpitations, and exophthalmos in 1835. Graves' disease is the most common cause of hyperthyroidism in both adults and children [35, 36]. Hyperthyroidism is relatively rare in children (yearly incidence of 8–9 per 1000,000 children less than 15 years old and 1 per 1000,000 children less than 4 years old) [37, 38]. Girls are affected

four to five times more frequently than boys, although no gender difference is noted under 4 years of age [37, 39].

18.3.1 Pathophysiology

Graves' disease shares many features associated with chronic autoimmune thyroiditis, including autoantibodies directed against thyroglobulin, thyroid peroxidase, and the sodium-iodine symporter. Graves' hyperthyroidism is caused by thyroid-stimulating antibodies which bind and activate the thyrotropin (TSH) receptor, leading to follicular cell hyperplasia and the hypersecretion of thyroid hormone. Lymphocytic infiltration of the thyroid is present, hence its classification as a form of thyroiditis. Occasionally, germinal centers form which can develop as major sources of intrathyroid autoantibodies. Lymphocytic infiltration and the accumulation of glycosaminoglycans in the orbital connective tissue and skin can also cause the extrathyroidal manifestations of Graves' ophthalmopathy and dermopathy, respectively.

18.3.2 Clinical Presentation

The clinical presentation of Graves' disease in childhood may be insidious, and a careful history will often reveal a several-month history of progressive symptoms. Common complaints include nervousness, hyperactivity, heat intolerance, sleep disturbances, and a decline in school performance (▶ Box 18.3). A goiter is palpable in the majority of cases, characterized by diffuse enlargement which is smooth, firm, and nontender. The pyramidal lobe is often palpable, and a bruit may be audible secondary to increased blood flow through the gland. Extrathyroidal manifestations such as ophthalmopathy and dermopathy are rare and tend to be less severe than in adults [39]. The pediatric literature cites a 25–60% frequency of ocular manifestations, but the majority have mild signs such as lid retraction, "staring," and slight proptosis that can be attributed to the pseudosympathetic hyperactivity of thyrotoxicosis rather than true infiltrative disease of the orbital structures [40]. As expected, these signs improve in most patients after restoration of euthyroidism, and conservative management is generally recommended so long as vision is not threatened [41]. Unique to pediatric Graves' disease is the acceleration of linear growth and bone maturation associated with prolonged hyperthyroidism [42].

Box 18.3 Symptoms and Signs of Hyperthyroidism in Children

- Goiter
- Exophthalmos
- Acceleration of linear growth
- Nervousness
- Increased irritability
- Decreased concentration and impaired school performance
- Headache
- Hyperactivity
- Fatigue
- Palpitations
- Tachycardia
- Increased pulse pressure
- Hypertension
- Heart murmur
- Polyphagia
- Increased frequency of bowel movements
- Weight loss
- Heat intolerance
- Increased perspiration
- Tremor

18.3.3 Diagnosis

The term thyrotoxicosis refers to the clinical symptomatology and manifestations of excessive circulating thyroid hormone. In contrast, hyperthyroidism refers only to the subset of thyrotoxic diseases which are due to the overproduction of hormone by the thyroid itself. Graves' disease is the most common etiology of hyperthyroidism, and the ability to accurately diagnose it is critical as antithyroid drugs have no role in the treatment of thyrotoxicosis secondary to thyroiditis. Thyrotoxicosis is recognized by an elevation of serum free T4 with a decreased serum TSH (typically less than 0.1 μU/ml). A determination of the free T3 concentration is warranted if TSH is suppressed and the serum free T4 is normal. In patients with early disease or in iodine-deficient patients, serum free T4 concentrations may be

normal or reduced despite elevated levels of triiodothyronine. These are the only situations in which a serum free T3 measurement is required to confirm to the diagnosis of thyrotoxicosis. Once biochemical derangement has been documented, it is helpful to address the duration of thyrotoxicosis to facilitate the differentiation of Graves' disease from painless thyroiditis. Onset may be documented by prior laboratory studies or inferred from the history.

The differential diagnosis of thyrotoxicosis includes transient thyroiditis, hyperfunctioning nodule(s), and thyrotoxicosis factitia. In the majority of cases, the presence of a symmetrically enlarged thyroid gland coupled with the chronicity of symptoms will be adequate to allow a diagnosis, but radionuclide studies using I-123 or technetium 99 can provide confirmatory data (▶ Box 18.4). If thyrotoxicosis has been present for less than 8 weeks, transient thyrotoxicosis secondary to subacute thyroiditis or the thyrotoxic phase of autoimmune/silent thyroiditis should be considered. These forms of thyroiditis are self-limited and refractory to therapy with thionamides. Results of scintigraphy will demonstrate low radioiodine or technetium uptake, distinguishing them from the more common Graves' disease. For thyrotoxicosis which has been present for more than 8 weeks, Graves' disease is by far the most likely etiology. The constellation of thyrotoxicosis, goiter, and orbitopathy is pathognomonic of this condition, and no additional laboratory tests or imaging studies are necessary to confirm the diagnosis.

Box 18.4 Differential Diagnosis of Thyrotoxicosis in Children

- Causes of thyrotoxicosis
  - Thyrotoxicosis associated with sustained hormone overproduction (hyperthyroidism) (high RAIU)
    - Graves' disease
    - Toxic multinodular goiter
    - Toxic adenoma
    - Increased TSH secretion
  - Thyrotoxicosis without associated hyperthyroidism (low RAIU)
    - Thyrotoxicosis factitia
    - Subacute thyroiditis
    - Chronic thyroiditis with transient thyrotoxicosis (painless thyroiditis, silent thyroiditis, postpartum thyroiditis)
    - Ectopic thyroid tissue (struma ovarii, functioning metastatic thyroid cancer)

If thyromegaly is subtle and eye changes are absent, diagnostic testing to confirm the diagnosis of Graves' disease should be considered. Depending on available expertise, appropriate options include the measurement of serum thyrotropin receptor antibody (TRAb) titers, the Doppler ultrasonography to document increased thyroidal blood flow, or the measurement of I-123 RAIU [43]. Recent pediatric studies report high diagnostic sensitivity (93%) and specificity (100%) with modern TRAb assays and even greater accuracy with novel chimeric TSHR bioassays [44]. For severe thyrotoxicosis (when decisions regarding the appropriateness of antithyroid medication are time-critical), we favor the use of radioactive iodine uptake using I-123 (I-123 RAIU) to rapidly document (or refute) the presence of true hyperthyroidism. As in adults, autonomous nodules must be large to cause hyperthyroidism (typically 2 cm or more in diameter), so radioiodine scanning can be reserved for patients in whom a discrete nodule(s) is palpable [45]. In patients with a toxic nodule, I-123 uptake will localize to the nodule, and the signal in the surrounding tissue will be low secondary to TSH suppression. Thyrotoxicosis factitia can be recognized by a low RAIU and serum thyroglobulin in the presence of thyrotoxicosis and a suppressed TSH.

There is a subgroup of patients who have a subnormal but not severely depressed TSH (usually between 0.1 μU/ml and 0.3 μU/ml) and normal serum concentrations of thyroid hormone. These patients are generally asymptomatic, and the term "subclinical hyperthyroidism" has been applied to their condition. In adults over 60 years of age, a low serum TSH concentration has been associated with an increased risk of atrial fibrillation, and some studies suggest that postmenopausal women are also at risk of bone loss [46, 47]. However, it is important to note that no similar risks have been identified in the pediatric population, and several studies indicate that a significant fraction of patients with subclinical thyrotoxicosis experience spontaneous remission [48]. Accordingly, in children, the initial detection of a suppressed TSH concentration without elevated levels of thyroid hormone or associated symptoms should be addressed simply by repeating thyroid function tests in 4–8 weeks. Assuming there are no specific risk factors such as a history of cardiac disease, asymptomatic children with

subclinical hyperthyroidism can be followed with the expectation that TSH suppression which is due to transient thyroiditis will resolve spontaneously and that which is due to Graves' disease or autonomous secretion will declare itself over time.

18.3.4 Antithyroid Medications

The treatment of Graves' hyperthyroidism may be divided into two categories, antithyroid medications and definitive therapy. The thionamide derivatives, Tapazole (methimazole: MMI) and propylthiouracil (PTU), are the most commonly used antithyroid drugs [49]. Both block thyroid hormone biosynthesis, and PTU, when used at doses over 450–600 mg/day, has the additional action of inhibiting the extrathyroidal conversion of T4 to T3 [18]. The recommended starting dose is 0.5–1.0 mg/kg/day for MMI and 5–10 mg/kg/day for PTU. In patients who present with severe thyrotoxicosis, inorganic iodine (SSKI three drops po bid for 5–10 days) may be added to speed the fall in circulating thyroid hormones.

In 2009, reports from the Food and Drug Administration's Adverse Event Reporting System and the United Network for Organ Sharing heightened awareness of the risk of PTU-related liver failure [50, 51]. These studies supported that severe thionamide-induced liver failure is specific to PTU and suggest that children are especially prone to this complication. Based upon these publications, it is now recommended that MMI be exclusively used as the first-line drug whenever antithyroid medications are initiated. In contrast, the use of PTU is now generally reserved for the specific situations of first-trimester pregnancy (due to concerns of MMI-associated birth defects), life-threatening thyroid storm (to inhibit T4-to-T3 conversion), and allergic reactions to MMI (when definitive therapies are inappropriate or declined). In these situations, families must be counseled regarding the risks of PTU-induced liver failure and provided clear instructions to discontinue PTU and immediately contact the prescribing physician if concerning symptoms such as jaundice, fatigue, malaise, or anorexia onset exist.

For adolescent patients, the following rule of thumb is helpful in the determination of a starting dose of Tapazole:

Starting dose of Tapazole for adolescent patients	
Free T4 index or free T4	**Tapazole dose**
<1.5 times the upper limit of normal range	10 mg qd
1.5–2 times the upper limit of normal range	10 mg bid
>2 times the upper limit of normal range	20 mg bid

Some authors have advocated a "block and replace" strategy of high-dose antithyroid medication (to suppress all endogenous thyroxine secretion) combined with levothyroxine replacement. While one report described a lower frequency of recurrence with this approach, all subsequent studies have failed to duplicate this finding [52–54]. This approach offers no therapeutic advantage and is more complicated. For the purpose of simplifying the patient's regimen and minimizing the risk of adverse drug reactions, monotherapy with MMI is preferable. After the serum free T4 has fallen to the upper end of normal range, the MMI dose should be decreased by one half or one third. Further dose adjustments are guided by serial thyroid function tests, initially relying upon the FT4I. After pituitary TSH secretion recovers from suppression, the goal of maintenance therapy is TSH normalization. Due to its long half-life, MMI can be administered once daily in most patients after the initial restoration of euthyroidism.

The first clinical response to medications is 2–4 weeks into therapy. Weight loss subsides and weight gain may occur. Beta-adrenergic antagonists may be used as an adjunct during this interval, but, as the cardiovascular manifestations of hyperthyroidism are generally well tolerated in the young, we reserve this therapy for symptomatically significant palpitations. Antithyroid drugs are usually well tolerated, but side effects are seen more commonly in children than in adults. Agranulocytosis (defined as a granulocyte count less than 500 per μl) is a serious idiosyncratic reaction that can occur with either MMI or PTU. For this reason, a baseline white count should be obtained prior to the initiation of antithyroid drugs since mild neutropenia may be present in the Graves' patient prior to the initiation of treatment [55]. Families should be counseled that fever, sore throat, and other serious

infections may be manifestations of agranulocytosis and therefore should prompt the immediate cessation of antithyroid drugs, the notification of the physician, and a determination of white blood cell count with differential.

Reports of long-term remission rates in children are variable, ranging anywhere from 30% to 60% [56–58]. One-year remission rates are considerably less in prepubertal (17%) compared to pubertal (30%) children, but a recent retrospective study of 76 pediatric patients describes a 38% rate of long-term remission achieved with more prolonged courses of antithyroid medication (mean treatment duration of 3.3 years) [59, 60]. If the dose of antithyroid medication required to maintain euthyroidism is 5 mg/day of Tapazole for 6 months to a year and the serum TSH concentration is normal, a trial off medication may be offered. Antithyroid drugs can be discontinued and TSH concentrations monitored at monthly intervals. If hyperthyroidism recurs, as indicated by a suppression of TSH, antithyroid medications should be resumed or definitive therapy provided [43, 61].

18.3.5 Definitive Therapy

The two options for the definitive treatment of Graves' disease are radioactive iodine (I-131) and thyroidectomy. Both are likely to result in lifelong hypothyroidism, and there is debate in the literature as to their indications [62–64]. Some centers consider these modalities as options for the initial treatment of pediatric hyperthyroidism [65–67]. However, as remission of Graves' disease occurs in a significant percentage of children, we recommend that antithyroid medications be offered as initial therapy. If patient non-adherance prevents the successful treatment of thyrotoxicosis or antithyroid medications must be discontinued secondary to serious drug reactions, definitive therapy is appropriate.

Thyroid destruction by I-131 is the definitive treatment of choice in adults, but concerns over the potential long-term complications of pediatric radiation exposure have made endocrinologists cautious in applying this approach to children. It is estimated that more than 1000 children have received I-131 for the treatment of Graves' disease, and a number of reports describe no increase in the incidence of thyroid carcinoma or leukemia in this population [43, 68–70]. Despite the reassurances of this literature, experience with X-rays and the Chernobyl Nuclear Power Plant accident indicate that the carcinogenic effects of radiation to the thyroid are highest in young children. This argues for continued surveillance and, for children who fail antithyroid medication, the provision of an I-131 dose adequate to destroy all thyroid follicular cells [71–73]. In addition, recent consensus guidelines recommend avoiding I-131 therapy in very young children less than 5 years of age and limiting the calculated I-131 administered activity to less than 10 mCi in children less than 10 years of age [43]. Some institutions administer an empiric dose of 3–15 mCi or a dose based upon the estimated weight of the gland (50–200 μCi/g of thyroid tissue) [68, 69, 74]. Efficacy is dependent upon both thyroid uptake and mass, and it is more logical to prescribe a dose which will provide approximately 200 μCi/g estimated weight in the gland at 24 h. Antithyroid drugs should be discontinued for 5 days prior to the administration of I-131. For children who are unable to swallow a capsule, a liquid preparation of I-131 is available.

$$\text{Dose I}-131 = \frac{\left(200\mu\text{Ci/gm} \times \text{estimated weight of thyroid in gm} \times 100\right)}{\left(\%\text{uptake at 24h}\right)}$$

The frequency of acute side effects is low although one recent paper describes vomiting in 4 out of 35 pediatric patients [67]. One prospective study of 443 patients ranging from 15 to 85 years of age has raised the concern that I-131 may worsen or precipitate the development of Graves' ophthalmopathy in approximately 15% of cases [75]. Severe ophthalmopathy is less common in pediatric Graves' disease, and the current pediatric literature suggests that the rate of ophthalmologic exacerbation is similar among the various treatment modalities: 3% after I-131, 2% with thionamide derivatives, and 9% after subtotal thyroidectomy [56]. A short course of glucocorticoids is appropriate if there is rapid progression of ophthalmopathy or prophylaxis in children with preexisting moderate to severe ophthalmopathy.

Thyroidectomy is rarely used electively for the definitive therapy of adults with Graves' disease in the United States except with massive thyromegaly (over eight times the normal size) or for patients in whom coexisting nodules are suspicious for

carcinoma by fine needle aspiration. A recent meta-analysis of the pediatric literature provided the following analysis of surgical treatment: subtotal thyroidectomy relieved hyperthyroidism in 80% of patients, with 60% becoming hypothyroid. Total thyroidectomy cured hyperthyroidism in over 97% of patients with nearly universal hypothyroidism. The overall complication rate in children included a 2% incidence of permanent hypoparathyroidism, a 2% incidence of vocal cord paralysis, and a 0.08% mortality [56]. In the author's opinion, these average complication rates limit the appropriateness of surgery as first-line therapy for pediatric Graves' disease, given the benign nature of the disease and the other therapeutic options available. One large institution has published a series of 82 children treated surgically over 14 years with much better results. Bilateral subtotal resection was the most frequently performed operation (86%), and, with a median follow-up of 8.3 years. They cite a recurrence rate of 6% and *no* cases of permanent recurrent laryngeal nerve palsy, permanent parathyroid disease, or death [76]. The difference between the average complication rate and those in a single institution emphasizes the importance of skill and experience in the performance of this procedure [77]. While we hesitate to apply average rates of postsurgical complications to every institution, it is clear that referral to a surgeon with a low personal complication rate and extensive experience with subtotal thyroidectomy is required if this is the desired procedure [78]. Postoperative hypothyroidism is expected and should be viewed as a relatively trivial complication as it is easily treated and all Graves' patients require lifelong monitoring. We suggest that thyroidectomy be considered only for patients who have persistently failed medical management or those whose parents or physicians do not wish to proceed with radioiodine therapy. Based on the results to date, I-131 therapy is an acceptable alternative if the surgical options are undesirable in a given community. I-131 is recommended for all patients who recur following surgery due to the high complication rate of secondary thyroidectomy [79].

18.3.6 Monitoring of Graves' Disease and the Transition to Adult Care

Given the documented risks of surgery and the theoretical risks of radioiodine, prolonged courses of antithyroid medication are appropriate in the treatment of pediatric Graves' disease, especially with the relatively high possibility of remission. Therefore, monitoring of thyroid function tests every 3 months in the growing child and 3–4 weeks after any medication adjustment with the goal of normalizing the TSH is recommended. Physical examination should focus upon heart rate, puberty, linear growth, and vision.

The transition to adulthood should prompt a re-discussion of therapy. For young adults with persistent hyperthyroidism, I-131 is our definitive treatment of choice. We perform an RAIU prior to treatment with the goal of delivering approximately 8 mCi of I-131 into the gland at 24 h. For glands larger than three times the normal size, about 11 mCi is required [80]. Definitive therapy typically results in permanent hypothyroidism but allows for a simpler regimen of medication and laboratory monitoring (daily levothyroxine and a yearly TSH measurement). Additionally, prior definitive therapy simplifies the management of female patients during pregnancy.

18.4 Neonatal Graves' Disease

Approximately 0.6% of infants born to mothers with a history of Graves' disease will develop neonatal hyperthyroidism due to the transplacental passage of thyroid-stimulating immunoglobulins. Even after definitive treatment by I-131 or thyroidectomy, women with a history of autoimmune thyroid disease are at risk for fetal and neonatal thyroid dysfunction secondary to the persistence of maternal autoantibodies. The care of such women must be coordinated between the high-risk obstetrician and an endocrinologist. Fetal heart rate and growth should be monitored by regular prenatal ultrasounds, and the measurement of anti-thyrotropin receptor antibodies in the third trimester of high-risk pregnancies has been recommended as a predictorfor the development of fetal/neonatal Graves' [81, 82]. Highly experienced ultrasonographers can often visualize the fetal thyroid. The presence of fetal goiter, tachycardia, and intrauterine growth retardation suggests fetal hyperthyroidism. In these rare patients, antithyroid drugs are administered to the mother to control fetal hyperthyroidism. Pediatricians should be aware

that the use of maternal antithyroid medications near the time of delivery or the co-transfer of maternal thyrotropin receptor blocking immunoglobulins may delay the appearance of neonatal Graves' [83, 84]. For high-risk infants, such as those born to mothers with high levels of thyrotropin-stimulating antibodies or those with a history of an affected sibling, it is our practice to obtain thyroid function tests at birth and at 1 and 2 months of age. An additional set of lab work at 1 week of age is indicated for infants who have been exposed to maternal antithyroid drugs in the third trimester.

Affected infants are often flushed, diaphoretic, and hyperkinetic. Goiter is common and, when severe, can endanger the infant's airway. Diarrhea, vomiting, poor weight gain, and a transient exophthalmos may be seen. Arrhythmias and/or congestive heart failure can develop and require treatment with digoxin. Serum for confirmatory thyroid function tests (TSH, free T4) should be obtained and treatment initiated immediately. Methimazole (0.25–1.0 mg/kg/day) or propylthiouracil (5–10 mg/kg/day) may be administered orally or per nasogastric tube in divided doses every 8 h. Inorganic iodine will speed the fall in circulating thyroid hormone, using SSKI (48 mg iodide/drop) at the dose of one drop per day. As in older patients, adjunctive therapy with beta-blockade (propranolol 2 mg/kg/day) and glucocorticoids (prednisone 2 mg/kg/day) may be helpful in severe cases. The cumulative morbidity of neonatal Graves' disease was estimated to be as high as 25% in the past although it appears to be considerably lower today [74]. Potential long-term morbidity includes growth retardation, craniosynostosis, impaired intellectual function, and central hypothyroidism [74, 85, 86].

The half-life of maternal immunoglobulin is approximately 14 days, so most cases of neonatal Graves' disease will resolve after 3–12 weeks (depending upon the initial levels of thyrotropin receptor autoantibodies). The differential diagnosis of neonatal thyrotoxicosis includes the McCune-Albright syndrome and activating mutations of the TSH receptor. These non-autoimmune etiologies are exceedingly rare but should be considered if thyrotoxicosis persists beyond 3 months of age.

18.5 Summary

Autoimmune thyroid diseases, both Hashimoto's thyroiditis and Graves' disease, are due to dysregulation of the immune system. In a genetically predisposed child, activated T cells infiltrate the thyroid gland which leads to B cell dysregulation and antibody production with loss of tolerance to multiple thyroid antigens, including thyroglobulin, thyroperoxidase, sodium-iodine symporter, and the TSH receptor. In Hashimoto's thyroiditis, there may be a waxing and waning of thyroid function with prolonged periods of euthyroidism and potential progression to overt hypothyroidism in the future. In general, the treatment of subclinical hypothyroidism has not been shown to be beneficial in the pediatric population. In Graves' disease, TSH receptor antibodies stimulate glandular growth and lead to the production of excess thyroid hormone and symptoms of thyrotoxicosis. It is important to differentiate between thyroiditis and Graves' disease because the use of antithyroid drugs is not indicated in the former. Treatment options for Graves' disease are individualized; however, the use of antithyroid drugs is typically the first line of therapy with radioiodine ablation or surgical thyroidectomy being utilized for refractory cases or in the event of an adverse side effect. Side effects of antithyroid drugs occur in up to 25% of patients, and therefore, it is important to educate families particularly to the risks of agranulocytosis, hepatitis, arthritis, and acute Stevens-Johnson syndrome. While adults have a more favorable remission rate, children require a more prolonged course of antithyroid drug to achieve remission. For those that don't remit, permanent therapy with either radioiodine ablation or surgical thyroidectomy in a high-volume center can be pursued.

Neonatal Graves' disease is a rare disorder that results in the transplacental passage of TSH receptor antibodies from a mother who has active Graves' disease or had been treated for Graves' disease in the past. It has a high morbidity rate, and therefore, prompt recognition and treatment are critical. Therapy consists of antithyroid drug, beta-blockade, inorganic iodine, and glucocorticoids dependent upon the degree of biochemical derangement. Fortunately, neonatal Graves' disease typically resolves within 3 months as the maternal TSH receptor antibodies clear the neonatal circulation.

Review Questions

1. A 12-year-old girl is referred with a 3-month history of fatigue, weight loss, and poor school performance. On physical examination, she is noted to have tachycardia, an enlarged thyroid gland, and mild proptosis. Results of her thyroid function tests are as follows: TSH 0.003 uIU/mL, free T4 6.4 ng/dL, and T3 550 ng/dL. Her TSH receptor antibody titer is >40 U/L (normal <1.75).

   Of the following options, the most appropriate initial action is:

   A. Observation with follow-up laboratory testing in 4–6 weeks
   B. Initiation of methimazole therapy
   C. Initiation of propylthiouracil therapy
   D. Plan for radioactive iodine ablation
   E. Consultation for surgical thyroidectomy

2. A 14-year-old boy is referred for the evaluation of abnormal thyroid function tests. His mother has noted a 10 pound weight gain and fatigue; however, they deny skin changes, cold intolerance, constipation, or muscle aching. He has been growing well and progressing appropriately through puberty. His family history is significant for a maternal aunt with hypothyroidism and a maternal grandfather with thyroid nodules. His physical examination is notable for normal vital signs with a BMI in the 85th percentile. He has Tanner stage III testicular and pubic hair development. His thyroid gland is firm and minimally enlarged, but there are no appreciable nodules or abnormal lymph nodes. The results of his laboratory evaluation are as follows:

   TSH, 6.5 uIU/mL; free T4, 1.2 ng/dL; thyroglobulin antibodies, <1 U/mL (normal 0–20 U/mL); and thyroid peroxidase antibodies, 250 U/mL (normal 0–30 U/mL).

   The most appropriate next step would be to:

   A. Recommend dietary and lifestyle modifications and return to pediatrician's care.
   B. Begin therapy with levothyroxine 2 mcg/kg/day and repeat thyroid function tests in 6–8 weeks.
   C. Observe clinically and repeat thyroid function tests in 4–6 months.
   D. Check a spot urine iodine level and encourage increasing iodized salt and cruciferous vegetables.
   E. Obtain an I-123 uptake and scan.

3. A mother with a distant history of Graves' disease and hypothyroidism due to radioiodine ablation gives birth to a full-term neonate. The pregnancy was uncomplicated, and the mother's thyroid hormone levels were well maintained on thyroid hormone replacement therapy. At 3 days of life, the neonate was found to be tachycardic and sluggish with feeding. Newborn screening results were notable for a suppressed TSH <0.01 uIU/mL and elevated T4 25 mcg/dL. Serum thyroid function tests are as follows:

   TSH <0.005 uIU/mL, free T4 6.61 ng/dL, and T3 585 ng/dL.

   The next step in medical management of this neonate is:

   A. Measure thyroperoxidase antibodies (TPO Ab) and begin therapy with a beta-blocker.
   B. Begin therapy with methimazole, beta-blocker, and inorganic iodine.
   C. Observe with close monitoring of thyroid function.
   D. Measure TSH receptor antibodies (TRAb) and begin methimazole.
   E. Begin an exchange transfusion, PTU, and glucocorticoids.

Answers

1. (B) Treatment options for children and adolescents with Graves' disease include an antithyroid drug or definitive therapy with near-total thyroidectomy or radioactive iodine. In children, antithyroid drug is usually recommended as the initial treatment. Antithyroid drugs inhibit thyroid hormone synthesis by interfering with the thyroid peroxidase (TPO) enzyme. Methimazole is most commonly used in children due to the potentially adverse hepatotoxicity associated with propylthiouracil (PTU). Indications for definitive treatment in children (RAI or thyroidectomy) include relapse after a

prolonged trial of drug, nonadherence, or adverse side effect. Radioactive iodine treatment (I-131) is effective in children and adolescents with hyperthyroidism caused by Graves' disease, and most patients can be successfully treated with a single ablative dose. RAI is contraindicated during pregnancy and is generally avoided in very young children because of an increased potential risk of neoplasia. Evidence of reproductive dysfunction or higher frequencies of secondary malignancies in treated patients is controversial. Surgery is most commonly reserved for patients with a large goiter or with severe ophthalmopathy.

2. (C) The patient has subclinical hypothyroidism with a mildly elevated TSH and normal free T4. In the setting of positive thyroperoxidase antibodies, the most likely etiology of his goiter and abnormal laboratory tests are due to autoimmune or Hashimoto's thyroiditis. Subclinical hypothyroidism is defined as a TSH concentration that is above the upper limit of the normal range, with free T4 that is still within the normal range, whereas overt hypothyroidism is defined as an elevated TSH with a low free T4 level. Typically, the TSH in subclinical hypothyroidism is only mildly elevated, usually between 5–10 μU/mL. Evidence-based data regarding the decision of whether to treat subclinical hypothyroidism in children is limited. In most studies, the rate of progression to overt hypothyroidism ranges from 15–30%. In addition, the effect of treatment with levothyroxine on growth and thyroid gland size is somewhat variable. In pediatrics, the general recommendation is observation with TSH levels less than 10 μU/mL with serial monitoring of thyroid function.
3. (D) Neonatal Graves' disease is a rare complication of neonates born to mother who have or had a history of Graves' disease. Affected neonates can be symptomatic at diagnosis; however, it may also take several days until antithyroid drugs clear the circulation for symptoms to present. Treatment should begin promptly after confirmatory serum studies have been obtained. The use of antithyroid drug, methimazole, and beta-blockers are the primary therapeutic options. Morbidity can be high if not recognized. However, typically neonatal Graves' disease is limited as it resolves after the maternal TSH receptor-stimulating antibodies clear the neonatal circulation within approximately 3 months.

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Non-thyroidal Illness Syndrome

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Key Points

- Non-thyroidal illness syndrome (NTIS) is characterized by decreased serum T3 and to a lesser extent T4 levels, increased reverse T3 (rT3) levels, variable serum free T3 and free T4 levels, and "inappropriately" normal or low TSH levels.
- There is evidence that acute illness results in diminished TRH production and TSH secretion, leading to decreased thyroidal T3 and T4 production. Decreased type 1 and type 2 deiodinase (D1 and D2) result in decreased extrathyroidal conversion of T4 to T3, while increased D3 activity increases T4 conversion to rT3. Decreased total T3 and total T4 are also the result of decreased thyroid hormone-binding proteins.
- While the changes in thyroid function in NTIS appear to be "adaptive," there is some evidence that thyroid hormone treatment in certain clinical situations may be beneficial, such as preterm infants <27 weeks' gestation.
- Postoperative pediatric cardiac surgery patients demonstrate marked suppression of T3 levels in combination with other findings of NTIS. These laboratory changes are associated with longer periods of mechanical ventilation and intensive care treatment and higher requirements for inotropic support, suggesting that NTIS in this setting may be a maladaptive response and may warrant intervention. Studies of postoperative T3 administration have demonstrated some improvement in postoperative cardiac function, but the evidence remains inadequate to support a recommendation for treatment.

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19.1 Introduction and Background

Non-thyroidal illness is the term used to describe the changes in thyroid hormone and thyroid-stimulating hormone (TSH) with acute illness not caused by an intrinsic abnormality of thyroid function [1]. In children, non-thyroidal illness is most commonly seen in acutely ill patients admitted to pediatric or neonatal intensive care units (ICUs). The characteristic decrease in thyroid hormone levels also can be seen with starvation, trauma, or surgical procedures. Non-thyroidal illness probably occurs with any severe illness, and the pattern of changes in thyroid hormones correlates with the severity of illness. Typically, the first changes are a decrease in serum triiodothyronine (T3) and a rise in reverse T3 (rT3) levels [2]. This disorder has been referred to as the low-T3 syndrome or the euthyroid sick syndrome. However, as there is disagreement about whether patients truly are "euthyroid," non-thyroidal illness syndrome (NTIS) is the term preferred at present [3].

This chapter will begin with a description of the changes in thyroid hormone and thyroid-stimulating hormone (TSH) levels that occur in NTIS. This will be followed by a brief review of what is known about the pathogenesis of NTIS in starvation and acute illness. This will include a discussion of changes in hypothalamic–pituitary–thyroid (HPT) function, changes in peripheral thyroid hormone metabolism (as regulated by the three deiodinase enzymes), changes in thyroid hormone binding in the circulation, thyroid hormone transport into the cell, and finally changes in thyroid action at the tissue level in NTIS. Next will be a discussion of some of the most common pediatric clinical disorders associated with NTIS, including "hypothyroxinemia of prematurity," acutely ill patients in an ICU setting, renal insufficiency (acute and chronic), cardiac surgery, and psychiatric illness. NTIS is characterized by changes in thyroid hormone levels and TSH consistent with central hypothyroidism. Discussion of the clinical disorders will review evidence that NTIS is either a beneficial "adaptive response" to starvation or acute illness or a "maladaptive response" that might be improved by thyroid hormone treatment.

19.2 Description of Thyroid Hormone Changes in NTIS

A fall in serum T3 accompanied by a rise in rT3 levels is the most common change in patients with NTIS (thus, as noted above, in the past NTIS has been referred to as the "low-T3 syndrome"). Simple fasting produces these changes within 24–36 h; refeeding (particularly with glucose) rapidly reverses these changes. Serum T4 levels

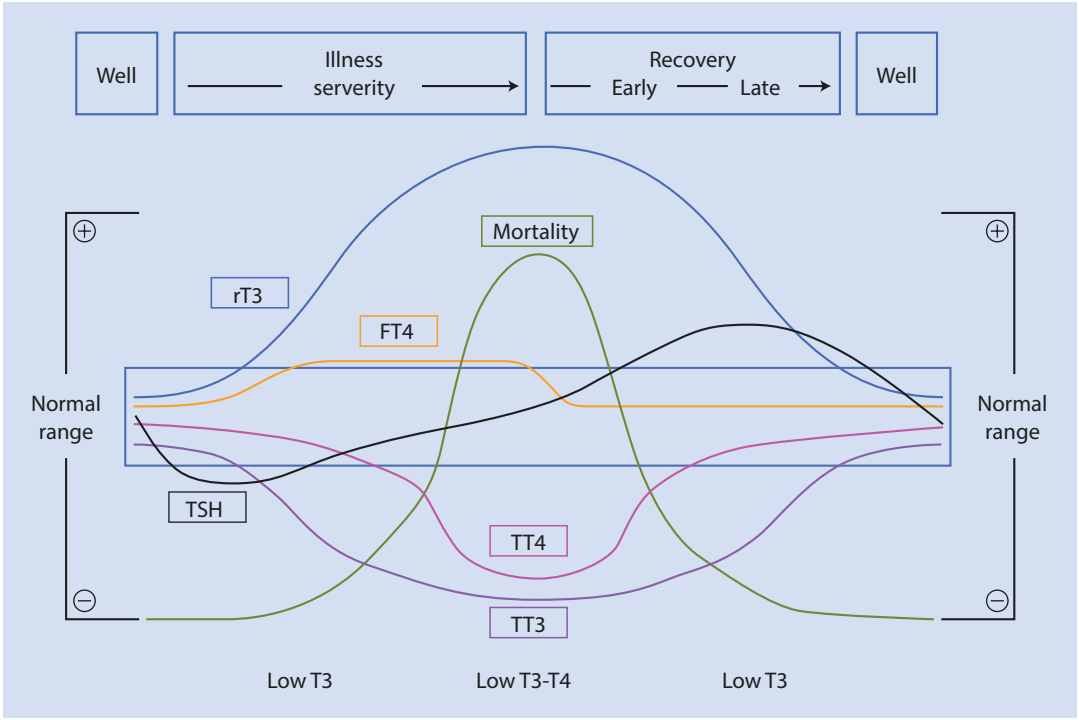


Fig. 19.1 Summary of the mechanisms that give rise to the serum thyroid hormone changes in the non-thyroidal illness syndrome

do not fall with fasting in healthy subjects. Many acute illnesses are associated with "starvation"; reduced caloric intake thus is likely one factor resulting in the initial changes in serum T3 and rT3.

Serum T4 levels tend to be normal in mild acute illness, but with increasing severity of illness, serum T4 levels will decrease. The degree of fall in serum T3 and T4 is related to the severity of the acute illness [4]. Changes in serum free T4 and free T3 levels are less certain, with results varying with the assay method. When free T4 is measured by most common "analogue" immunoassays, serum free T4 levels appear to decrease with acute illness [5]. If free T4 is calculated using a measure of total T4 and some measure of serum protein binding, e.g., T3 resin uptake, serum free T4 levels also appear to decrease. However, if free T4 is measured by methods that involve initial physical separation of free T4 from protein-bound T4, e.g., equilibrium dialysis or ultrafiltration, serum free T4 levels are usually normal or even increased in patients with acute illness [6]. A similar pattern is seen with free T3 measurements. If free T3 is measured by the more common commercial assays, the fall in serum free T3 parallels the fall in total T3. However, if free T3 is measured by a dialysis or filtration method, the decrease in serum free T3 does not match the fall in total T3. One study reported serum free T3 levels only 10% lower than a healthy control group [7].

TSH measurements generally are in the normal range, though serum TSH may decrease below normal in some patients with severe acute illness [8]. Thus, it is not uncommon to find low serum T3 and T4 levels, low free T4 levels (by commonly used assay methods), and normal or low TSH levels, results that are consistent with central hypothyroidism.

Patients with NTIS exhibit a typical pattern of thyroid hormone and TSH changes as they recover from their acute illness (Fig. 19.1). If serum TSH falls below the normal range, with recovery it rises back into the normal range and even mildly above the normal range in some patients (e.g., 10–20 mU/L) [9]. Serum T4 rises back to the normal range, followed by a rise in total T3 and a fall in rT3 back into the normal range. Serum free T4, if measured by an analogue immunoassay, will rise back into the normal range; if free T4

is measured by equilibrium dialysis or ultrafiltration, it typically remains normal throughout the NTIS. Serum free T3 follows a similar pattern.

19.3 Pathogenesis of NTIS–Thyroid Changes

19.3.1 Changes in the Central Hypothalamic–Pituitary–Thyroid Axis

Although serum T3 and T4 levels fall, there is no increase in serum TSH levels, and with increasing severity of illness, there may be a decrease in TSH levels. Frequent sampling studies show a decrease in the nocturnal TSH surge and amplitude [10]; similar changes occur in patients with central hypothyroidism. Evidence points to a decrease in thyrotropin-releasing hormone (TRH) as a cause of diminished TSH secretion, along with direct effects of acute illness on pituitary thyrotroph cell function. Postmortem studies in patients with NTIS show decreased TRH mRNA expression in the paraventricular nucleus (PVN), the main source of TRH [11]. Reduced intake of calories, either as part of an acute illness or with fasting, results in decreased leptin levels. In experimental animal studies, decreased leptin levels result in altered neuroendocrine regulation of TRH secretion [12]. Further, cytokines along with increased cortisol produced with acute illness appear to have a direct inhibitory effect on TSH secretion in the pituitary [13]. If acutely ill patients are treated with dopamine (to maintain cardiovascular function) or glucocorticoids, these drugs also inhibit TSH secretion. There is also one other proposed mechanism that may inhibit TSH secretion. Although PVN cells do not appear to directly sense circulating T3 or T4 levels, a unique glial cell with processes that extend into the hypothalamus, the tanycyte, provides a communication between the portal circulation and the hypothalamus. Studies in an animal model of NTIS show an increase in tanycyte deiodinase type 2 (D2) enzyme activity [14]. Increased D2 activity could increase T4 to T3 conversion, resulting in "local tissue hyperthyroidism" in the hypothalamus, which, by way of negative feedback, would inhibit TRH synthesis. In summary, current evidence supports diminished TRH production and decreased TSH secretion as the cause of decreased thyroid gland T4 production and secretion and perhaps also decreased T3 levels (see below: since the majority of T3 is produced by peripheral tissue deiodination of T4, changes in tissue deiodinases also appear to be a cause of lower serum T3 levels).

19.3.2 Changes in Peripheral Thyroid Hormone Metabolism

Thyroid hormone metabolism in extrathyroidal tissue is regulated by three deiodinase enzymes. Type 1 and type 2 deiodinase (D1 and D2) are the main activating enzymes, while type 3 deiodinase (D3) is the inactivating enzyme [15]. D1 is present in many extrathyroidal tissues, including the liver and kidney, while D2 is present in the pituitary, brain, and brown adipose tissue. The main action of D1 and D2 is to convert T4 to biologically active T3; D1, located on the plasma membrane, is the enzyme responsible for production of most of the T3 that enters the circulation. D3 is present in many extrathyroidal tissues, including the brain; the main action of D3 is to convert T4 to biologically inactive rT3 and, to a lesser extent, T3 to 3,3′-diiodothyronine (T2). The expression of the deiodinase enzymes is modified in acute illness and appears to be highly tissue specific. It is generally accepted that the fall in serum T3 levels is the result of decreased D1 and increased D3 activity [16]. There is some evidence that these changes in D1 and D3 are mediated by the increased levels of cytokines and glucocorticoids seen in acute illness. More recent evidence, however, including studies in D1/D2 and D3 knockout mice, find that the changes in serum T3 and T4 with induced acute illness are similar to wild-type animals. These studies suggest that the changes noted in deiodinase enzymes may be a consequence, not the cause of the NTIS [17]. Lastly, there is evidence of increased degradation of T4 and T3 via non-deiodination pathways, manifested by increased levels of T3 sulfate and triiodothyroacetic acid (TRIAC) and tetraiodothyroacetic acid (TETRAC) in patients with NTIS [1].

19.3.3 Changes in Thyroid Hormone-Binding Protein Kinetics

Thyroid hormone-binding proteins including thyroxine-binding globulin (TBG), transthyretin (formerly termed thyroxine-binding prealbumin), and albumin are "acute-phase" reactants and tend to fall with acute illnesses [2]. This appears to be the result of both impaired synthesis but also rapid breakdown and movement out of the plasma space. This is particularly true with sepsis and cardiopulmonary bypass. As the vast majority of serum T4 (99.97%) and T3 (99.70%) are bound, concentrations of serum total T4 and total T3 may be lower in large part as a result of lower binding protein levels. In addition, there is evidence of circulating inhibitors of T4 and T3 binding to their binding proteins in NTIS. Nonesterified fatty acids and certain drugs used to treat patients with NTIS appear to act as inhibitors, including heparin, furosemide, and salicylate. Heparin's action appears to be in vitro; it activates lipoprotein lipase which then breaks down triglycerides into glycerol and fatty acids, resulting in a dramatic release of bound T4 and false elevation of free T4 measurements [18].

19.3.4 Changes in Thyroid Hormone Transport and Action at the Tissue Level

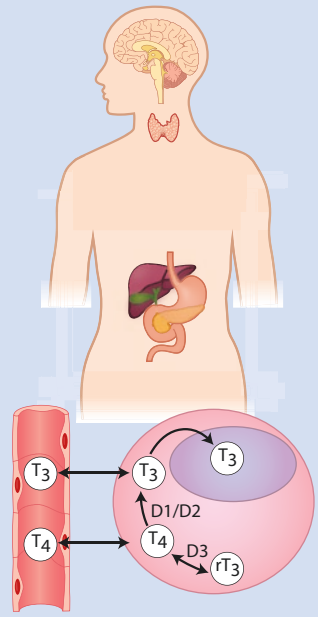
Entry of thyroid hormone into the cell is carried out by ATP-dependent transporters, of which monocarboxylate transporter 8 (MCT8) appears to be the most important. Most studies show no change in thyroid hormone transporter expression in NTIS, though some report an increase in MCT8 expression, likely a consequence of falling T3 and T4 levels [19]. Once thyroid hormone enters the cell, T3 (either from direct entry or via intracellular T4 deiodination to T3) binds to a specific thyroid hormone receptor (THR). Studies in humans with acute illness and animal models of NTIS yield conflicting information on changes in THR, with reports of increased, decreased, or no change in THR expression. THR action is regulated by coactivators and corepressors; there is some evidence that cytokines produced with acute illness compete with coactivators or corepressors and so may regulate tissue-specific THR action. It appears that THR expression is downregulated in acute illness and upregulated in chronic illness, though again this appears to be tissue specific and likely an overgeneralization [1].

19.3.5 Summary of Pathogenesis of NTIS–Thyroid Changes (Fig. 19.2)

Acute illness results in diminished TRH production and TSH secretion. The characteristic fall in serum T3 levels appears to be the result of decreased production in the thyroid gland, decreased D1 and D2 activity resulting in decreased extrathyroidal conversion of T4 to T3, and increased D3 activity with increased conversion of T4 to rT3. Decreased levels of total T3 and total T4 are also the result of decreased thyroid hormone-binding proteins. Levels of free T4 and free T3 are assay dependent; assays using physical separation techniques, such as equilibrium dialysis, tend to show normal serum free T4 and normal or only mildly low free T3 results. Changes in thyroid hormone transport into the cell and in thyroid hormone receptor expression appear to be more a consequence of thyroid hormone changes in NTIS.

19.3.6 Separating NTIS from True Thyroid Dysfunction

It can be difficult to separate the changes in serum thyroid hormone levels seen in patients with NTIS from those who have true thyroid dysfunction. While good data do not exist for children, studies in adult patients admitted to medical services report a low serum T3 level in 50%, a low serum T4 level in 15–20%, and an abnormal (low or high) TSH in 10% [2]. If there is a clinical suspicion of hypothyroidism, we recommend that patients undergo measurement of serum free T4 by equilibrium dialysis and TSH levels. In true hypothyroidism, patients will have a low free T4 level and elevated TSH level. Caution must be used, however, as patients recovering from NTIS may manifest a low free T4 and elevated TSH,



Hypothalamus	• Malnutrition→↓ Leptin→↓TRH • Sepsis/Inflammation→↑ D2 (tanycyte →↑ T_3→↓ TRH)
Pituitary	• Cytokines→↓ TSH
Plasma	• Acute phase responses →↓TBG →↓ TT_4 ↓ TT_3 • ↑Competitors for TH binding proteins →↓ TT_4 ↓ TT_3 • Free T_3 and free T_4 may also fall due to central hypothyroidism
Tissue uptake	• ↓T_4/T_3 uptake • ↑or unchanged thyroid hormone transporter expression
Intracellular deiodination	• ↓D1 Liver/kidney • ↑D2 Muscle, prolonged illness, LPS, turpentine • ↓D2 Muscle/pneumonia • ↑D3 Muscle/Liver
Nuclear TH receptors and coactivators	• ↑Chronic illness • ↓Acute illness

Fig. 19.2 Changes in thyroid tests during the course of NTI

though typically it is mild, in the 10–20 mU/L range. A TSH elevation >20 mU/L is suspicious for true hypothyroidism. As autoimmune thyroiditis is the most common cause of acquired hypothyroidism, finding positive anti-thyroglobulin and/or thyroid peroxidase antibodies would support a diagnosis of hypothyroidism. Clinicians should be aware that in patients with true hypothyroidism, elevated TSH levels may decrease, even into the normal range, particularly if patients are treated with drugs that inhibit TSH secretion such as dopamine or glucocorticoids. If thyroid hormone treatment is to be started, patients should undergo evaluation of pituitary–adrenal function first. Thyroid hormone treatment in the face of unrecognized adrenal insufficiency may precipitate adrenal crisis, certainly undesirable in the face of acute illness.

If there is a clinical suspicion of hyperthyroidism, we recommend measurement of serum free T4, free T3 (both by equilibrium dialysis), and TSH levels. In true hyperthyroidism, patients will have an elevated free T4 and free T3 level and a TSH suppressed below the normal range. Patients with severe NTIS may manifest a low TSH level, but this usually is not confused with hyperthyroidism as serum free T4 and free T3 are not elevated. Again, patients with true hyperthyroidism are likely to have not just a low TSH level but an unmeasurable TSH level (<0.01 mU/L). A normal TSH level excludes hyperthyroidism. Finding a positive thyrotropin receptor-stimulating antibody (e.g., thyroid-stimulating immunoglobulin [TSI]) would support the diagnosis of Graves' disease and hyperthyroidism.

As many clinical manifestations of acute illness overlap with thyroid dysfunction, often the best course is to recheck thyroid function tests after resolution of the acute illness.

19.4 Clinical Presentation and Diagnostic Evaluation of Pediatric NTI Syndromes

In the next section, we will review common pediatric NTI syndromes. This includes infants admitted to neonatal intensive care units (primarily infants born preterm), children admitted to pediatric intensive care units, children with congenital heart disease undergoing cardiac surgery,

and children with renal insufficiency (acute and chronic). Some psychiatric disorders appear to be associated with NTIS, although the changes in thyroid function tests may be more a result of drug treatment than the underlying psychiatric disorder. For each, we will summarize the clinical manifestations of these syndromes that may be the result of changes in thyroid function and the evidence that thyroid hormone treatment is beneficial, harmful, or neither.

19.4.1 Preterm Infants

The third trimester of pregnancy is an important period in the development of the thyroid gland and the maturation of the HPT axis [20]. Between 24 and 40–42 weeks' gestation, the following developmental steps are accomplished: an eight- to ten fold increase in thyroid gland volume, a three- to four fold increase in thyroid hormone reserve, increasing TSH secretion leading to increasing thyroxine secretion, as well as maturation of the negative feedback system of control of TSH. In addition, the profile of expression of deiodinase enzymes differs between the fetal and postnatal periods with approximately 75% lower levels of D1 and 10–15-fold higher levels of D3 in the fetus as compared to adults. This altered ratio of D1 to D3 results in a lower concentration of T3 and higher concentration of rT3 in the circulation of a preterm infant as compared to a term infant. Other manifestations of HPT axis immaturity in preterm infants include a decreased neonatal TSH surge, decreased thyroid reserve, and persistent production of inactive thyroid hormone metabolites. The degree of HPT axis immaturity is inversely related to gestational age, making the loss of transplacental maternal T4 increasingly critical with decreasing gestational age.

Preterm infants with decreased circulating T4, decreased circulating T3, increased circulating rT3, and inappropriately normal or even frankly low TSH are displaying a phenotype that closely resembles the non-thyroidal illness syndrome. This constellation of findings is also sometimes termed transient hypothyroxinemia of prematurity (THOP). In addition to developmental immaturity of the HPT axis, there are a number of acute events in the postnatal period as well as drugs used to treat these events that have been associated with decreased T4, T3, and TSH levels and/or with increased rT3 and inactive thyroid hormone metabolite levels in preterm infants. Williams et al., as part of the Scottish Preterm Thyroid Group, showed that relevant postnatal events include bacteremia, endotracheal bacterial colonization, persistent patent ductus arteriosus, necrotizing enterocolitis, acute intraventricular hemorrhage, or the development of periventricular leukomalacia and the development of chronic lung disease as evidenced by oxygen dependency at 28 days of age. Drugs associated with alterations in thyroid function in the same study include aminophylline, caffeine, dexamethasone, diamorphine, and dopamine [21].

Before considering the impact of transient hypothyroxinemia, it is important to understand how common this condition is among preterm infants. The majority of studies categorize infants according only to total T4 levels, and the cutoff values for hypothyroxinemia differ considerably among these studies. Hadeed et al. studied 215 preterm infants at 28–36 weeks' gestational age and found an overall incidence of hypothyroxinemia of 22% relative to term infants, with 52% of the hypothyroxinemic infants falling in the 28–30 weeks' gestation cohort, while 33% were within the range of 31–33 weeks' gestation and 12% were 34 weeks or greater [22]. A 1996 study by Reuss and colleagues defined mild hypothyroxinemia as a T4 level 1.3–2.6 standard deviations (SD) below the mean for the assay (with each assay including a significant number of normal term newborns) and severe hypothyroxinemia as a T4 level more than 2.6 SD below the mean for the assay. By this definition, 38–61% of infants ≤24–33 weeks' gestation exhibited mild hypothyroxinemia (with no clear increase or decrease in prevalence across the range of gestational ages included in the study), while 19–44% of infants ≤24–27 weeks' gestation showed severe hypothyroxinemia vs. 16–27% of infants 28–30 weeks' gestation and only 4–7% of infants >30 weeks' gestation [23]. The Scottish Preterm Thyroid Group has addressed this question in several different studies. Delahunty et al. used the group's data set to assess neurodevelopmental outcomes. For this purpose, they defined hypothyroxinemia as a T4 value below the 10th percentile of cord serum corrected for

gestational age, which is an attempt to normalize the value to a similar aged fetus still in utero. As such, 38% of infants 23–27 weeks' gestation, 23% of infants 28–30 weeks' gestation, and 10% of infants 31–34 weeks' gestation were classified as hypothyroxinemic [24]. Finally, in a more comprehensive interpretation of the Scottish Preterm Thyroid Group's data set that takes into account trends in T4, free T4, and T3 in preterm infants as compared to cord blood of similar gestational age and postnatal values in term infants, it appears that almost all infants <28 weeks' gestational age experience hypothyroxinemia as evidenced by total T4 values but may have preservation of free T4 levels [25].

The greatest concern regarding transient hypothyroxinemia in the preterm infant is its potential impact on neurocognitive development. While this was historically not believed to be a problem, several studies published in the last 15 years suggest an association between transient hypothyroxinemia and worsened developmental outcome at 2–5 years of age. The most recent of these studies is again a product of the Scottish Preterm Thyroid Group. Delahunty et al. showed that hypothyroxinemic preterm infants, defined as infants with a T4 < 10th percentile of cord sera of the same gestational age, scored significantly worse on cognitive and verbal scales than euthyroid preterm infants, even after adjusting for confounders of neurodevelopment such as parental intellect, maternal age, length of breastfeeding, significant postnatal events, and several others. Perceptual performance, memory, and motor scores were also lower in the hypothyroxinemic infants, but these differences fell away with adjustment for confounders [23]. In the 1996 study by Reuss et al. referenced above with regard to incidence of hypothyroxinemia in preterm infants, severe hypothyroxinemia was found to increase the risk of disabling cerebral palsy 4.4-fold compared to the risk in preterm infants with normal thyroxine concentrations. The severely hypothyroxinemic group also had mental development scores approximately seven points lower after adjustment for confounders than did the euthyroid group [22].

Given the prevalence of hypothyroxinemia in preterm infants, particularly those below 28–30 weeks' gestational age, and the apparent association of this finding with neurodevelopmental deficits later in childhood, it is reasonable to question whether supplementation of thyroid hormone might be beneficial for this population. Several trials have been undertaken without a clear consensus of benefit, but sample sizes and study design have demonstrated some limitations.

The largest study of thyroid hormone supplementation in preterm infants <30 weeks' gestation was conducted by van Wassenaer et al. This group conducted a prospective, randomized, double-blind, placebo-controlled trial of thyroxine supplementation in 200 infants, 100 of whom received treatment and 100 placebo. The study group was not limited to those infants with hypothyroxinemia but included all newborns 25–30 weeks' gestation without severe congenital malformations, maternal endocrine disease, or maternal drug use. Follow-up developmental assessments included the Bayley Developmental Index and neurological examinations at 24 months of age as well as a more detailed follow-up at 5.7 years of age. No overall difference in developmental outcome was seen; however, those born at <27 weeks' gestational age did have an 18-point improvement in developmental quotient as assessed by the Bayley index with thyroxine treatment, while those born at 29 weeks' gestation or later actually scored worse on all measures of developmental outcome if treated with thyroxine [26, 27]. The Cochrane Database of Systematic Reviews concluded that there is insufficient evidence to determine whether use of thyroid hormones for treatment of preterm infants with transient hypothyroxinemia results in changes in neonatal morbidity or mortality or reductions in neurodevelopmental impairments [28]. Significantly more research is needed to determine if there is a population within the greater group of preterm infants that should routinely receive supplementation with thyroxine and, if so, how that treatment should be accomplished.

Case Study

You are asked to consult on an 800 gm, 27-week preterm infant at 7 days of age for evaluation of possible hypothyroidism; newborn screening tests show a total T4 = 2.7 ug/dL (<10th percentile) and TSH = <2 mIU/L. After birth, this preterm baby developed respiratory distress syndrome and was intubated and placed on a ventilator with supplemental oxygen. Pulmonary function gradually improved, such that the baby was extubated at 5 days of age. However, he has been slow to establish oral feeding, and he has temperature instability, edema, and hypotonia. His neonatologists are concerned that many of these features may be explained by hypothyroxinemia. Although the thyroid test results are compatible with central hypothyroidism, you are suspicious that NTIS is a more likely explanation. You therefore recommend measurement of serum total T3, free T4, TSH, and reverse T3. At 8 days of age, serum total T3 = 45 ng/dL (50–200), free T4 = 0.5 ng/dL (0.8–1.8), TSH = 1.2 mIU/L (0.6–6.0), and reverse T3 = 260 ng/dL (90–250). Although you believe these results are most consistent with NTIS, there is still concern about central hypothyroidism due to the low free T4 (measured by an analogue assay method). You therefore recommend rechecking serum free T4 by equilibrium dialysis; at 14 days of age, free T4 = 1.4 ng/dL (0.8–2.2). The infant is now feeding better, no longer has temperature instability, and edema and muscle tone have improved. In summary, this baby had a combination of hypothyroxinemia of prematurity and NTIS, with gradual resolution over the first few weeks of life. There is no clear indication for thyroid hormone treatment.

19.4.2 Acutely Ill Children

While there is a fair amount published on NTIS in preterm infants (see above) and children undergoing cardiac surgery (see below), there is a paucity of data in children admitted to pediatric intensive care units (PICUs). Hebbar et al. from Emory University studied 73 children admitted to their PICU (ages 3 months to 19 years) [29]. In blood samples obtained in the first 12 h of admission, the mean serum T3 = 59 ng/dL (normal range 60–160 ng/dL), mean T4 = 7.2 μg/dL (4.9–11.7 μg/dL), and mean TSH = 0.58 μIU/mL (0.30–5.0 mU/L). Mean serum rT3 was elevated at 52.5 ng/dL (10–50 ng/dL). Patients with sepsis had an even lower mean serum T3 level (47 ng/dL) and higher rT3 level (70.5 ng/dL). As might be expected, thyroid results were influenced by drug therapy (vasopressors, including dopamine and steroids) and low serum albumin levels.

Children with diabetic ketoacidosis (DKA) often are admitted to a PICU for management. In a retrospective case-control study from China, Hu et al. reported that the subgroup of children judged to have NTIS, as compared to a euthyroid subgroup, had lower serum free T4 (0.88 ng/dL vs. 1.33 ng/dL), free T3 (171 pg/dL vs. 323 pg/dL), and TSH (1.77 mIU/L vs. 2.56 mIU/L) [30]. Guidelines recommend obtaining screening thyroid function tests in children with type 1 diabetes mellitus; however, it may be misleading to assess thyroid function while they are in DKA.

Our search of the literature did not turn up any clinical trials of thyroid hormone treatment in children admitted to PICUs (other than for cardiac surgery). Brent and Hershman undertook a randomized trial of l-thyroxine treatment in 23 men admitted to their medical ICU at the Wadsworth Veterans Hospital in Los Angeles [31]. Patients were selected for inclusion if they had a serum T4 level < 5 μg/dL. Half were randomized to l-thyroxine 1.5 mcg/kg IV daily for 2 weeks. While serum T4 and free T4 levels rose into the normal range, serum T3 levels remained low. Serum TSH levels were significantly decreased. By day 7, serum T3 levels rose in the control group, but this rise was delayed in the l-thyroxine-treated group, perhaps related to the decreased TSH concentration. Mortality was similar in the two groups (75% control, 73% treatment). Brent and Hershman concluded that there was no benefit in their patient population, and potentially some harm might come from the delayed rise in T3 levels, as is normally seen in patients recovering from NTIS. This raises the possibility that T3 may be the treatment of choice. Becker et al. carried out a T3 vs. placebo treatment trial in 36 men with burn injuries at the Brooke Army Medical Center in Texas [32]. Patients randomized to T3 received 200 mcg daily until their wounds were healed. T3 treatment raised the free T3 index into the normal range, but it did not affect resting metabolic rate or survival. In summary, studies in children admitted to PICUs demonstrate the same pattern of thyroid hormone changes seen in adults. Treatment trials of T4 or T3 (again, in noncardiac patients), while limited to adults, generally have not shown any benefit.

Case Study

You admit a 12-year-old girl with new onset type 1 diabetes mellitus in moderate DKA to the PICU. After presenting with a 3-week history of polyuria and polydipsia, a 2-day history of vomiting, and a 6 pound weight loss, the emergency department obtained laboratory studies showing a blood glucose of 645 mg/dL and a bicarbonate of 6 mmol/L. She received fluid resuscitation in the ED and is admitted to the PICU for initiation of an insulin drip. Her diabetic ketoacidosis resolves over the course of 24 h, and she is transferred to the pediatric ward on subcutaneous insulin. Two days later, just prior to discharge, screening laboratory studies are obtained to rule out other autoimmune disorders associated with type 1 diabetes. Results of thyroid function testing are as follows: free T4 0.9 ng/dL (0.6–1.2) and TSH 7.4 mIU/L. The blood sample is inadequate to run the thyroid antibody studies that you ordered. Given the timing of the laboratory studies in relation to this patient's recent acute illness, you cannot immediately distinguish between recovery from NTIS and true hypothyroidism, but the patient is clinically euthyroid, so you elect to wait to repeat these studies at the time of outpatient follow-up. When the patient returns to your clinic 1 month after discharge, you repeat the thyroid function tests and find that TSH has normalized and thyroid antibody studies are negative. You therefore conclude that the mild elevation of TSH was almost certainly due to recovery from NTIS.

19.5 Cardiac Surgery in Children

It is well established that infants and children who undergo cardiac surgery, with or without cardiopulmonary bypass, display significant HPT axis suppression consistent with NTIS. The most profound and consistent changes in this population are reduced total and free T3 levels, with free T3 falling as much as 80% from preoperative levels by 12–48 h postoperatively [33–35]. TSH and total T4 are also suppressed after cardiac surgery, reaching a nadir within the first 24 h [34, 35]. Free T4 rises initially when bypass is initiated, likely due to displacement of thyroxine from its binding globulin as a result of exposure to heparin. Levels then return to baseline in most studies and remain there throughout the postoperative period [34]. Reverse T3 rises postoperatively, reaching a peak at about 24 h [34, 35]. All of the changes in the HPT axis resolve gradually over the course of 5–7 days [34, 36].

The etiology of NTIS following cardiac surgery is multifactorial. Fasting and the physiologic stress of surgery contribute to the suppression of the HPT axis. Depth of hypothermia, duration of cardiac arrest, and hemodilution during cardiopulmonary bypass also have been associated with the development of NTIS. Many medications used in the perioperative management of cardiac surgery patients are also known to have a suppressive effect on thyroid function, including dopamine, glucocorticoids, anesthetic agents, and iodinated antiseptics [33, 34, 36].

Reduced circulating thyroid hormone in the postoperative period is associated with unfavorable physiologic changes including decreased cardiac output, left ventricular dysfunction, increased vascular resistance, and impaired ventilatory drive [33]. Children who have significant thyroid suppression after cardiac surgery have been shown to require longer periods of mechanical ventilation and intensive care treatment and to have higher requirements for inotropic support [33, 36], all suggesting that NTIS in this setting may be a maladaptive response and may warrant intervention.

Given the rapid onset and transient nature of thyroid suppression following cardiac surgery, intervention studies have focused on the use of T3, which has a <24-h half-life in infants and young children, as opposed to thyroxine which may take up to 2 weeks to reach steady state. T3 supplementation in adults has been fairly well studied, and there are data to support improved cardiac function postoperatively as well as decreased need for inotropes, decreased rate of arrhythmias, and decreased length of hospital stay with T3 treatment [36]. A recent meta-analysis, however, showed no evidence of alteration in postoperative mortality in the adult population with T3 supplementation [37]. Studies in children are still somewhat limited and have been less conclusive. Only the results of randomized, double-blind, placebo-controlled trials will be summarized below. In all cases, T3 levels were significantly increased by T3 administration.

Bettendorf et al. studied 40 children aged birth to 10 years. The study intervention was a once-daily infusion of T3 beginning on postoperative day 1 and continuing until subjects were

weaned off dopamine support or until postoperative day 12, whichever came first. Neither thyroid hormone levels nor cardiac function postoperatively was considered in subject selection. Treated subjects showed improved cardiac index and decreased need for intensive care services. Improved cardiac function was most pronounced in those with longer bypass time and lower cardiac output postoperatively. No adverse events were seen [38]. Portman et al. studied 14 subjects <1 year of age. The study intervention was a T3 bolus immediately before bypass initiation and with reperfusion. Heart rate was transiently elevated in the treatment group. Treated subjects had an increased peak systolic pressure rate product, suggesting improved cardiac function [39]. Chowdhury et al. evaluated 75 subjects aged birth to 18 years and subsequently randomized 29 subjects with significantly reduced postoperative T3 levels and a need for mechanical ventilation to the treatment or placebo arm of the study. The study intervention was continuous T3 infusion. There were no adverse events. Only subjects <1 month of age showed a significant effect of T3 therapy with reduced need for inotropes and lower therapeutic intervention scores indicating a decreased need for intensive care. Neither neonates nor older children showed any difference in need for diuretics, days of mechanical ventilation, or length of hospital stay [40]. Finally, Mackie et al. enrolled 42 neonates randomized to continuous T3 infusion vs. placebo for 72 h postoperatively. Neonates in the study demonstrated negative fluid balance more quickly in the treated group than in the placebo group, but neither clinical outcome scores nor cardiac index values were significantly different. Treatment was discontinued in two subjects due to hypertension and arrhythmia [41]. A Cochrane review encompassing three of the above studies concluded that there was insufficient evidence to support a positive effect of T3 supplementation in infants undergoing cardiac surgery [33].

19.6 Renal Insufficiency (Acute and Chronic)

Children with chronic renal insufficiency (CRI) have many, but not all, of the changes in thyroid function tests summarized above for NTIS. They typically have low serum T4 and T3 levels, while TSH levels are not elevated [42]. Conversion of T4 to T3 by the kidney is reduced. However, patients do not have elevated rT3 levels. Serum free T4 and free T3 levels are found to be normal in some reports and low in others, even when determined by equilibrium dialysis technique. Some children with CRI have reduced binding protein levels, as the result of either malnutrition or protein-losing nephropathies. In addition, some uremic factors appear to inhibit binding of T4 and T3 to their binding proteins. All of these effects contribute to low serum total T4 and T3 concentrations.

Children with CRI manifest some of the clinical symptoms and signs seen in hypothyroidism, including growth retardation, lethargy, poor appetite, and a puffy appearance. The prevalence of goiter is increased, present in up to 50% of children with CRI in some reports [43]. Studies report increased plasma iodine concentrations, most likely the result of decreased renal iodine clearance with CRI. Increased iodine concentrations likely play a role in goiter formation.

Some children with CRI have thyroid function tests consistent with central hypothyroidism (low free T4, inappropriately "normal" TSH). In addition, some studies report a "prolonged" TSH response to TRH stimulation and a subnormal nocturnal TSH surge [44]. However, given that studies also report that TRH degradation is decreased in children with CRI [45], these results may be an indirect effect of CRI on TRH clearance rather than clear evidence of abnormal hypothalamic–pituitary–thyroid function. While a low free T4 in the face of a normal TSH level would appear to be consistent with central hypothyroidism, the fact that free T4 levels are reported to normalize immediately after hemodialysis is difficult to reconcile with true hypothalamic–pituitary–thyroid dysfunction [44].

Patients with acute renal failure (ARF) have thyroid function tests similar to NTIS, with low T4, normal TSH, and elevated rT3 levels. A trial of thyroid hormone treatment was undertaken by Acker et al. to determine whether it might help recovery from ARF [46]. Patients (adults) received either l-thyroxine 150 mcg or placebo IV every 12 h for a total of 48 h. Thyroid hormone treatment resulted in a decrease in TSH (vs. a rise in the control group), but it had no effect on any measure of ARF. Mortality was higher in the thyroxine-treated vs. control group (43% vs. 13%). Acker et al. undertook a randomized, double-blind, placebo-controlled trial of T3 treatment in patients

(adults) with ARF due to acute tubular necrosis undergoing kidney transplantation to determine whether it might improve "delayed graft function" [47]. Patients received T3 0.2 mcg/kg IV bolus and a second infusion of 0.2 mcg/kg over 6 h. T3 treatment had no effect on percentage requiring dialysis, time to recovery of renal function, or percentage recovering function. At 1-year follow-up, graft function was similar in both groups.

In summary, the changes seen in thyroid function tests in children with CRI are confounded by the effects of decreased metabolic clearance of iodine, thyroid hormone and its metabolites, and TSH and TRH; decreased T4 to T3 conversion in the kidney; uremic factors that appear to inhibit binding of T4 and T3 to their binding proteins; and drugs used to treat patients after kidney transplant, including glucocorticoids that lower TSH levels. Many or most of these changes in thyroid function tests revert to normal after hemodialysis, arguing against true central hypothyroidism. The few treatment studies carried out in adult patients with ARF do not show benefit and may show harm.

19.7 Cystic Fibrosis

Thyroid dysfunction has been recognized as a potential comorbidity in cystic fibrosis patients for many years. This was commonly seen in the past in association with iodine-based expectorants and took the form of goiter and hypothyroidism. With the decline in the use of these iodine-based expectorants, there has been a change in the character of thyroid disease in this population. A recent study by Lee et al. utilized repository samples obtained from both inpatients and outpatients with CF to examine the nature and prevalence of CF-related thyroid dysfunction [48]. Among 89 subjects with a mean age of 24.4 years, they identified only five patients with elevated TSH (one case of overt hypothyroidism and four cases of subclinical hypothyroidism). Seventeen additional subjects displayed isolated mild hypothyroxinemia thought to represent NTIS. T3 levels were not measured. Neither hospitalization status nor forced expiratory volume in 1 s (FEV1), both potential markers of disease acuity, was predictive of free T4 levels, suggesting that in this case NTIS results from the chronic disease state rather than acute exacerbation.

19.8 Psychiatric Disorders

Changes in thyroid function are reported in patients admitted to inpatient psychiatric services, but the pattern is not classic for NTIS. In one study, children with bipolar disorder had higher TSH levels than controls, though the TSH levels were still in the normal range (2.59 mU/L vs. 2.08 mU/L); T4 and T3 levels were normal [49]. Children with bipolar disorder treated with lithium or divalproex sodium (Depakote) may have elevated TSH levels; these are drugs associated with the development of hypothyroidism. In one study, one-quarter of such children treated for only 3 months had TSH levels >10 mU/L [50]. Female offspring of parents with bipolar disorder appear to have a higher prevalence of autoimmune thyroid disease (16% vs. 4% in controls) [51].

Patients with depression tend to have normal to high T4 or free T4 levels and low TSH concentrations [52]. The high T4 levels combined with hypercortisolism in depression are speculated to inhibit brain D2 activity, resulting in low brain intracellular T3 content. This is hypothesized to be the mechanism explaining why administration of T3 may be effective in patients with depression refractory to tricyclic antidepressants alone [53].

Reports have associated attention-deficit hyperactivity disorder (ADHD) in children with generalized resistance to thyroid hormone (GRTH). Children with GRTH have elevated T4 and T3 levels but normal TSH levels and are at higher risk for ADHD [54]. However, when this question was examined from the perspective of children generally referred with ADHD, thyroid function tests were not suggestive of GRTH [55].

Children with epilepsy treated with certain anticonvulsants may show alterations in thyroid function tests. Children treated with phenytoin (Dilantin) or carbamazepine (Tegretol) may have low serum T4 and T3 and increased TSH levels; these drugs stimulate hepatic P450 metabolism of thyroid hormones [56]. On the other hand, valproate does not appear to alter thyroid function.

In summary, psychiatric disorders are associated with normal or high T4 and TSH levels, the opposite of the pattern of thyroid function test changes seen in NTIS. Some of these changes are medication-induced.

19.9 Summary and Conclusions

NTIS is a common clinical syndrome affecting infants and children with a broad spectrum of acute and chronic illnesses. It is characterized by reduced T3 and T4 levels, increased rT3 levels, variable free T3 and free T4 levels, and inappropriately normal TSH. These changes are the result of alterations in regulation of the HPT axis, peripheral thyroid hormone metabolism as regulated by deiodinase enzymes, thyroid hormone-binding proteins, and thyroid hormone action at the cellular level. The traditional view of NTIS is that it is an adaptive mechanism, protecting the body from high metabolic demands in the face of acute illness. There is, however, evidence that in certain clinical situations, the alterations in thyroid hormone secretion and action seen in NTIS in fact may be detrimental. Particularly in preterm infants who may have worse neurologic outcomes if they have experienced significantly reduced thyroid hormone levels in the neonatal period and cardiac surgery patients who may have prolonged intensive care unit stays, prolonged need for mechanical ventilation and inotropic support, and worse cardiac function in the immediate postoperative period, it is tempting to think that intervention with thyroid hormone may be warranted in NTIS. Treatment studies to date, however, have not shown clear benefit and in some subgroups (preterm infants older than 27 weeks, adult acute renal failure patients) may demonstrate harm. Significantly more research is needed to understand the true impact of NTIS and the appropriate interventions, if any.

Review Questions

1. Which of the following patterns of thyroid function tests is most commonly seen in children with non-thyroidal illness syndrome (NTIS)?

	Total T3	Free T4	TSH	Reverse T3
A	Normal	Low	Normal	Normal
B	Low	Normal	Reduced	Normal
C	Low	Normal	Normal	Normal
D	Low	Normal	Reduced	High
E	Normal	Low	Normal	High

2. Which of the following statements correctly describes the pathophysiological changes underlying NTIS?
   A. Diminished TRH production, decreased D2, decreased D3, decreased thyroid hormone-binding proteins
   B. Diminished TRH production, decreased D2, increased D3, decreased thyroid hormone-binding proteins
   C. Diminished TRH production, decreased D2, increased D3, increased thyroid hormone-binding proteins
   D. Increased TRH production, decreased D2, increased D3, decreased thyroid hormone-binding proteins
   E. Increased TRH production, decreased D2, increased D3, increased thyroid hormone-binding proteins
3. Which of the following patterns of thyroid function tests most likely represents true thyroid dysfunction, even if discovered in the setting of acute illness?
   A. Normal free T4, normal total T3, TSH >5 but <15 mIU/L, negative thyroglobulin and thyroid peroxidase antibodies
   B. Normal free T4, low total T3, low TSH, positive thyroglobulin and thyroid peroxidase antibodies
   C. Low free T4, low total T3, TSH >20 mIU/L, positive thyroglobulin and thyroid peroxidase antibodies
   D. Normal free T4, low total T3, low TSH, negative thyroglobulin and thyroid peroxidase antibodies
   E. Normal free T4, normal total T3, TSH >5 but <10 mIU/L, positive thyroglobulin and thyroid peroxidase antibodies
4. For which of the following pediatric patient populations is there some evidence that NTIS is maladaptive and may warrant treatment?
   A. Preterm infants <27 weeks' gestation
   B. Children with chronic renal insufficiency
   C. Children with diabetic ketoacidosis
   D. Noncardiac PICU patients
   E. Critically ill term neonates

Answers

1. D
2. B
3. C
4. A

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Resistance to Thyroid Hormone (RTH) and Resistance to TSH (RTSH)

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Key Points

- Resistance to TH (RTH) refers to a group of disorders manifesting as impaired sensitivity to thyroid hormone.
- The classical form of RTH is caused in the majority of cases by mutations in the TH receptor beta (*THRB*) gene and is classified as RTH-beta. 15% of patients with this phenotype do not have a detectable mutation in the *THRB* gene, and this entity is known as non-TR-RTH.
- Mutations in the *THRA* gene have been recently reported to cause RTH-alpha. Patients with this defect are phenotypically different from the RTH patients with *THRB* gene mutations in terms of thyroid function tests and clinical features.
- Other disease entities reported are the TH cell membrane transport defect caused by *MCT8* gene mutations and TH metabolism defect caused by *SBP2* gene mutations. These disorders can be distinguished by their characteristic abnormalities of thyroid function.
- Resistance to TSH (RTSH) manifests with high serum TSH of normal biological activity in the absence of goiter. Several genetic defects have been reported to present the phenotype of RTSH, including inactivating mutations in the TSH receptor *TSHR* gene, mutations in the *PAX 8* gene, and a dominantly inherited form without TSHR and PAX8 mutations that is linked to a region on chromosome 15.

20.1 Resistance to Thyroid Hormone (RTH)

20.1.1 Introduction and Background Information

Resistance to thyroid hormone (RTH) refers to impaired sensitivity to thyroid hormone (TH).

The classical syndrome, now known as RTH-beta, is characterized by reduced intracellular action of the active TH, triiodothyronine (T3), in target tissues. This syndrome was first reported in 1967 [1] and was subsequently associated with mutations in the gene encoding the beta isoform of the TR [2], *THRB*. Biochemically, the syndrome is characterized by high serum concentrations of free T4 and usually free T3 as well, accompanied by normal or slightly high serum TSH levels. The reduced sensitivity to TH in the hypothalamus and pituitary leads to mildly elevated TSH levels, which stimulate the thyroid gland to increase production of TH. The severity of TH resistance varies among tissues in an affected individual, due to differences in the relative expression of TR-beta and TR-alpha in different tissues [3] (Fig. 20.1). Thus, patients with RTH-beta may have variable symptoms or signs of hypothyroidism and/or hyperthyroidism. Clinical features of hypothyroidism may include growth retardation, delayed bone maturation, learning disabilities, mental retardation, sensorineural deafness, and nystagmus. Clinical features of hyperthyroidism may include tachycardia, hyperactivity, and increased basal metabolic rate. The majority of patients with the RTH-beta phenotype have autosomal dominant mutations of the *THRB* gene. Patients have been identified from a wide range of races and ethnic groups; the exact geographic distribution of the disorder

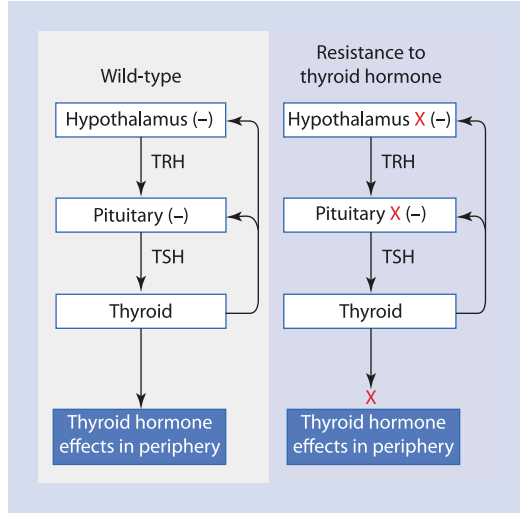

Fig. 20.1 The hypothalamic-pituitary-thyroid axis in RTH-beta patients. TRβ mutations or other (as yet undefined) defects in patients with RTH lead to reduced thyroid hormone responsiveness in the hypothalamus and pituitary, resulting in increased production of thyroid hormone. Impaired thyroid action elsewhere in the body results in the clinical phenotype seen in patients with RTH-beta. However, increased thyroid hormone action on TRα receptors leads to selective tissue hyperthyroidism (e.g., in the heart conduction system)

is unknown, but it has been estimated that RTH occurs in about 1 case per 50,000 live births [4]. Individuals without an identifiable *THRB* mutation but with thyroid function tests consistent with RTH-beta may have non-TR-RTH, and dynamic testing is required to confirm or exclude the diagnosis. Therapeutic strategies for RTH-beta are not well-defined and treatment (if any) must be individualized. Most studies of patients with RTH-beta have been performed in adults, and approaches for the pediatric population may need to be deduced in the absence of firm data. In the past several years, additional syndromes of reduced sensitivity to thyroid hormone, distinct from the classic RTH-beta, have been described, and these will be discussed below.

Until recently, mutations in the *THRA* have remained elusive. In fact, because *THRA* mutations were not previously identified, they were believed to be extremely rare, potentially lethal, or subclinically expressed [5]. Mouse models of TRα defects (knockouts [6, 7] and knock-ins [8]) were investigated in an attempt to infer the phenotype of human *THRA* mutations. The first patients with *THRA* mutations were reported in 2012 [9, 10], and their phenotype is now classified as RTH-alpha. Because TRα is not involved in the feedback regulation of the hypothalamic-pituitary-thyroid axis, their TFTs are distinct from RTH-beta, namely, they have low serum T4, borderline high T3, and very low rT3, with normal to elevated TSH levels. The clinical manifestations in this syndrome are variable but consistent with the manifestations of untreated congenital hypothyroidism in peripheral tissues expressing predominantly TRα such as bone, GI, and CNS tissues [5].

20.1.2 Etiology

The thyroid hormone receptor (TR) is a member of the nuclear hormone receptor (NHR) family of transcription factors. These proteins directly bind DNA to modulate gene transcription [11, 12]. NHRs contain a number of important domains: an N-terminal transactivation or AF-1 domain (A/B domain); a central DNA-binding domain (DBD); and a C-terminal ligand-binding domain (LBD) with ligand-dependent activation (AF-2) function. In addition to binding ligand, the LBD is involved in the recruitment of key nuclear cofactors such as corepressors and coactivators.

TRs and other NHRs bind sequences within regulatory regions of genes; for the TR, these regions are termed thyroid hormone response elements (TREs) [13, 14]. When TRs bind to "positive" TREs (pTREs) in the presence of TH, gene transcription is increased; in contrast, "negative" TREs (nTREs) are involved in thyroid hormone-mediated repression of transcription. Negative TREs have been identified in the promoters of TRH and TSH subunit genes [15–17], but their regulation is less clear. When recruited to pTREs, TRs bind nuclear proteins termed corepressors, including the nuclear corepressor protein (NCoR) and the silencing mediator of retinoid and thyroid hormone receptors (SMRT) [18–23] (Fig. 20.2). These cofactors, in turn, recruit a protein complex with histone deacetylase function [24–26], leading to gene silencing. The binding of T3 leads to a conformational change in the TR, loss of corepressor binding, and subsequent recruitment of coactivators [27]. Coactivators stimulate gene expression by increasing the degree of histone acetylation, modulating interactions with general transcription factors, and other mechanisms [28–36]. More recently, rapid non-genomic actions of thyroid hormone have been identified (independent of transcription) [11], although it is currently unclear how these effects relate to syndromes of RTH.

There are two major isoforms of the TR, termed TRα and TRβ, which are encoded by genes on different chromosomes: *THRA* on chromosome 17 and *THRB* on chromosome 3 [37, 38]. Additional isoforms of TRα and TRβ are generated by alternative splicing or differential promoter usage. Although TRα1 is a true thyroid hormone receptor, TRα2 is an alternatively spliced isoform that does not bind thyroid hormone [39]. In contrast, TRβ1, TRβ2, and the more recently described TRβ3 isoform [40] all bind thyroid hormone and differ only in their N terminus. The function of TRβ3 in vivo remains unknown.

Mutations in the *THRB* gene have been identified in many patients with RTH, and are now classified as RTH-beta. In the vast majority of cases, this syndrome is inherited in an autosomal dominant fashion. A subset of classical RTH patients, however, does not exhibit *THRB* mutations [41], and this entity is known as non-TR-RTH. These patients may have defects in other

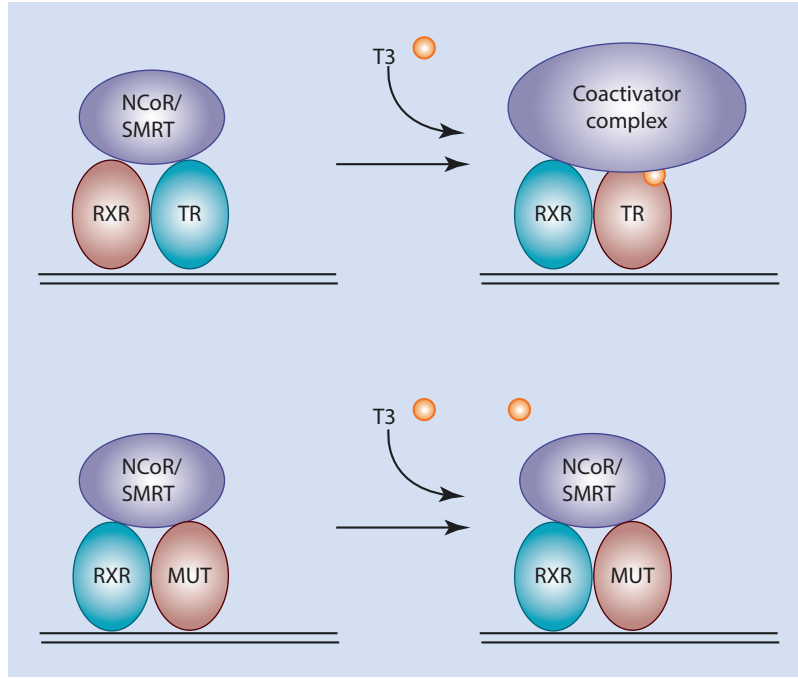

Fig. 20.2 Role of abnormal TR-corepressor interactions in the pathogenesis of RTH. The thyroid hormone receptor (TR) binds DNA as a TR-retinoid X receptor (RXR) heterodimer (or potentially as a TR-TR homodimer). The presence of ligand (T3) results in a conformational change in the TR, leading to dissociation of corepressors (CoR) and subsequent recruitment of coactivators. Mutations of the TR that abolish T3 binding result in constitutive CoR recruitment and loss of T3-mediated stimulation of gene transcription

proteins involved in thyroid hormone action [42], but such mutations have not yet been identified [43]. This is an ongoing area of research, and novel mutations are being sought. A recent search for mutations in the retinoid X receptor gamma (*RXRγ*) gene was not successful [44]. It has been hypothesized that some non-TR-RTH patients may exhibit mosaicism for de novo *THRB* mutations [45].

Mutations of *THRB* that cause RTH-beta generally cluster in three "hot spot" regions of the gene [46, 47]. Most of these mutations interfere with the binding of T3 to the receptor. In these cases, the mutant receptor strongly binds corepressors such as NCoR or SMRT even in the presence of T3 (Fig. 20.2). In a few patients though, the defect does not affect ligand binding, but results in altered corepressor and/or coactivator recruitment [48, 49]. Interestingly, it has been shown that mutant TRs interfere with wild-type TR function, an effect that has been termed "dominant-negative inhibition" [50, 51]. Recently, mouse models have clarified the role of the mutant TRs in the pathogenesis of RTH. Although complete knockout of TRβ produces mice with thyroid function tests consistent with RTH-beta [52], modeling "knock-in" of *Thrb* mutations found in patients with RTH-beta yields mice with more severe resistance [53, 54].

RTH-beta can be subdivided into (i) generalized resistance to thyroid hormone (GRTH) and (ii) pituitary resistance to thyroid hormone (PRTH). In GRTH, the elevated thyroid hormone levels generated by resistance at the level of the hypothalamus and pituitary have diminished activity in the periphery; thus, there is a variable degree of generalized resistance. In contrast, in PRTH (also called central resistance to thyroid hormone, or CRTH), there is resistance solely (or at least primarily) at the level of the hypothalamus and pituitary. This resistance leads to elevated levels of thyroid hormone, but in contrast to GRTH, sensitivity to thyroid hormone is maintained in peripheral tissues, causing thyrotoxicosis. Patients with GRTH frequently exhibit tachycardia due to the high levels of thyroid hormone stimulating intact TRα1 receptors in the heart; thus, tachycardia should not be used to differentiate GRTH and PRTH. Some investigators do not recognize the existence of PRTH as a distinct clinical entity [55], arguing that the same mutations have been reported to cause both GRTH and PRTH [56]. However, a careful evaluation of clinical and biochemical indices in a patient with RTH suggested that PRTH may very well exist [57]. In addition, experiments have revealed that mutant TRs of patients with PRTH behave differently than TRs of patients with GRTH, particularly with respect

to the TRβ2 isoform [58, 59]. A mouse model of the R429Q mutation (which has been reported to cause PRTH) showed that the mutant TR selectively interferes with negative regulation by thyroid hormone [60]. Another study identified differential recruitment of nuclear cofactors by a different TRβ defect associated with PRTH [61].

In contrast, knockout of *Thra* in mice causes a syndrome of hypothyroidism with growth arrest [6, 7]. Mice with heterozygous knock-in *Thra1* mutations are viable and have a heterogeneous phenotype, depending on the severity and the location of the mutation [8]. Most adult *Thra1* mutant mice have a mildly elevated TSH. Various knock-in mouse models manifest other features, such as delayed endochondral ossification resulting in dwarfism, disturbed behavior, memory impairment, locomotor dysfunction, mild bradycardia, and insulin resistance [8]. Thus, a patient with a dominant-negative *THRA* mutation was expected to have a different phenotype from the classical RTH-beta phenotype. In fact, because *THRA* mutations were not identified until recently, they were considered to be extremely rare, lethal, or subclinically expressed [5].

In 2012 the first patients with *THRA* mutations were reported [9, 10], and their phenotype was classified as RTH-alpha. Since these initial reports, additional patients have been reported [62–65], bringing the number of patients known to date to 14, belonging to 9 families. There are eight known mutations affecting the *THRA* gene in patients with RTH-alpha, and they are located in the ligand-binding domain. Four mutations are frame shifts with early termination resulting in a truncated receptor, and the other four are point mutations in the ligand-binding domain and in the C-terminal helix. Six of the eight mutations are located in the part of the gene specific only to the TRa1 isoform, while the other two are located in the part of the protein common between the TRa1 and TRa2 isoforms. As in the case of *THRB* mutations, this results in three different mechanisms causing functional impairment: (i) reduced affinity for T3, (ii) interference of the normal TR-alpha allele by the mutant TR alpha, resulting in a dominant negative effect; and (iii) defective coactivator recruitment to the ligand bound receptor. Interestingly, some of the mutations identified in the *THRA* gene in cases of RTH-alpha were also identified in the *THRB* gene in cases of RTH-beta. Comparison of the alpha and beta forms of RTH for equivalent mutations enables the study of the distinctive roles of the different receptors [5].

20.1.3 Clinical Presentation

20.1.3.1 RTH-Beta Phenotype

The clinical presentation of RTH-beta is variable (► Box 20.1). The initial family described by Refetoff et al. [1] included an 8 1/2-year-old girl and a 12 1/2-year-old boy, both of whom were overall clinically euthyroid but exhibited goiter, deaf-mutism, stippled epiphyses on radiological skeletal survey, and elevated protein-bound iodine (PBI) levels. In contrast to most cases of RTH-beta, this family was shown to have an autosomal recessive pattern of inheritance, and affected family members were later found to have a complete deletion of the *THRB* gene [66]. The heterozygous parents were phenotypically normal, suggesting that a single wild-type TR (in the absence of a mutant TR) may be sufficient for thyroid hormone action. In contrast, most cases of RTH-beta are inherited in an autosomal dominant fashion because mutant TRs exhibit dominant-negative inhibition over wild-type alleles.

Box 20.1 Clinical Characteristics of RTH-Beta in Children

1. Physical exam
    1. Goiter
    2. Tachycardia
    3. Short stature
    4. Low body weight
2. Associated symptoms
    1. Neurological
        1. Developmental delay
        2. Attention deficit hyperactivity disorder (ADHD)
        3. Low IQ
    2. ENT
        1. Deafness
        2. Speech impediment
        3. Recurrent ear, nose, and throat infections
3. Radiological findings
    1. Delayed bone age
    2. Increased thyroid ^{123}I uptake

Derived from data in Refs. [2, 68]

Clinical findings found in patients with resistance to thyroid hormone

Patients with RTH-beta come to medical attention of a variety of reasons. Goiter is the presenting sign in about 38% of cases; less common reasons include learning disabilities, developmental delay, tachycardia, suspected thyrotoxicosis, and elevated thyroxine levels at birth [4]. Thyroid function tests reveal elevated thyroid hormone levels in the setting of a non-suppressed TSH (see "Diagnostic Guidelines" below). Infants with RTH-beta may have congenital deafness, congenital nystagmus, neonatal jaundice, and hypotonia [2]. Patients with RTH-beta may have an increased risk of developing autoimmune thyroid disease [67]. Individuals with RTH-beta who inappropriately undergo thyroidectomy or radioactive iodine treatment will exhibit signs and symptoms of hypothyroidism despite thyroid hormone levels in the normal range following replacement therapy.

A National Institutes of Health study [68] evaluated prospectively a cohort of 42 kindreds manifesting the RTH-beta phenotype. There was autosomal dominant transmission in 22 kindreds, sporadic transmission in 14, and an unknown transmission in 6. This last group was characterized as non-TR-RTH, as they manifested the classical RTH-beta like phenotype without a detectable *THRB* mutation. A palpable goiter was identified in 74% of females and 53% of males. Attention-deficit hyperactivity disorder (ADHD) was present in 72% of the males and 43% of females. IQ was about 13 points lower in patients with the RTH-beta phenotype compared to controls, and one-third of patients had an IQ <85. In contrast, only a few patients had actual mental retardation. Patients with the RTH-beta phenotype had a higher incidence of speech delay (24%), stuttering (18%), and hearing loss than controls. Although resting pulse was higher in patients with RTH-beta, in this particular study, the correlation did not persist after adjustment for age (though it has been noted by other groups). Children with the RTH-beta phenotype exhibited delayed bone maturation. Bone age was delayed in 29% of patients, and 18% had short stature, though another study suggested that the RTH-beta phenotype is not associated with decreased final adult height [69].

As noted above, certain tissues demonstrate increased thyroid hormone-mediated effects in patients with RTH-beta. This is presumably caused by thyroid hormone stimulation of TRα1 in these (TRα1-predominant) tissues. The classic example of this phenomenon is tachycardia, which has been reported in many patients with GRTH. More recently, Mitchell et al. reported that patients with RTH also exhibit increased energy expenditure, muscle mitochondrial uncoupling, and hyperphagia [70].

Findings of abnormal IQ and ADHD in patients with RTH suggest the importance of thyroid hormone in CNS development and function. Matochik et al. used positron emission tomography (PET) scans to study CNS activity in patients with RTH [71]. This study showed that RTH patients have higher cerebral metabolism in certain key areas of the central nervous system (CNS) during a continuous auditory discrimination task, including the anterior cingulate gyrus and the parietal lobe. While PET scanning techniques remain a research tool for RTH-beta, these results suggest an important role for thyroid hormone in these CNS regions. A study of children with ADHD with and without coexisting RTH-beta examined the role of thyroid hormone therapy (in this case, L-T3) in ADHD [72]. The majority of patients with RTH-beta and ADHD improved when placed on T3 therapy, whereas patients with ADHD (in the absence of RTH) deteriorated or remained stable. Thus, ADHD in patients with RTH-beta appears to be distinct from ADHD in patients without RTH [73].

While a number of unusual coexisting conditions in patients with RTH have been reported, some of these may have occurred by chance. These include a birdlike appearance of the face, various vertebral and other skeletal anomalies, short fourth metacarpals, patent ductus arteriosus, and non-communicating hydrocephalus [2].

20.1.3.2 RTH-Alpha Phenotype

The first patient identified with RTH-alpha presented with delayed linear growth, delayed tooth eruption and severe constipation, decreased muscle tone and impaired gross and fine motor skills [9]. Additional defects in the cardiovascular system, including decreased heart rate and blood pressure, were also reported [9]. Currently, 14 patients belonging to 9 families and harboring

8 different *THRA* mutations have been reported in the literature [5]. Clinical data for only 13 of them are available, as the genetic identification of the remaining patient was through whole genome sequencing in subjects with familial forms of autism [64]. The only information about this case was that the female patient was autistic and had a brother who was also autistic but did not harbor the *THRA* mutation identified in his sister [64]. Overall 9 women and 5 men have been reported, but it is too early to define a sex ratio. The cases included de novo and familial mutations. The age at molecular diagnosis varied (7 children or adolescents, and 7 adults), which contributes to certain variations in the clinical descriptions. Further, in the adult patients reported, there is some uncertainty regarding the description of the phenotype in childhood. The clinical presentation of RTH-alpha includes to varying degrees the combination of a dysmorphic syndrome and psychomotor development disorders [5]. The absence of goiter, which is among the typical manifestations of RTH-beta, is particularly notable.

20.1.4 Diagnostic Considerations

20.1.4.1 RTH-Beta

The initial testing of a patient suspected to have RTH-beta phenotype should include routine thyroid function tests. Patients with RTH-beta phenotype have elevated free thyroid hormone levels in the setting of non-suppressed (normal or elevated) TSH levels. Other causes of "euthyroid hyperthyroxinemia" should be excluded (▶ Box 20.2), including methodological laboratory artifacts due to the presence of heterophile antibodies [74]. Such patients may actually be hyperthyroid, with elevated thyroid hormone levels and (appropriately) suppressed TSH levels when measured accurately. This problem has been decreased, but not eliminated, with improvements in the TSH assay. Reevaluation of TSH levels after serial dilutions may be useful. Similarly, patients with autoimmune hypothyroidism occasionally exhibit falsely elevated thyroid hormone levels due to the presence of antibodies interfering with the measurement of T4 and/or T3.

Box 20.2 Causes of Euthyroid Hyperthyroxinemia

1. Methodological artifacts
   1. Antibodies to thyrotropin (TSH)
   2. Antibodies to thyroid hormones (T4, T3)
2. Binding protein abnormalities
   1. Acquired forms of increased TBG
      Estrogen use/pregnancy
      Liver disease
      Acute intermittent porphyria
      Other drugs (methadone, perphenazine, 5-FU)
   2. Inherited
      TBG excess
      Familial dysalbuminemic hyperthyroxinemia (FDH)
      Mutant transthyretin variants
3. T4 to T3 conversion defects
   1. Acquired
      Amiodarone
      Propranolol (high doses)
      Oral cholecystographic contrast agents
   2. Inherited (SBP2 mutations, possibly deidonase defects)
4. Miscellaneous causes
   1. Acute psychiatric illness
   2. High altitude
   3. Amphetamine use
   4. Thyroxine therapy
   5. Non-steady-state conditions of thyroid hormone testing
5. Resistance to thyroid hormone (RTH)

Causes of euthyroid hyperthyroxinemia in the differential diagnosis of resistance to thyroid hormone. Thyrotropin-secreting pituitary adenomas are not included, as they are generally associated with hyperthyroidism

Patients with defects in thyroid hormone-binding proteins, such as TBG, transthyretin, and albumin, can also exhibit abnormal levels of total T4 and T3. Euthyroid patients with TBG excess, which can be congenital or acquired (e.g., in pregnancy [75] and liver disease [76]), have elevated total T4 levels in the setting of a non-suppressed TSH. These patients have normal free T4 levels, when measured directly or estimated based on T3 resin uptake, T3RU. Familial dysalbuminemic hyperthyroxinemia (FDH) is a syndrome caused by the production of albumin variants with Arg-His or Arg-Pro mutations at codon 218 [77, 78]. These albumin variants have increased affinity for

T4. Therefore, measurement of total serum T4 is elevated; correction of T4 based on T3RU may also yield abnormally high results. Free T4 levels are falsely elevated when measured by certain analog measurements, but a free T4 level measured by dialysis will be normal. Serum T3 levels are normal in FDH and exclude the diagnosis of RTH.

Certain medications such as amiodarone [79] and propranolol (at high doses) inhibit T4 to T3 conversion. Euthyroid patients with T4 to T3 conversion defects may have elevated T4 levels and inappropriately normal TSH levels; however, these patients have low-normal or low T3 levels, excluding the diagnosis of RTH. Finally, a few other conditions such as acute psychiatric illness [80] can also cause abnormal thyroid function tests that can occasionally be confused with RTH (► Box 20.2).

Once these various conditions are excluded, the diagnosis is generally one of RTH-beta vs. thyrotroph adenoma [81]. Differentiation between these two disorders can be difficult. In general, TSH adenomas are associated with hyperthyroidism, whereas patients with RTH-beta have variable degrees of compensated thyroid hormone hyporesponsiveness. In addition, patients with thyrotroph adenomas generally have higher serum levels of the glycoprotein alpha subunit than patients with RTH-beta [81], though there is significant overlap.

In terms of radiology studies, patients with TSH adenomas generally have pituitary findings on MRI scans, but it should be noted that patients with RTH-beta may develop incidental pituitary adenomas, mimicking a thyrotroph adenoma [82]. A study used Doppler ultrasonography to determine whether thyroid blood flow distinguishes between RTH-beta and thyrotroph adenomas [83]. These investigators showed that parameters of thyroid blood flow normalized in T3-treated RTH-beta patients, but not in those with TSH-secreting adenomas, but this evaluation is not common practice.

An effective method to confirm a diagnosis of RTH-beta (at least GRTH) is to administer graded doses of T3 and measure a battery of thyroid hormone-responsive tests. Although patients with thyrotroph adenomas have impaired TSH responses to T3, they retain intact peripheral responses to T3. In contrast, patients with GRTH have impaired TSH and peripheral responses to exogenous T3. Patients are generally admitted to a clinical research center for the duration of the protocol. A few different protocols have been described, during which time exogenous T3 is given in an escalating regimen, and various thyroid hormone-responsive measurements are taken [2, 57, 84].

Most cases of RTH-beta are associated with mutations in the TRβ gene. Ultimately, the most secure way to make a diagnosis of RTH-beta is to demonstrate (a) elevated thyroid hormone levels in the setting of a non-suppressed TSH, (b) thyroid hormone hyporesponsiveness, and (c) a *THRB* gene mutation.

20.1.4.2 Neonatal Considerations

If a child is born to a parent with RTH with a known *THRB* mutation, the most straightforward way to confirm or exclude the diagnosis in the infant is to sequence the known mutation. There are two other ways infants with RTH frequently come to medical attention: (a) symptoms consistent with RTH and (b) abnormal thyroid screening tests. Infants with RTH may have congenital deafness, congenital nystagmus, neonatal jaundice, and hypotonia. In addition, screening programs that are in place to identify infants with congenital hypothyroidism occasionally identify RTH instead. A fetus with suspected RTH can be tested for TR mutations by chorionic villus sampling or amniocentesis and DNA analysis [85], though the benefits of making the diagnosis at this stage of development have not been clearly established.

20.1.4.3 RTH-Alpha

As RTH-alpha patients present primarily with dysmorphic features and GI symptoms in the context of only mild thyroid test abnormalities, they might not present early to the attention of the endocrinologist. There is no standard testing recommended for these patients at this point. Astute clinicians who identify the syndromic features of this defect and review the literature will be able to consider *THRA* mutations as a possible diagnosis. The typical pattern of TFTs includes low or normal T4, high normal T3, and normal or slightly elevated TSH. Identification of more patients will allow further insight into this novel defect.

20.1.5 Outcomes and Possible Complications

20.1.5.1 RTH-Beta

There is no reason to treat patients with RTH-beta who have elevated levels of TH that are appropriate for the degree of both thyrotroph and peripheral tissue resistance. In fact, the mainstay of the management of asymptomatic RTH-beta patients is to recognize the correct diagnosis and avoid antithyroid treatment. Attempts aimed to decrease the circulating TH levels, either with antithyroid drugs, or with ablative treatment by surgery or radioiodide, will result in objective findings of TH deprivation and administration of supraphysiological doses of TH is required. The dose of TH needs to be uptitrated in order to normalize the serum TSH levels and high doses of L-T4 be necessary, sometimes as high as 500–1000 μg/d.

20.1.5.2 RTH-Alpha

Mutations in THRA gene have been only recently recognized, and limited data is currently available to accurately assess long-term outcomes and possible complications. Based on the few patients known to date, it seems that L-T4 treatment during childhood has some benefits; however, there is the risk of making the TR-beta predominant tissues hyperthyroid. Lack of intervention has an overall poor outcome in patients with severe *THRA* mutations.

20.1.6 Treatment

20.1.6.1 RTH-Beta

No specific therapy is available to correct the underlying defect in RTH. Frequently, patients with RTH are in a clinical state of compensated thyroid hormone hyporesponsiveness. In these patients, no specific therapy is indicated. In those few patients who have greater peripheral hyporesponsiveness and thus clinical hypothyroidism, treatment with thyroid hormone (e.g., levothyroxine) may be considered. If used, the specific dosage must be individualized based on markers of thyroid hormone action (such as SHBG, cholesterol, ferritin, BMR, and bone density) [56]. The use of TRIAC (3,5,3′-triiodothyroacetic acid), a thyroid hormone analog with relative specificity toward the TRβ receptor [86, 87], has been advocated for use in patients with RTH, but its specific role has not been clearly defined. D-Thyroxine has also been used [88], though one study suggested it was less effective than TRIAC [89]. Novel TR analogs hopefully will be developed that activate mutant receptors [90]. Overall, therapy (or lack thereof) must be individualized for each patient. Of course, for patients who have had their thyroid glands inappropriately ablated for misdiagnosed hyperthyroidism, treatment with thyroid hormone will be necessary.

In patients with PRTH, beta blockers have been used to control symptoms; the use of antithyroid drugs in this situation is controversial, and these medications are not indicated in patients with GRTH. Agents that have been used to decrease TSH levels include somatostatin analogs and bromocriptine, but these have had only limited success.

The care of RTH patients during pregnancy needs to be individualized as well and depends on the genotype of both the fetus and mother [91]. High miscarriage rates of wild-type fetuses of pregnant RTH mothers has been suggested to be due to high circulating levels of thyroid hormone [92]. A prenatal diagnosis of RTH was made [85] in the fetus of a 29-year-old pregnant woman at 17 weeks' gestation. The fetus and mother were both found to harbor the *THRB* mutation (T337A), and the pregnant woman was treated with TRIAC with beneficial effects on maternal symptoms and fetal goiter size. Cordocentesis was performed to evaluate effects of the medication on fetal thyroid function tests. However, an accompanying editorial to the report [56] points out some potential dangers of this approach, since cordocentesis led to the need for emergency C-section.

In children with RTH, special care should be directed toward issues of growth and mental development. Patients with delayed bone age may be candidates for therapy. One approach is to consider treatment in children with the following signs and symptoms: (a) elevated serum TSH levels, (b) unexplained failure to thrive, (c) unexplained seizures, (d) developmental delay, and (e) history of growth or mental retardation in other affected members of the family [56]. As noted above, patients with RTH and coexisting ADHD may improve when treated with thyroid hormone [72]. In any case, patients who require treatment

should be followed closely, with careful evaluation of growth, bone age, and thyroid-responsive biochemical indices.

20.1.6.2 RTH-Alpha

L-T4 therapy has been shown to have beneficial effects on certain components of the phenotype, however, cognitive and fine motor skill defects do not improve [5]. During L-T4 treatment, serum T4 and rT3 normalized while serum T3 remained elevated, and this resulted in suppressed TSH. However, some peripheral markers of TH action have been reported to respond to L-T4 therapy, and markers of bone turnover rose progressively upon L-T4 treatment, with certain markers, including procollagen type 1 N-propeptide, and C-telopeptide cross-linked collagen type I, becoming frankly elevated. Some of the patients reported being more energetic and showed greater alertness after the initiation of L-T4 therapy, and L-T4 therapy induced an initial catch-up growth in one patient. Of note, in all patients, L-T4 treatment had a beneficial effect on constipation. However, L-T4 therapy may be of limited benefit in RTH-alpha, as the resulting hyperthyroidism in TRβ-expressing tissues may preclude the use of higher doses of L-T4. L-T3 treatment was tried in only one case and led to a reduction in TSH and consequently T4 levels, with an increase in heart rate; thus, L-T3 treatment might be difficult to implement [5]. It is possible that different treatment regimens could be used in children vs. adults, considering the important role of TH in growth and development. Thus judicious supplementation may be necessary in young patients, while the L-T4 doses used in adults could be lower. As only a few patients with RTH-alpha are known, there is limited information about treatment of this condition. Alternative therapeutic strategies could involve development of TRα1 subtype-specific hormone analogues.

Case Study

A 1-year-old baby presents for genetic evaluation of his thyroid condition. He is the first child born to unrelated parents. He was premature due to the mother developing preeclampsia and C-section was performed at 32 weeks. Apgar score was 9/9, BW 1320 g, BL 46 cm, he was diagnosed as being small for gestational age, and heart rate was 130/min. Due to initial hypoglycemia, GH, insulin, cortisol, TSH, and fT4 were tested. TSH and fT4 were found to be elevated at 7.1 mIU/L and 51 pmol/L, respectively. Thyroid ultrasound showed a mildly enlarged gland (right lobe of 0.76 × 1.05 cm and left lobe of 0.84 × 0.71 cm), with normal structure. He had no signs of hypo- or hyperthyroidism, and heart rate was 134/min even when asleep. He was initially treated with PTU and propranolol; however a pediatric endocrine consult recommended stopping this regimen. He was seen later in Pediatric Endocrine Division at 4.5 months, and at that time his weight was 5.380 kg, length 59.7 cm, head circumference 41 cm, and HR 160/min, ranging from 130/min to 160/min, even during sleep. Thyroid gland was easily palpable and TSH was 5.53 mIU/L (0.62–8.05) and fT4 60.10 pmol/L. A complete panel of thyroid function tests showed markedly elevated TH levels TT4 18.8 mcg/dL (5–11.6), TT3 418 ng/dL (90–195), while TSH 2.9 mU/L (0.4–3.6). This phenotype was indicative of RTH-beta, and sequencing of the *THRB* identified a de novo mutation in the patient, predicted to be a pathogenic variant. This case illustrates a case of early diagnosis of RTH-beta prompted by abnormal blood tests in perinatal period. It also illustrates an initial incorrect approach attempting to decrease TH levels although TSH was not suppressed. Symptomatic evaluation of these patients over time will dictate what intervention is needed if at all.

20.1.6.3 Summary

RTH is an inherited syndrome characterized by reduced responsiveness of target tissues to thyroid hormone caused by mutations in the TH receptor genes.

Mutations in the *THRB* cause the classical form of RTH known as RTH-beta and patients present with elevated TH levels with unsuppressed TSH. The reduced sensitivity to TH at the level of the hypothalamus and pituitary leads to elevated TSH levels, which stimulate the thyroid gland to increase production of TH. Both hyperthyroid and hypothyroid signs or symptoms may be present depending on the relative expression of TR-β and TR-α in different tissues.

Identification of *THRA* mutations has led to the characterization of RTH-alpha. These patients are phenotypically different from the RTH-beta patients in both TFTs and clinical features. Typical TFTs include low serum T4, borderline high T3, and very low rT3, with normal to elevated TSH. The resistance to TH is limited to tissues in which TH action is predominately mediated by TR-α such as bone, the GI tract, and the CNS.

20.2 Additional Thyroid Hormone Insensitivity Syndromes

In recent years, additional genetic syndromes have been identified that are associated with decreased thyroid hormone sensitivity in one form or another (but distinct from RTH). Such syndromes generally involve defects in thyroid hormone metabolism or transport.

For many years, it was thought that thyroid hormone diffused passively through cell membranes. We now know that thyroid hormone is transported into cells by a variety of transporter proteins [93]. One of these transporters is MCT8 and its gene is located on the X chromosome. Multiple patients with *MCT8* mutations have now been identified that exhibit X-linked mental retardation, dysarthria, athetoid movements, muscle hypoplasia, spastic paraplegia and hypotonia presenting in infancy or childhood [94, 95], also called the Allan-Herndon-Dudley syndrome. In these patients, serum T3 levels are elevated (from increased Type 1 deiodinase deficiency), free T4 levels are low, and TSH is normal or mildly increased. The severe CNS symptoms are due at least in part to impaired transport of thyroid hormone in the brain. Although brain T3 levels have been documented to be low, liver T3 levels are high [96], so that patients have a complex mix of hypothyroid and hyperthyroid symptoms. While current therapeutic options are limited to supportive measures, a recent study identified a thyroid hormone analog (3,5-diiodothyropropionic acid (DITPA)) that did not require MCT8 for transport and may represent a novel modality for patients suffering from this syndrome [97].

While mutations in deiodinase genes have not been identified, a recently described syndrome identified mutations in *selenocysteine insertion sequence-binding protein 2 (SECISBP2 or SBP2)*. SBP2 is involved in the incorporation of the rare amino acid selenocysteine to generate selenoproteins. Since deiodinase enzymes are selenoproteins, these recessive mutations result in abnormalities in thyroid hormone metabolism. Affected patients exhibit low T3 levels, high T4 levels, and normal or slightly elevated TSH levels [98, 99]. Recently, other kindreds with *SBP2* mutations were found to have coexisting azoospermia, axial muscular dystrophy, photosensitivity, abnormal immune function, and insulin sensitivity [100], suggesting that SBP2 deficiency produces a complex, systemic selenoprotein deficiency syndrome.

20.3 TSH Receptor Mutations

20.3.1 Introduction and Background Information

Thyrotropin (TSH) is a member of the glycoprotein family of hormone secreted by the anterior pituitary, along with luteinizing hormone (LH) and follicle-stimulating hormone (FSH) [101]. These hormones along with chorionic gonadotropin consist of noncovalently linked α and β subunits, with linked carbohydrate chains. While the β subunit of each hormone is unique, all share a common α subunit. TSH stimulates the growth and function of thyroid follicular cells, leading to the production of thyroid hormone. Thus, resistance to TSH (RTSH) ranges from a compensated state of euthyroid hyperthyrotropinemia to frank hypothyroidism. The first report of a patient with resistance to the biological properties of TSH was in 1968 [102], but it was not until 1995 that the first patient with a *TSH receptor (TSHR)* mutation was firmly documented [103].

20.3.2 Etiology

In 1995, Sunthornthepvarakul et al. documented the first case of a *TSHR* mutation leading to TSH resistance [103]. The index case was an infant born to unrelated parents found to have an elevated TSH level on routine neonatal screening. Two siblings were also found to have high TSH levels and normal thyroid hormone levels. All were clinically euthyroid and found to be compound heterozygotes for mutations in exon 6 of the *TSHR* corresponding to a region in the TSH extracellular domain [103]. In vitro data confirmed the mutant

receptors exhibited decreased biological activity. Since that time, a number of other patients have been identified with *TSHR* mutations and TSH resistance, though additional patients have been identified without identifiable mutations. Most patients with *TSHR* mutations are either homozygotes or compound heterozygotes.

The TSHR is a transmembrane G-coupled receptor (Fig. 20.3). It contains a large extracellular domain with three regions—the middle of these regions (approximately amino acids 58–288) contains the most significant homology to FSH and LH receptors [104]. The extracellular domain may inhibit constitutive activity of the receptor [105]. The carboxy-terminal portion of the TSH receptor includes the transmembrane domain, which spans the plasma membrane seven times, and an 82 amino acid cytoplasmic tail [105]. The gene encoding the TSH receptor has been localized to chromosome 14q31 [106, 107].

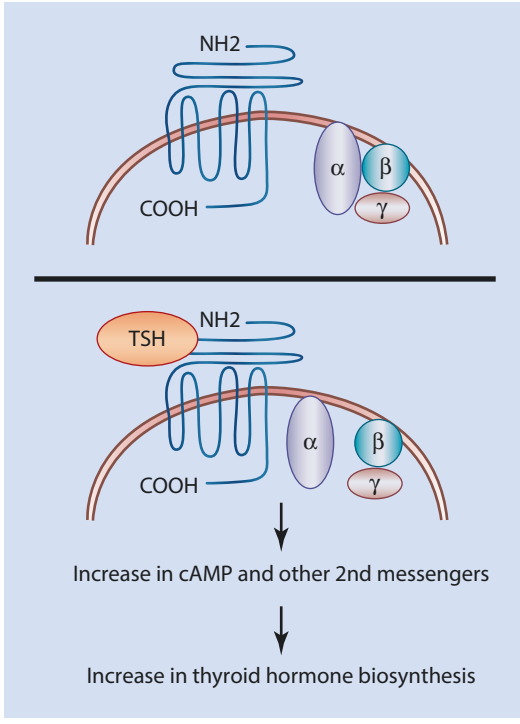

Fig. 20.3 Schematic diagram of the TSHR. The TSH receptor is composed of an N-terminal extracellular domain, a transmembrane region (which spans the plasma membrane seven times), and a cytoplasmic tail. Stimulation of the TSH receptor leads to G-protein dissociation and activation

20.3.3 Clinical Presentation

There are two general modes of presentation for patients with loss-of-function germline *TSHR* mutations. The first is similar to the family identified by Sunthornthepvarakul et al. [103]. In these patients, high TSH levels are necessary to overcome partial TSH resistance, and patients remain euthyroid (compensated euthyroid hyperthyrotropinemia). Four additional families with these clinical characteristics were identified by de Roux et al. [108]. One patient had a homozygous mutation in codon 162 of the *TSHR*; the other three were compound heterozygotes. Interestingly, one of the mutations (C390W) caused loss of TSH binding, whereas another (D410N) resulted in normal TSH binding but an inability to activate the second messenger adenylate cyclase. Mutations affecting signal transduction were also found in extracellular (D410N) and intracellular (F525 L) domains [108]. Additional mutations have been identified from patients with euthyroid hyperthyrotropinemia [109, 110].

In contrast, other *TSHR* mutations cause more extreme hormone resistance. Patients with these mutations present with hypothyroidism and may be identified by neonatal screening. Abramowicz et al. reported two such patients, a brother and sister, who were diagnosed with congenital hypothyroidism [111]. Ultrasound evaluation revealed hypoplastic thyroid glands. A homozygous mutation of the *TSHR* in the fourth transmembrane domain (A553T) was identified; the parents and unaffected siblings were heterozygous for the same mutation. In vitro analysis suggested that there was decreased expression of the mutant receptor at the cell surface [111]. Severely affected patients have been identified by other groups as well [112–114].

Not all patients with resistance to TSH have mutations in the *TSHR* [115]. Patients with pseudohypoparathyroidism caused by mutations in *GNAS* may exhibit resistance to a variety of hormones including TSH [116]. Mutations in transcription factors involved in thyroid gland development such as Pax8 [117] and TITF1 (Nkx2.1) [118] have been reported to cause resistance to TSH. Finally, Grasberger et al. identified multiple kindreds with resistance to TSH inherited in an autosomal dominant fashion without identifiable mutations [119]. However, the

phenotype in these patients was linked to a locus on chromosome 15 [120].

Recently, *DUOX2* genetic defects have been recognized to manifest a phenotype of TSH resistance. *DUOX2* mutations have been shown to cause elevated TSH at neonatal screening in Korean and Chinese patients that were found to be either euthyroid or have subclinical hypothyroidism, transient, or permanent congenital hypothyroidism [121, 122]. In this population, pathogenic variants in other genes known to manifest as TSH resistance were also identified, namely, *TSHR* and *PAX8*. The coexistence of multiple pathogenic variants seemed correlated to the severity of the hypothyroid condition.

20.3.4 Diagnostic Considerations

Mild TSH resistance (euthyroid hyperthyrotropinemia) is easily confused with subclinical hypothyroidism, since both present with elevated TSH levels in the setting of normal free thyroid hormone levels. Most cases of subclinical hypothyroidism are due to underlying autoimmune thyroid disease, which is generally absent in resistance to TSH. Patients with more severe TSH resistance present with thyroid function tests consistent with primary hypothyroidism. Patients with resistance to TSH, however, do not have a goiter, and the disorder is usually (but not always) inherited in an autosomal recessive pattern.

TSH resistance may be detected by neonatal screening programs. Since congenital hypothyroidism is not generally inherited, a significant family history of congenital hypothyroidism is suggestive for TSH resistance. A recent study in Japan of congenital hypothyroid infants found that 4.3% had biallelic TSH receptor mutations; the authors estimated that the frequency of *TSHR* heterozygous carriers to be 1 in 172 in that population [123]. Thus, the prevalence of TSH resistance may be higher than previously appreciated.

20.3.5 Outcome and Possible Complications

Outcome is generally good for these patients as they compensate for the TSH resistance with increased TSH levels. Only in rare instances of severe TSH resistance that go undetected at neonatal screening does one see the natural history of congenital hypothyroidism. However this occurrence is rare as detection at the neonatal screen prompts treatment with L-T4.

The opposite situation can occur when a patient with mildly elevated TSH and normal TH levels gets treated with L-T4 in an attempt to normalize TSH. In this case the identification of the genetic defect in the *TSHR* can prevent further unnecessary treatment.

20.3.6 Treatment

Patients with mild TSH resistance and mild hyperthyrotropinemia are clinically euthyroid and do not require treatment, although they should receive genetic counseling. Patients with more severe TSH resistance and frank hypothyroidism are treated with levothyroxine.

Case Study

A 7-year-old boy presents for genetic evaluation of his thyroid condition. He had elevated TSH at newborn screening, and without initial thyroid imaging he was started on L-T4. Reportedly his TSH was increased at every trial made to discontinue L-T4, while TT4 and freeT4 were normal. At 2 years of age, thyroid uptake and scan I-123 showed activity bilaterally, with mildly asymmetric increased activity on the right, no nodules, and uptake of 12.2% at 2 h (5–10%) and 6.8% at 24 h (6–33%). Thyroid ultrasound showed normal size and echogenicity with the right lobe measuring 1.6 × 0.8 × 0.6 cm, left lobe 0.6 × 1.3 × 0.4 cm, and isthmus 1 mm. A complete panel of thyroid function tests performed in the patient and his family showed mild elevation of TSH in the patient, his sister and father ranging from 5.3 mU/L to 11.6 mU/L (normal values 0.4–3.6). T4, T3, and rT3 levels were in the normal range and anti-TG and anti-TPO antibodies were negative. Sequencing of the *TSHR* gene identified a missense mutation in exon 10 inherited from the father and present in the heterozygous state in all three individuals. This case illustrates that occasionally even heterozygous *TSHR* mutation can manifest a typical phenotype of resistance to TSH, mostly depending on the consequence of the mutation on the function of the TSHR protein.

20.3.7 Summary

Resistance to TSH is characterized by high serum TSH and normal or low serum T4 and T3 in the setting of normal or hypoplastic thyroid glands.

The phenotype of RTSH can be caused by inactivating autosomal recessive or rarely autosomal dominant mutations in the *TSH receptor* gene and autosomal dominant mutations in the *PAX 8* gene. Most recently also *DUOX2* gene mutations have been recognized as a possible genetic defect resulting in this phenotype. Other cases have been linked to a locus on the chromosome 15.

RTSH should be suspected in patients who have high serum TSH concentrations, normal or low serum free T4 and T3 concentrations, and a normally located thyroid gland.

For RTSH patients in whom the impaired response to TSH is fully compensated by the increased TSH and who are euthyroid, no TH treatment is needed

If the elevated serum TSH cannot fully compensate for the defect, the patient should be treated with thyroid hormone, similar to treatment of hypothyroid patients.

Review Questions

1. A 10-year-old boy presents for evaluation of goiter and growth delay. Previously he was diagnosed with ADHD. His thyroid function tests were checked, and free T4 was found elevated at 4.6 mcg/dL (0.9–1.7), and TSH was 5 mU/L (0.4–3.6). Thyroid antibodies and TSI were negative. Thyroid ultrasound showed an enlarged gland. What is the most likely diagnosis?
   A. TSH secreting pituitary adenoma
   B. Activating *TSHR* mutation
   C. RTH caused by mutations in the *THRB* gene
   D. RTH caused by mutations in the *THRA* gene
2. A baby boy born to non-consanguineous parents living in the USA presents with an elevated TSH of 30 mU/dL at neonatal screening. On day 7, T4 and T3 levels are in the normal range, thyroid gland is normal on ultrasound, and he does not have hypothyroid symptoms. What is the diagnosis?
   A. Iodine deficiency
   B. TSH secreting pituitary adenoma
   C. *TSH receptor* mutations
   D. Resistance to thyroid hormone

Answers

1. C
2. C

20.4 Conclusions

Hormone resistance leading to thyroid dysfunction can occur at multiple levels of the hypothalamic-pituitary-thyroid axis. Care of patients with RTH must be individualized, and the endocrine status of the patient must be determined. In children, special attention must be paid to growth, bone development, and mental development. Further studies in children should be performed so that medical care can be optimized in these patients. RTH is usually caused by autosomal dominant mutations of the *THRB* gene. In contrast, resistance to TSH is usually caused by autosomal recessive mutations in the *TSH receptor* gene. However, patients with both disorders have been identified without identifiable mutations. These patients probably harbor mutations in other important endocrine genes. Further evaluation of these patients will be important not only to optimize their medical care but also to gain fundamental insights into the mechanisms of action of thyroid hormone, TSH, and other hormones.

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Thyroid Neoplasia

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Key Points

- Thyroid nodules are common among children with autoimmune thyroid disease and goiter and in cancer survivors who received radiation therapy.
- Evaluation using ultrasound and fine needle aspiration is critical for identifying thyroid cancer.
- Papillary thyroid cancer is the most common form of thyroid cancer in children.
- Overall survival is excellent, but therapy must be individualized in order to reduce the risk of complications from treatment.

21.1 Background

The World Health Organization divides thyroid neoplasms into thyroid carcinomas, thyroid adenomas, and other thyroid tumors [1]. In this chapter, we will review thyroid nodules, differentiated thyroid cancers (papillary thyroid cancer, PTC; follicular thyroid cancer, FTC), and medullary thyroid cancer (MTC). The latter is usually associated with multiple endocrine neoplasia (MEN) type II in children [2]. Much has been learned about pediatric thyroid tumors since this material was last published, including publication of the inaugural guidelines for the management of thyroid nodules and cancers in children [3]. This chapter will focus on recent updates in the field and includes a case study that demonstrates application of these new guidelines to patient care.

21.2 Thyroid Nodules

Thyroid nodules (TN) are discrete lesions that are distinct from the surrounding thyroid. Nonpalpable TN may be found during imaging for other reasons ("incidentalomas") but have the same risk for differentiated thyroid cancer (DTC) as do palpable TN of similar size [3]. Estimates from ultrasound (US) and postmortem examination suggest that 0.2–5% of children and 13% of adolescents have thyroid nodules [4], and thyroid cysts occur in 57% of children screened using US [5]. However, it remains unclear how many of these lesions would ever grow to reach a threshold for clinical detection or require treatment.

Factors that increase the risk for TN to develop in children include family history of TN or thyroid cancer, iodine deficiency, radiation exposure, autoimmune thyroid disease (AIT), goiter, elevated serum thyrotropin (TSH), and several genetic syndromes.

The management of children with TN has changed over the past decades. Prior to the 1980s, virtually all thyroid nodules in children were removed. By the 1990s, high-resolution US and fine needle aspiration (FNA) were performed to identify DTC preoperatively so that an appropriate "cancer surgery" could be performed. However, benign TN were also removed due to concerns about possible malignancy. Now, US and FNA are used to identify DTC but also to diagnose benign TN. "Apparently benign" TN are not summarily removed but are expectantly monitored for growth or the development of concerning US changes. The challenge is to distinguish those TN that should be removed from those that can be followed.

21.2.1 Evaluation of Thyroid Nodules

In adults, 7–15% of TN are malignant, but the risk for DTC in children is higher (range 3–70%, average between 22% and 26%) [6]. The American Thyroid Association (ATA) Guidelines for Managing Thyroid Nodules and Cancer in Children suggests that palpation of the thyroid by experienced examiners is sufficient screening to identify DTC that require evaluation and treatment [3]. However, 40% of DTC in children are discovered by the parent suggesting that palpation of the thyroid by primary care providers is either insufficient or too infrequently performed to allow timely detection of many DTC [7]. For that reason, children at high risk for DTC are commonly evaluated by US and, when indicated, FNA.

21.2.1.1 Family and Individual Medical History

A detailed history of familial thyroid disorders, iodine intake, radiation exposure, and antecedent thyroid disease should be obtained. The risk for DTC is 2.5-fold higher for children with a family history of benign thyroid disease and 4-fold higher for children with a family history of DTC [8–15]. US surveillance of at-risk children detects

DTC of smaller size and with a lower incidence of lymph node metastasis (23.2% vs. 65.6%) and extrathyroidal extension (20.9% vs. 56.2%) suggesting that US screening of children with a family history of thyroid cancer may offer benefit [15].

In addition, there are several specific genetic syndromes that are associated with TN and DTC. Benign and malignant thyroid tumors can occur in patients with familial adenomatous polyposis [FAP] [16, 17], Carney complex [18], PTEN [phosphatase and tensin homolog] hamartoma tumor syndrome [PTHS; Cowden syndrome] [19, 20], Werner syndrome/progeria [21], and DICER1 syndrome [22]. There are no clear recommendations for screening except for DICER1-related disorders, PTHS, and FAP [23–25].

21.2.1.2 Iodine Intake

Chronic iodine deficiency is associated with an increased risk of thyroid dysfunction, thyroid nodules, and DTC [26, 27]. An estimated 5% of children in the United States have iodine deficiency, spanning all socioeconomic groups [28].

21.2.1.3 Radiation Exposure

TN develop in childhood cancer survivors who received radiation therapy (RT) at a rate of about 2% annually and reach a peak incidence after 15–25 years [29–31]. The risk is greatest following RT at younger age and with doses up to 20–29 Gy [32–34]. Up to 20% of long-term cancer survivors have a TN > 1 cm detected by US that is not apparent on physical exam [3, 35, 36]. The risk for DTC is increased in survivors who had Hodgkin lymphoma, leukemia, or central nervous system tumors [32, 37], but there is also a fivefold greater risk following treatment for neuroblastoma which suggests the possibility of shared genetic risk factors [38].

21.2.1.4 Autoimmune Thyroid Disease

AIT, Graves' disease, and goiter are associated with an increased risk for TN and DTC in children [39–42]. In one prospective study, US identified TN in 31.5% of children with AIT, but only 1/3 of these could be palpated [39]. There were 11 PTC, of which only 4 could be palpated. In a retrospective review of patients referred for thyroidectomy, Kovatch et al. identified thyroid nodules in 41% and DTC in 22% of children and adolescents with Graves' disease [42]. A recent study evaluated 113 Korean children with goiter and found nodules in 63.7% [40]. The prevalence of nodules in this cohort was much greater than that of a previous US cohort (17%) suggesting there may be regional, genetic, or environmental confounders [41]. Routine US screening remains controversial, and the ATA guidelines recommend an US should be performed whenever the gland feels suspicious of if abnormal lymphadenopathy is found on physical exam [3].

21.2.1.5 A Serum Thyrotropin (TSH) Level Should Be Obtained

Patients with a TN and serum TSH in the upper tertile may be at increased risk for DTC [43–46], although this was not confirmed by the European Prospective Investigation into Cancer and Nutrition Cohort (EPIC) [47]. Conversely, children with suppressed TSH are more likely to have a hyperfunctioning (hot) thyroid nodule with a lower risk of malignancy [48].

21.2.1.6 A Dedicated Thyroid and Neck US Should Be Performed on All Children with a Suspected or Incidental TN [3]

The US should describe the TN composition (solid, cystic, or spongiform), echogenicity, margins, presence or absence of calcifications, shape, vascularity, and presence or absence of suspicious cervical lymphadenopathy [49]. For adults, a stratification system was published in the 2015 American Thyroid Association Guidelines for Management of Adults with Thyroid Nodules and Cancer which correlates US features with malignancy risk and identifies TN that require FNA [50]. However, there are differences in the prevalence of subtypes of DTC in children that limit extrapolation of these criteria to children. In addition, children have a greater overall probability of DTC than adults [6, 51, 52], and children may have a widely invasive form of PTC called diffuse sclerosing variant PTC (dsvPTC) [53–61]. DsvPTC does not usually present as a discrete TN but does have abundant microcalcifications that are visualized on US. Typically, dsvPTC presents as diffuse enlargement of a thyroid lobe or the entire gland, which is often associated with cervical lymphadenopathy and elevated anti-thyroglobulin antibody titers [62, 63]. For these reasons, in children, a combination of patient history, US characteristics,

and clinical context is used to identify TN that warrant FNA. Sonographic features of TN that are associated with PTC in children include hypo-echogenicity, invasive margins, increased intra-nodular blood flow, microcalcifications, and abnormal cervical lymph nodes [64, 65]. Unfortunately, none of these are sufficiently robust to identify all malignant TN in children, and for that reason, FNA is warranted for almost all TN in children (see below).

21.2.1.7 US-Guided FNA Is the Most Accurate Method to Identify DTC (Fig. 21.1)

In children, an US-guided FNA should be performed on TN > 10 mm unless the lesion is purely cystic and on TN 5–10 mm that show any of the suspicious US features listed above [46]. FNA should also be performed on any suspicious lymph nodes in the lateral neck compartments. Thyroglobulin [Tg] measured from the FNA needle washout may be added if the cytology from the lymph node is indeterminate for metastatic disease [66, 67]. This is required to confirm the presence of DTC prior to a lateral neck lymph node dissection [3]. The sensitivity, specificity, and accuracy of FNA in children are similar to that of adults [overall accuracy (91%), sensitivity (100%), and specificity (88%)] [4], but there is a greater risk for false-negative FNA in large (> 4 cm) lesions [68–71], and there appear to be differences in the risk of DTC for some classes of cytopathology [50, 72, 73].

All FNA results are categorized according to The Bethesda System for Reporting Thyroid Cytopathology [74], which includes six tiers:

1. Nondiagnostic or unsatisfactory
2. Benign
3. Atypia or follicular lesion of undetermined significance (AUS/FLUS)
4. Follicular/Hürthle neoplasm or suspicious for follicular/Hürthle neoplasm (FN or SFN)
5. Suspicious for malignancy (SUSP)
6. Malignant

A recent study in children found DTC in 10% (4/41) of TN with benign FNA cytology, 50% (3/6) of TN with SFN cytology, 86% (6/7) of TN with SUSP cytology, and 100% (7/7) of cases with malignant cytology [73]. The risk of malignancy in each Bethesda Class appears to be higher in children than in adults (Table 21.1) [72–79].

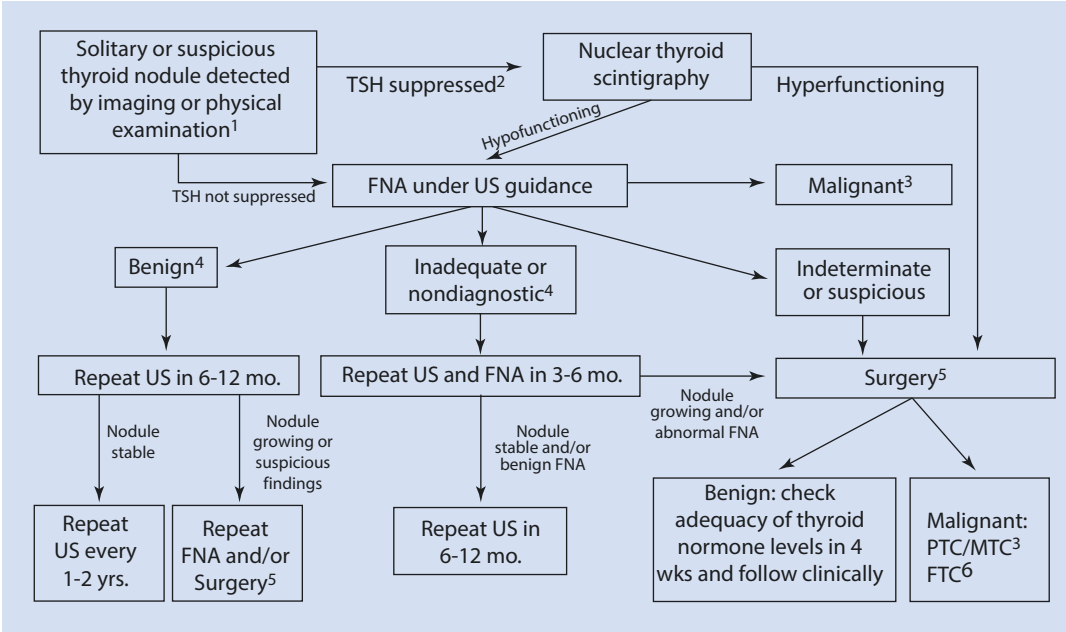

Fig. 21.1 Evaluation and treatment for thyroid nodules. Fine needle aspiration is the cornerstone to diagnosis and management. [1]Risk for cancer is higher in children with radiation exposure. This algorithm may not apply to irradiated patients . [2]See Fig. 21.2 (PTC algorithm) . [3]Surgery can always be considered if concerning clinical features, size >4 cm, compressive symptoms, and/or patient preference . [4]Completion thyroidectomy and possible RAI

Table 21.1 Risk of malignancy in Bethesda cytopathology of FNA classes

Pediatric[a]		Adult[b]	
FNA cytopathology	**Cancer risk**	**FNA cytopathology**	**Cancer risk**
Nondiagnostic	"Usually benign"	Nondiagnostic	1–4%
Benign	5–10%	Benign	0–3%
AUS/FLUS	28%	AUS/FLUS	5–15%
Follicular neoplasm or suspicious for follicular neoplasm	>50%	Follicular neoplasm or suspicious for follicular neoplasm	15–30%
Suspicious for malignancy	86–100%	Suspicious for malignancy	60–75%
Malignant	100%	Malignant	97–99%

[a]Based on limited studies in children [72, 73] and multiple studies in adults[b] [50]

The recommendation for and extent of surgery are based on the individual DTC risk, the likelihood of false-negative FNA, the risks of surgery, and the tolerance for uncertainty in the patient and family.

For children with a TN showing nondiagnostic cytopathology (Bethesda Class I), repeat FNA is an option but may have limited acceptability in children and should not be performed any sooner than 3 months to avoid atypical cellular features that arise during the reparative process that follows FNA [79]. Continued follow-up and removal are options.

For children with benign cytopathology (Bethesda Class II), follow-up is warranted. Surgery may be considered for growth, compressive symptoms, or patient/parent choice as there may be a false-negative rate of about 5% [3]. Given the higher false-negative rate for large lesions (> 4 cm), lobectomy should be considered in children with TN > 4 cm even if the FNA reveals benign cytopathology.

As the likelihood of DTC increases (AUS/FLUS or FN/SFN), the potential need for completion thyroidectomy similarly increases. Lobectomy with intraoperative frozen section may help diagnose classic PTC but has less benefit for follicular variant PTC (fvPTC) and no benefit for FTC, which requires evaluation of the entire lesion to detect the vascular and/or capsular invasion that is required to diagnose FTC [80, 81].

In children with AUS/FLUS (Bethesda Class III), repeat FNA or review by a high-volume cytopathologist may yield a definitive diagnosis, but 10–30% of AUS/FLUS in adults remain AUS/FLUS during repeat examination [82–84]. Molecular testing with oncogene panels may aid in the diagnosis of AUS/FLUS cytology in adults [85–92]. Preliminary reports have begun to explore the utility of these oncogene panels in children and adolescents with indeterminate cytology [93–95], but none have been validated on sufficient numbers of patients to support widespread use. The data so far suggest that 17–39% of pediatric FNAs contain a mutation or rearrangement; however, *BRAF* is the only mutation with 100% positive predictive value for DTC [73, 94]. Based on the increased risk for DTC in children with AUS/FLUS, lobectomy is recommended for patients with low-risk US, while total thyroidectomy might be considered for patients with bilateral nodules or if oncogene testing reveals a BRAF mutation or gene fusion, including RET/PTC or NTRK fusion [96–100]. Total thyroidectomy can also be considered for patients with AIT to prevent leaving a contralateral lobe that requires continued surveillance.

In children suspicious for follicular neoplasm FNA (Bethesda IV), there appears to be ≥50–58% risk for DTC [72, 73, 94, 101]. Lobectomy has been the standard of care, but molecular testing is being used to assess DTC risk in adults [86, 102]. However, TN lacking known mutations still carry a substantial DTC risk, and, therefore, lobectomy continues to be recommended for all children with FN/SFN [86]. Total thyroidectomy should be considered if the US is highly suspicious or if there are bilateral thyroid nodules.

For children with FNA showing either suspicious for malignancy (Bethesda V) or malignant (Bethesda VI) cytopathology, the risk for DTC is near 100% [72, 73], and for that reason, total thyroidectomy +/− central neck dissection is recommended [3]. The surgical risks of total thyroidectomy are greater than those for lobectomy [103] but are reduced if the surgery is performed by a high-volume thyroid surgeon (who performs at least 30 or more thyroid surgeries annually) [104, 105]. Total thyroidectomy requires levothyroxine (LT4) replacement, while lobectomy rarely does [106].

21.3 Differentiated Thyroid Cancers

Only 1.8% of thyroid cancers develop in children and adolescents, but the incidence has increased 2.3-fold over the last 40 years [107, 108]. This increase cannot be entirely explained by detection of small, incidental lesions during unrelated imaging. If this were the explanation, mainly non-palpable thyroid cancers would rise in incidence. In adult patients, Chen et al. found an increase in all stages of DTC in the Surveillance, Epidemiology, and End Results (SEER) database, while Morris and Myssiorek found a twofold increase in large DTC with extrathyroidal extension and cervical metastases [109, 110]. Within pediatrics, differentiated thyroid cancer (DTC) is now the eighth most common cancer among 15–19-year-olds and the second most common cancer of adolescent girls [111]. Adolescents have a tenfold greater incidence of DTC and a female/male preponderance (5:1) that are not seen at younger ages [108, 111, 112]. Estimates from the SEER database report 5-year survival for 99.8% of children and adolescents with DTC confined to the thyroid and 97.1% for those with metastasis to regional lymph nodes [113]. Overall cure rates are high [114–117], but children diagnosed prior to age 10 years may have a higher risk of recurrence [118], although not all studies confirm this [119].

The treatment of children with DTC has evolved over time. In 1934, Schreiner and Murphy described DTC in children as a "fatal disease with few exceptions" [120]. Radioactive iodine (RAI) first came into use to treat DTC in 1946 [121]. In the 1980s, children with DTC were sent for total thyroidectomy but without preoperative staging, and lymph node dissection generally consisted of palpation to identify suspicious nodes that were removed by "berry picking," leaving many with persistent disease. Essentially all children received RAI ablation, and the end point for treatment was to achieve no evidence for disease (NED) based upon a negative RAI scan. Such treatment was effective, but was associated with a high risk for complications and a risk of second malignancies. This high rate of complications and second malignancies along with other important differences between DTC in children and adults prompted the creation of pediatric-specific treatment guidelines [3].

The major differences between pediatric and adult DTC are:

1. Thyroid nodules are more likely to be malignant in children than in adults [51, 52].
2. Children with PTC are more likely to have regional lymph node involvement, extrathyroidal extension, and pulmonary metastases [114–117, 122, 123].
3. Children are less likely to die from disease (2% long-term cause-specific mortality) [115–117].
4. Many children with pulmonary metastases (30–45%) develop stable yet persistent disease following RAI therapy [124, 125].

Long-term (40 year) follow-up studies indicate that children with DTC have an increase in mortality attributed to second malignancies that appear to be possibly related to the use of radiation and/or RAI [115, 126, 127]. Adding to the caution regarding the routine use of RAI in low-risk patients is the fact that recurrence risks are similar whether RAI is almost always prescribed (16.6%) or not prescribed (20.8%) as long as the surgery was performed by an experienced thyroid surgeon [128]. For these reasons, the ATA treatment guidelines for children with thyroid nodules and cancer now suggest that total thyroidectomy remains the initial surgery of choice but should be based upon preoperative staging. Compartment-focused lymph node dissection should be performed instead of "berry picking." RAI therapy is reserved for children at high risk for persistent or recurrent disease, and the end point of therapy may not require NED (Fig. 21.2) [3].

DTC that arise from thyroid follicular cells include papillary thyroid cancer (PTC) and PTC variants [follicular variant (fvPTC) , diffuse sclerosing variant (dsvPTC), cribriform-morula

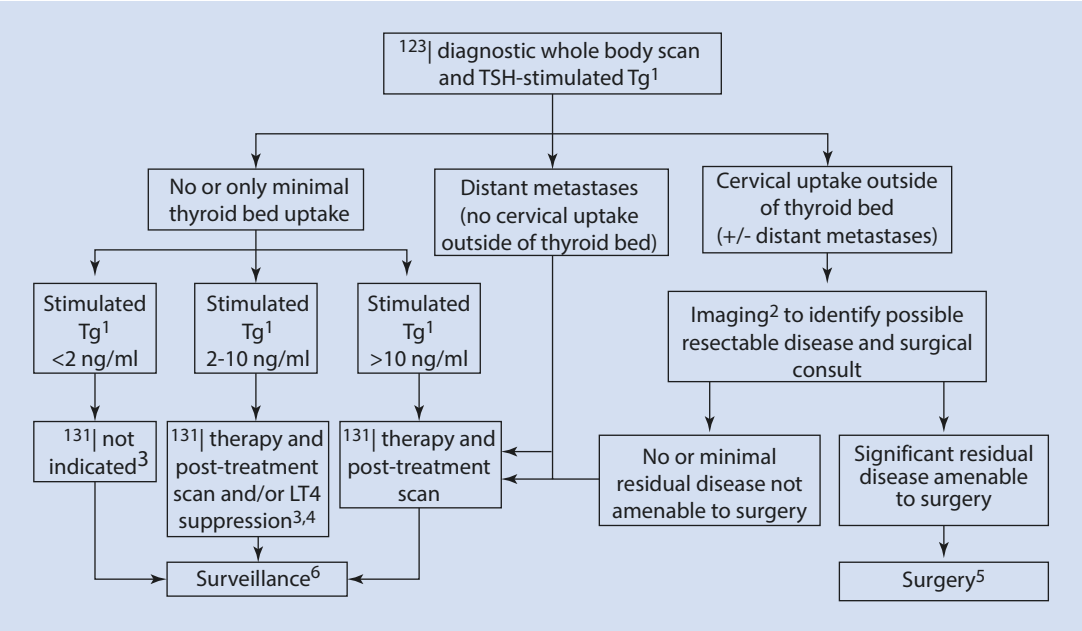

Fig. 21.2 Initial management for papillary thyroid cancer in children. Preoperative staging is vital to determine therapy. RAI ablation is indicated for high-risk patients but may be deferred for low-risk patients if close follow-up is assured (see text for details). [1]Assumes a negative TgAb and a TSH >30 mIU/L; in TgAb-positive patients, consideration can be given (except in patients with T4 tumors or clinical M1 disease) to deferred evaluation to allow time for TgAb clearance ("delayed" staging). [2]Imaging includes neck ultrasonography ±SPECT/CT at the time of the diagnostic thyroid scan. [3]Consider ^{131}I in patients with thyroid bed uptake and T4 tumors or known residual microscopic cervical disease. [4]While there are no prospective studies in patients ≤18 years of age, the use of ^{131}I remnant ablation may not decrease the risk for persistent or recurrent disease. Consider surveillance rather than ^{131}I with further therapy determined by surveillance data. [5]Repeat postoperative staging 3–6 months after surgery (Reprinted with permission from Mary Ann Liebert, Inc., 140 Huguenot St., New Rochelle, NY 10801–5215)

variant (usually associated with *APC* mutations), solid variant, and tall cell variant], as well as follicular thyroid cancer (FTC) which is divided into minimally invasive and widely invasive forms.

21.3.1 Papillary Thyroid Carcinoma (PTC)

PTC are generally well differentiated in children (Fig. 21.3) [116, 117]. The major risk factor for developing PTC is radiation exposure to the thyroid [37, 129]. *RET/PTC* rearrangements involving the *RE*arranged during *T*ransfection (*RET*) proto-oncogene are the most common molecular changes in PTC from children (10–80%) [130, 131]. With newer molecular technologies, there is also increasing evidence of point mutations in the v-raf murine sarcoma viral oncogene homolog B1 (*BRAF*) gene [96–100, 131–133]. Up to 5% of patients have a family history of PTC [134], which may present earlier in life and may be more aggressive [135].

PTC most commonly presents as a palpable thyroid nodule, but PTC can also present as cervical adenopathy or a diffusely enlarged gland (especially with dsvPTC). PTC is frequently multifocal and bilateral, and it metastasizes to regional neck lymph nodes prior to the lung. Distant metastases occur in 5–10% of children but typically only after extensive regional lymph node disease [136, 137].

21.3.1.1 Initial Therapy for PTC in Children

1. *Preoperative staging is required to direct the initial management* (Fig. 21.2) *and generally includes comprehensive neck US to interrogate the contralateral thyroid lobe and the lymph nodes in the central and lateral neck. Chest radiograph (CXR) or computerized tomography (CT) scan may be considered for patients found to have extensive lymph node metastasis* [138, 139].

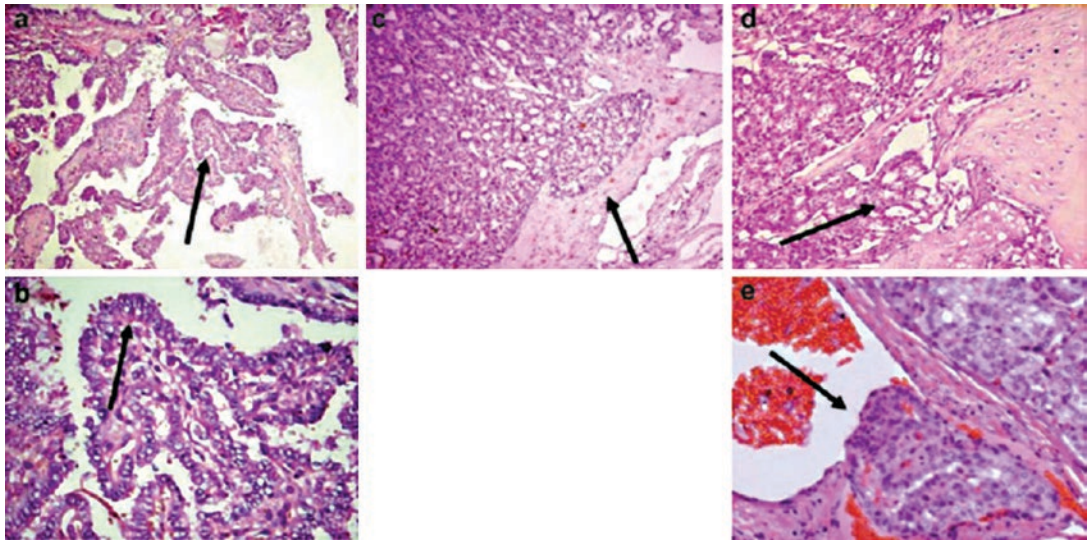

Fig. 21.3 Typical histology for DTC in children. Hematoxylin and eosin staining of **a** typical PTC showing prominent papillae with fibrovascular cores (100×); **b** typical PTC at high power (400×) showing overlapping nuclei with intranuclear inclusions; **c** minimally invasive FTC showing invasion into the tumor capsule (100×); **d** widely invasive FTC showing invasion into tracheal cartilage (100×); and **e** widely invasive FTC at high power (400×) showing vascular invasion

Because most children with PTC have cervical node involvement [138], preoperative US is necessary to identify the lymph node compartments that require dissection. Contrast-enhanced neck CT or MRI should be considered for locally extensive disease. However, if iodinated contrast agents are used, therapeutic RAI may need to be delayed 2–3 months until the urine iodine excretion is low normal, ideally below 75 μg/L at the time of administration of radioiodine [140, 141]. Due to robust RAI uptake by childhood PTC, nuclear scintigraphy is not useful in the child with an intact thyroid and a normal TSH. Due to the well-differentiated and usually indolent nature of pediatric PTC, ^{18}fluorodeoxyglucose positron emission tomography (FDG-PET) scanning is also not useful for detection or prognostication at this stage.

2. *Total thyroidectomy and central compartment lymph node dissection are generally recommended for PTC in children with the possible exception of incidental micro-PTC (PTMC).*

Total or near-total thyroidectomy (TT) is recommended for children with confirmed PTC. Recurrence risks are tenfold greater if lesser surgery is performed and threefold greater if inadequate lymph node dissection is performed [123]. In both procedures, the left and right thyroid lobes, the pyramidal lobe (when present), and the isthmus are resected. However, in near-TT, a small amount of thyroid tissue (<1–2%) is frequently left at the entry of the recurrent laryngeal nerve (RLN) and/or superior parathyroids in an effort to decrease the risk of complications. TT is recommended because children frequently have bilateral (30%) and multifocal (65%) disease [142–145] and an increased risk for recurrence when less extensive surgery is performed [115, 144].

All lymph node dissections should be comprehensive and compartment focused because recurrence rates are higher when "berry picking" is performed [146]. Although total thyroidectomy and central compartment dissection (CND) are associated with greater risks of hypoparathyroidism and recurrent laryngeal nerve injury [142, 147], the risks are minimized when surgery is performed by a high-volume surgeon [104, 148]. Lobectomy and isthmusectomy may be adequate for unifocal PTMC (<1 cm) but only if US shows absence of disease in the contralateral lobe and normal regional lymph nodes [148, 149].

Lymph node dissection can include the central neck (levels VI and VII), lateral neck (levels II–V), or both (Fig. 21.4). Central neck

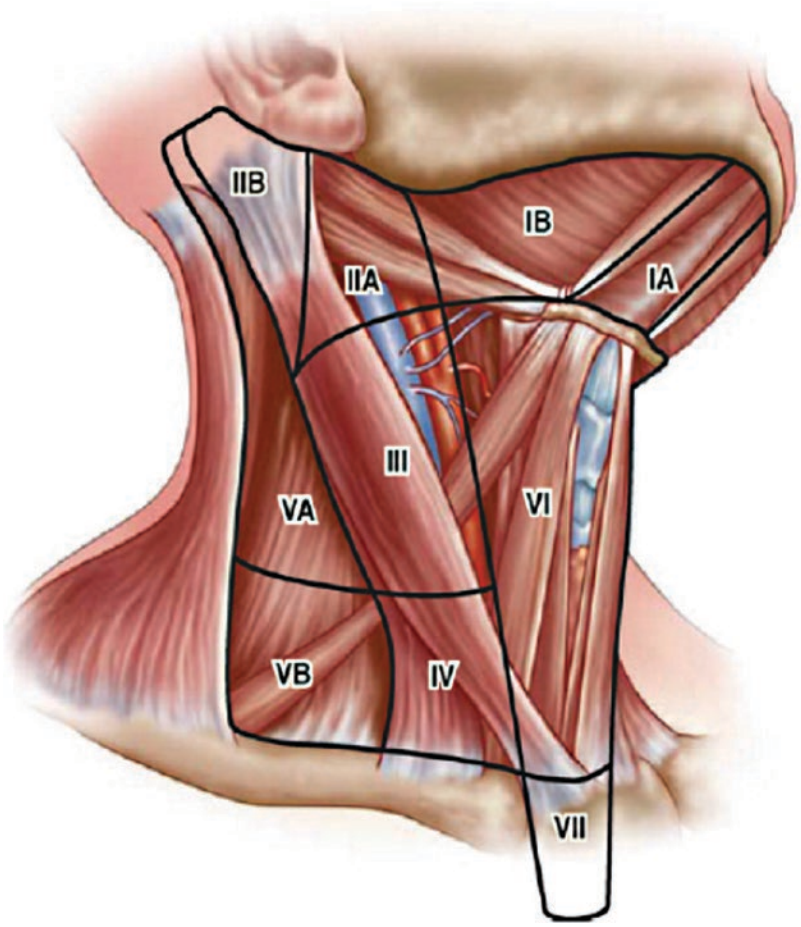

Fig. 21.4 Central and lateral cervical lymph nodes. Lymph nodes of the neck are divided into regions I through VII. Level I is submental and submandibular; level II is upper jugular; level III is midjugular; level IV is lower jugular; level V is posterior triangle and supraclavicular; level VI is Delphian, prelaryngeal, pretracheal, and paratracheal; and level VII is superior mediastinal. A bilateral central compartment lymph node dissection (level VI dissection) removes the nodes from one carotid artery to the other and down into the superior mediastinum (With permission: Mary Ann Liebert, Inc., 140 Huguenot St., New Rochelle, NY 10801–5215)

dissection involves resection of lymphatic tissue from the hyoid bone to the left innominate vein and bilaterally to the carotid sheaths and is routinely considered for pediatric patients with confirmed DTC [150]. A lateral neck dissection usually removes the lymphatic tissue from zones II through V while preserving critical structures.

A therapeutic central neck dissection (CND) should be performed in patients with preoperative evidence of central and/or lateral neck metastasis in order to reduce the risk of persistent or recurrent disease [3, 143]. Prophylactic CND should also be considered for children with PTC since decreased disease-free survival (DFS) is correlated with persistent or recurrent locoregional disease [115, 143, 144]. The extent of initial surgery appears to have the greatest impact on improving long-term disease-free survival (DFS) [115, 144]. However, one must weigh the risks of more extensive surgery with the potential benefit of decreasing persistent/recurrent disease. In children, TT with prophylactic CND is associated with DFS as high as 95% at 5 and 10 years [151, 152]. Some experts routinely perform prophylactic CND, particularly for larger tumors [153, 154], while others make this decision based on intraoperative findings [155]. In patients with unifocal disease, data from adults suggest that ipsilateral, prophylactic CND may provide the same benefit while decreasing the rate of complications [156].

Due to the increased risk for complications associated with lateral neck dissection, lateral neck dissection should only be performed if disease is identified by US in these compartments and confirmed with FNA. It is common with PTC to resect only the anterior portion of zone V rather than extending the dissection posteriorly to the trapezius muscle. When indicated, compartment-oriented lateral neck dissection (levels II, III, IV, and anterior V) also improves DFS [143, 144].

Thyroid surgery must be performed with attention to detail by an experienced thyroid surgeon

as complication rates can be higher in children [157]. A study evaluating over 5800 patients showed a significant association between surgeon experience, complication rates, and length of stay [158]. Because the number of pediatric thyroid surgeries is small, surgeons may need to gain this experience from adult procedures and develop a collaborative team approach for the care of young patients.

It is well recognized that the risks of thyroidectomy are greater for the youngest children; the risks are less clear for adolescents. Earlier series reported higher complication rates in the pediatric population, but there are now single center studies that report complication rates similar to those in adults. In one single center study where surgery was performed by high-volume surgeons, the overall complication rate was only 9% and there were no permanent complications [159]. This is most likely attributed to surgeon experience. Adjuncts to surgery such as laryngeal nerve monitoring and vessel sealing devices may help decrease complications, but there are no conclusive results in children.

Complications such as bleeding and infection are rare following thyroid surgery. The low infection rate is most likely related to the lack of contamination from the respiratory or gastrointestinal tracts. One risk of thyroid surgery is voice change. These can result from injury to any of the external branches of the superior laryngeal nerves and/or the recurrent laryngeal nerves and can affect the strength, projection, and quality of the voice. Bilateral nerve injury may compromise the airway. Careful visualization of all nerve branches and the use of electrodes to identify and monitor nerve function during the procedure may help reduce the risk of nerve injury [160]. The risk of injury to the recurrent laryngeal nerve should be less than 3% with total thyroidectomy.

Due to their small size and delicate blood supply, ischemic injury to the parathyroid glands may also occur. The risk of permanent hypocalcemia is rare with lobectomy and 1.5–6.5% with total thyroidectomy in children [159, 161–167]. Using an intracapsular approach for TT allows the superior parathyroid glands to be more easily preserved [168]. If the parathyroid glands appear threatened during surgery, autotransplantation into a sternocleidomastoid muscle should be performed at that time.

Perioperative parathyroid hormone (PTH) levels may help to identify those at greatest risk for postsurgical hypoparathyroidism [169, 170]. Postoperative hypocalcemia (occurring in up to 50% of patients) is treated with oral calcium and vitamin D and resolves in 80% of cases [157, 171].

Lymph node dissection can involve the central neck (levels VI and VII), lateral neck (levels II–V), or both (Fig. 21.4). Central dissection includes resection of lymphatic tissue from the hyoid bone to the left innominate vein and bilaterally to the carotid sheaths [150]. A lateral neck dissection usually removes lymphatic tissue from zones II through V while preserving critical neurovascular and muscular structures, in addition to the thoracic duct. It is common with PTC to resect only the anterior portion of zone V rather than extending the dissection posteriorly to the trapezius muscle.

Total thyroidectomy is performed through a low transverse neck incision. Patients are usually discharged home the same or next day and may resume their normal activity within 1–2 weeks. However, it is critical that the correct operation be performed the first time because the risk of complications is significantly increased during reoperative neck surgery [172].

3. *After surgery, patients are restaged based upon the operative findings to identify those with persistent disease and those at intermediate or high risk for recurrence* (Fig. 21.5).

Children with palpable cervical node metastases are more likely to recur (53% vs. 0%), persist (30% vs. 0%), or have multifocal disease (89% vs. 16%) and pulmonary metastases (20% vs. 0%) than are children without nodal disease [123, 137]. Therefore, absence of cervical node disease is a strong indicator of low recurrence risk.

The ATA guideline uses standard American Joint Committee on Cancer (AJCC) tumor-node-metastasis (TNM) nomenclature to stratify children into low, intermediate, and high risk for recurrence based on TNM descriptions (Table 21.2) [3]. These three groups are:

1. ATA Pediatric Low Risk

Pediatric low-risk category includes disease grossly confined to the thyroid with no cervical node disease (N0) or unknown but unsuspected cervical node disease (NX) or patients with incidental node involvement of the central

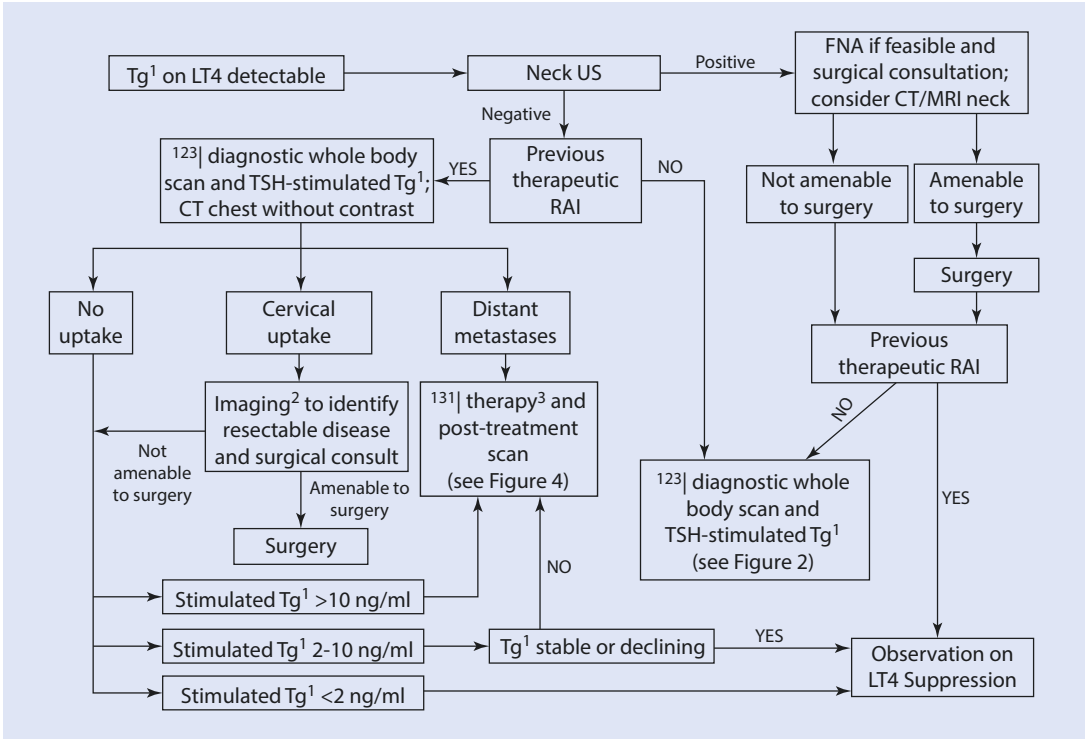

Fig. 21.5 Postoperative staging for persistent disease and risk for recurrence. [1]Assumes a negative TgAb; in TgAb-positive patients, the presence of TgAb alone cannot be interpreted as a sign of disease unless the titer is clearly rising. [2]Imaging includes SPECT/CT at the time of the diagnostic thyroid scan and/or contrast-enhanced CT/MRI neck. [3]Repeat ^{131}I therapy in patients previously treated with high-dose ^{131}I should generally be undertaken only if iodine-avid disease is suspected and a response to previous ^{131}I therapy was observed (Reprinted with permission from Mary Ann Liebert, Inc., 140 Huguenot St., New Rochelle, NY 10801–5215)

compartment (N1a) where "incidental" is defined as the presence of microscopic metastasis to a small number of central neck (level VI) lymph nodes. These patients appear to be at lowest risk for distant metastasis but may still be at risk for residual cervical disease, especially if the initial surgery did not include a central node dissection (CND).

2. ATA Pediatric Intermediate Risk

Pediatric intermediate-risk category includes extensive N1a or minimal lymph node involvement beyond the central compartment (N1b). These patients appear to be at low risk for distant metastasis but are at an increased risk for incomplete lymph node resection and therefore persistent cervical disease. The impact of microscopic extrathyroidal extension (ETE) (T3 disease) on management and outcome has not been well studied in children with PTC, but patients with minimal ETE are probably either ATA pediatric low or intermediate risk, depending on other clinical factors.

3. ATA Pediatric High Risk

Pediatric high-risk category includes regionally extensive disease (extensive N1b) or locally invasive disease (T4 tumors), with or without distant metastasis. Patients in this group are at the highest risk for incomplete resection, persistent disease, and distant metastasis.

For ATA pediatric low-risk patients, initial postoperative staging includes a TSH-suppressed serum Tg assuming that thyroglobulin antibodies (TgAb) are negative (see section below for management of TgAb-positive patients).

The interpretation of serum Tg is based not only on the magnitude of serum Tg but its trend over time. The majority of children who have undergone total thyroidectomy by an experienced thyroid surgeon will have an undetectable TSH-suppressed Tg and will remain assigned to

Table 21.2 TNM classification of DTC in children

Primary tumor (T)		
TX		Size not assessed, limited to the thyroid
T1	T1a	≤ 1 cm, limited to the thyroid
	T1b	> 1 cm but ≤2 cm, limited to the thyroid
T2		> 2 cm but ≤4 cm, limited to the thyroid
T3		>4 cm, limited to the thyroid, or any tumor with minimal extrathyroid extension
T4	T4a	Tumor extends beyond the thyroid capsule to invade subcutaneous soft tissues, larynx, trachea, esophagus, or recurrent laryngeal nerve
	T4b	Tumor invades prevertebral fascia or encases carotid artery or mediastinal vessels
Lymph nodes (N)		
NX		Regional lymph nodes not assessed
N0		No regional lymph node metastasis
N1	N1a	Metastasis to level VI (pretracheal, paratracheal, and prelaryngeal/Delphian lymph nodes)
	N1b	Metastasis to unilateral, bilateral, or contralateral cervical (levels I, II, III, IV, or V) or retropharyngeal or superior mediastinal lymph nodes (level VII)
Distant metastasis (M)		
MX		Distant metastasis not assessed
M0		No distant metastasis
M1		Distant metastasis

the low-risk category [173–175]. Periodic follow-up by neck US and TSH-suppressed Tg levels is indicated. An increase in serum Tg over time indicates possible recurrent disease that should ultimately be identified by anatomic localization [neck US, chest CT, whole body diagnostic RAI scan (DxWBS) with single photon emission computed tomography with integrated conventional CT (SPECT/CT) as indicated to better localize RAI uptake]. Treatment for recurrent disease is based on anatomic localization with preference being given to surgical removal if possible. RAI therapy is indicated for pulmonary metastases and when surgery is unlikely to succeed or associated with unacceptable risks.

In contrast, for ATA pediatric intermediate- and high-risk patients, a TSH-stimulated Tg and diagnostic RAI whole body scan (DxWBS) are generally recommended to determine if treatment with ^{131}Iodine is warranted.

In patients without TgAb, the TSH-stimulated Tg is a reliable marker for residual disease. In a study examining 218 consecutive adult DTC patients across all ATA risk strata, a TSH-stimulated Tg < 2 ng/mL had a 94.9% predictive value for absence of disease [176].

It is not yet clear if a DxWBS might image disease that is not identified through neck US or suspected from the serum Tg, and there are few data in children to address this issue. In one pediatric study, US and DxWBS equally identified lymph node metastases in the majority of patients (35/45); however, in six patients, lymph node metastases were found only with a posttreatment RAI WBS [177]. Two of the patients were TgAb positive, reinforcing the potential benefit of DxWBS in patients who are TgAb positive [178]. DxWBS may also visualize disease in the lungs or elsewhere that would not otherwise be shown by neck US or other cross-sectional imaging [179].

For patients with cervical RAI uptake, the addition of hybrid imaging using SPECT/CT offers improved anatomic imaging to determine whether cervical uptake is secondary to remnant thyroid tissue or metastasis to regional lymph nodes [180–182].

A potential drawback of DxWBS imaging is that, if ^{131}Iodine is used, the small diagnostic activity may theoretically "stun" the iodine-avid tissue and reduce subsequent ^{131}Iodine uptake if high-activity ^{131}Iodine treatment is prescribed [183]. This possibility can be reduced by selecting the lowest possible activity of ^{131}Iodine (2.7 to 4.0 mCi = 100–148 MBq) or by using ^{123}Iodine for the DxWBS [184]. Due to its lower cost, ^{131}Iodine is more commonly used, but ^{123}Iodine provides superior imaging quality and generates lower absorbed doses of radiation to the tissues [185], which favors its use for diagnostic imaging in children [186].

4. *Postoperative staging is used to identify children who may or may not benefit from additional treatment with surgery and/or RAI therapy* (Fig. 21.5).

Residual cervical disease should be removed if this can be safely accomplished. Patients with small cervical foci of disease (i.e., <1 cm) or patients with cervical disease that cannot be visualized with cross-sectional imaging may be considered for treatment with therapeutic ^{131}Iodine, which may reduce future recurrence risk but is unlikely to reduce the already low mortality rate [187]. Although repeat surgery is another option, finding a small cervical recurrence intraoperatively may be difficult. In most cases, children with small-volume residual disease <1 cm can be safely observed while continuing TSH suppression. Given the excellent overall prognosis, and the low risk for clinical progression, the risk-to-benefit ratio for treatment of small-volume disease in a child who has already undergone surgery and ^{131}Iodine is unfavorable. On the other hand, in patients with structural disease greater than 1 cm that is visualized by US, CT, or MRI and confirmed by FNA, surgical resection is preferable to ^{131}Iodine especially when surgery is performed by a high-volume surgeon [188, 189].

For patients with pulmonary metastases or cervical disease that is not resectable, ^{131}Iodine therapy is indicated [187]. Some experts also advocate routine ^{131}Iodine therapy for children with T3 tumors or following resection of extensive regional nodal involvement (extensive N1a or N1b disease) (Fig. 21.6) [187].

Published studies show that children with iodine-avid pulmonary metastases benefit from ^{131}Iodine treatment and that complete remission is achievable for many, particularly those with microscopic and small-volume lung disease [125, 190]. However, with increasing disease burden, multiple treatments may be required [191].

The difficulty is to determine if and when additional therapy is warranted. Serial ^{131}Iodine treatments can induce remission in many, but not all, children with pulmonary metastases. However, many will develop stable disease that still has low disease-specific mortality [192, 193]. The optimal frequency of ^{131}Iodine treatment has not been determined, and the maximal clinical and biochemical response from administered ^{131}Iodine may not be reached for up to 15–18 months [194]. In fact, continual reduction in tumor burden as reflected by serum Tg levels may continue over several years [124]. Given the fact that a majority of children with pulmonary metastases will not have a complete response to therapy and because it may take years to see the full response of ^{131}Iodine therapy, an undetectable Tg level should no longer be the sole goal of treatment for children with pulmonary metastases. Furthermore, longer intervals between ^{131}Iodine therapy would seem prudent for children who do not have progressive disease. For patients with persistent pulmonary metastases who have already received treatment with ^{131}Iodine, the decision to retreat should be individualized. One should monitor the TSH-suppressed Tg and structural imaging in these children and defer repeat treatment with ^{131}Iodine until the effect of ^{131}Iodine appears to have worn off as evidenced by progression of disease (either an increase in serum Tg or increase in visualized tumor). In the rare case where disease continues to progress despite ^{131}Iodine, further ^{131}Iodine therapy is unlikely to be helpful. In these cases, continued observation and TSH suppression are indicated, and alternative therapies may be considered if iodine-refractory disease becomes clinically significant (see section on alternative therapies).

Children with diffuse pulmonary metastases and high RAI uptake in the lung are at risk for posttreatment pulmonary fibrosis [195, 196]. In these cases, lower ^{131}Iodine activities and using

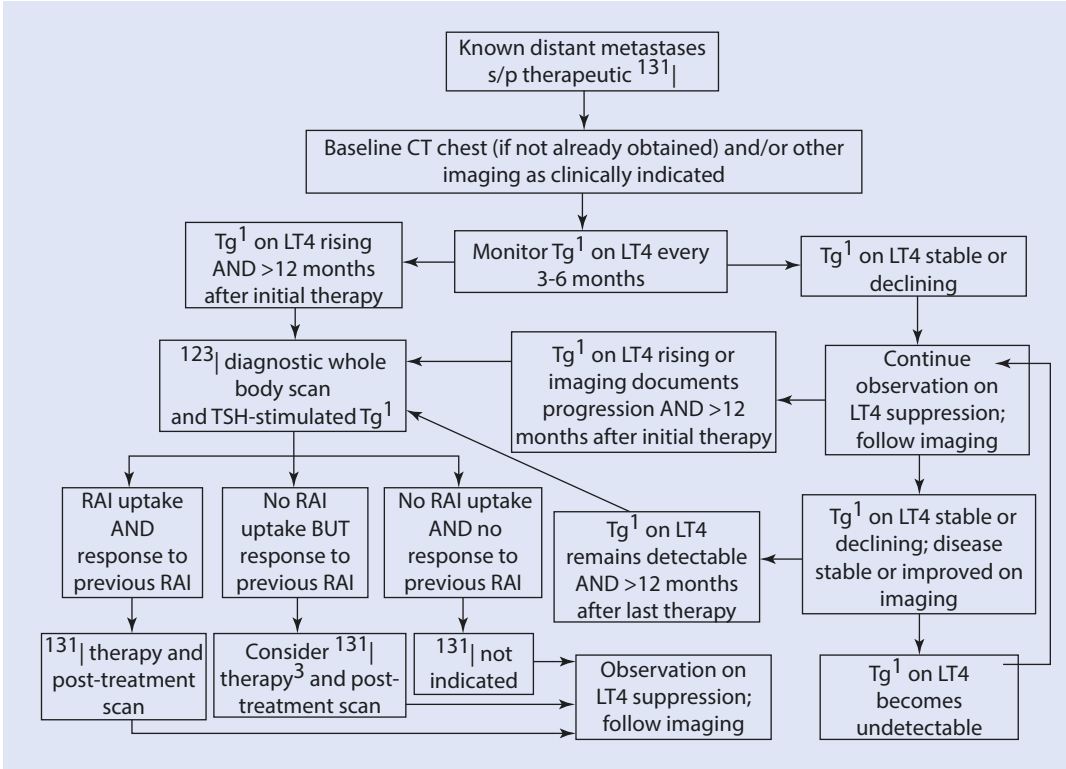

Fig. 21.6 Treatment of known metastatic PTC in children. [1]Assumes a negative TgAb; in TgAb-positive patients, the presence of TgAb alone cannot be interpreted as a sign of disease unless the titer is clearly rising. [2]Tg can transiently rise after ^{131}I therapy and should not be misinterpreted as evidence for progression. [3]Repeat ^{131}I therapy in patients previously treated with high-dose ^{131}I should generally be undertaken only if iodine-avid disease is suspected and a response to previous ^{131}I therapy was observed (Reprinted with permission from Mary Ann Liebert, Inc., 140 Huguenot St., New Rochelle, NY 10801–5215)

dosimetry to help select appropriate doses may help limit radiation exposure to the adjacent normal lung parenchyma [195, 197]. The utility and optimal intervals at which to perform pulmonary function testing (PFT) in children with lung metastases have not been studied, but many experts recommend that PFT be obtained intermittently in children with pulmonary metastases, especially if multiple ^{131}Iodine treatments are planned.

5. *Irrespective of initial postoperative risk stratification, all patients enter into surveillance, ensuring that appropriate therapy will be given if disease is ultimately detected.*

As long as the patient is maintained on levothyroxine to suppress the TSH, and a proper surveillance plan is followed, delayed treatment is not expected to alter the already low disease-specific mortality of PTC in children. Furthermore, a more individualized and conservative approach to postoperative staging and treatment will decrease unnecessary exposure to ^{131}Iodine in children without evidence of disease.

Patients at low risk for recurrence (e.g., small unifocal tumors without lymph node disease) may be evaluated by US and a TSH-suppressed serum Tg. They may then be followed in an expectant fashion and evaluated should the serum Tg increase.

In conjunction with neck US, the serum Tg is a critical component in the management of DTC, both at the initial postoperative staging and during long-term surveillance. At the same degree of TSH suppression, the Tg level obtained while the patient is on levothyroxine is thought to be the best predictor of changes in tumor mass [198, 199]. Therefore, monitoring of the TSH-suppressed Tg along with neck US is the ideal approach to detect recurrence or disease progression despite

the fact that an undetectable Tg on levothyroxine therapy may not always predict an undetectable TSH-stimulated Tg [200–202]. This is true even for patients with pulmonary metastases in whom the majority of recurrence is in the cervical lymph nodes [190].

Based on adult studies, an undetectable TSH-stimulated Tg after surgery and ^{131}Iodine therapy identifies patients with a high probability of remaining disease-free [203–205]. An undetectable TSH-stimulated Tg in children is similarly considered to indicate remission. A minimally elevated TSH-stimulated Tg (<10 ng/ml) in children should not automatically require therapy unless there is other evidence for active disease (anatomic localization of disease or an increasing trend in serum Tg). This is due to multiple factors, including the fact that some adult patients with a TSH-stimulated Tg > 2 ng/mL but <10 ng/ml remain free of clinical disease and have stable or decreasing TSH-stimulated Tg levels over time [198, 206, 207]. Furthermore, patients with DTC can have a continual decline in Tg over many years without additional therapy [115, 194, 198, 206, 207].

An increase in serum Tg while the patient is on levothyroxine indicates that disease is likely to become clinically apparent [198, 206, 208–210]. In that case, restaging and appropriate treatment will be based on the degree of Tg elevation, the trend in Tg over time, and the results of imaging studies. When imaging fails to confirm disease, the clinical importance of a low-level disease burden identified only by Tg in children is not yet clear, and there is no absolute serum Tg value above which empiric treatment is indicated [198, 206].

The serum Tg level while on TSH suppression is still a reliable indicator of disease progression in patients who were initially treated with total thyroidectomy but not RAI [173–175, 211], assuming the initial surgery was done by an experienced thyroid surgeon. A TSH-stimulated Tg is of no value in this situation outside of its use in initial postoperative staging.

21.3.1.2 Children with Thyroglobulin Autoantibodies

TgAb are detected in up to 20–25% of patients with DTC, primarily PTC, and interfere with Tg measurements, rendering the Tg level uninterpretable [212–214]. Antibody interference with the most commonly used Tg immunometric assays (IMA) always results in underestimation of Tg (i.e., a potentially false-negative test), whereas interference with radioimmunoassay (RIA) has the potential to cause either under- or overestimation of Tg, depending on the characteristics of the patient-specific TgAb and the RIA reagents [213, 214]. All specimens sent for Tg measurement require concomitant TgAb testing because TgAb status can change over time and even very low TgAb concentrations can interfere [213, 214]. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) can be used to measure Tg in TgAb-positive patients and is not affected by TgAb [215]. The sensitivity does not appear to be as robust as that of conventional IMA, but the trend in Tg levels over time appears to be a reliable disease indicator.

Because TgAb concentrations respond to the level of Tg antigen and indirectly reflect changes in tumor mass, the TgAb level can also serve as a surrogate marker for DTC [197, 216]. Most studies have reported that the de novo appearance, persistence, and a rising trend in TgAb are risk factors for persistent or recurrent disease [217–220]. However some patients will have persistent TgAb during the first year after diagnosis and may even exhibit a rise in TgAb during the 6 months following ^{131}Iodine treatment due to release of Tg antigen [213, 218, 221]. A decline in TgAb titer reflects a declining disease burden, but it takes a median of 3 years in adults to clear TgAb following apparent cure of DTC [222].

21.3.2 Children and Adolescents with Pulmonary or Non-resectable Metastases Are Treated with RAI (Fig. 21.6) [3]

In order for RAI to be effective, the TSH should be above 30 μIU/ml to facilitate uptake, and this can be induced by ≥14 days of thyroid hormone withdrawal [223]. Recombinant human TSH (rhTSH) using the typical adult dose can be used for remnant ablation in low-risk patients and may result in a lower absorbed radiation dose [224–226]. However, data on rhTSH in children are limited and retrospective. A low-iodine diet is prescribed for 2 weeks prior to therapy. For

children who received intravenous contrast during CT, it is advisable to wait 2–3 months or to confirm appropriate 24-h urine iodine levels first.

There are no standardized doses of RAI for children. Some adjust ^{131}I dose according to weight or body surface area (BSA) and give a fraction (e.g., child's weight in kg/70 kg) based on the typical adult dose used to treat similar disease extent [227, 228]. Others suggest that ^{131}I doses should be based entirely on body weight (1.0–1.5 mCi/kg) [187]. Dosimetry may be used to limit lung retention to <80 mCi at 48 h and blood/bone marrow exposure to <200 cGy [229–231] and is useful in small children, children with diffuse lung uptake or significant distant metastases, children who received chemotherapy or radiation therapy for other malignancies, and those undergoing multiple treatments. Lesional dosimetry can be performed in children with substantial lung involvement or large tumor burden at other sites, such as bone [232, 233]. A posttreatment scan, with the addition of SPECT/CT as indicated, to localize any metastatic disease should be obtained 5–8 days after all RAI treatments [97].

21.3.3 Papillary Thyroid Microcarcinoma (PTMC)

The prevalence of papillary thyroid microcarcinoma (PTMC, < 1 cm in diameter) in children is unknown, but detection is increasing. In adults, one third of PTC are PTMC and detected by imaging or surgery for unrelated conditions. The natural history of PTMC is not well defined, but patients are commonly considered low risk [3]. A recent study in adults found that PTMC <0.575 cm were at low risk whereas PMTC >0.575 cm were at increased risk for lymph node metastases [234]. Very few data address PTMC in children, but a recent study found lower rates of extrathyroidal extension (8.8% vs. 33%) and cervical lymph nodal disease (60% vs. 95.2%) compared with larger tumors [235]. The percentage of PTMC also increased with age (< 15 years of age, 7.1%; 15–18 years of age, 32%; and >18 years of age, 48.1%) [235].

Based on the limited available data in children with PTMC, many clinicians perform US of the contralateral lobe and cervical lymph nodes. Those without involvement can be treated with lobectomy and followed expectantly.

21.3.4 Follicular Thyroid Carcinoma (FTC)

FTC are uncommon in children. The diagnosis of FTC is based on the pathologic identification of capsular and/or vascular invasion. FTC are subdivided into those with only minimal capsular invasion (minimally invasive FTC) and those with more widespread capsular or vascular invasion (Fig. 21.3). Several genetic alterations have been reported in FTC, including rearrangements of the peroxisome proliferator-activated receptor gamma (*PPARγ*) and the paired box gene (*PAX-8*) [236–239]. FTC are typically unifocal and rarely metastasize to regional lymph nodes. However, FTC primarily develop hematogenous metastases, usually to bone and lungs, even without cervical node involvement. Therefore, most children with angioinvasive FTC are treated with total thyroidectomy and RAI [154, 240–242]. Treatment of minimally invasive FTC is controversial for adults and children [243]. In a study of young patients (< 20 years of age) with minimally invasive FTC, none died of disease [244], suggesting that minimally invasive FTC might be less aggressive in young patients and that lobectomy followed by close follow-up and possible TSH suppression may be sufficient. However, another recent study by Enomoto et al. evaluated 20 children with FTC including 16 with minimally invasive and 4 with widely invasive tumors [245]. Vascular or lymphatic invasion was seen in 9/20 tumors, and 3 went on to recur. All 3 were minimally invasive, but all 3 had vascular invasion suggesting that minimally invasive FTC with vascular invasion might also require more aggressive therapy. Thirty-year disease-specific survival was 100%, and disease-free survival was 62.8%.

21.3.4.1 Thyroid Hormone Suppression and Follow-Up

Postoperative TSH suppression is almost always prescribed for DTC in children, but optimal suppression is debated [246]. Some recommend initial TSH suppression to <0.1 μIU/ml and relaxation to 0.5 μIU/ml following remission [3]. However, none of these recommendations has been validated in large numbers of children or in lengthy studies. Potential risks of TSH suppression (such as negative effects on growth, cognition, bone mineralization, and the heart) are unstudied but are presumed to be minimal in otherwise healthy children [247, 248]. Follow-up

for recurrence should be lifelong as all series have some recurrence after 20–30 years [115–117]. However, patients with a negative stimulated Tg and negative US are defined as having "no evidence of disease" (NED), and TSH suppression and follow-up can be relaxed. In adults, an undetectable stimulated serum Tg is generally associated with remission, while Tg levels >10 ng/ml (off thyroid hormone) likely indicate residual disease [198, 206, 207, 249, 250]. Most patients with an rhTSH-stimulated Tg value of >2 ng/ml will have disease identified within 5 years, although some spontaneously resolve without additional therapy, and a significant increase in serial Tg levels indicates disease that might achieve clinical importance [198, 206, 207, 249, 250].

It is not yet clear if these same Tg levels have a similar prognostic value for children. Older data regarding disease status were generally based on negative dxWBS in children and not the serum Tg levels. Also unknown is how aggressive we should be in treating disease detected solely by abnormal Tg levels. It is possible for Tg levels to slowly decline in children who were previously treated with RAI, and undetectable Tg levels may not be achieved in all children with pulmonary metastases who may develop stable but persistent disease after ^{131}I therapy [124, 125].

6. *For these reasons, all patients with DTC undergo sequential staging following any diagnostic, therapeutic, or surveillance procedure* [50].

This begins with preoperative staging to inform the extent of initial surgery and postoperative staging to inform the potential need for additional surgery or RAI. Subsequently, all patients are restaged to reassess the individual risk for persistent/recurrent disease after each treatment. It is possible for patients who were initially ATA high risk for recurrence to become low risk for recurrence following appropriate surgery and RAI. Conversely, some patients who were initially categorized as ATA low risk for recurrence may become intermediate or even high risk for recurrence based on serial Tg and US follow-up.

21.3.4.2 Alternative Therapy for Progressive Non-iodine-Avid Disease

A small proportion of children will have progressive disease following total thyroidectomy and RAI therapy. Patients who present with distant metastasis appear to be at the greatest risk. The decision to pursue additional treatment, including additional surgery, RAI, or systemic therapy, is based on distinguishing stable, persistent disease from progressive disease. In a systematic review of pediatric patients with pulmonary metastasis, only 47% achieved a complete response to therapy, while 53% had persistent disease, including 14% who showed no reduction in pulmonary metastasis following the initial RAI treatment [191]. Many patients with distant metastasis will have stable, persistent disease; however, overall progression-free survival is reduced as shown in one large study that reported progression-free survival of 76% at 5 years and 66% at 10 years from diagnosis [125].

In adults, this "iodine-refractory disease" is defined as [251]:

1. Disease does not take up RAI at known sites of metastatic disease.
2. Disease that grows despite ^{131}I and confirmed uptake.
3. Disease that grows over a 1-year period after ^{131}I.
4. The patient has received a cumulative dose of ^{131}I > 600 mCi and has persistent disease.

The ^{18}FDG-PET/CT scan is used in adults with iodine-refractory disease [252–254] and appears to offer prognostic information [255]. However, iodine-refractory disease has not yet been defined for children, and there are limited data regarding ^{18}FDG-PET/CT in children [256]. Reports suggest low sensitivity for ^{18}FDG-PET/CT to identify disease in children, and it is unclear whether or not the ^{18}F-FDG-PET/CT has prognostic value in children. For these reasons, it is difficult to identify children who require alternative therapies.

Prior to treating children with persistent disease, one must carefully weigh the potential benefits and risks of treatment. The overall disease-specific mortality for DTC in children is less than 3%, and long-term survival is >97% [108, 115, 125, 191]. Because ^{131}Iodine may continue to have an effect for many years, the ATA pediatric thyroid cancer guidelines state that an undetectable Tg level should no longer be the sole goal of treatment for children with pulmonary metastases and continued RAI therapy should be considered only in those who show signs of progression and who are believed to have previously received clinical benefit from RAI [3]. If the child

has no evidence of cervical disease and still shows no uptake of ^{131}Iodine into pulmonary lesions with concurrent low urine iodine excretion, it is unlikely that additional doses of ^{131}Iodine would benefit. At that point, TSH-suppressive therapy is continued, and, if the disease is progressive based on radiological evidence of anatomic changes in the size or number of metastasis, one may consider alternative systemic approaches.

For adult patients with iodine-refractory disease, there are an increasing number of agents that target protein tyrosine kinase-dependent pathways and are collectively referred to as multi-kinase inhibitors (TKIs) [257]. TKIs have been studied in adults with advanced PTC, FTC, MTC, and anaplastic thyroid carcinoma [257]. For adults, where the risk of mortality is much greater than in children, the benefit of extending progression-free survival (PFS) even without cure is significant. The multicenter phase 3 sorafenib trial (DECISION) reported a 12% response rate and an increase in median PFS from 5.8 to 10.8 months [258], while the phase 3 SELECT trial using lenvatinib also increased PFS for FTC and PTC [259]. Almost 75% of patients responded and 4/169 patients achieved complete radiographic remission. Additional TKIs are currently in phase 2 clinical trials [260, 261], but only four TKIs have so far received FDA approval for use in adults with advanced thyroid cancer [262].

Phase 1 and phase 2 trials have been conducted to determine the safety and efficacy of sorafenib in pediatric patients with refractory solid tumors or leukemias [263, 264], but no children with PTC were enrolled into either trial, and there is only limited or anecdotal experience using molecular therapies in children with DTC [265, 266].

An alternative use of kinase inhibitors is redifferentiation therapy, which is designed to increase expression of the sodium-iodine symporter (NIS). The MEK 1/MEK 2 inhibitor (selective mitogen-activated extracellular signal-regulated kinase), selumetinib, was found to increase RAI uptake to the threshold required for ^{131}Iodine treatment in 8 of 12 patients, but this use has not yet been studied in children [267].

All of these agents have the potential for significant toxicities, and even in adults none of these agents have yet been shown to improve overall survival [257]. National clinical trials are needed to determine which pediatric patients would benefit from systemic therapy as well as the best strategy for their use. In the meantime, a conservative approach to initiating systemic therapy is recommended, including consultation with providers who are experienced in the use of systemic therapy for progressive disease in children.

21.3.5 Medullary Thyroid Carcinoma (MTC)

Medullary thyroid carcinoma (MTC) is a rare neuroendocrine malignancy that arises from the calcitonin-secreting, parafollicular C cells of the thyroid and comprises 5% or less of pediatric thyroid cancers and has an annual incidence of 0.3 cases/million/year [108]. Since it does not derive from the thyroid follicular epithelium, MTC does not produce Tg or concentrate iodine. However, MTC secretes calcitonin and carcinoembryonic antigen (CEA), both of which serve as excellent tumor markers that are utilized in presymptomatic screening, the assessment of disease burden, and monitoring for recurrence/progression in patients with treated disease.

In children, MTC is almost always a monogenic disorder caused by a dominantly transmitted or de novo gain-of-function mutation in the *RE*arranged during *T*ransfection (*RET*) proto-oncogene and associated with the clinical syndromes of multiple endocrine neoplasia (MEN) 2A or MEN2B, depending on the specific mutation [2, 268–274]. MEN2B is characterized by early-onset MTC, as early as the first few months of life, whereas individuals with MEN2A often have a relatively late onset of MTC, especially with American Thyroid Association (ATA) moderate-risk mutations [2, 272, 274, 275] (Table 21.3).

Hereditary MTC is characterized by a predictable, stepwise progression from benign C-cell hyperplasia to noninvasive, microscopic MTC and ultimately to frankly invasive disease that gives rise to distant metastases [274, 276–278]. Genetic testing for *RET* mutations has been incorporated into routine clinical care and has ushered in an era wherein children and young adults with MEN2 rarely present with clinical MTC, unless they are members of a newly identified MEN2A kindred or if they have MTC associated with MEN2B. Thus, most children with MEN2 (with the notable exception of MEN2B; see below) will be diagnosed via the presymptomatic identification of a pathogenic *RET* mutation.

Table 21.3 *RET* mutations and associated phenotype, ATA risk levels, timing of thyroidectomy, and surveillance

Exon	Codon	Associated syndrome[a]	ATA risk level	Age for thyroidectomy	Age for PHEO[b] and PHPT[c] screening
8	533	MEN2A	MOD	Calcitonin/preference	By 16 years
10	609 611 618 620	MEN2A MEN2A MEN2A MEN2A	MOD MOD MOD MOD	Calcitonin/preference Calcitonin/preference Calcitonin/preference Calcitonin/preference	By 16 years By 16 years By 16 years By 16 years
11	630 631 634 666	MEN2A MEN2A MEN2A MEN2A	MOD MOD H MOD	Calcitonin/preference Calcitonin/preference Age 5 years Calcitonin/preference	By 16 years By 16 years By 11 years By 16 years
13	768 790	MEN2A MEN2A	MOD MOD	Calcitonin/preference Calcitonin/preference	By 16 years By 16 years
14	804	MEN2A	MOD	Calcitonin/preference	By 16 years
15	883 891	MEN2B	H MOD	Age 5 years Calcitonin/preference	By 11 years By 16 years
16	912 918	MEN2A MEN2B	MOD HST	Calcitonin/preference <1 year	By 16 years By 11 years

Adapted from the 2015 ATA guidelines [243]
Abbreviations: *ATA* American Thyroid Association, *CLA* cutaneous lichen amyloidosis, *Ctn/pref* calcitonin/preference (denoting that timing of thyroidectomy can be determined by Ctn level and parent/patient preference), *FMTC* familial medullary thyroid carcinoma, *H* high risk, *HST* highest risk, *HSCR* Hirschsprung disease, *MTC* medullary thyroid carcinoma, *MOD* moderate risk, *PHEO* pheochromocytoma, *PHPT* primary hyperparathyroidism
[a]Classical MEN2A is found primarily in Exon 10, 11, and 14 mutations; MEN2A + CLA is seen in Codon 634 (primarily) and 804 mutations, MEN + HSCR is seen in Exon 10 mutations (primarily codon 620), and FMTC is seen in codon 768 and 912 mutations
[b]Fractionated plasma or 24-h urine metanephrines
[c]Albumin-adjusted calcium or ionized calcium

In contrast with adults, in whom most MTC cases are nonfamilial, sporadic MTC (which will not be discussed further) is extremely uncommon in children; in these cases, somatic mutations in *RET* and *RAS* are most often identified [2].

Mutations in *RET* are transmitted with an autosomal dominant mode of transmission and have an extremely high penetrance. Once a *RET* mutation is identified, strong genotype-phenotype relationships allow for prediction of the rapidity with which an individual may develop MTC as well as the risk for developing other MEN2-related clinical manifestations [2, 270, 272, 274, 275] (Table 21.3). The disease-causing *RET* mutations have been stratified into risk categories that help to define the MTC risk, the age for initial clinical testing, and the timing of prophylactic thyroidectomy in children before clinical MTC [2, 274, 275, 279, 280]. Most recently, the ATA divided the most common *RET* mutations into three major risk categories: highest risk, high risk, and moderate risk [2] (Table 21.3). Although genotype-phenotype correlations are typically strong in MEN2, it is important to note that the age of onset and aggressiveness of MTC may not always hold true to the predicted phenotype [281–283].

21.3.5.1 Multiple Endocrine Neoplasia 2A

MEN2A represents 95% or more of MEN2 cases, with de novo cases representing 10% or less of MEN2A. Classical MEN2A is characterized by the uniform presence of MTC coupled with

pheochromocytoma (PHEO) and/or primary hyperparathyroidism (PHPT) [284]. Rarer variants of MEN2A include MEN2A with cutaneous lichen amyloidosis (CLA), MEN2A with Hirschsprung disease (HSCR), and familial medullary thyroid carcinoma (FMTC) [2, 275]. Primarily diagnosed in adults, CLA is a skin disorder usually located in the interscapular region of the upper back that is associated with intense pruritus and, as a result of scratching, secondary skin changes and dermal amyloid deposition. HSCR is caused by the complete absence of neuronal ganglion cells (aganglionosis) from the myenteric (Auerbach) and submucosal (Meissner) plexuses in variable lengths of the distal gastrointestinal tract. In MEN2A patients, HSCR is typically diagnosed during infancy, but milder cases may not be recognized until adulthood. FMTC is limited to rare moderate-risk mutations in which MTC has been the only reported manifestation of MEN2A.

The extracellular, cysteine-rich domain of *RET* (Exons 10 and 11) is the primary location for most MEN2A-causing mutations, but mutations can also be found in the intracellular tyrosine kinase domain in exons 13, 14, 15, or 16 [2, 270, 272, 285, 286] (◘ Table 21.3). Codon 634 (exon 11) mutations account for the vast majority of classical MEN2A cases, but more recently, the prevalence of other mutations has increased [286]. The greatest risk of developing early MTC is seen with codon 634 mutations followed by those with mutations in codons 609, 611, 618, 620, or 630, whereas mutations in other *RET* codons impart the lowest risk for clinically aggressive MTC [2, 274, 275, 287, 288].

21.3.5.2 Multiple Endocrine Neoplasia 2B

MEN2B represents 5% or less of MEN2 cases and is characterized by the uniform early onset of MTC, a 50% lifetime risk of PHEO, and a pathognomonic physical phenotype that includes oral and conjunctival mucosal neuromas, thickened lips, intestinal ganglioneuromatosis and secondary megacolon, musculoskeletal abnormalities (Marfanoid body habitus, high-arched palate, narrow long facies, pectus excavatum, scoliosis, pes cavus, joint laxity, hypotonia, or proximal muscle weakness), and ophthalmologic issues (mild ptosis, thickened and everted eyelids, and medullated corneal nerve fibers) [2, 274, 289–292]. Patients with MEN2B may also experience pubertal delay and slipped capital femoral epiphysis. Other signs and symptoms that begin in infancy include constipation, feeding problems, and an inability to make tears (alacrima) [290]. PHPT is not a component of MEN2B.

MEN2B is due to a de novo *RET* mutation in >90% of cases [281, 286], and >95% of MEN2B patients will have the pathognomonic M918 T *RET* mutation. Rarer MEN2B mutations include double *RET* mutations involving codon 804 and the A883F mutation, which may be associated with less aggressive MTC [2, 293].

21.3.5.3 Early Thyroidectomy in MEN2

After MTC metastasizes beyond the thyroid, it most often becomes an incurable disease that may ultimately shorten survival. Given our ability to diagnose MEN2 via predictive genetic testing, hereditary MTC has become one of the rare malignancies that can be prevented (via prophylactic thyroidectomy) or cured (via early thyroidectomy) before it becomes clinically apparent. When total thyroidectomy is performed by an experienced surgeon prior to the onset of metastatic disease, children with MEN2 have an excellent long-term outcome [2, 211, 274, 281, 294–297].

Contemporary guidelines (◘ Table 21.3) suggest performing a total thyroidectomy within the first year of life in carriers of the highest risk mutation (codon 918) and at or before age 5 years for those with a high-risk mutation (codons 634 and 883) [2]. With all other moderate-risk *RET* mutations, the detection of an elevated calcitonin level can be used to determine the timing of surgery, recognizing that the ultimate decision should be made by a multidisciplinary team experienced in MEN2 management and the child's parent(s) or guardian, who may decide to intervene earlier [2]. Notably, an elevated calcitonin level does not always portend malignant C-cell disease [295] and can also be found in nonneoplastic conditions such as chronic kidney disease, autoimmune thyroiditis, and hyperparathyroidism [2]. Conversely, MTC can be identified pathologically even in the presence of a normal calcitonin level [298].

At the time of total thyroidectomy, a central neck dissection is recommended for patients whose basal calcitonin is >40 pg/ml or for those in whom there is a clinical suspicion for lymph node metastases [2, 274, 275]. Lateral neck disease would be extremely unlikely in a patient who is undergoing routine early thyroidectomy for MEN2, and hence lateral neck lymph node dissection is not usually required.

21.3.5.4 Management of MTC in MEN2

The first clinical manifestation of MEN2, MTC, is most commonly multifocal and bilateral and located in the C-cell rich, middle to upper regions of the thyroid lobes [276, 278]. In MEN2A, there is a well-described, age-related progression of malignant disease, with lymph node and distant metastases usually occurring years after the onset of tumorigenesis [277]. Lymph nodes in the neck and mediastinum are the most common sites of metastatic disease; distant sites of metastases due to hematogenous spread include the lungs, liver, and bone/bone marrow. MTC occurring in the clinical context of MEN2B has an accelerated tempo of disease progression, with metastatic lymph node disease documented as early as 3 months of life [299]. The average age of onset of MTC in MEN2B is in the second decade of life, about 10 years earlier than that seen in MEN2A [279, 281, 290, 295, 300]. Individuals with hereditary MTC have a better prognosis than patients with sporadic MTC, while individuals with MEN2B have the worst outcome [301]. However, it remains unclear if MEN2B-associated MTC is inherently more biologically aggressive [302], given that overall survival may be more impacted by the extremely early onset of MTC in MEN2B coupled with its frequently delayed diagnosis [290, 303].

As discussed above, total thyroidectomy +/− central neck dissection remains the mainstay of treatment for MTC. The exact surgical approach depends on the specific *RET* mutation as well as the primary tumor size, the calcitonin level, and the extent of neck disease identified pre- and intraoperatively [2, 274]. In reality, many MEN2A thyroidectomy specimens harbor microscopic, non-metastatic MTCs that are often cured by early thyroidectomy [304–306]. Cure in MEN2B is most often an unattainable goal due to the fact that most surgeries in this population are therapeutic (instead of prophylactic or early), an unfortunate truth resulting from the delayed diagnosis of MEN2B in most cases [290, 303].

After initial surgical therapy, levothyroxine replacement is given to normalize, not suppress, the TSH. Calcitonin and CEA levels should initially be monitored at least every 6 months, as should neck US in patients with persistently detectable tumor markers or initial nodal metastases. If there is documentation of a rapid calcitonin and/or CEA doubling time or if these markers remain significantly elevated over time, additional imaging studies should be obtained. Other recommended tests include chest CT, contrast-enhanced liver MRI/CT, bone scan, and MRI of the axial skeleton/pelvis; FDG-PET/CT has limited value in the follow-up of MTC [2, 307]. The lifelong management of an individual with hereditary MTC also includes continued genetic counseling, psychological support, and prospective screening for PHEO (MEN2A and MEN2B) and PHPT (MEN2A) (◘ Table 21.3).

When not diagnosed, and treated before the onset of metastatic disease, hereditary MTC is generally incurable yet may demonstrate an indolent clinical course with stable disease over decades. A more aggressive clinical course and poorer outcome can be predicted by the inability of the MTC cells to produce calcitonin, a rapidly rising CEA out of proportion to calcitonin and a fast tumor marker doubling time [2, 308]. For patients with a significant MTC burden and symptomatic or progressive metastatic disease, molecular targeted therapies that inhibit RET and other receptor tyrosine kinases involved in angiogenesis should be considered. Two such therapies, vandetanib and cabozantinib, have been FDA-approved for the treatment of adults with progressive, metastatic MTC, and vandetanib appears to be safe and effective in children with advanced MTC in the setting of MEN2B [309].

Case Study

A 13-year-old male was noted to have an enlarged posterior cervical lymph node following a viral upper respiratory illness. He was treated with 2 courses of antibiotics and a short course of oral glucocorticoids. Despite resolution of the illness, the lymphadenopathy persisted for the following year. At that time he was referred for evaluation and physical examination revealed a right-sided 2 cm mobile, non-matted level V lymph node along with a few other "appropriate feeling" anterior cervical lymph nodes. Thyroid palpation was normal as were the serum TSH (1.16 μIU/ml; reference range = 0.35–4.94 μIU/ml) and total T4 (6.7 μg/dl; reference range = 4.9–11.7 μg/dl).

Neck ultrasound (Fig. 21.7) was performed and revealed asymmetric enlargement of the thyroid (right lobe = 1.3 × 1.7 × 4.6 cm, volume = 5.4 cm^3 and left lobe = 1.4 × 1.2 × 3.6 cm, volume = 3.1 cm^3). The entire right lobe showed heterogeneous abnormal echogenicity and a more focal heterogeneous mass within the posterior aspect of the right lobe of the thyroid gland measuring approximately 0.9 × 0.8 × 1.2 cm. There was poor delineation between this mass and the remainder of the right thyroid lobe; the margins appeared slightly lobulated. There were extensive punctate microcalcifications throughout the majority of the right lobe of the thyroid and extending into the isthmus, which was also thickened on the right side. There were also microcalcifications within the inferior aspect of the left thyroid lobe, but there was no discrete mass seen within the left thyroid lobe. Vascularity within the thyroid gland was grossly normal. The findings were concerning for diffuse infiltrative PTC.

US examination of the cervical lymph nodes (Fig. 21.8) revealed numerous enlarged cervical lymph nodes, most prominent in the right anterior cervical chain, and superior mediastinum, involving at least levels III, IV, VI and VII. There was a right supraclavicular node partially compressing the internal jugular vein and several enlarged midline lymph nodes inferior to the thyroid gland. All the enlarged lymph nodes demonstrated abnormal architecture with loss of the normal fatty hila, heterogeneous cortical echogenicity, and microcalcifications consistent with metastatic disease.

FNA was performed on the right level V lymph node showing involvement by a low-grade epithelioid tumor. Immunohistochemical staining was positive for thyroid transcription factor-1 (TTF-1), thyroglobulin, cytokeratin-19 (CK19), and epithelial cell adhesion molecule (Ber-EP4) but negative for calcitonin supporting the diagnosis of metastatic thyroid carcinoma. Thyroglobulin level on the FNA needle washout was strongly positive (861.9 ng/ml), also consistent with DTC.

This case was selected as it represents a common presentation for PTC in children where a distinct thyroid nodule is not always seen, but instead, one encounters diffuse infiltration of the entire lobe or gland. Typical US features for PTC included microcalcifications, which are highly suspicious for malignancy. Persistent lymphadenopathy (especially in levels III and/or IV) with or without a

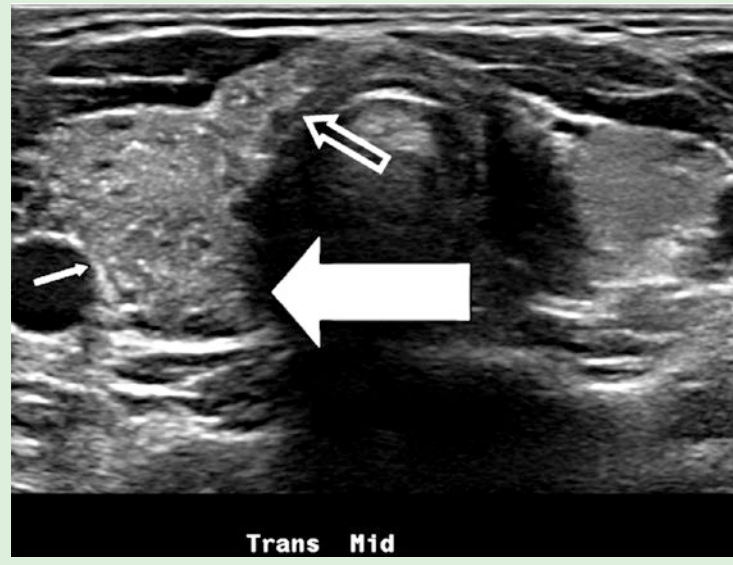

Fig. 21.7 Ultrasound findings suspicious for PTC. The right lobe (*large solid arrow*) is enlarged and heterogeneous and contains multiple microcalcifications (*small solid arrow*). The isthmus is also enlarged (*small open arrow*)

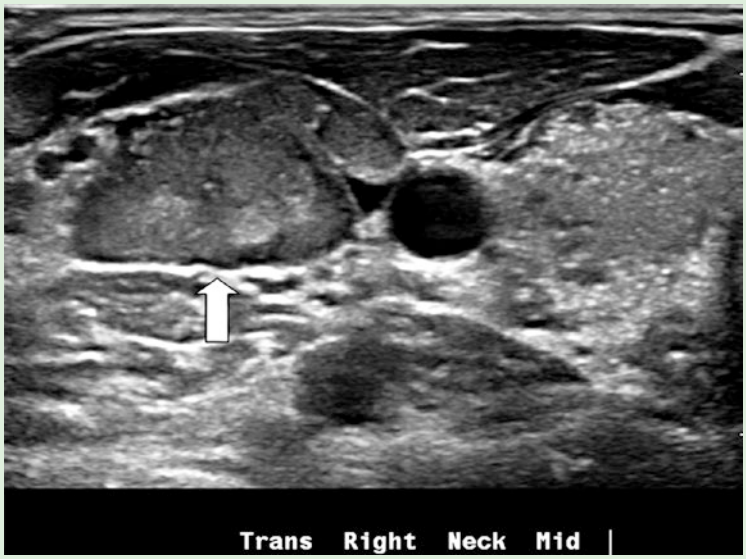

Fig. 21.8 Lymphadenopathy with features concerning for malignancy (*solid arrow*): rounded shape, loss of central hilum, and presence of microcalcifications

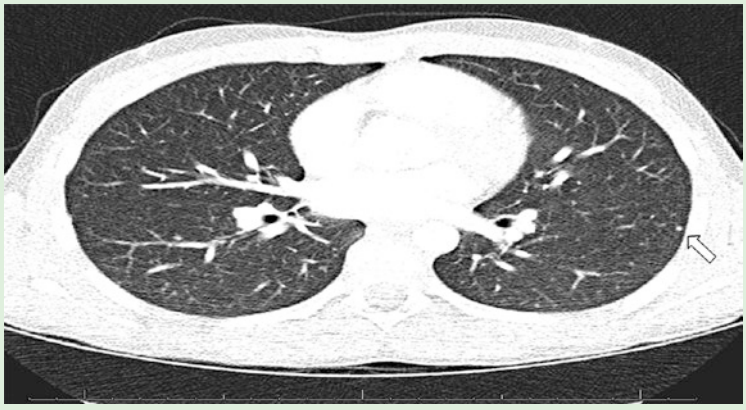

Fig. 21.9 Chest computerized tomography showing one of many foci of suspected metastatic PTC in the lung (*arrow*)

palpable abnormality of the thyroid is a common presentation for PTC in children. The lymph nodes demonstrated typical findings for metastatic disease including rounded shape, loss of the central hilum, and microcalcifications.

This case showed extensive cervical lymph node involvement including levels III, IV, V, VI, and VII. Widespread cervical disease at diagnosis is also common for PTC in children. US identification of PTC beyond the central compartment (level VI) with a suggestion of mediastinal disease was an indication for cross-sectional imaging prior to surgery in order to better define the surgical approach.

The patient underwent computerized tomography (CT) confirming involvement of multiple lymph nodes in the right cervical, supraclavicular, and thoracic inlet areas. Chest CT revealed no definite upper paratracheal, lower mediastinal, axillary, or hilar lymphadenopathy, and the thymic tissue was normal. However, there were innumerable suspicious pulmonary nodules (< 5 mm in diameter) scattered throughout all lobes of the lungs bilaterally (Fig. 21.9).

In this case, there is an increased risk for pulmonary metastases based on the widespread cervical involvement, which generally precedes distant metastases. Serum Tg level was markedly elevated (475 ng/ml), but the TgAb was positive, potentially interfering with the Tg assay. For that reason, Tg was measured by tandem mass spectrometry (MS/MS) which is not affected by the presence of TgAb. The Tg level by MS/MS (184 ng/ml) was also

elevated. In adults, the magnitude of serum Tg correlates with the histological type and location of disease [310]. However, such correlations have not yet been validated for children. Nevertheless, the magnitude of serum Tg in this case is typical of metastatic disease.

He underwent total thyroidectomy with bilateral cervical lymph node dissection of levels II–VII. A total of 47/123 lymph nodes were involved. His tumor was classified as PTC (classic variant), pT3N1b clinical M1.

This placed the patient in the ATA pediatric high risk for persistent disease and recurrence category for which RAI therapy is warranted. However, due to the iodinated contrast given for his CT scan, RAI was deferred pending 24-h urine iodine measurement. Two months after surgery, the urine iodine was still elevated 651.8 μg/24 h (100–460 μg/24 h) but declined to 104.0 μg/24 h at the third month, and he was scheduled for ^{131}Iodine therapy and placed on a low-iodine diet for 2 weeks.

Pre-ablation whole body scan (WBS) was performed following thyroid hormone withdrawal (3 weeks). At that time, the serum TSH was 61.26 uIU/ml and the stimulated Tg was 1006 ng/ml. This Tg was measured using an immunometric assay (IMA) as the Tg antibody was negative and demonstrates the robust Tg response to TSH stimulation typical of PTC in children. The WBS scan revealed 2.8% uptake in the thyroid bed (Fig. 21.10). During previous decades, the WBS was used as a "gold standard" to identify children who were free from disease, but this case demonstrates the reduced sensitivity of the diagnostic WBS when compared to the serum Tg for detection of disease. Unlike the serum Tg, however, the WBS provides anatomic localization of disease. The WBS will also identify lesions that should be surgically removed prior to RAI therapy. Based on the serum Tg and WBS results, he was classified as having persistent disease and received a therapeutic dose of ^{131}Iodine (136.0 mCi). Seven days later a post-therapy scan (Fig. 21.11) confirmed uptake in the thyroid bed, bilateral cervical lymph nodes at level II, and bilateral uptake in the lungs. He returned to levothyroxine suppressive therapy and will have serum Tg measures every 3 months to follow the biochemical response (trend in the TSH-suppressed Tg) to the initial treatment. Repeat neck ultrasound and non-contrast chest CT will be performed approximately every 6–12 months after the initial treatment to assess for persistent anatomic disease. Patients with pulmonary metastasis often require more than one administration of RAI therapy, and the timing for additional RAI will be determined based on the follow-up biochemical and anatomic data.

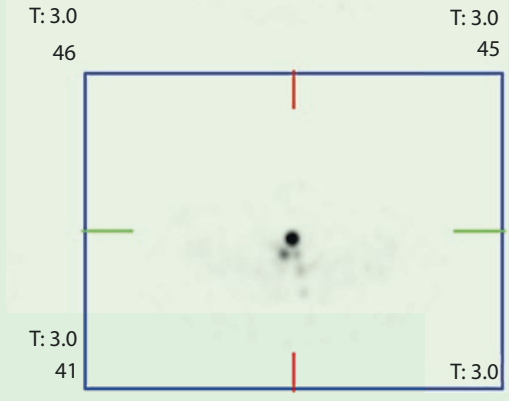

Fig. 21.10 Whole body ^{123}Iodine scan (1.38 mCi) showing uptake only in the thyroid bed

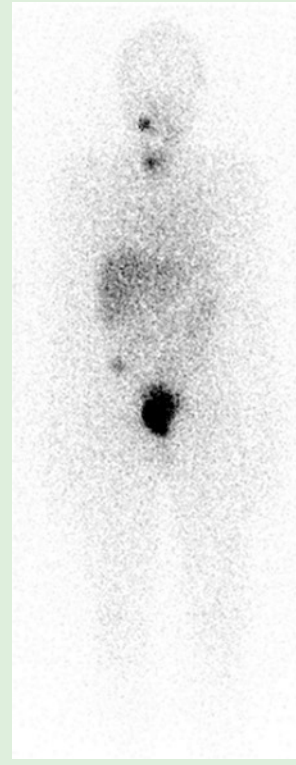

Fig. 21.11 Whole body scan performed 7 days after administration of 136.0 mCi ^{131}Iodine confirms bilateral uptake in the neck at level II nodes, uptake in thyroid bed, and absent uptake in lungs

21.4 Summary

Survival for children with differentiated thyroid cancer is excellent, but therapy must be individualized in order to reduce the risk of treatment-associated complications. Individual therapy is determined by thorough staging at diagnosis and after all diagnostic, therapeutic, and surveillance procedures. The majority of children with DTC require total thyroidectomy and central compartment lymph node dissection. However, preoperative staging will identify children with lateral neck disease who require more extensive lymph node dissection. Postoperative staging will identify children with persistent disease and those who are at increased risk for recurrence. Follow-up staging will determine the response to therapy and identify children who will require additional treatments. Almost one half of children with pulmonary metastases develop stable but persistent disease following surgery and RAI therapy and should have repeated treatment when there is evidence for progression and only if they benefited from previous RAI.

Review Questions

1. A 10-year-old female was involved in a motor vehicle collision and underwent head and neck CT. Incidental finding was a 1.5 cm lesion in the left lobe of the thyroid. Based on this information, you would do which of the following:
   A. Hemi-thyroidectomy to remove suspected PTC
   B. Serum thyroglobulin level to determine if this is PTC
   C. An ^{123}Iodine whole body scan to look for PTC
   D. A serum TSH and a high-resolution thyroid and neck ultrasound
2. A 10-year-old male was diagnosed with PTC and underwent total thyroidectomy and central compartment lymph node dissection (classic PTC, T3N1aMx). Postoperative serum thyroglobulin was 2.3 ng/ml, and Tg antibody was 400 IU/ml (reference range < 0.9 IU/ml). Based on these data, you would do which of the following:
   A. Repeat the serum Tg level in 3 months to determine if this increases or decreases.
   B. Perform an FDG-PET scan to look for persistent disease.
   C. Perform an ^{123}Iodine whole body scan to look for iodine-avid metastases and serum Tg using radioimmunoassay or tandem mass spectrometry.
   D. Place on TSH-suppressive therapy and follow the Tg antibody trend.
3. A 12-year-old female has just undergone total thyroidectomy and central compartment lymph node dissection for PTC and is found to have PTC in the right lobe of the thyroid and in 5/25 central compartment lymph nodes. Her postoperative staging includes a neck ultrasound that shows no evidence for disease and a serum thyroglobulin obtained while the TSH is suppressed (Tg, 124 ng/ml; TSH, 0.06 uIU/ml). Based on these data, you would do which of the following:
   A. Repeat the serum Tg level in 3 months to determine if this increases or decreases.
   B. Perform an FDG-PET scan to look for RAI-resistant disease.
   C. Perform an ^{123}Iodine whole body scan to look for iodine-avid pulmonary metastases.
   D. Ask the surgeons to perform a lateral neck dissection on the right to remove suspected lateral neck metastases.

Answers

1. (D) The serum TSH will help to determine if this is a hyperfunctioning lesion. The US will help detect any suspicious cervical lymph nodes and determine the US characteristics of the lesion. FNA should be performed on any suspicious lymph node, and the US characteristics of the lesion would help target a specific portion of the lesion for fine needle aspiration. Although the size meets criteria for performing FNA in children, US may reveal calcification or blood flow in a portion of the lesion that would be most concerning.

2. (C) A serum Tg level may be falsely low in the presence of anti-Tg antibodies even if one uses a radioimmunoassay or tandem mass spectrometry rather than immunochemiluminometric assay. Thus, the low Tg is less informative in the presence of elevated TgAb. With the pathology revealing extrathyroidal extension (T3) and metastasis to central neck lymph nodes (N1a), a whole body radioactive iodine scan should be considered. The WBS is not affected by the presence of anti-Tg antibodies and may be informative to determine if the patient would benefit from radioiodine therapy. If the extrathyroidal extension was minimal and there were less than 5 central compartment lymph nodes positive for micrometastatic disease, one could also consider placing the patient on TSH-suppressive therapy and following the Tg and anti-Tg antibody titers to assess for evidence of persistent disease. In this situation, a stimulated Tg and whole body scan could be performed at a later time if the anti-Tg antibody titer remained elevated or showed an upward trend.
3. (C) Perform an ^{123}Iodine whole body scan to look for iodine-avid pulmonary metastases. This patient has a marked elevation in Tg while the TSH is suppressed that is highly suggestive of residual disease. Neck US fails to localize disease so the most likely site is the lung. The whole body scan will help to localize disease in lung and also neck/mediastinal nodes if there are additional metastases to lymph nodes that cannot be visualized by routine neck US.

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Mineral and Bone Disorders

Abnormalities in Calcium Homeostasis

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Key Points

- Hormonal regulation of serum calcium in fetal, neonatal, and childhood periods
- Diagnosis, evaluation, and treatment of hypocalcemia
- Diagnosis, evaluation, and treatment of hypercalcemia

22.1 Introduction

Calcium plays an important role in a number of physiological processes as diverse as bone formation and turnover, neuronal cell excitability, muscle contractility, and blood clotting. Significant shifts in serum calcium concentration have adverse effects on these physiological functions. In children, maintenance of adequate calcium balance is particularly important since bone accrual and growth are closely linked to the availability of calcium. Higher organisms have developed mechanisms to regulate the extracellular concentration of calcium, normally affected by intermittent changes in calcium absorption in the gut, continuous mineral bone turnover, and calcium losses in urine. Extracellular calcium levels are set within a very narrow range by the concerted action of several regulatory "calciotropic" hormones on calcium handling in the gastrointestinal tract, bone, and kidney. The abnormal function of calciotropic hormones or the failure of any of these organs to handle calcium properly can cause either hypo- or hypercalcemia.

Calcium is among the most abundant mineral ions in the body. Greater than 98% of total calcium is present as mineral salts in bone but can be mobilized as part of a continuous exchange of calcium between bone and the extracellular compartment during bone remodeling. The remaining fraction of calcium is distributed between the intracellular and extracellular compartments. Calcium in serum exists in three forms: (1) a protein-bound fraction (30–50% of total serum calcium), primarily bound to albumin; (2) complexed with serum anions like phosphate, citrate, and bicarbonate (5–15%); and (3) ionized Ca (Ca^{2+}) (40–60%). Ca^{2+} is the metabolically active form and is the soluble fraction that is tightly regulated. As a result, the concentration of serum Ca^{2+} remains relatively constant with age and dietary intake.

22.2 Hormonal Regulation of Serum Ca^{2+}

22.2.1 Parathyroid Hormone

Changes in serum Ca^{2+} are rapidly sensed by the parathyroid glands [1]. There are four paired parathyroid glands, usually positioned in the superior and inferior poles of the thyroid, derived from the 4th and 3rd pharyngeal pouches, respectively. In response to a decrease in serum Ca^{2+}, they secrete parathyroid hormone (PTH), an 84-amino acid polypeptide synthesized and stored in secretory granules. The net effect of PTH on calcium homeostasis is to activate mechanisms that increase serum Ca^{2+} levels [2]. PTH promotes calcium mobilization from bone by osteoblast-mediated activation of bone-resorbing osteoclasts. In the kidney's proximal tubule, PTH activates 1-α-hydroxylase to synthesize calcitriol ($1,25(OH)_2D$) and increases the absorption of sodium, calcium, and bicarbonate while inhibiting phosphate transport and promoting phosphaturia. PTH has its most significant effect in the distal convoluted tubule where it activates calcium absorption. In the gut, PTH indirectly promotes, through the action of $1,25(OH)_2D$, the absorption of both calcium and phosphate.

The overall effect of changes in calciotropic hormones on calcium handling by the kidney, gastrointestinal tract, and bone is to maintain the extracellular Ca^{2+} concentration around the normal range (usually 1.12–1.23 mmol/L). This is primarily achieved by the regulation of PTH secretion in parathyroid cells. Ca^{2+} sensing is mediated by a G protein-coupled, calcium-sensing receptor (CaSR) [3] expressed in parathyroid cells. Elevations in serum Ca^{2+} activate the CaSR which, in turn, mediates the inhibition of PTH secretion. Although Ca^{2+} is the major regulator of PTH secretion, other calciotropic factors also affect its secretion. The active form of vitamin D, calcitriol, inhibits PTH synthesis, while high serum phosphate has been shown to stimulate PTH secretion [4]. Profound hypomagnesemia inhibits both PTH secretion and action by affecting intracellular signaling function. Hypermagnesemia also inhibits PTH secretion, a process likely mediated by the CaSR, since magnesium is also a ligand for this receptor [1]. PTH is exquisitely sensitive to degradation both intracellularly in the parathyroid cell and in serum, especially as it traverses

the liver and kidney; its serum half-life is less than 8 min. Thus, an accurate measurement of active PTH requires an immunoassay that measures intact PTH, presently achieved by a sandwich immunoradiometric assay (IRMA) or an immunofluorometric assay.

The bioactive site in PTH resides within the first 27 amino acids of the peptide [2]. PTH binds to a G protein-coupled receptor (PTH1R) that activates the production of cAMP and, in some cells, the release of intracellular calcium stores via activation of phospholipase C. This receptor is present in osteoblasts and kidney tubular epithelium, cells that play a direct role in calcium homeostasis. Two additional receptors (PTH2R and PTH3R) with some homology to the first characterized receptor have been recently described [5], but their role in calcium homeostasis may not be significant.

At least another peptide has been shown to have PTH-like effects. PTH-related protein (PTHrP) was initially characterized as causing hypercalcemia when secreted by some malignant tumors [6]. The amino terminus of this peptide has high homology with the bioactive amino terminus of PTH and binds the PTH receptor. Besides its role as a calciotropic hormone when present in serum in high concentration, PTHrP serves important functions in cartilage formation, in the growth plate, and the differentiation of several organs where it is expressed during fetal and postnatal development [7]. In the placenta, active transplacental transport of Ca^{2+} appears to be mediated by PTHrP binding to an unidentified receptor [8].

22.2.2 Vitamin D

Vitamin D_3 (cholecalciferol) is produced by photolysis of cholesterol to 7-dehydrocholesterol under UVB irradiation (280–305 nm wavelength) followed by isomerization in the skin [9]. It is hydroxylated to 25-hydroxyvitamin D (25OHD) in the liver, a step that is largely substrate dependent, making 25OHD levels a useful index of vitamin D stores. Its serum half-life is 2–3 weeks. An additional hydroxylation step by 1-α-hydroxylase in the renal proximal tubule produces the bioactive form of vitamin D, $1,25(OH)_2D$. PTH, hypocalcemia, and hypophosphatemia are the major inducers of 1-α-hydroxylase activity in the proximal tubule. FGF23, a regulator of phosphate excretion by the renal tubule, appears to inhibit $1,25(OH)_2D$ production [10]. An increase in $1,25(OH)_2D$ production becomes apparent hours after exposure to a stimulus, and the half-life of $1,25(OH)_2D$ is only several hours. The proximal tubule also has 24-hydroxylase activity; hypercalcemia, hyperphosphatemia, and $1,25(OH)_2D$ induce this enzyme, promoting the production of $24,25(OH)_2D$, an inactive metabolite. 1-α-hydroxylation activity is not limited to the proximal tubule. 1-α-Hydroxylase is expressed in the placenta, a significant source of calcitriol for the fetus, in keratinocytes, and activated mononuclear cells. Excess 1-α-hydroxylase activity in mononuclear cells is thought to be responsible for the hypercalcemia and elevation of $1,25(OH)_2D$ levels seen in granulomatous disorders [11].

Vitamin D and its metabolites are transported in serum bound to vitamin D-binding protein (DBP), showing greatest affinity for 25OHD. This protein provides a reservoir of vitamin D metabolites and prevents its rapid clearance in the urine. Megalin, a lipoprotein-like receptor that binds DBP, has been shown to mediate the uptake of vitamin D metabolites in the proximal tubule, suggesting a role for this protein in ensuring 25OHD availability for 1-α-hydroxylation in the kidney [12].

Calcitriol promotes the rise of both calcium and phosphate levels in serum [9]. $1,25(OH)_2D$ binds to vitamin D receptors (VDR), a member of the retinoid family of nuclear receptors, expressed in the intestine, distal renal tubular cells, osteoblasts, parathyroid cells, and other tissues not directly involved in calcium homeostasis. In bone, binding to VDR promotes the activation of osteocalcin and alkaline phosphatase production by osteoblasts and the differentiation of osteoclast precursors, having a net effect in mobilizing calcium and phosphate from bone. In the kidney, $1,25(OH)_2D$ facilitates the action of PTH on distal tubule calcium absorption. The major impact of $1,25(OH)_2D$ is in the small intestine where it promotes the absorption of calcium and phosphate in the duodenum and jejunum.

22.2.3 Calcitonin

Calcitonin is a 32-amino acid peptide produced by thyroid parafollicular C cells and in lesser amounts by other neuroendocrine cells [13]. High Ca^{2+} elicits a rise in calcitonin secretion in parafollicular cells, a process mediated by the same

CaSR expressed in parathyroid cells [3]. In almost all instances, calcitonin antagonizes the effect of PTH on the bone and kidney, via its binding to a G protein-coupled receptor of the same family as the PTH receptor. Calcitonin has no measurable effects on intestine handling of mineral ions. Paradoxically, calcitonin levels rise abruptly at birth, despite a drop in serum Ca^{2+} normally seen during the same period, and decrease rapidly after birth [14]. In children older than 3 years, normal serum levels are often below detection unless elicited by hypercalcemia or in the setting of medullary thyroid carcinoma. The role of calcitonin in normal calcium homeostasis is uncertain, since in the absence of parafollicular cells (i.e., thyroidectomy), no significant alterations in calcium homeostasis have been observed; however, it has a pharmacological role in the acute treatment of hypercalcemia and osteoporosis as a promoter of calcium deposition in bone.

22.2.4 Calcium Homeostasis During Fetal and Early Neonatal Period

During fetal development, calcium homeostasis is affected by maternal Ca^{2+} levels [14]. Serum Ca^{2+} in the fetus is set at a higher concentration (≈ 0.25 mmol/L higher) than the mother. There is active transport of calcium across the placenta to sustain this gradient, a process that appears to be mediated by both PTH and PTHrP (likely the midregion fragment of PTHrP) which is secreted by the fetal parathyroid among other fetal organs during pregnancy. Although the parathyroid glands are present as early as the first trimester in gestation, PTH levels are low because its secretion is normally suppressed during fetal development as fetal serum Ca^{2+} levels remain elevated in utero. In the fetus, bone mass accretion occurs primarily from 24 weeks to full gestation. Maternal serum Ca^{2+} levels and, less significantly, vitamin D status affect the extent of mineralization during this period, when the mother is the primary source of vitamin D. Both maternal 25(OH)D and $1,25(OH)_2D$ cross the placenta, while the placenta also produces $1,25(OH)_2D$.

At birth, there is a fall in serum Ca^{2+} levels, reaching a nadir (1–1.17 mmol/L) in the first 24–48 h of life [15]. PTH levels are low at birth but rise with the decrease in serum Ca^{2+}. Serum PTHrP levels decrease rapidly in the first day of life. $1,25(OH)_2D$ levels increase concomitantly with the increase in serum PTH. Milk intake provides the primary source of serum Ca^{2+} during the neonatal period. During the initial neonatal period, intestinal calcium absorption is not significantly regulated by $1,25(OH)_2D$; instead, passive absorption mechanisms enhanced by the presence of lactose in milk predominate at this stage [14]. The intestine progressively develops increased sensitivity and dependency on vitamin D for adequate calcium absorption. Vitamin D levels in infants correlate best with supplementation and sun exposure and not with breast milk intake, regardless of maternal vitamin D status.

22.3 Hypocalcemia

Hypocalcemia develops as a consequence of either reduced influx of calcium from the gastrointestinal tract or bone into the extracellular space or excessive loss of calcium from this space into urine, bone, or stool. Causes of hypocalcemia include abnormalities in calciotropic hormone production and action or improper calcium handling by organs targeted by these hormones.

Differential Diagnosis of Hypocalcemia

Alterations in hormonal response

- Hypoparathyroidism
  - Abnormal PTH production
    - Parathyroid agenesis/dysfunction
    - Familial forms of isolated PTH deficiency
    - DiGeorge syndrome
    - Kenny-Caffey syndrome
    - HDR syndrome
    - Dyshormonogenesis
  - Acquired hypoparathyroidism
    - Polyglandular autoimmune disease type I
    - Mitochondrial myopathies (Kearns-Sayre syndrome, MELAS)
    - Disorders of metal ion deposition
    - Radiation exposure
    - Idiopathic
    - Thyroid and parathyroid surgery
  - Abnormal PTH secretion
    - Hypomagnesemia
    - Autosomal dominant hypocalcemia
    - Critical illness

- Peripheral resistance to PTH
  - Pseudohypoparathyroidism types IA, IB, II
  - Pseudo-pseudohypoparathyroidism
- Vitamin D
  - Vitamin D deficiency
    - Nutritional deficiency
    - Liver disease
    - Iatrogenic (e.g., phenobarbital use)
  - Vitamin D resistance
    - Hydroxylase deficiencies
    - Vitamin D receptor dysfunction

Alterations of organs involved in calcium homeostasis
- *Kidney*: Renal failure, renal tubular acidosis
- *Intestine*: Malabsorption
- *Skeleton*: Hungry bone syndrome

Other causes of hypocalcemia
- High phosphate load
  - Tumor lysis syndrome
  - High phosphate formula
  - Rhabdomyolysis
- Calcium sequestration or clearance
  - Acute pancreatitis
  - Drugs: Furosemide, calcitonin, bisphosphonates
- Decreased ionized calcium
  - Exchange blood transfusion
  - Alkalosis

22.3.1 Alterations in Calciotropic Hormones Causing Hypocalcemia

22.3.1.1 Hypoparathyroidism

Lack of adequate PTH production is a frequent cause of hypocalcemia in neonates and early childhood. In hypoparathyroidism, decreased PTH levels cause hypocalcemia and hyperphosphatemia. There are sporadic and familial forms of hypoparathyroidism caused by parathyroid agenesis or dysfunction [16]. Autosomal dominant, autosomal recessive, and X-linked recessive patterns of inheritance have been described for familial forms of hypoparathyroidism. Mutations of GCM2, a protein linked to parathyroid differentiation, are a recently identified etiology of parathyroid agenesis [17]. Point mutations of the PTH gene in chromosome 11p15 lead to inappropriate expression of PTH and dyshormonogenesis [18]. A form of autosomal dominant hypoparathyroidism in HDR syndrome, linked to mutations in GATA-binding protein 3, is associated with sensorineural deafness and renal dysplasia [19]. DiGeorge syndrome and its variants are a more generalized embryological abnormality that occurs either sporadically or with variable autosomal dominant penetrance, involving the development of the third and fourth branchial pouches. This complex malformation is associated with dysmorphic facial features and anomalies of the heart and great vessels with variable defects in thymic and parathyroid gland function, often showing dysgenesis of both glands. Deletions and translocations of chromosomes 22q11 and 10p13 have been detected and can be screened in suspected cases [20]. Hypoparathyroidism is also common in patients with mutations in tubulin folding cofactor E linked to Sanjad-Sakati and Kenny-Caffey syndromes, the latter characterized by medullary stenosis of the long bones, short stature, hyperopia, and basal ganglia calcifications [21]. Hypoparathyroidism has also been reported in a number of mitochondrial myopathies (i.e., Kearns-Sayre syndrome) where PTH secretion appears affected by the intracellular metabolic abnormality [22].

Acquired forms of hypoparathyroidism often occur later in infancy and adolescence. Infiltrative processes such as excess deposition of iron (thalassemia and hemochromatosis) and copper (Wilson's disease) in the parathyroid can impair the secretion of PTH. Exposure to radiation as part of therapy for hyperthyroidism or lymphoma has been linked to the onset of hypoparathyroidism, as has surgical removal or compromise of the vascular supply to the parathyroid glands. Autoimmune destruction of the parathyroid gland can be an isolated process or as part of polyglandular autoimmune disease type I, an autosomal recessive disorder, linked to mutations of the *AIRE* gene, which is also associated with mucocutaneous candidiasis, hypoadrenocorticism, hypogonadism, thyroid disease, type I diabetes mellitus, pernicious anemia, chronic active hepatitis, malabsorption, and manifestations such as alopecia, vitiligo, keratopathy, and enamel hypoplasia [23]. In this disorder, chronic oral candidiasis is the first manifestation, usually in early infancy. The average age of onset for mucocutaneous candidiasis, hypoparathyroidism, and adrenal insufficiency is 5 years, 9 years, and 14 years of age, respectively. About half of all affected children end up having at least these three manifestations. The presence of intestinal malabsorption complicates the treatment of hypocalcemia as calcium and vitamin D absorption is often impaired.

Several conditions are characterized by impaired PTH secretion despite the presence of viable parathyroid tissue and PTH synthesis. PTH secretion can be impaired in the presence of severe hypomagnesemia. Hypomagnesemia may be secondary to intestinal malabsorption or excessive renal wasting as seen in Bartter syndrome and renal tubular acidosis [24]. In autosomal dominant hypocalcemia, activating mutations of the CaSR increase the inhibition of PTH secretion and a sufficient enough decrease in serum Ca^{2+} concentrations to elicit adverse effects. Correction of hypocalcemia causes significant hypercalciuria as the ability of CaSR to decrease tubular absorption of calcium is also increased, augmenting the risk of urinary stones compared to other forms of hypoparathyroidism. Finally, PTH secretion has been shown to be impaired in critical illness, perhaps by an interleukin-mediated overexpression of CaSR [25].

Tissue insensitivity to PTH has a clinical presentation very similar to hypoparathyroidism. Pseudohypoparathyroidism (PHP) includes various familial disorders that are characterized by a resistance to PTH [26]. Hypocalcemia occurs despite very elevated PTH levels but without a concomitant elevation of $1,25(OH)_2D$ levels or increased renal phosphaturia. In patients with type IA PHP or Albright hereditary osteodystrophy, the characteristic phenotype is short stature, stocky habitus, developmental delay, round face, short distal phalanx of the thumb, brachymetatarsias and brachymetacarpals, dental hypoplasia, and subcutaneous calcifications. Hypocalcemia is often not diagnosed until the mid-childhood years. PTH resistance is characterized by the absence of an increase in urinary cAMP excretion following administration of PTH (normally elevated when the kidney is responsive to PTH). Inactivating mutations in the α subunit of the stimulatory protein G_s are responsible for PTH resistance in this condition by preventing the activation of adenylyl cyclase by the PTH receptor. These patients may show additional deficiencies due to the defective action of other peptide hormones that use the same stimulatory G_s to enhance cAMP production. In particular, thyrotropin action is often affected, and occasionally hypothyroidism is diagnosed before the hypocalcemia is noted. The actions of corticotropin, gonadotropin, glucagon, and GH-releasing hormone, among other hormones, have been shown to be affected. Pseudopseudohypoparathyroidism is used to describe patients with the Albright osteodystrophy phenotype without the biochemical abnormalities of PHP and may represent the inheritance of the defective gene from the father, suggesting the presence of imprinting in the inheritance of this disorder. Type IB PHP resembles type IA except that the $G_{s\alpha}$ subunit is normal, pointing to a defect in another step of the pathway that stimulates cAMP. Type II PHP is yet another variant where the phenotypic features are absent and infusion of PTH induces the normal increase in urinary cAMP excretion but without the expected phosphaturia, suggesting a defect distal to cAMP production.

22.3.1.2 Vitamin D Deficiency or Resistance

If vitamin D stores are markedly depleted, intestinal calcium absorption can decrease sufficiently to cause hypocalcemia. In growing children, the negative calcium and especially phosphorus balance has deleterious effects on mineral deposition, and particularly on the growth plates, resulting in rickets. The parathyroid response to hypocalcemia is intact, but the elevated levels of PTH cannot compensate for the absence of substrate necessary to produce $1,25(OH)_2D$. Inadequate sun exposure or lack of vitamin D intake can cause a decrease in vitamin D levels. Children with liver disease or taking drugs that enhance the activation of liver hydroxylating enzymes (i.e., phenobarbital) may have impaired 25OHD production or increased turnover to inactive metabolites of 25OHD, respectively. In rare occasions, a deficiency in 1-α-hydroxylase activity in the kidney or the presence of abnormal receptors for $1,25(OH)_2D$, conventionally classified as vitamin D-dependent rickets (VDDR) I and II, respectively, can have the same biochemical consequences and clinical presentation as vitamin D deficiency, including hypocalcemia [27]. Patients with VDDR-I do not respond to massive doses of vitamin D or 25OHD. Interestingly, alopecia is often seen in VDDR-II, suggesting a role of vitamin D receptors in hair development and growth.

22.3.2 Other Causes of Hypocalcemia

When calcium handling by the gastrointestinal tract, bone, or kidney is abnormal or not responsive to calciotropic hormones, hypocalce-

mia can persist despite an appropriate hormonal response (i.e., increased PTH secretion and calcitriol production). The hyperphosphatemia that ensues with renal failure causes hypocalcemia, as excess phosphate complexes with Ca^{2+}, reducing its serum concentration. The lack of calcitriol production in advanced renal failure further aggravates the risk for hypocalcemia by decreasing intestinal calcium absorption. In disorders that have intestinal malabsorption as one of their manifestations or in cases of short gut syndrome, calcium absorption can diminish sufficiently to cause hypocalcemia. In conditions where calcium deposition in bone exceeds nutritional intake (i.e., hungry bone syndrome), as occasionally seen during the treatment phase of severe rickets or following parathyroid surgery for hyperparathyroidism, acute onset of hypocalcemia is not uncommon.

Hypocalcemia can occur in settings where there is a high influx of phosphate or another anion into the extracellular space to complex with Ca^{2+}. The release of high loads of phosphate in tumor lysis syndrome and rhabdomyolysis can cause severe hypocalcemia with deposition of calcium phosphate salts in various tissues. Likewise, an exogenous source of phosphate as in high phosphate content formula can have a similar effect in small infants. In acute pancreatitis, calcium is sequestered by free fatty acid complexes decreasing its effective concentration in serum, while the presence of citrate in exchange blood transfusions or alkalosis can decrease serum Ca^{2+} acutely.

22.3.3 Classification of Neonatal Hypocalcemia

Neonatal hypocalcemia has been traditionally described as "early" when it occurs in the first 72 h of life or "late" when it occurs beyond that period of time [15] (see below). Infants that are born prematurely or experience asphyxia are particularly prone to experience a period of hypocalcemia in the early neonatal period. Preterm infants may have a deficient increase in PTH secretion to counteract the normal drop in serum Ca^{2+} after birth. In addition, calcium intake is often suboptimal, increasing the risk for hypocalcemia. The role of asphyxia in causing hypocalcemia is poorly defined but may be similar to the hypocalcemia seen in acute illness. Infants of diabetic mothers are also prone to develop hypocalcemia early in the neonatal period. Although both a history of prematurity and asphyxia are usually present in these babies, magnesium deficiency has also been invoked as a likely cause of hypocalcemia since maternal glycosuria is accompanied by significant magnesium losses predisposing the fetus to total body magnesium deficiency.

Common Causes of Neonatal Hypocalcemia

Early
- Asphyxia
- Prematurity
- Maternal gestational diabetes
- Hypomagnesemia

Late
- Maternal hyperparathyroidism
- Hyperphosphatemia
- Transient hypoparathyroidism
- Congenital forms of hypoparathyroidism

Late neonatal hypocalcemia encompasses most of the etiologies described earlier that are commonly seen in childhood. A common cause of hypocalcemia is a transient form of hypoparathyroidism that lasts from a few days to several weeks. These infants appear to have a deficient PTH response to hypocalcemia that improves slowly with time. In some instances, this transient deficiency is due to exposure to maternal hypercalcemia in utero. Maternal serum Ca^{2+} should be measured to rule out this possibility. Infants with transient hypoparathyroidism have been shown to have a higher risk to develop hypocalcemia later in life, suggesting that a mild abnormality in parathyroid function may be present.

22.3.4 Diagnosis and Evaluation of Hypocalcemia

Hypocalcemia can be asymptomatic in children and adolescents, especially when it is longstanding, and is often diagnosed in the setting of a routine biochemical screen. Abrupt decreases in serum Ca^{2+} predispose children to more severe symptoms, mostly neurological in nature, that require prompt medical attention. Early neuromuscular symptoms include numbness around the mouth, tingling, paresthesias, muscular cramping (especially after

vigorous exercise), and carpopedal spasm. More severe symptoms include seizures, tetany, laryngospasm, and mental status changes. In neonates, symptoms can be more subtle, and the only manifestation may be poor feeding and vomiting; however, acute presentations are usually characterized by a history of recurrent seizures, twitching of the extremities, agitation, high-pitched voice, tachypnea, or apnea. In some instances, neonates with acute hypocalcemia may present in cardiac failure.

Infants with acute symptomatic hypocalcemia frequently show hypotonia, tachycardia, and a bulging fontanelle on physical examination. In older asymptomatic children, the physical examination usually reveals no striking abnormality other than hyperreflexia, a positive Chvostek sign (twitching of facial muscles after tapping the facial nerve just in front of the ear) and/or a Trousseau sign (carpopedal spasm with hypoxia after maintaining a blood pressure cuff above the systolic blood pressure for 3–5 min). These findings are not exclusively present in hypocalcemic states; the Chvostek sign can be present in normal adolescents, and other ionic abnormalities such as hypokalemia, hyperkalemia, hypomagnesemia, and severe hypo- or hypernatremia can cause tetany. Hypocalcemia affects cardiac function by impairing myocardial contractility and prolonging the QTc interval, increasing the predisposition to cardiac arrhythmias. Ophthalmologic findings can include papilledema, optic neuritis, and subcapsular cataract formation. Calcium deposition in intracranial locations with a preference for basal ganglia is not uncommon in chronic hypocalcemia. Other physical findings in chronic hypocalcemia include coarse hair, dry skin, brittle nails, and defective dentition, all the consequence of inadequate serum Ca^{2+}. When hypocalcemia is accompanied by vitamin D deficiency and decreased intestinal calcium absorption, the bony abnormalities commonly seen in rickets are a prominent feature of the physical presentation.

Other findings in the history and physical examination frequently prove useful in the determination of the etiology of hypocalcemia. If the phenotypic features of type I PHP are present, PTH resistance should be suspected, whereas the presence of facial anomalies (i.e., mandibular hypoplasia, hypertelorism, short philtrum, and low-set ears), a heart murmur, or a history of recurrent infections suggests DiGeorge syndrome. The absence of a thymus shadow on a chest X-ray in a neonate with hypocalcemia should point to this syndrome. A history of mucocutaneous candidiasis, vitiligo, or alopecia may suggest the presence of autoimmune polyendocrinopathy type I.

Serum calcium concentration should always be obtained and compared to normal values to confirm hypocalcemia. Since calcium is found in both protein-bound and ionized forms in serum, conditions that alter protein content and binding affinity affect the Ca^{2+} concentration in serum. In acidic states, calcium is dissociated from albumin, and the concentration of serum Ca^{2+} increases, while the reverse occurs in alkaline conditions. An ionized measurement is the more accurate assessment of serum Ca^{2+} concentration and has currently become more routinely available, especially in the hospital setting. Normal values range from 1.12 to 1.23 mmol/L in most laboratories. Adequate sampling is imperative to prevent excessive exposure to air or to high amounts of heparin since, in both circumstances, readings are artificially lower.

As part of a complete evaluation of mineral ion homeostasis, both serum phosphate and magnesium levels should be obtained. Phosphate levels should be compared to normal values adjusted for age. Vitamin D stores can be measured by obtaining 25OHD levels, while $1,25(OH)_2D$ levels provide a good measure of PTH activity. The bone-derived serum alkaline phosphatase level is a measure of osteoblast activity and bone turnover. It is usually elevated in states of high bone turnover as seen in hyperparathyroidism and rickets. Renal function can be adequately screened by measurement of total protein, electrolytes, bicarbonate, BUN, and creatinine. In addition, urine calcium, phosphate, and creatinine levels provide a measure of mineral ion handling by the kidney, especially in conjunction with serum measurements.

Several useful calculations provide a measure of calcium handling before and during therapy:

- Ca × Phosphate, if >60 there is a high predisposition to insoluble mineral deposition in joints and tissues.
- Urine calcium/urine creatinine, if >0.2 the risk of nephrocalcinosis increases. In healthy neonates and infants, this ratio may be higher; hence the urine calcium/creatinine ratio should be interpreted in the context of the age of the infant. Spot measurements are usually adequate, especially if obtained early in the morning and fasting.

- TRP (tubular reabsorption of phosphate) = 1 − (urine phosphate × serum creatinine/serum phosphate × urine creatinine). This measure provides a measure of phosphate retention by the kidney. TmP/GFR = TRP × serum phosphate (normal range 2.5–4.2 mg/dL), TRP adjusted for glomerular filtration rate.

In most instances, when hypocalcemia has been confirmed, a concomitant measure of serum Ca^{2+} and intact PTH provides an adequate assessment of parathyroid function. In hypocalcemic states, PTH levels should be elevated when parathyroid function is normal. In most laboratories, the normal range of serum intact PTH values falls between 10 and 65 pg/mL. If PTH values are below detection level or inappropriately normal for the degree of hypocalcemia, a form of primary hypoparathyroidism is the likely diagnosis. Elevations in serum phosphate would also support this diagnosis. If serum magnesium levels are low, usually below 1.5 mg/dL, hypocalcemia may be due to impaired PTH secretion and action; restoration of normal serum magnesium levels and monitoring of serum Ca^{2+} should be considered before diagnosing an intrinsic abnormality in parathyroid function.

When the PTH level is appropriately elevated in the presence of hypocalcemia, a form of PTH resistance or PHP is the likely diagnosis. In PHP, PTH levels are frequently very elevated, while calcitriol levels are generally in the normal range or even low despite normal vitamin D stores. To distinguish between different types of PHP, in addition to careful description of the physical phenotype, a PTH infusion with concomitant measurement of urinary cAMP would be required, a test that is seldom performed because PTH is not readily available in most clinical centers. Fortunately, the treatment is currently similar for all forms of PHP, and their clinical classification is less critical for adequate management.

If hypocalcemia is accompanied with normal or low serum phosphate levels, a form of vitamin D deficiency should be suspected, a diagnosis that would be supported by physical findings of rickets and an elevated alkaline phosphatase level. Low 25OHD levels would suggest a dietary deficiency, an intestinal malabsorptive process, or improper processing by the liver. Normal 25OHD levels would point to a defect in calcitriol production or action. It is not unusual to see very high levels of $1,25(OH)_2D$ in patients with vitamin D receptor defects.

22.3.5 Management of Hypocalcemia

22.3.5.1 Acute Hypocalcemia

In a symptomatic patient, the initial goal is to take the appropriate steps to eliminate symptoms associated with hypocalcemia. In patients whose acid-base status or the infusion of agents that may complex with calcium is responsible for the hypocalcemia, adequate steps to ameliorate these causes should be taken. In acute symptomatic cases or in neonatal hypocalcemia, an intravenous infusion of calcium is the most effective intervention. Calcium gluconate (10% calcium gluconate = 9.3 mg Ca/mL), 2 mL/kg, can be administered slowly, over a 10-min period to avoid cardiac conduction problems while monitoring the ECG. The dose can be repeated every 6–8 h.

To maintain normocalcemia, it is occasionally necessary to start a continuous intravenous infusion of calcium (20–80 mg Ca/kg/24 h). The infusion rate should be titrated to achieve a low normal serum Ca^{2+} level. Hypomagnesemia should be corrected when present. $MgSO_4$ (50% solution) may be administered at a dose of 25–50 mg Mg^{2+}/kg in intravenous or intramuscular form every 4–6 h, 10–20 mg Mg^{2+}/kg for the neonate. A maintenance dose of 30–60 mg Mg^{2+}/kg/day as an oral or continuous intravenous infusion could also be given if necessary.

It is preferable to transition patients to oral therapy as soon as possible. In asymptomatic patients, it is likely that the hypocalcemia, even when very severe, has been longstanding and oral therapy should be the first line of therapy. Several forms of calcium supplements [calcium salts of carbonate (40% Ca), citrate (21% Ca), lactate (13% Ca), gluconate (9.4% Ca), glubionate (6.6% Ca)] are available to be used for this purpose. The dose of oral calcium should provide 25–100 mg Ca/kg/day divided every 4–6 h. Milk is also a good source of calcium (119 mg Ca/100 mL), but not necessarily appropriate in hyperphosphatemic states since its phosphate content is high (93 mg/100 mL). Both forms of therapy should be adjusted as needed with monitoring, paying attention to serum Ca^{2+} levels, Ca × phosphate,

and urine Ca/urine creatinine to avoid the deposition of calcium salts in the peripheral tissues and kidney.

22.3.5.2 Chronic Hypocalcemia

The overall goal in management of chronic hypocalcemia is to achieve a serum Ca^{2+} level that does not cause symptoms while avoiding hypercalcemia or excessive hypercalciuria (i.e., urine Ca/urine creatinine >0.2), the latter being particularly difficult to achieve in hypoparathyroidism as the absence of PTH limits calcium absorption in the renal distal tubule. In hypoparathyroidism, serum Ca levels <9 mg/dL limit the degree of hypercalciuria. In some patients in whom normocalcemia has been difficult to achieve without significant hypercalciuria, the addition of a thiazide diuretic may limit hypercalciuria while increasing serum Ca^{2+} significantly. Correction of hypocalcemia does not need to be so stringent in most forms of PHP since hypercalciuria is rarely seen even when calcium levels reach high normal values. It is not unusual to require relatively high doses of calcium to overcome long-standing hypocalcemia, especially in PHP; however, calcium requirements are frequently reduced once normocalcemia has been achieved and the degree of hyperphosphatemia has been reduced.

In all forms of hypoparathyroidism, vitamin D administration is an integral part of the therapy once oral supplementation of calcium is initiated. Calcitriol, in most instances, is the adequate choice due to its short half-life and high activity, which limits its toxicity and increases efficacy, respectively. The standard dose of 10–50 ng/kg/day is usually sufficient to promote adequate calcium absorption, but the dose is often increased further if the hypocalcemia remains recalcitrant to oral therapy. Calcitriol is also the adequate choice in the treatment of hypocalcemia secondary to renal failure, liver disease, or defects in 1-α-hydroxylase function. In intestinal malabsorption syndromes where there is a deficiency in fat absorption, calcidiol (1–3 mcg/kg/day), the more polar vitamin D metabolite, can be used. When hypocalcemia is caused by poor vitamin D stores, vitamin D, 2000 U/day, or 50,000 U IM once weekly for at least 6 weeks followed by maintenance therapy of 600–1000 U/day, should be quite adequate to achieve 25OHD levels above 30 ng/mL since calcitriol production and action are not defective. Finally, patients with 1-α-hydroxylase deficiency or VDDR-I respond well to calcitriol therapy, while VDDR-II patients with an abnormal vitamin D receptor usually require an exceedingly high dose of calcitriol (up to 1000 mcg/day) or chronic parenteral calcium to maintain normal serum Ca^{2+}. For the treatment of hypoparathyroidism, replacement therapy with PTH has been investigated in both adults and children with clinical trials showing a reduction in calcium and calcitriol requirements without changing serum and urinary calcium levels significantly. Further studies are needed to establish the long-term safety of treatment with PTH for hypoparathyroid patients [28].

In general, phosphate binders are not required to manage hyperphosphatemia in hypoparathyroidism; moreover, the use of calcium alone limits intestinal phosphate absorption. The intake of phosphate-rich foods (i.e., dairy products) should not be encouraged. The use of a nonabsorbable antacid when serum phosphate levels remain greater than 6 mg/dL in the older child may be useful to prevent metastatic calcifications.

In chronic forms of hypoparathyroidism, frequent follow-up (i.e., every 3–4 months) to ensure adequate calcium balance may be adequate as is periodical screening of kidney function by urine analysis and ultrasound to rule out the presence of hematuria, kidney stones, and nephrocalcinosis.

22.3.5.3 Neonatal Hypocalcemia

The initial treatment of hypocalcemia in neonates with hypothyroidism should be approached as described for all children. As a large proportion of these infants ultimately have a form of transient hypoparathyroidism, initial treatment should be limited to calcium supplementation alone without addition of calcitriol. Since infants depend on maternal or formula milk for their nutrition, a useful approach is to supplement their milk with calcium. When hyperphosphatemia is significant, the use of a low phosphate content formula (i.e., PM60/40) supplemented with calcium to bring the calcium/phosphate ratio to 4:1 is often sufficient to limit phosphate absorption while supplying sufficient calcium to achieve normocalcemia. The amount of calcium can be slowly tapered as long as the infant remains normocalcemic, with serum Ca^{2+} measured following each decrease in dose. When a permanent form of hypoparathyroidism has been confirmed (i.e., clear features of DiGeorge syndrome are present or PTH measure-

ments are persistently low) or the hypocalcemia is resistant to oral calcium treatment, calcitriol could be administered to enhance calcium absorption.

22.4 Hypercalcemia

Hypercalcemia develops when either there is an increased influx of calcium from the gastrointestinal tract or bone into the extracellular space that exceeds the renal excretory capacity or when there is enhanced renal tubule absorption of calcium. Causes of hypercalcemia can be divided into etiologies that involve abnormalities in calciotropic hormones or defects in calcium handling by organs targeted by these hormones.

Differential Diagnosis of Hypercalcemia

Alterations in hormonal response

- Hyperparathyroidism
  - Excessive PTH production
    - Primary Hyperparathyroidism
      - MEN (types I, IIA)
      - Sporadic forms
    - Secondary/tertiary hyperparathyroidism
      - Renal failure
      - Renal tubular acidosis
      - Treatment of hypophosphatemic rickets
    - Transient hyperparathyroidism
      - Neonatal hyperparathyroidism (secondary to maternal hypoparathyroidism)
  - Excessive PTH secretion
    - Lithium toxicity
    - Calcium-sensing receptor inactivating mutations
      - Familial hypocalciuric hypercalcemia (FHH)
      - Neonatal severe hyperparathyroidism
  - Excessive PTH receptor activity
    - Jansen's metaphyseal chondrodysplasia
  - Vitamin D excess
    - Excess nutritional intake
    - Granulomatous disorders
    - Neoplasms and lymphomas

Alterations of organs involved in calcium homeostasis

- Skeleton
  - Immobilization
  - Hyperthyroidism
  - Neoplastic bone metastasis

Other causes of hypercalcemia

- Hypercalcemia of malignancy
  - PTHrP excess
  - Excess cytokine and osteoclast-activating factors
- Hypophosphatemia
- High calcium load (milk-alkali syndrome)
- Vitamin A intoxication
- Drugs (e.g., thiazides)
- Williams syndrome
- Hypophosphatasia
- Subcutaneous fat necrosis
- Adrenal insufficiency
- Pheochromocytoma
- Vasoactive intestinal peptide-secreting tumor

22.4.1 Alterations in Calciotropic Hormones Causing Hypercalcemia

22.4.1.1 Hyperparathyroidism

Hyperparathyroidism (HPT) is diagnosed when hypercalcemia is accompanied by elevated PTH levels. HPT is one of the most common causes of hypercalcemia in adults, but it is a relatively uncommon disorder in children and neonates. Less than 20% of pediatric cases are diagnosed in children younger than 10 years. Most cases of HPT (80%) represent a sporadic adenomatous change in one of the parathyroid glands, but a subset of patients show generalized hyperplasia of all glands that can occur sporadically or as part of the multiple endocrine neoplasia (MEN) types I and IIA. Parathyroid carcinoma is an even less common but more indolent form of parathyroid cell neoplasia. Parathyroid adenomas show a marked decrease in sensitivity to elevations of serum Ca^{2+}, while hyperplastic glands remain sensitive to Ca^{2+} but secrete more PTH by virtue of the increased cell number.

The underlying cause for sporadic primary HPT is not known, but most tumors are monoclonal in origin; the genetic defect in some of them has been allocated to translocation of cyclin D1 to the proximity of the PTH gene promoter inducing its overexpression [29]. Familial forms of HPT, accounting for about 10% of all cases and comprising most cases of hyperplasia, are usually transmitted in autosomal dominant fashion. Hyperparathyroidism-jaw tumor syndrome is a rare autosomal dominant condition linked to mutations of the *HRPT2* gene and characterized by a combination of parathyroid neoplasm, ossifying fibromas of the mandible and maxilla, and renal manifestations including cysts, hamartomas, Wilms tumors, and uterine tumors. HPT

is the most prominent manifestation and may develop as early as the first decade of life. In type I MEN, the affected gene, *Menin*, has been mapped to chromosome 11q13 [30]. HPT is associated with almost all affected members and is often the first manifestation of the disorder; pancreatic tumors, pituitary adenomas, and neuroendocrine tumors of the gastrointestinal tract are other common manifestations. MEN type IIA is also an autosomal dominant disorder in which HPT occurs in association with medullary carcinoma of the thyroid and pheochromocytoma. The incidence of HPT is only 10–30% and is rarely the first manifestation of the syndrome. The typical presentation is hyperplasia of all glands, but adenomatous changes are not uncommon, especially in type IIA. The affected gene is the *RET* proto-oncogene in chromosome 10q11.2 [31].

In conditions where a normal parathyroid is exposed to chronic hypocalcemia (e.g., renal failure, renal tubular acidosis, therapy for hypophosphatemic rickets), the gland can undergo hyperplastic changes with concomitant increases in PTH secretion that cause hypercalcemia and secondary HPT. In severe cases, often in the setting of renal failure, adenomatous changes can also occur (tertiary HPT). A similar but usually less severe and transient form of HPT has been observed in neonates born to mothers with hypoparathyroidism and exposed to low serum Ca^{2+} in utero.

Hypercalcemia has been observed in patients treated with lithium [32]. PTH levels are elevated, suggesting a form of HPT. Lithium has been shown to decrease the sensitivity of the parathyroid cell to serum Ca^{2+}, by interfering with the signaling mechanisms utilized by the CaSR.

In Jansen syndrome, children present with hypercalcemia, a metaphyseal chondrodysplasia, and other skeletal findings consistent with HPT. The genetic defect has been identified as a mutation of the PTH receptor that renders it constitutively active [33]. These children have undetectable PTH levels as their parathyroids respond appropriately to hypercalcemia.

22.4.1.2 Familial Hypocalciuric Hypercalcemia

Familial hypocalciuric hypercalcemia (FHH) is an autosomal dominant disorder characterized by mild, asymptomatic hypercalcemia, increased tubular reabsorption of calcium, and inappropriately normal PTH values, caused by the presence of an inactivation mutation in one of the alleles coding for the CaSR or a dominant negative heterozygous mutation [34]. Affected individuals often go undiagnosed until a laboratory screen reveals the hypercalcemia. They do not have the common skeletal and gastrointestinal manifestations seen in primary hyperparathyroidism and are not at risk to develop urinary calcium stones or pancreatitis. The parathyroid glands are normal in appearance and do not show significant hyperplasia in mild forms of the disorder. There is, nevertheless, a broad spectrum of the disorder ranging from mild hypercalcemia to severe, life-threatening hypercalcemia that typically presents in the neonatal period. This severe form, classically described as neonatal severe hyperparathyroidism, is either homozygous for inactivation mutations of the CaSR or heterozygous for a very severe inactivation mutation aggravated by exposure to low Ca^{2+} in fetal development. These infants have very elevated PTH levels and all the manifestations of HPT including hyperplasia of the parathyroid glands. Removal of most parathyroid tissue is often necessary.

22.4.1.3 Vitamin D Excess

Excessive exposure to vitamin D in the diet or for therapeutic reasons will cause an increase in intestinal calcium absorption and hypercalcemia. In this setting, phosphate absorption is also increased, and PTH levels are appropriately suppressed. Hypercalcemia is similarly present in a number of granulomatous disorders (i.e., sarcoidosis, tuberculosis, leprosy), chronic collagen vascular inflammatory disorders, and some neoplastic diseases (Hodgkin B cell lymphoma), where there are proliferation and activation of monocytic cells. Production of $1,25(OH)_2D$ is increased due to the unregulated expression of 1-α-hydroxylase in these cells [35].

22.4.2 Other Causes of Hypercalcemia

As bone is the repository of greater than 98% of the body's calcium, increased or unregulated bone turnover can easily overcome the renal excretion capacity for calcium. Excess thyroid hormone can promote a disproportional stimulation of osteoclast function causing increased

bone resorption and hypercalcemia [36, 37]. Immobilization, particularly in adolescents and when prolonged for more than 2 weeks, results in decreased bone accretion and increased bone resorption that is initially noted as hypercalciuria, but when persistent, frank symptomatic hypercalcemia can occur requiring immediate treatment [38]. Increased prostaglandin E secretion by renal tubular cells in Bartter syndrome has been suggested to promote bone resorption [12]. Vitamin A excess has been shown to cause hypercalcemia, likely from the activation of osteoclast-mediated bone resorption [39].

Malignancy is a rare cause of hypercalcemia in children. When it occurs, it can be the result of metastases to bone with concomitant dissolution of mineral content or the production of lytic factors by the original tumor that promote the mobilization of calcium (i.e., PTHrP, IL-1, IL6, TNF, prostaglandins).

Excessive intake of calcium in milk, calcium-containing antacids, and alkali can result in absorptive hypercalcemia. Conversely, severe hypophosphatemia associated with parenteral nutrition and prematurity is associated with a reciprocal increase in serum Ca^{2+} concentration, partly due to increased calcitriol levels and intestinal calcium absorption. Hypercalcemia has also been observed in adrenal insufficiency, pheochromocytoma, and vasoactive polypeptide-secreting tumors by mechanism(s) that have not been well defined.

Hypercalcemia is present transiently during infancy in 15% of children with Williams syndrome, a sporadic disorder linked to the loss of the elastin gene in chromosome 7 characterized by defined facial features (e.g., dolichocephaly, periorbital prominence, bitemporal depression, long philtrum with prominent lips and nasal tip, full cheeks, epicanthal folds, and periorbital prominence) among other physical features. More prominently up to 30% of affected children have supravalvular aortic stenosis. The etiology of hypercalcemia is unknown; however, mildly elevated calcitriol and calcidiol levels have been reported [40, 41]. The hypercalcemia often resolves before the first year of life; however, hypercalciuria often persists.

Hypercalcemia, sometimes very severe and life-threatening, has been seen with subcutaneous fat necrosis, a condition seen in neonates, often premature, that have had traumatic births or a history of critical illness with significant poor peripheral perfusion. Subcutaneous fat undergoes necrosis, showing a significant infiltration by mononuclear cells. Although the etiology of hypercalcemia is not known, excessive prostaglandin E production and mononuclear-derived calcitriol, which in some cases have been mildly elevated, have been invoked as the cause of hypercalcemia [42, 43].

22.4.3 Diagnosis and Evaluation of Hypercalcemia

Children with mild (total calcium <12 mg/dL) or chronic hypercalcemia frequently go undiagnosed unless a routine biochemical screen reveals the elevation of serum calcium. The predominant manifestation may be a failure to thrive with arrest of weight gain and linear growth. In mild hypercalcemia (total calcium 12–13.5 mg/dL), generalized weakness, anorexia, constipation, and polyuria are usually present. In severe hypercalcemia (total calcium >13.5 mg/dL), nausea, vomiting, dehydration, and encephalopathic features including coma and seizures may occur. Neonates with severe hypercalcemia often present in respiratory distress and have hypotonia and apnea. It is not uncommon for relatives and patients to note significant psychological changes ranging from depression to paranoia and obsessive-compulsive behavior.

The physical examination is usually normal in hypercalcemic patients. In patients with MEN type IIB, a marfanoid habitus is often present. A parathyroid mass is rarely palpable. When not dehydrated, hypertension may be noted, and a cardiac evaluation may show shortened QTc intervals in ECG tracings. In chronic hypercalcemia, a survey of soft tissues may reveal calcifications in the kidney, skin, SQ tissues, cardiac arteries, and gastric mucosa. In untreated patients with prolonged HPT, and occasionally reported in untreated children where the diagnosis was never suspected, distinctive skeletal findings showing subperiosteal resorption of the distal phalanges, tapering of the distal clavicles, salt and pepper appearance of the skull, bone cysts, and brown tumors (hemosiderin deposition in areas of active osteoclast-mediated osteolysis with accumulation of fibrous tissue, woven bone, and supporting vasculature) are the constellation of findings that describe osteitis fibrosa cystica. These findings are readily visible by conventional radiography.

The evaluation of hypercalcemia should include a thorough medical history searching for exposure to drugs, agents, and conditions that can cause hypercalcemia and a family history of hypercalcemia or other associated medical conditions. The approach to the biochemical evaluation is similar to the evaluation described for hypocalcemia and should initially include the measurement of serum intact PTH levels, calcium, phosphate, and magnesium together with measurements of urine calcium excretion. Renal function should also be assessed to rule out renal insufficiency, and a urine analysis is useful to look for the presence of hematuria or calcium salt residue.

HPT is diagnosed when hypercalcemia is noted in conjunction with elevated PTH levels. In the absence of secondary causes of HPT, the presence of hypercalciuria is consistent with primary HPT. Hypercalciuria is usually present in HPT since the PTH-mediated increase in tubular calcium resorption does not fully compensate for the increase in calcium concentration in the glomerular filtrate. The degree of hypercalciuria has significant diagnostic value, especially when trying to distinguish mild HPT from FHH, since mild elevations of PTH are often seen in both cases [34]. The calculation of 24-h urinary calcium clearance provides a measure of calcium handling by the kidney. Decreased urinary calcium excretion in the presence of mild hypercalcemia should raise the possibility of inactivating mutation of the CaSR and FHH. A better measure of hypercalciuria that takes into account changes in glomerular filtration is the calcium clearance ratio ([urine Ca × serum creatinine]/[urine creatinine × serum Ca]). The clearance ratio in FHH is one-third of that in typical primary HPT, and a value less than 0.01 is virtually diagnostic of FHH. Unfortunately, FHH patients do not always show significant hypocalciuria. Mild elevations of magnesium can sometime distinguish FHH from HPT, since serum magnesium is usually in the low normal range in HPT. A family history of asymptomatic hypercalcemia would provide further support for a diagnosis of FHH. Both parents should be evaluated when the diagnosis is suspected in a child. Adequate distinction between HPT and FHH is not trivial since hypercalcemia in FHH has not been associated with any long-term adverse outcome and requires no treatment. Furthermore, the surgical removal of parathyroid tissue in FHH, in cases that were thought to represent HPT, does not correct the hypercalcemia. Genetic testing for known inactivating mutations of CaSR could help distinguish FHH from mild forms of hyperparathyroidism.

When PTH levels are adequately suppressed in the presence of hypercalcemia, elevated 25OHD levels would suggest vitamin D intoxication. Elevated $1,25(OH)_2D$ without a concomitant elevation of 25OHD points to an ectopic source of 1-α-hydroxylase. In both settings, hyperphosphatemia and marked hypercalciuria are usually present greatly increasing the predisposition to calcium toxicity. In the absence of elevated PTH and vitamin D metabolites, hypercalcemic patients that have not been exposed to high calcium ingestion or prolonged immobilization should be screened for the secretion of other hypercalcemic factors (i.e., PTHrP, prostaglandin E).

22.4.4 Management of Hypercalcemia

The management of hypercalcemia depends on the severity and cause of the elevation of serum Ca^{2+}. When hypercalcemia is mild and the patient is asymptomatic, no initial treatment may be necessary, and medical efforts to reach a diagnosis should be given preference.

When hypercalcemia is severe (total serum calcium >14 mg/dL) or when there are symptoms and signs of cardiac, gastrointestinal, and central nervous system dysfunction, prompt intervention is appropriate. Since patients are usually dehydrated because of the polyuria and anorexia associated with severe hypercalcemia, the initial step is to provide adequate hydration, preferably in the form of isotonic saline at 3000 ml/M^2 for the first 24–48 h, to restore vascular volume, increase glomerular filtration rate, and dilute serum Ca^{2+}. After hydration, the loop diuretic furosemide (1 mg/kg every 6 h) can further inhibit the reabsorption of calcium, especially in the presence of sodium, further promoting calciuresis. In comatose patients, hemodialysis should be considered as a means to decrease serum Ca^{2+} more aggressively.

If hypercalcemia does not respond to these initial measures, agents that block bone resorption may be useful as adjuvant therapy. Calcitonin 4 U/Kg SQ q 12 h is commonly used

for this purpose; however, its efficacy diminishes with continuous administration due to tachyphylaxis. Bisphosphonates, analogues of pyrophosphate that inhibit osteoclast action, have been used, especially when hypercalcemia is primarily driven by the mobilization of calcium from bone as in cases of tumor-induced hypercalcemia, severe HPT, or immobilization. Both etidronate and pamidronate could be used, the latter given as a single-dose intravenous infusion.

When hypercalcemia is due to excess vitamin D ingestion or activity, glucocorticoids (prednisone 1 mg/kg/day) can be very effective since they inhibit both 1-α-hydroxylase activity and intestinal calcium absorption. Ketoconazole (3 mg/kg/day divided in three doses) is also a very effective inhibitor of 1-α-hydroxylase activity, but its use may be associated with significant gastrointestinal side effects and liver toxicity, as well as adrenal insufficiency.

Pharmacological agents have become available that can suppress PTH secretion in affected glands. Calcimimetics that activate the CaSR and suppress the secretion of PTH may be used to treat hypercalcemia secondary to HPT; however, in young patients with well-described HPT, preferably confirmed by several measurements of serum calcium and PTH, the surgical removal of the affected gland is ultimately required to control hypercalcemia. A number of imaging techniques (i.e., neck ultrasound, computed tomography, magnetic resonance imaging, and radionuclide scanning) have been used to detect a hyperfunctioning gland; however, the reported sensitivities have ranged between 40 and 90% and may be more informative when used in combination. ^{99m}Tc-sestamibi scanning has shown some promise, especially in the visualization of adenomas [44]. Intraoperative measurements of PTH are now feasible, aiding the surgeon in the search for hyperplastic or adenomatous tissue, as successful removal is reflected by a rapid drop in PTH levels [45]. In cases of an isolated adenoma, its resection is usually curative. In cases of isolated hyperplasia or secondary HPT, removal of three and one-half glands is recommended. Total parathyroidectomy is recommended with autotransplantation of minced parathyroid tissue in the forearm for patients with MEN, where it can easily be removed in cases of recurring hypercalcemia. Postsurgical hypocalcemia is common and treated with calcium supplements. In cases of severe HPT, hypocalcemia can be more severe and prolonged due to hungry bone syndrome. These patients have severe phosphate and calcium deficits as mineral bone deposition occurs following resolution of hyperparathyroidism. The use of both calcium and phosphate supplements together with calcitriol is recommended to manage this profound hypocalcemia. In some instances, permanent hypoparathyroidism ensues, requiring lifelong therapy.

Case Study

A 3-month-old girl presented to the emergency room after an episode of generalized tonic-clonic seizure that had subsided prior to arrival. She had a fixed stare and was lethargic. She was not febrile and had no other abnormal symptoms. She was the product of a fraternal twin pregnancy, born at term with a weight of 2700 grams and length of 48 cm. There were no other relevant findings in the medical history. Physical examination showed a 5 kg, afebrile pale girl with peculiar facies, heart rate over 100/min with regular cardiac rhythm, shallow breathing, soft non-tender abdomen without masses or organomegaly, and normal fontanel without meningeal signs.

In the emergency room, she had a recurrent seizure requiring airway and oxygen support while receiving rectal diazepam in an attempt to stop clonic activity. Once transferred to the intensive care unit, blood screen shows ionized calcium (iCa) level of 0.72 mmol/L (1.12–1.23), at which time she received an intravenous infusion of 10% calcium gluconate to acutely correct the hypocalcemia and stop seizure activity. Further laboratory workup showed that renal function was normal; capillary glucose, within normal range; total calcium, 6.4 mg/dl (8–10.3); iCa, 0.67 mmol/L (1.12–1.23); phosphate, 6.4 mg/dL (4.4–6.9); and PTH, 45 pg/mL (10–65). The inappropriately normal PTH level despite the severe hypocalcemia suggested a state of hypoparathyroidism that is often associated with elevations in serum phosphate levels, not present in this case. Since she had an abnormal facies and the hypocalcemia was difficult to normalize, the possibility of DiGeorge syndrome was initially entertained.

Further workup showed a mildly elevated alkaline phosphatase level for age and 25OHD, 4.3 ng/mL (20–62), and 1,25$(OH)_2$D, 17.8 pg/mL (18.5–42.3). Wrist X-ray showed epiphyseal flaring consistent with rickets. Chest X-ray showed absence of thymus shadow. Echocardiogram showed a small atrial septal defect.

Karyotype: 46, XX. Chromosome 22 study showed deletion of the *TUPLE 1* gene in one of the chromosome 22 pairs.

The clinical presentation is consistent with severe symptomatic hypocalcemia due to hypoparathyroidism associated with DiGeorge syndrome and aggravated by vitamin D deficiency. Following vitamin D supplementation, phosphate levels increased to above the normal range consistent with the diagnosis of hypoparathyroidism. Initial screening of her twin sibling and mother showed decreased 25OHD levels in both, suggesting that maternal vitamin D deficiency was responsible for the initial low stores in her newborns.

22.5 Summary

Extracellular calcium level is maintained within a very narrow range to ensure adequate regulation of important calcium-mediated metabolic processes. The proper interplay of adequate sensing of extracellular calcium levels by the parathyroid gland and the role of primarily PTH and vitamin D metabolites in the regulation of calcium handling by the intestinal tract, bone, and kidney ensure that adequate feedback mechanisms are present to ensure only minimal variations in extracellular calcium levels. Abnormalities in extracellular calcium sensing, hormonal regulation, or function of the different organs involved in extracellular calcium regulation lead to acute or chronic states of either hypo- or hypercalcemia. Appropriate treatment requires a proper diagnosis of the etiology responsible for the deregulation in order to take the necessary steps to either restore normal regulation or to compensate for the abnormal handling of calcium by the intestinal tract, bone, or kidney.

Review Questions

1. Which organ does not have an important role in the regulation of extracellular calcium levels?
   A. Gastrointestinal tract
   B. Kidney
   C. Skeleton
   D. Liver
2. What is a distinguishing feature that differentiates extracellular calcium regulation during the fetal period from other periods in the life cycle?
   A. Extracellular calcium under maternal PTH regulation
   B. Vitamin D-dependent calcium resorption from bone
   C. PTHrP-mediated placental calcium transport
   D. Calcitonin-mediated hypercalcemia
3. What laboratory parameters are useful to follow in the treatment of chronic hypocalcemia secondary to hypoparathyroidism?
   A. 25OHD levels
   B. Urinary calcium clearance
   C. Serum phosphate
   D. Alkaline phosphatase
4. Which laboratory screen can be useful to distinguish between HPT and FHH?
   A. PTH levels
   B. 25OHD levels
   C. Urinary calcium clearance ratio
   D. $1,25(OH)_2D$ levels

Answers

1. D
2. C
3. B
4. C

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Rickets: The Skeletal Disorders of Impaired Calcium or Phosphate Availability

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Key Points

- Rickets results from various impairments in calcium or phosphate accrual or retention.
- The most common cause of rickets is nutritional in nature, predominantly due to vitamin D deficiency.
- Many rare hereditary forms of rickets exist due to abnormalities in various defects in vitamin D or phosphate metabolism.
- Careful evaluation should seek the underlying cause of rickets to guide specific treatment.

23.1 Introduction and Background Information

Rickets derives from the old English word "*wrikken*" meaning to twist or bend and refers to conditions of impaired mineralization of growing bones, ultimately resulting in their bowing and twisting. Rickets and *osteomalacia* refer to similar processes occurring in different compartments of the bone. Rickets is evident histologically and radiographically as a disrupted and expanded growth plate (physis) of growing bone together with the accompanying *osteomalacia* (accumulation of excess unmineralized osteoid matrix) of trabecular and cortical bone. Children with untreated rickets may develop severe curvature deformities of the lower extremities, primarily due to the effect of weight bearing on the poorly mineralized skeleton. *Osteomalacia* is specifically identified histologically by a lag in mineralization of the osteoid in cortical or trabecular bone tissue, independent of growth plate abnormalities. Osteomalacia accompanies rickets in childhood, but similar pathophysiologic mechanisms in adults are usually described as osteomalacia, rather than rickets, because epiphyses have fused to the metaphyseal front of previously growing bone, such that growth plate manifestations of rickets no longer can develop (although bowing may still develop in mature long bones).

The terms rickets and osteomalacia should be distinguished from *osteopenia*, a general term referring to the appearance of diminished bone density on radiographs. Osteopenia and osteoporosis represent degrees of bone deficits, and have a variety of causes, but generally result from an imbalance between osteoblastic bone formation and osteoclastic bone resorption. *Osteopenia* has also come into standard use in adults to describe bone mineral density between 1 and 2.5 standard deviations (SD) below the young adult mean using dual-energy X-ray absorptiometry. *Osteoporosis* is defined as reduced bone tissue per unit volume of whole bone or in adult clinical practice to describe bone density more than 2.5 SD below the young adult mean. However, these bone density criteria are *not* considered appropriate for children, and the preferred terminology in children for those with a bone density more than 2 SD below the mean for age, race, and sex is "low for chronological age" [1]. In children, a diagnosis of osteoporosis requires low bone density along with the presence of fragility fractures. In contrast to rickets or osteomalacia, a pure osteoporotic lesion is not characterized by excess unmineralized bone matrix or delayed mineralization time. Of note, some of the underlying causes of rickets in children, or osteomalacia in adults, may also cause a radiographic appearance of osteopenia (however the histologic features differ). To further confuse this distinction, increased bone density may occur in the setting of some inherited rachitic conditions such as X-linked hypophosphatemia (XLH), despite the lag in mineralization time [2].

Rickets may be conveniently classified as either *calciopenic*—predominantly resulting from reduced availability of calcium to the mineralizing skeleton—or *phosphopenic*, resulting from a reduced availability of phosphate. These should be distinguished from several *rickets-like* disorders, such as hypophosphatasia and certain skeletal dysplasias. Moreover, both the calciopenic and phosphopenic forms of rickets may be subclassified as those resulting from nutritional, inherited, or other causes (Table 23.1). However, studies of the murine growth plate suggest that local hypophosphatemia is common to most forms of rickets, including vitamin D deficiency [3].

In general, most instances of nutritional rickets are calciopenic, whereas heritable causes are usually phosphopenic. Identification of the cause of rickets is important, since treatment strategies for the various forms differ. The underlying cause of rickets can generally be determined from the medical and family history, physical examination, and appropriate laboratory evaluation. This review describes the various forms of rickets and offers a practical approach to the evaluation and management of this disorder.

Table 23.1 Classification scheme for calciopenic and phosphopenic rickets

	Calciopenic	Phosphopenic
Nutritional	Dietary vitamin D deficiency	Dietary phosphate deficiency
	Dietary calcium deficiency	Malabsorption of phosphate
	Malabsorption of vitamin D and calcium	Neocate formula
	Cystic fibrosis	Phosphate binders
	Inflammatory bowel disease	
	Celiac disease	
	Short bowel syndrome	
Inherited	1α-hydroxylase deficiency (*CYP27B1*) (vitamin D-dependent rickets type I)	FGF23-mediated hypophosphatemic rickets:
		X-linked hypophosphatemia (*PHEX*)
	Hereditary resistance to $1,25(OH)_2D$ (*VDR*) (vitamin D-dependent rickets type II)	Autosomal dominant hypophosphatemic rickets (*FGF23*)
		Autosomal recessive hypophosphatemic rickets (*DMP1*, *ENPP1*, *FAM20C*)
	25-hydroxylase deficiency (*CYP2R1*)	Non-FGF23-mediated hypophosphatemic rickets:
		Hereditary hypophosphatemic rickets with hypercalciuria (*SLC34A3*)
		Dent's disease, X-linked hypercalciuric nephrolithiasis, X-linked recessive hypophosphatemic rickets (*CLCN5*)
		Linear sebaceous nevus syndrome
Other	Impairment of hydroxylation of vitamin D	Tumor-induced osteomalacia
	Severe hepatobiliary disease	Fibrous dysplasia of the bone
	Severe renal disease	Liver disease
	Increased catabolism of vitamin D	
	Anticonvulsant therapy	Fanconi syndrome (a variety of causes)

23.2 Etiology

Despite the general perception that nutritional rickets has been long eradicated, the incidence of this disorder remains more common than rachitic disease due to other etiologies. Nutritional deficiency must always be excluded as the etiology of any case of rickets under evaluation. Both vitamin D and calcium deficiency are significant factors in nutritional rickets, and children often have a mixed dietary deficiency. Both vitamin D and calcium deficiency are associated with macrobiotic and vegan diets and with other forms of dairy avoidance. The increasing intake of carbonated beverages (replacing milk) in children contributes to greater risks of both calcium and vitamin D deficiency.

In contrast to calcium deficiency, nutritional phosphorus deprivation is rare. Prior to the 1990s, breastfed premature infants commonly developed phosphate deficiency [4]. However, once the limited phosphate content of human breast milk was identified as the cause, human breast milk fortifiers were given to breast milk-fed premature

infants, to ensure that the higher mineral needs of premature infants were met. The abuse or overuse of phosphate binders can impair intestinal phosphate absorption and result in phosphate deficiency but is rarely encountered in children. Recently, the use of certain amino acid-based elemental formulas has been associated with the development of phosphopenic rickets. Exposure to heavy metals or toxic agents may result in phosphate-wasting tubulopathies and should be considered when sporadic renal phosphate losses are evident. Many drugs may cause hypophosphatemia, including some antiretroviral agents [5]. Fat malabsorption, with resultant fat-soluble vitamin malabsorption, and underlying renal or liver disease may be important factors in the development of nutritional rickets and should be identified in the history. It is particularly important to obtain a detailed family history with attention to bone and mineral diseases and fractures. A history of inborn errors such as those associated with renal Fanconi syndrome should be sought. Finally, in patients with apparent sporadic phosphopenic rickets, causes such as tumor-induced osteomalacia (TIO) or fibrous dysplasia may need to be considered, especially if the patient presents as an older child or adult (◘ Table 23.2)

23.2.1 Differential Diagnosis of Rickets

23.2.1.1 Nutritional Rickets

The incidence of nutritional rickets from vitamin D deficiency in the United States remains surprisingly greater than that of inherited forms of rickets. Consequently, although other forms of rickets are generally described as having normal 25OHD concentrations, we occasionally encounter vitamin D deficiency in the setting of inherited forms of rickets. Despite vitamin D fortification of milk and infant formulas, numerous reports describe nutritional rickets occurring with regularity in African-American children with a history of (often exclusive) breastfeeding [6]. Vitamin D content of human breast milk is relatively low under normal circumstances and is even lower if the mother is vitamin D insufficient. Such children usually present in the late winter or early spring following a season of limited sunlight exposure, when no vitamin D supplementation has been given. Moreover, in our experience many such children are weaned to diets with low calcium intake. It is likely that following the shift in diet, the inadequate dietary calcium compounds the vitamin D deficiency, resulting in accelerated turnover of vitamin D.

Rickets due to isolated calcium deficiency (with apparently normal vitamin D metabolism) is not commonly reported in North America, and most publications describe this condition in children in Africa and Asia. Nigerian children with this disorder are reported to have low dietary calcium intake, adequate sunlight, and 25OHD levels higher than expected for causing rickets (most in the normal range). Some affected children are older than typically reported for vitamin D-deficiency rickets in the United States [7]. One study of Gambian children suggested that the phosphate-regulating hormone, FGF23, may be involved in the pathogenesis of the disease, as hypophosphatemia is described [8]. However, in contrast to most forms of FGF23-mediated rickets, circulating $1,25(OH)_2D$ levels were elevated, likely accounting for a secondary increase in FGF23 levels.

In contrast to the often limited calcium supply in many diets, phosphate is nearly ubiquitous in human food, and nutritional phosphate deficiency is relatively rare. Patients abusing phosphate binders such as antacids may develop hypophosphatemia. In addition, while human milk is an ideal food for term infants, it is insufficient in phosphate for preterm infants, leading to rickets of prematurity in exclusively breast milk-fed premature infants (◘ Fig. 23.1) [4]. While this condition has become less common since the practice of adding breast milk fortifiers containing increased phosphate (among other nutrients) has become more common, we continue to see cases of rickets of prematurity when total nutritional intake is severely compromised due to comorbid conditions limiting the ability to adequately provide phosphate and calcium to such infants.

23.2.1.2 Heritable Forms of Calciopenic Rickets

Heritable forms of calciopenic rickets are much rarer than nutritional rickets. Heritable calciopenic rickets results from defects in the vitamin D metabolic pathway, including inadequate activation of vitamin D and abnormalities of the vitamin D receptor.

Table 23.2 Expected laboratory values (untreated) for different etiologies of rickets

		Serum Pi	Serum calcium	Serum ALP	Serum PTH	Serum 25OHD	Serum $1,25(OH)_2D$	Serum FGF23	TmP/GFR	Urine calcium
Calciopenic rickets	Vitamin D deficiency	L, N	L, N	H	H	L	L, N, H	L, N, H	L, N, H	L
	25-hydroxylase deficiency (*CYP2R1*)	L, N	L, N	H	H	L	L, N, H	L, N, H	L, N, H	L
	1α-hydroxylase deficiency (*CYP27B1*)	L	L, N	H	H	N	L	L	L, N, H	L
	Vitamin D receptor (*VDR*) mutation and related calcitriol resistance syndromes	L	L, N	H	H	N	H	L	L, N, H	L
	Calcium deficiency	N	L, N	H	H	N	L, N, H	L, N, H	L, N, H	L
Phosphopenic rickets	Renal: Increased excretion									
	FGF23-mediated[a]	L	N	H	N, H	N	L, N	H	L	N, L
	Non-FGF23 mediated[b]	L	N, H	N, H	N, H	N	N, H	L	L	N, H
	Gut: Impaired absorption									
	Impaired absorption[c]	L	L, N	N, H	N, H	L, N	L, N, H	L	H	L

In general, values provided are based on published values when available or expectations based on known physiology and animal models; values may vary in individual patients

Legend: *L* below normal range, *N* within normal range, *H* above normal range, ALP indicates alkaline phosphatase

[a]Including XLH (*PHEX*), ADHR (*FGF23*), ARHR (*DMP1*, *ENPP1*, *FAM20C*), tumor-induced osteomalacia, fibrous dysplasia, linear sebaceous nevus syndrome, post-renal transplantation hypophosphatemia

[b]Including HHRH (*SLC34A3*); in other tubulopathies, there may be urinary wasting of other electrolytes, glucose, and amino acids

[c]Elemental infant formula (decreased bioavailability), premature infants, phosphate binders; in isolated phosphate deficiency from any of these causes, $1,25(OH)_2D$ may be upregulated causing increased calcium absorption and hypercalciuria with or without hypercalcemia. In chronic kidney disease patients treated with phosphate binders, calcium and alkaline phosphatase may be low, while phosphorus, FGF23, and PTH are usually high

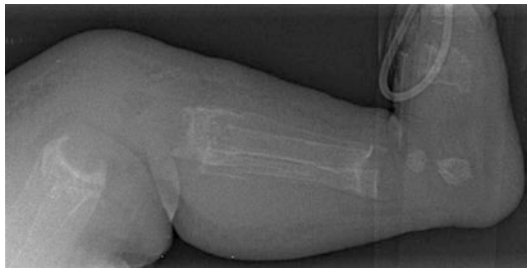

Fig. 23.1 A 25-week gestational age infant presented at 3 months of age with rickets of prematurity due to very low mineral and overall nutritional intake secondary to multiple comorbidities, including necrotizing enterocolitis and bowel resection, with intolerance of adequate enteral and parenteral nutrition volumes. This image demonstrates fraying and cupping of metaphyses as well as fractures. Bowing is not usually evident due to lack of weight bearing (X-ray from the files of Erik A. Imel)

1α(Alpha)-Hydroxylase Deficiency (Vitamin D-Dependent Rickets Type IA)

$1,25(OH)_2D$ is the most potent and active metabolite of vitamin D and circulates at concentrations 300–1000-fold lower than 25OHD. An autosomal recessive form of calciopenic rickets results from loss of function of the renal 25OHD 1α-hydroxylase enzyme system that converts 25(OH)D to $1,25(OH)_2D$ [9]. Mutations in *CYP27B1*, the gene encoding the cytochrome P450 moiety of the enzyme complex, render a dysfunctional enzyme unable to donate electrons to 25OHD [10]. This disorder usually presents with typical features of rickets at 4–12 months of age. The serum 25OHD level is typically normal, but the $1,25(OH)_2D$ is low to low normal. This form of rickets is resistant to even pharmacologic doses of vitamin D or 25OHD, due to the inability to convert 25OHD to the active $1,25(OH)_2D$ metabolite. Treatment with calcitriol ($1,25(OH)_2D_3$) or other analogs of activated vitamin D is indicated.

25-Hydroxylase Deficiency (Vitamin D-Dependent Rickets Type IB)

Defects in *CYP2R1*, encoding the hepatic vitamin D 25-hydroxylase enzyme, can lead to a disorder of vitamin D metabolism that mimics vitamin D deficiency [11, 12] and is referred to as vitamin D-dependent rickets type 1B. Homozygous subjects in families with identified missense mutations show a minimal increase in circulating 25OHD levels after oral bolus doses of vitamin D (50,000 IU), whereas heterozygous subjects demonstrate blunted responses compared to controls, indicating a gene dose effect. High vitamin D intake can be used as therapy for this disorder.

Hereditary Vitamin D Receptor Resistance (Vitamin D-Dependent Rickets Type II)

Another heritable cause of calciopenic rickets is target tissue resistance to $1,25(OH)_2D$ due to receptor or post-receptor defects. Hereditary vitamin D resistance (also known as vitamin D-dependent rickets type II) is a rare autosomal recessive disorder. A variety of mutations in the gene coding for the vitamin D receptor have been described, including missense mutations in the DNA- or steroid hormone-binding domains of the receptor [13]. In addition, mutations resulting in inappropriate truncation of the vitamin D receptor have been identified. In some families with vitamin D resistance, no genetic defect has been clearly identified.

Hereditary vitamin D resistance typically manifests between 6 months and 3 years of age, and the clinical, radiologic, and biochemical features are similar to those observed in 1α-hydroxylase deficiency, except that the circulating levels of $1,25(OH)_2D$ are elevated. Some kindreds with this disorder have total body alopecia due to the necessary effect of the VDR in keratinocytes for normal hair follicles [14], and some patients may demonstrate oligodontia.

23.2.1.3 Phosphopenic Rickets

X-Linked Hypophosphatemic Rickets and Other FGF23-Mediated Disorders

Phosphopenic rickets is most frequently due to renal tubular phosphate wasting. The most common of these disorders is X-linked hypophosphatemic (XLH) rickets. As this condition is transmitted in an X-linked dominant fashion, family history aids in distinguishing between various forms of heritable phosphopenic rickets; e.g., male-to-male transmission is inconsistent with XLH. All daughters and no sons of an affected male should be affected; and approximately one-half of all children (male or female) of an affected female would be expected to have the disorder. Sporadic cases occur as well, and clinical severity varies widely even within a kindred [15]. Affected individuals usually present between 1 and 3 years of age, with bowed legs, other signs of rickets, and short stature. The disorder can be detected

earlier when screening within an affected family is performed. Bowing usually does not occur until after an affected child is walking but may present earlier. Patients are at high risk for recurrent dental abscesses, and many require repetitive pulpectomy procedures. Though not a prominent feature of the disease, a defect in the regulation of vascular tone also occurs in some patients with XLH, as evidenced by abnormal blood pressure response to exercise and mild ventricular hypertrophy [16]. Affected patients have low serum phosphate levels, low indices of renal tubular phosphate threshold, and inappropriately low or normal $1,25(OH)_2D$ levels [17].

Patients with XLH have mutations in *PHEX* [18], which encodes an endopeptidase expressed in osteoblasts and osteocytes, as well as in odontoblasts. Through as yet unclear mechanisms, PHEX mutations lead to overexpression of fibroblast growth factor 23 (*FGF23*) [19]. FGF23 is an O-glycosylated peptide hormone that has critical roles in phosphate and vitamin D homeostasis. O-glycosylation is necessary at certain sites to prevent cleavage (and inactivation) of FGF23 at a subtilisin-like proprotein convertase site prior to secretion [20]. Bone-produced FGF23 circulates and interacts with FGF receptors in the renal tubule, in conjunction with the co-receptor, klotho [21]. Downstream activation of this FGFR pathway inhibits expression of sodium-phosphate cotransporters, causing increased urinary phosphate excretion. In addition, FGF23 downregulates the vitamin D 1α-hydroxylase, while upregulating the vitamin D 24-hydroxylase, thereby resulting in decreased $1,25(OH)_2D$ levels by affecting its production and degradation. Thus, FGF23 actions account for the characteristic biochemical phenotype of XLH [22]. Conversely, FGF23 is itself regulated through feedback mechanisms by both phosphate and $1,25(OH)_2D$ [23].

Other inherited renal phosphate wasting disorders are less common. Autosomal recessive hypophosphatemic rickets (ARHR) have been associated with biallelic mutations in several genes which regulate FGF23 expression. Those with mutations in *Dentin matrix protein 1 (DMP1)* have features similar to those with XLH including the characteristic hypophosphatemia, phosphaturia, and low or normal $1,25(OH)_2D$, due to FGF23 excess [24]. Studies in dmp1 null mice suggest a maturational defect in osteocytes, and the histologic appearance of osteocytes in dmp1 null mice is similar to that observed in Hyp mice, suggesting that the defects resulting from deficient DMP1/dmp1 and PHEX/Phex share a common pathway [24]. Another form of autosomal recessive hypophosphatemic rickets has been attributed to inactivating mutations in ENPP1 (ectonucleotide pyrophosphatase/phophodiesterase 1), thought to regulate local concentrations of pyrophosphate at mineralization sites [25, 26]. The loss of function of this enzyme is also associated with generalized arterial calcification of infancy [27]. Recessive *FAM20C* mutations have been demonstrated to cause FGF23-mediated hypophosphatemia, along with ectopic calcification and dental anomalies [28]. The protein product of FAM20C is a Golgi casein kinase which phosphorylates FGF23 at serine 180 (part of a cleavage motif), preventing O-glycosylation and leading to increased cleavage of FGF23 [29]. The lack of FAM20C thus results in impaired cleavage of FGF23, increased FGF23 concentrations, and hypophosphatemia.

Activating mutations in *FGF23* are responsible for autosomal dominant hypophosphatemic rickets (ADHR) [30], another disorder with features similar to those of XLH but with more variability in clinical presentation. ADHR-causing mutations in FGF23 alter the subtilisin-like proprotein convertase cleavage site, impairing FGF23 cleavage and leading to increased circulating FGF23 concentrations [31, 32]. Individuals affected with ADHR can be clinically indistinguishable from those with XLH and may similarly present in early childhood. However, ADHR patients may also demonstrate delayed onset of clinical features (i.e., normal phosphate and growth in childhood with subsequent hypophosphatemia and osteomalacia developing as an adolescent or adult), resolution of the biochemical phenotype, or waxing and waning of the phenotype [32, 33]. The characteristic biochemical phenotype is identical to that of XLH, including increased FGF23 concentrations [32]. However, whereas the mutation causes resistance of FGF23 to proteolytic cleavage, the changes in the severity of the clinical features of the disease correspond to increases and decreases in FGF23 concentrations, suggesting that regulation of FGF23 may intermittently function appropriately.

Recent studies have indicated an important role for iron in the pathophysiology of ADHR and in FGF23 metabolism. During conditions of iron deficiency, *FGF23* gene expression is

increased [34]. This phenomenon occurs both in normal individuals and in patients with ADHR, with resultant increased production of FGF23 protein during iron deficiency in both settings. However, when only the normal allele is present, the increased FGF23 produced can efficiently be cleaved, with maintenance of appropriate intact FGF23 concentrations [34, 35]. Thus, serum levels of FGF23 measured using C-terminal assays are elevated (because fragments are detected), but intact FGF23 is normal. In contrast, FGF23 species with an ADHR mutation resist proteolytic cleavage, and consequently, the intact FGF23 accumulates, causing hypophosphatemia and osteomalacia [35]. When this occurs in early childhood, the clinical presentation is similar to that of XLH. Later onset of ADHR disease may occur, in relation to onset of iron deficiency, potentially confusing the diagnosis with acquired disorders such as tumor-induced osteomalacia. In XLH, patients' C-terminal FGF23 measurements may also increase during iron deficiency, but there is no association between iron status and intact FGF23 or disease severity [36].

To complicate this scenario, specific formulations of intravenous iron may cause transient acute increases in serum intact FGF23 and hypophosphatemia when administered to severely iron-deficient patients [37–40]. However, only some intravenous iron formulations (e.g., ferric carboxymaltose) raise intact FGF23, while others do not (e.g., iron dextran), suggesting that this may be due to differences in the carbohydrate moieties being used. The data suggest that FGF23 levels increase due to the extreme upregulation of *FGF23* gene expression in the setting of iron deficiency and that cleavage of FGF23 is somehow transiently impaired by certain intravenous iron formulations [40].

Acquired phosphopenic rickets may present at any age due to tumor-induced osteomalacia (TIO). This rare condition is biochemically similar to XLH. Causative tumors are usually benign but secrete factors that lead to hypophosphatemia, and although many types of tumors have been reported, most are mesenchymal tumors and can be classified into variants of "phosphaturic mesenchymal tumor of a mixed connective tissue type" [41]. Rarely are such tumors malignant, though malignant and metastatic tumors are reported. Multiple potential phosphaturic factors have been identified from these tumors including MEPE, FRP4, and FGF7, but the best characterized is FGF23 [42–44]. In one recent series, 60% of phosphaturic mesenchymal tumors causing TIO were found to harbor a somatic mutation producing a novel *FN1-FGFR1* fusion protein incorporating the N-terminus of fibronectin and the FGF1 receptor. This genetic rearrangement has been proposed as a genetic mechanism of tumorigenesis for TIO producing tumors, in which FN/FGFR1 initiates a "feed-forward" loop by stimulating FGF23 secretion in the affected tumor cells. The FGF23 is thought to serve as an additional agonist for the fusion protein, thereby amplifying FGF23 production [45].

Tumors causing TIO are often small and may be difficult to detect radiographically, and many techniques are reported, including computed tomography, magnetic resonance imaging, octreotide-based scintigraphy, and positron emission tomography with co-registered computed tomography. However, in clinical practice the true sensitivity of any individual method is lower than would be hoped, and if clinical suspicion is present, multiple techniques may be required to determine the location of a tumor. They may be found at any anatomic locus but frequently occur in the sinuses and in the extremities. These tumors usually secrete FGF23 in sufficient amounts to cause hypophosphatemia, and selective venous sampling has been reported to assist in localization of some tumors [46, 47]. However, in one study, among those subjects without clear tumors on diagnostic imaging, no tumors could be localized using selective venous sampling [46]. Thus, this technique is not recommended for routine use. Upon complete resection of causative tumors, clinical and biochemical abnormalities typically resolve, though they may recur many years later; hence, long-term monitoring of phosphate is necessary even after apparent surgical cure [48].

Additional disorders may cause hypophosphatemia due to FGF23 excess. Patients with fibrous dysplasia with or without confirmed McCune-Albright syndrome may develop hypophosphatemia due to production of FGF23 within lesions. In these patients FGF23 correlates with total body burden of fibrous dysplasia lesions [49]. Patients with linear sebaceous nevus syndrome also may develop FGF23 excess [50].

Although the primary site of FGF23 production is bone, the liver may be also an important source in certain diseases. Hypophosphatemia secondary to renal phosphate losses has been noted to occur

following hepatic resection. Previous reports indicated that this was not mediated by FGF23, given declines in intact FGF23 during this process [51]. However, recently hypophosphatemic rickets was reported to occur in two infants with end-stage cholestatic liver disease caused by biliary atresia [52]. These infants had severalfold elevations in C-terminal FGF23 measurements and hypophosphatemia, and both normalized after liver transplantation. Immunostaining of the hepatocytes for FGF23 was confirmed in a liver biopsy specimen of one of these children [52], further supporting the liver disease as the cause of this patient's FGF23-mediated hypophosphatemia. Studies in adult end-stage liver disease patients from a variety of causes have also indicated increased C-terminal FGF23 levels, and mice with induced liver damage demonstrated increased liver *FGF23* gene expression and serum levels [53].

Hereditary Hypophosphatemic Rickets with Hypercalciuria

In contrast to XLH, hereditary hypophosphatemic rickets with hypercalciuria (HHRH) is an autosomal recessive disorder in which a primary renal phosphate leak occurs but, in contrast to XLH, maintains the capacity to appropriately increase $1,25(OH)_2D$ levels in response to the ambient hypophosphatemia. Consequently, increased intestinal calcium absorption occurs, and hypercalciuria becomes evident. PTH levels are appropriately suppressed. Mutations in *SLC34A3*, encoding the renal sodium-phosphate cotransporter, NaPi2c, have been identified to cause HHRH [54–56]. Many cases have been described in North African and Middle Eastern populations. In addition to rickets and osteomalacia, nephrolithiasis and nephrocalcinosis occur in some patients and may be associated with specific mutations [57, 58]. In contrast to XLH, the FGF23 concentration is reported as low normal in response to the hypophosphatemia in this disorder [54].

Fanconi Syndrome and X-Linked Recessive Hypercalciuric Nephrolithiasis

Phosphopenic rickets may accompany the Fanconi syndrome, a heterogeneous group of solute wasting disorders characterized by variably excessive urinary losses of glucose, phosphate, bicarbonate, and amino acids. This renal tubular dysfunction represents a primary proximal tubulopathy or results from exposure to certain drugs or other toxins. Additionally, inborn errors of metabolism such as cystinosis, tyrosinemia, galactosemia, Wilson's disease, hereditary fructose intolerance, or type 1 glycogen storage disease may result in Fanconi syndrome. Recently mutations in NaPi2a, another member of the type II class of renal sodium-phosphate cotransporters, have been shown to result in renal phosphate wasting [59].

Mutations in a renal tubular chloride channel, CLCN5, may result in a family of conditions referred to as Dent's disease, hypophosphatemic hypercalciuric rickets, and X-linked hypercalciuric nephrolithiasis (XLHN) [60]. Lowe (oculocerebrorenal) syndrome, due to mutations in OCRL, which encodes an inositol polyphosphate 5-phosphatase [61], is usually manifested by Fanconi syndrome together with severe mental retardation and cataracts. Patients with Dent's disease and Lowe syndrome have abnormalities in intracellular membrane trafficking affecting endocytic pathways and lysosomal pathways [61]. In both conditions, as with other forms of the Fanconi syndrome, phosphate wasting occurs, often resulting in hypophosphatemia and occasionally overt rickets.

As noted above, we recently encountered a novel association of hypophosphatemia occurring in certain children with complex gastrointestinal disorders fed with amino acid-based formulas (particularly Neocate®) as a sole source of nutrition. This phenomenon, which has been noted in infants as well as older children, was found upon routine biochemical evaluation or, in some cases, after presenting with fracture and overt rickets. The typical biochemical profile is manifested by hypophosphatemia, with elevated serum levels of $1,25(OH)_2D$ and alkaline phosphatase. Renal phosphate excretion is minimal (often undetectable), indicating appropriate renal conservation of phosphate. Thus enteral bioavailability of phosphorus appears to be compromised in this setting, despite normal phosphorus content of the formula. Phosphorus supplementation or changing the formula will usually correct the biochemical findings; however, such measures must be implemented carefully and under very close observation as precipitous decreases in the serum calcium level may occur [62]. After correction of biochemical values is observed, eventual healing of the skeletal lesions usually follows. Intermittent monitoring of serum phosphorus is indicated in children receiving such feeding regimens.

23.2.1.4 Rickets-like Disorders

Several related conditions manifest rachitic-like deformities that require specific identification, since usual therapy for rickets will not improve these conditions. These include *hypophosphatasia*, a rare disorder characterized by deficiency of tissue-nonspecific alkaline phosphatase (TNSALP). Over a hundred different *TNSALP* mutations and both autosomal dominant and recessive inheritance are reported [63]. Four clinical forms of hypophosphatasia have been described, with the severity of the disease being inversely related to the age at presentation: a perinatal lethal form; an infantile form presenting within the first 6 months of life with rachitic-like skeletal defects resulting in recurrent respiratory tract infection, poor growth, increased intracranial pressure, and death in 50% of cases; a milder childhood type presenting after 6 months of age with premature loss of deciduous teeth, rachitic-like lesions, and craniosynostosis; and an adult-onset form with recurrent fractures and pseudofractures. Skeletal disease results from the absence of TNSALP and subsequent impaired ability to initiate mineralization. The accumulation of inorganic pyrophosphate, a known mineralization inhibitor, occurs in the absence of alkaline phosphatase, thereby limiting growth of hydroxyapatite crystals [63]. Patients with hypophosphatasia may have high normal or high serum calcium and phosphate levels and may be hypercalciuric. This condition is not related to abnormal vitamin D levels and should not be treated with vitamin D supplementation because treatment with vitamin D may increase urinary calcium excretion. Hypophosphatasia is distinguished from rickets by a low serum alkaline phosphatase activity for age. Patients with hypophosphatasia also accumulate other metabolites including phosphoethanolamine (measured in urine) and pyridoxal 5'phosphate or vitamin B6 (measured in the blood), which may aid diagnosis. Radiographs may show "tongues" of lucency extending from the growth plate into metaphyses [63]. Recent trials of a recombinant, bone-targeted alkaline phosphatase enzyme replacement (asfotase alfa, Strensiq®) have shown remarkable improvement in skeletal abnormalities in children with the infantile form of hypophosphatasia [64]; this biologic has recently been approved by the FDA for this purpose.

Other skeletal dysplasias include the *Schmid-type metaphyseal dysplasia*, an autosomal dominant disorder due to a mutation in the type X collagen (COL10A1) gene [65]. This disorder may present as rickets because of its clinical and radiographic similarities. Type X collagen expression is restricted to hypertrophic chondrocytes in areas undergoing endochondral ossification. The deficiency in growth plates can result in a lesion resembling rickets; however, there are no abnormalities in serum calcium, phosphate, and alkaline phosphatase activity. These conditions do not respond to treatment with vitamin D metabolites, calcium, or phosphate.

23.3 Clinical Presentation

Patients with rickets are often first recognized due to skeletal abnormalities found by parents or physicians, including varying degrees of bowing or knock-kneed deformities of the legs, wide-based gait, and short stature. Genu varum or valgum is more likely to develop after 1 year of age once patients are bearing weight on their legs. Patients may also present with additional exam findings of rickets (described below). Some patients have delayed gross motor milestones, including delayed development of walking or running, or rarely older patients may manifest muscle weakness. Patients with calciopenic rickets at the extreme end may have hypocalcemia and associated symptoms and signs as tetany and seizures. Rickets may occasionally be discovered during evaluation of fractures or injury, though most children with rickets do not have fractures, or found incidentally on radiographs performed for other purposes. Rickets may also be discovered due to abnormal alkaline phosphatase, calcium, or phosphorus detected on laboratory testing performed for other reasons. Inherited forms of rickets may present due to family history prior to the development of frank physical signs.

23.3.1 Physical Examination

The physical examination should focus on height, evidence of rachitic bone deformities, and dentition. Short stature is a common finding, particularly

in certain types of rickets, generally reflecting the extent and duration of lower extremity involvement. Rachitic bone deformities vary, depending on age at onset and the relative growth rate of different bones. The most rapidly growing bones during the first year of life are the skull, the ribs, and the upper limbs. Rickets presenting at this age may manifest craniotabes (generalized softening of the calvaria), frontal bossing, widening of the cranial sutures, flaring of the wrists, rachitic rosary (bulging of the costochondral junctions of the ribs), and Harrison grooves (groove extending laterally from the xiphoid process across the ribs corresponding to the diaphragmatic attachment). After the first year of life, lower limb deformities such as genu varum (bowing) or genu valgum (knock-kneed deformity) occur. Parallel deformities described as "windswept" legs may occur when one leg has a varus deformity and the other manifests a valgus deformity. Bone pain is common in children, and palpable enlargement of the ends of the long bones occurs notably in the wrists, ankles, and knees.

Proximal muscle weakness can occur due to vitamin D- or to calcium-deficiency rickets as well. Hypocalcemia, if present, can result in carpopedal spasm, laryngeal stridor, seizures, and paresthesias. Though less likely, myopathy may also occur in hypophosphatemic forms of rickets but is generally limited to adults and severe cases of TIO in children.

Unique physical findings may accompany rickets in certain specific disorders, such as the alopecia and oligodontia associated with hereditary vitamin D resistance due to vitamin D receptor mutations. In XLH, the skull is often scaphocephalic, and Chiari I malformation, possibly related to severe calvarial osteomalacia and thickening, has been described [17, 66]. Adults with XLH may manifest vertebral anomalies such as thickening of the spinous processes, fusion of vertebrae, thickening of facet joints, and spinal canal stenosis. Calcification of tendons and ligaments (termed enthesopathy) is also common in XLH, which is often seen at the Achilles tendon, around the hip or knee, and at the anterior or posterior vertebral ligaments, leading to decreased range of motion [67]. Dental abscesses are a frequent occurrence in patients with XLH, due to the undermineralization and expansion of the pulp chamber from the low phosphate content of dentin, as well as effects on the cementum layer [68]. This phenomenon results in a diminished barrier to the exterior surface of the teeth and easy access for oral fluids and bacteria to pass through the outer enamel layer and initiate abscess formation. Early deciduous tooth loss, with characteristic sloughing of the entire tooth, including the root, can be a sign of hypophosphatasia.

23.4 Diagnostic Evaluation

23.4.1 Radiographic Assessments

Radiographic findings vary based on the age of the patient and the duration and severity of the rachitic defect. The earliest radiographic change of rickets is slight widening of the growth plate. In more severe rickets, fraying, cupping, and widening of the metaphyses occur. In infants, abnormalities are best seen at the costochondral junctions, wrists, and ankles; in older children, the distal femur is likely to exhibit major changes. In adolescence, the epiphyses begin to fuse to the larger corresponding bone segments as the growth plate resolves. During this time, the iliac crest may continue to show rachitic changes, as it is the last epiphysis to fuse. In older children with XLH, asymmetry of the growth plate results, due to altered weight-bearing forces through the bowed physis. Osteomalacic changes of the diaphyses include shaft deformities (bowing and torsion), decreased bone density, coarse spongiosa, variably thinned cortices depending on the severity and type of rickets, and pseudofractures. As healing of rickets begins, radiodense lines are detectable adjacent to the metaphyses representing rapid calcification of the cartilage.

23.4.2 Laboratory Assessments

Adequate testing to identify the cause of rickets should ideally be made prior to initiation of treatment, since treatment may alter some of the diagnostic parameters. Exceptions may be necessary in the setting of symptomatic hypocalcemia, in which some form of treatment with calcium and vitamin D will need to be initiated immediately.

Initial biochemical evaluation should include assessment of serum calcium, phosphate, and alkaline phosphatase activity. With most causes of rickets, elevated bone turnover is present, reflected in increased serum alkaline phosphatase activity. However, alkaline phosphatase activity is normal in most skeletal dysplasias and low for age in hypophosphatasia. Calciopenic forms of rickets are usually associated with alkaline phosphatase activity 3–10 times higher than the normal range, whereas heritable phosphopenic rickets are often associated with lesser (1.5–3-fold) degrees of elevations. Alkaline phosphatase activity generally decreases with therapy for rickets, although early in the therapeutic course, this parameter may briefly increase. Complete normalization may not occur despite aggressive therapy in some forms of rickets. Adults with XLH may have normal alkaline phosphatase levels despite impressive osteomalacia; thus, monitoring levels in this age group is not always a good index of disease status or response to therapy.

In all forms of calciopenic rickets, serum calcium and phosphate levels both tend to be in the low to low-normal range. Measurement of vitamin D metabolites serves to identify the specific cause. The primary indicator of vitamin D status is measurement of 25-hydroxyvitamin D (25OHD), the most abundant circulating vitamin D metabolite. Low serum concentrations of 25OHD indicate vitamin D deficiency and may result from insufficient dietary intake or malabsorption, among other causes (typically combined with insufficient sunlight exposure). Children with nutritional rickets often have a mixture of calcium and vitamin D deficiency, and in some regions, calcium may be the predominantly deficient nutrient. Partial treatment of vitamin D deficiency may increase circulating 25OHD levels into the normal range if treatment is initiated prior to the time of assessment, yet radiographic evidence of skeletal abnormalities may remain. Serum $1,25(OH)_2D$ is not a useful test for vitamin D deficiency; we generally only perform this measurement after excluding vitamin D deficiency, when investigating other suspected etiologies. In the setting of vitamin D deficiency, serum PTH rises, stimulating 1α-hydroxylase activity, and the resulting $1,25(OH)_2D$ level may be high, normal, or low. Thus, $1,25(OH)_2D$ levels do not clearly represent vitamin D status. In the setting of 1α-hydroxylase deficiency, normal levels of 25OHD will be accompanied by a low $1,25(OH)_2D$ level and hypocalcemia. However, in the same clinical situation (normal 25OHD with hypocalcemia), an increased $1,25(OH)_2D$ level suggests vitamin D resistance. Notably, although hypoparathyroidism causes hypocalcemia, it does not cause rickets, likely due to the elevations in phosphate that accompany this disorder.

A low serum phosphate level with normal serum calcium and normal 25OHD levels suggests the diagnosis of primary hypophosphatemic rickets and should be followed by an accurate assessment of renal phosphate handling. For this assessment, timing of the urine collection is important. Ideally, a 2 h urine collection following an overnight fast (or for infants, fasting at least 4 h) is performed, with a blood sample collected midway through the urine collection. If a 2 h collection is not possible, a single fasting urine sample that is not the first morning void (thus excluding residual urine produced overnight) can be used, with a simultaneous blood collection. Any questionable results should be followed up with a fasting 2 h urine collection, before treatment decisions are made. Measurements are made for serum and urine creatinine and phosphate which allows for calculation of the tubular reabsorption of phosphate (%TRP). A nomogram [69] is then used to determine the renal tubular threshold maximum for phosphate, as expressed per glomerular filtration rate (TmP/GFR). This nomogram may overestimate the effect of GFR in young children and does not fully incorporate the upper normal range of phosphate values and TmP/GFR seen in healthy young children and however does identify the *low* TmP/GFR values characteristic of phosphate wasting disorders. An alternate calculation (termed TP/GFR for distinction) can be determined [TP/GFR = serum phosphate – (urine phosphate x serum creatinine/urine creatinine)] and has less deviation between fasting and phosphate loading conditions, but fasting conditions are still preferred [70–72]. The TmP/GFR (or TP/GFR) is an indication of the serum phosphate level above which the tubule loses phosphate in the urine.

In evaluating any pediatric condition, age-appropriate normal values are essential for interpretation. Many reference laboratories do not provide age-appropriate normal ranges, which may lead to misinterpretations and incorrect diagnoses. Both serum phosphate and TmP/GFR (or TP/GFR) are higher in infants and young

children than in adults. Such differences in phosphate metabolism are critical to healthy growing bone, as infants and young children having low phosphate levels for age (but within the adult normal range) may develop rickets. As a rule of thumb, the normal TmP/GFR for age roughly approximates the normal range for serum phosphate at that age. A low TmP/GFR in the setting of a low serum phosphate indicates inappropriate renal phosphate losses as opposed to nonrenal causes of hypophosphatemia.

Several phosphate wasting disorders need to be considered, as treatment differs. To distinguish XLH from hypercalciuric variants of hypophosphatemia, hereditary hypophosphatemic rickets with hypercalciuria (HHRH), and X-linked hypercalciuric nephrolithiasis (XLHN), 24 h urinary calcium excretion should be determined if possible, although a briefer collection period may suffice if adequate urine volume is obtained. Urinary calcium excretion tends to be low in untreated XLH and in the calciopenic forms of rickets. Fanconi syndrome may be associated with glycosuria and aminoaciduria, along with phosphaturia and hypercalciuria. Hypercalciuria is defined as urinary calcium greater than 4 mg/kg per 24 h; normal values for the fasting morning urinary calcium/creatinine in children greater than 4 years of age are less than 0.21 mg/mg, whereas in infants this value is higher and can vary considerably based on the nature of the diet [73]. In addition, proteinuria or microglobulinuria is often present in patients with XLHN. TIO cannot be distinguished biochemically from hereditary hypophosphatemic rickets due to FGF23 excess, and it must be suspected in all sporadic cases of hypophosphatemic rickets presenting in late childhood or adulthood. To complicate matters, autosomal dominant hypophosphatemic rickets (ADHR) may also present with acquired hypophosphatemia in adolescents or even adults [33].

Furthermore, sporadic cases of heritable rickets do occur. In apparent sporadic cases, testing serum phosphate concentrations in family members may be revealing, as the diagnosis is not always known by affected family members [15]. Genetic testing should ideally be reserved for those cases in which confirmation of a mutation is expected to affect clinical management (such as when uncertainty exists about whether a patient's presentation represents TIO or a form of heritable phosphopenic rickets).

Measurement of PTH levels is useful in the diagnostic evaluation. Moderate to severe secondary hyperparathyroidism is characteristic of calciopenic rickets. On the other hand, in untreated XLH, PTH levels may be normal or modestly elevated, though more severe secondary (and tertiary) hyperparathyroidism is later encountered as a complication of phosphate therapy. Other findings of XLH include normal serum 25OHD levels and normal or somewhat low levels of $1,25(OH)_2D$ (inappropriately low in the setting of hypophosphatemia). By contrast, in hypercalciuric variants of hypophosphatemic rickets, the circulating $1,25(OH)_2D$ level appropriately increases in response to hypophosphatemia, resulting in normal to high serum calcium levels and suppressed PTH levels.

23.5 Treatment, Clinical Course, and Complications

23.5.1 Calciopenic Rickets

The immediate goal of medical treatment is to heal the active growth plate lesions. Medical therapy usually prevents progression of bow or knock-kneed deformities, which in untreated disease may become irreversible and require corrective orthopedic intervention. Medical therapy often resolves the deformities in some forms of rickets, especially nutritional rickets, over time. Furthermore, in untreated nutritional (and heritable) rickets, short stature often results and is associated with body disproportion, manifest by an increased upper segment (trunk) to lower segment ratio. It should be noted that while this review focuses on the treatment of rickets, efforts need to be expended as well on *primary* prevention by educating the public and the general pediatric community about the importance of adequate dietary intake and supplementation with vitamin D and calcium. S*econdary prevention* of nutritional calciopenic rickets has also been proposed in select groups at higher risk for vitamin D deficiency, such as those with malabsorptive conditions.

Some advocate screening for vitamin D deficiency prior to the onset of rickets or other long-term consequences of vitamin D deficiency. Although recently much has been discussed regarding the frequency of vitamin D deficiency or insufficiency in both the medical literature and the

lay press, there is no data that would currently justify widespread screening with blood levels of vitamin D metabolites. We believe, rather, that public education regarding the nutritional recommendations for calcium and vitamin D stands to offer the most broad-reaching benefits. However, screening should be considered for high-risk groups, such as exclusively breastfeeding infants (especially if dark-skinned, which limits dermal vitamin D production), children with dairy product avoidance, and unsupplemented infants living in areas where limited sunlight exposure due to higher latitudes or extremes of weather may limit vitamin D production. Interestingly the promotion of breastfeeding may incur increased risks for development of vitamin D-deficiency rickets, as breast milk is quite low in its natural content of vitamin D. The "Healthy People 2000" initiative set a target to achieve at least 6 months of breastfeeding in 75% of American infants. If this goal is attained in the absence of vitamin D supplementation, especially in higher-risk groups such as African-Americans, then a significant increased risk for rickets will result.

Thus the American Academy of Pediatrics recommended in 2008 that all children receive 400 units of vitamin D daily, beginning shortly after birth and continuing through adolescence, attained either through supplementation or dietary sources [74]. Similar recommendations were recently made by the Institute of Medicine in their 2010 updated report, "Dietary Reference Intakes for Calcium and Vitamin D" [75]. An intensive review of the available evidence was performed, providing an estimation of the population requirements for people living in North America. The report upwardly revised recommendations of vitamin D intake compared to the previous (1997) guidelines. The 2010 report advised 400 IU daily beginning in infancy, 600 IU units daily beyond 12 months of age, and 800 IU daily recommended for the elderly population. Upper limits of safe intake were estimated and vary based on age, from 1000 IU daily for infants to 4000 IU daily from ages 9 years through adulthood. Moreover, the report cautioned against oversupplementation of both vitamin D and calcium, as risks of harms related to hypercalcemia and hypercalciuria are clearly documented [75]. In 2016, the Global Consensus Recommendations on Prevention and Management of Nutritional Rickets was published [76]. The authors concurred with IOM recommendation for vitamin D daily intake targets in infancy and childhood, but did not recommend screening 25OHD levels in healthy children. While adequate vitamin D synthesis can be accomplished with sunlight exposure, there is an accompanying increased risk of skin cancer. Vitamin D deficiency was defined as levels <30 nmol/L (12 ng/ml), while sufficiency was defined as >50 nmol/L (20 ng/ml). Finally, although many published studies indicate a potential impact of vitamin D on a wide variety of non-skeletal conditions, there is insufficient evidence to justify basing dietary recommendations on non-skeletal targets of vitamin D at this time.

Both the IOM and the Global Consensus recommended that to prevent rickets, infants under 6 months required 200 mg calcium per day and infants 6–12 months 260 mg per day [75, 76]. The Global Consensus recommended calcium intake of over 500 mg per day for children 1–18 years of age [76], though the IOM recommendations ranged from 700 to 1300 mg daily for this age group [75].

23.5.1.1 Vitamin D-Deficiency Rickets

Treatment of overt vitamin D-deficiency rickets requires higher doses of vitamin D for a temporary period. A wide range of doses and schedules are reported, and all generally will heal rickets over time. Liquid oral preparations are commonly available in concentrations of 8000 units per ml, but other formulations exist, and the concentration should be confirmed. Vitamin D-deficiency rickets is often treated with 1000–2000 units per day of vitamin D. Some prefer the administration of higher amounts of oral vitamin D for 1 week or 1 month followed by lower doses, but this must be approached cautiously, if at all. If the patient fails to return for follow-up while taking higher doses, this increases the risks of overtreatment which can cause hypercalciuria and nephrocalcinosis even without overt hypercalcemia. Renal failure may result from vitamin D toxicity. Failure to follow-up can result in significant harm from either overtreatment or undertreatment. Recently, the Global Consensus Recommendations included treating active nutritional rickets with 2000 IU daily for 3 months in infants <12 months old and 3000–6000 units daily for 3 months for older children [76]. If there is a concern about compliance, a single observed oral or IM dose of 300,000 or 600,000 units of vitamin D (also called stoss therapy) may be given. Alternatively, this large oral dose may be divided into two or three doses given

over a 1–3 day period. However, an increased risk of hypercalcemia exists with stoss therapy, and the Global Consensus Recommendations listed daily oral therapy as the preferred route [76].

Children with vitamin D-deficiency rickets require supplemental calcium as well, as these children often have a low dietary intake of calcium. Moreover, "hungry bone syndrome" may develop during early vitamin D repletion, due to the rapid uptake of calcium into the bone as osteoid mineralizes with the application of therapy. In the setting of an inadequate calcium supply, hypocalcemia may result as the extracellular fluid compartment becomes depleted. Finally, it has been shown that vitamin D stores may be depleted rapidly during periods of low dietary calcium intake [77]. We recommend either calcium glubionate (6.4% elemental calcium) or calcium carbonate (40% elemental calcium) in two to four divided doses for a total daily intake of 30–50 mg/kg/day of elemental calcium. Hypocalcemia may necessitate higher intakes of calcium transiently. Although phosphate is the counterpart to calcium in the mineralized structure of the bone, most children consume a phosphate-sufficient diet. As vitamin D will also increase phosphate absorption, phosphate supplementation is not required during treatment of vitamin D-deficiency rickets. Radiographic imaging can be repeated 2 or 3 months following initiation of therapy to confirm healing of rickets. Serum alkaline phosphatase concentrations will often increase further during the initial phases of healing but usually decrease to normal over a few months. Urine calcium (or urine calcium/creatinine ratio) will increase as rickets resolves, as less calcium is being used to heal the bones. After radiographic evidence of healing of rachitic lesions, the vitamin D dosage can be decreased to an age-appropriate range of 400–600 units per day indefinitely, consistent with the recommended daily allowance. After healing of rickets, routine calcium intake consistent with dietary recommendations for age is appropriate. However, if an underlying cause for vitamin D deficiency such as malabsorption is present, then somewhat higher doses of vitamin D may be required to maintain normal levels.

23.5.1.2 Calcium-Deficiency Rickets

Although children with primarily calcium-deficiency rickets significantly increase $1,25(OH)_2D$ in response to administered vitamin D, fractional absorption of calcium does not appear to change [78]. In fact, fractional calcium absorption was not positively related to baseline 25OHD concentrations in this population [7]. Calcium repletion (1000 mg/day), with or without vitamin D, has been shown to be more effective than vitamin D alone in achieving improvement of biochemical and radiographic measures of rickets in these cases [79]. Thus the primary treatment for this disorder is adequate calcium supplementation (30–50 mg/kg/day of elemental calcium).

1α-Hydroxylase Deficiency

Patients with 1α-hydroxylase deficiency cannot convert 25OHD to $1,25(OH)_2D$ and are best treated with activated vitamin D metabolites or analogs. High dosages of inactive metabolites were used previously, but the activated compounds have a wider therapeutic index and can be more precisely titrated to control calcium levels. Such patients treated with calcitriol prior to the pubertal growth spurt have better height outcomes than those treated with nonactivated vitamin D metabolites through childhood [80]. Typically calcitriol is started at diagnosis in dosages ranging from 0.5 to 3.0 microgram per day. Once normocalcemia and healing are attained, lower maintenance dosages of 0.25–2.0 microgram per day are often used. Adequate dietary or supplemental calcium is necessary, but patients require monitoring for development of hypercalciuria and hypercalcemia, an indication of overtreatment, and a need for dose reduction [80].

25-Hydroxylase Deficiency

Doses of vitamin D can be titrated to achieve normal levels of 25-OHD in family members with CYP2R1 mutations. There may be a need to supplement both heterozygote and homozygous subjects based on the ambient vitamin D supply.

Hereditary Vitamin D Receptor Resistance

Patients with vitamin D receptor mutations have been treated with extremely large dosages of vitamin D or vitamin D metabolites, with variable responses to therapy. The disorder may vary in severity, from mild with response to high-dose oral calcium to more severe requiring continuous parenteral calcium administration [81], depending on the degree of the functionality of the VDR. However, parenteral administration of

calcium has been shown to completely correct the skeletal abnormalities in severe forms of this disorder [81, 82]. Treatment is very challenging and should be performed by a pediatrician experienced in the management of metabolic bone disease.

23.5.1.3 Other Causes of Calciopenic Rickets

For disorders affecting *vitamin D absorption* in the proximal small intestine and disorders of fat malabsorption, higher than usual oral vitamin D may be required with doses up to 5000–10,000 units of vitamin D daily. Higher-dose therapy may even be necessary but should be accompanied by at least monthly or bimonthly monitoring of mineral and vitamin levels so that appropriate adjustments in dosing can be made. Others have suggested the use of the more polar $1,25(OH)_2D_3$, which is, in part, absorbed in water-soluble form and is therefore less dependent on intact fat absorption.

Vitamin D-deficiency rickets resulting from *impairment of 25-hydroxylation* in severe hepatobiliary disease may be treated with high doses of vitamin D (up to 50,000 units/day). Addition of calcitriol may also be useful.

Chronic kidney disease is associated with complex metabolic bone disease that encompasses a wide spectrum from osteomalacia to low turnover states. Rickets and osteomalacia associated with chronic kidney disease require use of calcitriol in oral dosages of up to 1.5 microgram/day, since endogenous 1α-hydroxylase is impaired. However, monitoring for development of hypercalcemia is required. Calcitriol is also useful to suppress the hyperparathyroidism seen in CKD. In the setting of CKD, other vitamin D analogs, such as doxercalciferol and paricalcitol, may be useful alternatives to calcitriol. In addition, frank vitamin D deficiency does occur in these patients, and supplementation with routine vitamin D doses in addition to calcitriol may also be indicated, consistent with recommendations for the general population.

Phosphate binders are generally needed in CKD along with dietary phosphate restriction, but in the presence of concomitant rickets, if a phosphate binder is needed to control hyperphosphatemia, a calcium-containing phosphate binder (such as calcium carbonate) may be preferable. Alternate phosphate binders such as sevelamer or aluminum hydroxide will not help rickets. However, CKD itself also causes extreme elevations in FGF23, and in adults with CKD, FGF23 level is associated with long-term mortality risk [83], though the precise role of FGF23 in CKD-related bone disease is not clear. Both calcium-based and non-calcium-containing phosphate binders can decrease FGF23 concentrations similarly in CKD dialysis patients [84, 85], though some studies suggest calcium acetate may increase intact FGF23, while sevelamer decreased levels in non-dialysis CKD patients [86]. Overall in the absence of rickets, sevelamer may be the agent of choice for managing hyperphosphatemia in CKD. However, care should be taken to avoid causing hypophosphatemia.

Individuals receiving anticonvulsant therapy should receive the recommended dietary allowance of vitamin D (600 units) from the usual sources for prevention of vitamin D-deficiency rickets, and pharmacologic supplementation may be necessary if overt vitamin D-deficiency rickets results.

23.5.1.4 Monitoring and Complications of Therapy for Calciopenic Rickets

Overall, children with nutritional calciopenic rickets should be seen initially every few weeks, with close monitoring of serum calcium, phosphate, alkaline phosphatase, and vitamin D metabolite levels. Alkaline phosphatase levels may rise during the initiation of therapy, before declining to normal ranges. Radiographic evidence of healing of rachitic lesions may be seen within several weeks to months. Higher doses of calcium and vitamin D are not needed indefinitely for the most common (nutritional) causes of rickets. Eventually patients should heal their rickets and just require usual daily doses of calcium and vitamin D, except for rare situations, such as genetic causes of calciopenic rickets. Severe complications can occur with the use of vitamin D metabolites in an unmonitored fashion. Most commonly, sequelae from vitamin D intoxication are related to hypercalcemia and hypercalciuria, which may increase the calcium x phosphate product and precipitate soft-tissue calcification. Acute kidney injury often accompanies the hypercalcemia of vitamin D toxicity. Nephrolithiasis may occur, and nephrocalcinosis, if severe, may have long-term effects on renal function. Therefore, sampling of serum and urinary calcium and creatinine is warranted

within 2 weeks of the initial dosing and after dose adjustments are made. Patients on stable doses with long-term treatments may have these tests performed every 3–4 months. If serum calcium becomes greater than normal or the urinary calcium/creatinine ratio is greater than 0.35 mg/mg, decreases in doses are warranted. Finally, if severe hypercalcemia is present, vitamin D administration should be discontinued, and specific measures for treatment of hypercalcemia should be instituted (mainly oral and intravenous fluids) until hypercalcemia is resolved. We emphasize the long-term implications of such toxicity: as vitamin D is stored in fat, toxic levels may require months to correct after cessation of the excessive intake, given the massive storage that may occur after administration of excessive doses.

23.5.2 Phosphopenic Rickets

23.5.2.1 Nutritional Phosphate Deprivation

Premature infants are the primary population at risk for dietary phosphate deficiency. Prevention of nutritional phosphopenic rickets by routine use of breast milk fortifier is recommended in premature infants. Monitoring of mineral levels is critical as some of these products have been associated with hypercalcemia. If rachitic disease related to nutritional phosphate deprivation develops in the premature infant, it can be treated with 20–25 mg of elemental phosphorus/kg body weight per day, given as an oral supplement in 3–4 divided doses. However premature infants may also be at risk for other forms of rickets, including vitamin D deficiency, typically due to maternal deficiency, and potentially other inherited forms of rickets. Therefore, ascertaining vitamin D levels and ensuring adequate intake of calcium and vitamin D are still necessary in this population. Limitations in enteral intake secondary to necrotizing enterocolitis and other comorbidities may limit the ability to provide adequate mineral. If necessary, mineral supplementation may be administered parenterally, and newer amino acid formulations supplemented with the sulfur-containing amino acids taurine and cysteine allow greater quantities of calcium and phosphate to remain in solution in TPN formulations. Bone disease in the premature infant may be complex and include a component of osteoporosis as well. However, in most situations, infants recover well and correct their bone defect when provided with adequate therapy.

When hypophosphatemic rickets occurs associated with elemental formula feedings, caution must be applied with addition of phosphate or a change in formula. After long-term deprivation of phosphate, it is likely that the phosphate transporters at both the intestine and kidney are significantly upregulated. Thus any newly provided bioavailable phosphorus may be subject to massive intestinal absorption as well as impaired renal excretion of the phosphate load, resulting in potential severe toxicity with acute hyperphosphatemia and hypocalcemia. Indeed we have observed hypocalcemia to occur after phosphate supplementation or formula change in this setting [62]. We recommend close observation and incremental steps with frequent monitoring when introducing phosphate supplements or a new formula to these patients.

23.5.2.2 X-Linked Hypophosphatemic Rickets (XLH)

Early treatment of XLH may be associated with improved long-term outcomes [87]; however, this requires early diagnosis, which is most likely when screening infants from known affected kindreds. Screening is recommended with determination of serum calcium and phosphate levels and alkaline phosphatase activity and determination of TRP and TmP/GFR at 1–2 months of age and, if unrevealing, at 3–4 month intervals during the first year of life. The most likely source of error in screening is the inappropriate use of adult reference ranges for phosphate in infants, leading physicians to think that the child is normal, when in fact they are hypophosphatemic.

Treatment of XLH consists of the administration of phosphate salts in conjunction with calcitriol, a regimen which has been demonstrated to improve the rachitic lesions at the growth plates and mineralization in the trabecular bone [88, 89]. Because of the propensity to develop secondary and, in some instances, tertiary hyperparathyroidism with large doses of phosphate, and because of the concern for complications of hypercalcemia, hypercalciuria, and nephrocalcinosis from vitamin D intoxication, careful attention must be paid to dosing regimens. Note that these are considered pharmacologic therapy rather than supplementation.

In XLH phosphate is generally provided as 20–40 mg/kg per day of elemental phosphorus in three or four divided doses [90]. In early infancy, this amounts to a dose of 250–375 mg of elemental phosphorus, usually provided in two or three divided dosages, with the dosing interval limited by the ability to give smaller doses accurately. Parents have found it convenient to add the daily dose of phosphate to formula feedings; it is important to assure complete ingestion of feedings or to adjust for decreased intake of the supplemented formula, as necessary. A useful preparation for infants is an oral Phospho-Soda solution containing 127 mg of elemental phosphorus per ml. Recently a new formulation has been marketed at a lower concentration, 104 mg of elemental phosphorus per ml. Alternately a specialty pharmacy, usually at a children's hospital, may be able to compound oral phosphate solutions similar to Phospho-Soda or the traditional Joulie's solution containing 30 mg elemental phosphorus per ml. It is sometimes possible to use K-Phos Original (114 mg phosphorus per tablet) or K-Phos MF (128 mg phosphorus per tablet) using half tablets crushed and dissolved in liquid in older infants, if it is difficult to obtain the liquid formulations. In older children, Neutra-Phos or Neutra-Phos K powder (250 mg elemental phosphorus per packet) can be dissolved in water. When the child is old enough to chew or swallow a tablet, K-Phos Neutral, which contains 250 mg of elemental phosphorus per tablet, is preferred. In the older child, an average of 1 g of elemental phosphorus per day is used; it is seldom necessary to prescribe more than 2 g per day. Note that the different available phosphate preparations are not interchangeable, generally, and require recalculation of the amount of phosphorus in order to make sure the proper amount is given.

A common difficulty among children relates to disliking the taste of medications, and the use of a stronger-tasting beverage may be beneficial to "hide" the flavor of the medication. Almost all children will complain of abdominal discomfort or manifest diarrhea soon after the initiation of phosphate therapy, but this usually resolves within days to weeks. Occasionally, administration of phosphate must be suspended and restarted at lower dosages; and very rarely, diarrhea, bloody stools, or persistent dose-related abdominal pain occurs with this therapy, indicating a need for a substantial decrease in the dose.

Although the phosphate dose needs to be divided throughout the day, rather than taken all at once, we do not find it essential for good clinical outcomes for parents to wake children in order to administer phosphate throughout the night. It is clear from nocturnal monitoring studies that serum phosphate levels rise at night, independent of phosphate administration [91], and nocturnal dosing might increase the risk of hyperparathyroidism, in addition to being inconvenient for the patient and family.

It has long been known that phosphate therapy alone is insufficient, and some complications such as tertiary hyperparathyroidism were more common when attempting to treat with phosphate alone. Early attempts at treating XLH with ergocalciferol led to the description of XLH as vitamin D-resistant rickets. In the past, XLH patients were treated with extremely high doses of ergocalciferol, which had a higher risk of toxicity resulting in hypercalcemia and consequences of hypercalciuria, compounded by the long half-life of storage forms of vitamin D. More recently, administration of calcitriol has been recognized as important for a successful healing of osteomalacia in XLH [88, 89]. This metabolite enhances calcium and phosphate absorption and dampens phosphate-stimulated PTH secretion; however, the mechanism for its skeletal action in XLH is not entirely understood. FGF23 inhibits production and stimulates degradation of $1,25(OH)_2D$; thus, treatment with calcitriol addresses one of the pathophysiologic effects of FGF23 excess.

Although published doses vary widely, we have generally targeted doses of calcitriol at 20–30 ng/kg per day [90]. Other active vitamin D analogs such as alfacalcidol may be used, but there is no clear dose equivalency. In infancy it is easiest to use liquid preparations of calcitriol, or the intravenous preparation can be administered orally. If liquid preparations are completely unavailable, the oil solvent within the calcitriol capsule can be withdrawn with a needle and given to the child (after removal of the needle). Starting doses for an infant in the first 2 years of life are generally 0.25 micrograms once or twice daily, though liquid formulation may allow more precise dosing. Generally, children are switched to capsules as soon as practical. The liquid formulation of dihydrotachysterol is also a reasonable alternative for this very young age group.

Several investigators have examined human growth hormone as a therapeutic agent in XLH. However, despite several small, mostly non-randomized, trials of growth hormone therapy in XLH, the long-term impact of GH treatment on

final adult height in patients with XLH remains unknown [92]. Some have shown that growth hormone in combination with standard therapy results in improved circulating phosphate concentrations and an improvement in height velocity in the short term [93–95]. However, there is also potential for increasing the trunk to limb length discrepancy or worsening lower extremity deformity during treatment with growth hormone as well [93, 96]. One randomized controlled trial of growth hormone in prepubertal children with XLH indicated improvement in linear growth velocity without worsening of leg bowing [97]. However, since the mean height standard deviation scores did not differ between the growth hormone and control groups before or after 3 years of treatment, the benefit remains uncertain [97]. Some clinicians consider this approach for children that are extremely short and have suggested that reasonable growth outcomes occur only with very early implementation of therapy. A more aggressive therapy with calcitriol and phosphate may be required after implementation of growth hormone therapy, as there is potential for increasing mineral demands. Considering the expense and the unclear risk\benefit ratio, at present GH therapy is generally not recommended as a routine approach in children with XLH. If this measure is applied, it may be important to carefully monitor skeletal age in the patient.

As excess FGF23 activity is a central mediator of XLH, strategies to inhibit the effect of FGF23 have been attempted. Importantly administering a neutralizing antibody to FGF23 to a mouse model of XLH has resulted in the correction of hypophosphatemia and skeletal improvements in osteomalacia and bone growth [98]. More recently a monthly dosing regimen of anti-FGF23 monoclonal antibody has demonstrated correction of biochemical parameters including TmP/GFR and serum phosphorus and $1,25(OH)_2D$ in adults with XLH [99, 100]. Preliminary findings from trials in affected children indicate a similar biochemical response and radiographic improvement in rickets after switching from conventional therapy [101].

23.5.2.3 Monitoring and Complications of XLH

The goal of therapy is not specifically to normalize serum phosphate, as this is difficult to do consistently and safely with current therapy. However, the primary goals of treatment are to improve long-term skeletal growth and minimize skeletal deformities. Children with XLH should be seen every 3–4 months with concomitant monitoring of serum calcium, phosphate, and alkaline phosphatase activity. Calcium and creatinine excretion should be determined in a fasting or, if not possible, a randomly voided urine sample. Circulating PTH should be measured at least twice a year. Monitoring of alkaline phosphatase activity allows for a biochemical indicator of bone healing, though as in other causes of rickets, alkaline phosphatase activity may transiently increase shortly after starting therapy. Nevertheless, this measure often does not entirely normalize even with optimal therapy in childhood.

Phosphate is primarily monitored to prevent overcorrection of serum phosphate level. Phosphate dose should *not* be increased solely due to a low serum phosphate, in part because the cause may be secondary hyperparathyroidism, which is likely to be exacerbated by increasing phosphate dosages. The development of secondary hyperparathyroidism is an indication to alter the calcitriol/phosphate balance by decreasing phosphate or increasing the calcitriol dosage. However, when clinical response is poor, in terms of growth rate or epiphyseal radiographic response, a careful increase in the phosphate dose with a concomitant increase in the calcitriol dose is indicated.

Accurate height measurements and assessment of the bowing defect should be performed at all visits. During growth, radiographs of the epiphyses of the distal femur and proximal tibia are obtained every 2 years or more frequently if bow deformities fail to correct or if progressive skeletal disease is grossly evident. Short stature is common, so monitoring of weight needs to include determination of BMI, since being within the normal range on the weight curve may still indicate overweight status.

An oral exam for dental abscesses should be performed at each visit. Good oral hygiene is generally recommended to decrease the risk of abscess formation, and a dental specialist is needed to monitor for and treat dental complications. However the efficacy of this measure or of medical treatment of XLH on the prevention of tooth abscesses is not clear. Observational data suggest that treatment with calcitriol and phosphate might decrease the risk of severe dental disease in XLH [102].

Complications of therapy for XLH include hyperparathyroidism, soft-tissue calcification, and hypervitaminosis D. In order to prevent these

complications, children should be appropriately monitored every 3–4 months. Treatment must provide adequate mineral to improve rachitic lesions, but must not be so excessive that soft-tissue calcification or derangement of parathyroid hormone function will occur. Hyperparathyroidism occurs frequently in XLH [91]; thus, PTH levels must be routinely monitored during therapy. The parathyroid glands in XLH have a propensity to hypersecrete PTH, and circulating PTH levels are often somewhat elevated even before the initiation of any therapy in the disease. After treatment with calcitriol, initial PTH elevations may decline. However, further stimulation of PTH secretion by phosphate intake may occur over time. Phosphate supplementation should therefore never be given as a single therapy in XLH but always in combination with calcitriol. Prolonged or severe hyperparathyroidism may further compromise renal phosphate retention, provoke hypercalcemia, or enhance calcification of soft tissues. It is possible that chronic exposure to high concentrations of PTH may adversely affect the skeleton.

Parathyroidectomy is warranted for a more severe hyperparathyroidism, especially with hypercalcemia. We have demonstrated that the vitamin D analog, paricalcitol, can effectively suppress circulating PTH levels when added to the medical regimen for 1 year in XLH patients with secondary hyperparathyroidism. This effect is accompanied by a modest rise in serum phosphorus and TmP/GFR [103]. Calcimimetics may also prove useful modifiers of this process, but data using this agent in long-term studies for XLH are not available. Short-term studies have indicated potential effects of calcimimetics to limit the PTH increases triggered by an oral phosphate dose [104]. Furthermore, anecdotally, calcimimetics have been used to ameliorate secondary and tertiary hyperparathyroidism in XLH patients [105, 106]. Calcimimetics may prove useful as adjunctive therapy in the future, though proper studies need to be performed.

Soft-tissue calcification of the renal medullary pyramids (nephrocalcinosis) is common. Small studies suggest that nephrocalcinosis does not develop in patients who have never been treated for XLH [107, 108]. Nephrocalcinosis seems to be related more to the oral phosphate dose than the calcitriol dose though both contribute to the overall mineral load for renal excretion. Nephrocalcinosis often develops within the first 3 or 4 years of therapy. While progressive or moderate to severe nephrocalcinosis can lead to chronic kidney disease, significant renal impairment is not usually seen with more mild degrees of nephrocalcinosis. In one long-term survey of several patients with long-standing low-grade nephrocalcinosis, a mild, urinary concentrating defect of limited clinical significance was the only identified abnormality [109]. However, more significant (including end-stage) renal disease can occur with more severe nephrocalcinosis, and this is one reason for careful biochemical monitoring of therapy and intermittent ultrasonographic imaging for progression of nephrocalcinosis. Because of the relatively high radiation exposure from computed tomographic methods, renal ultrasonography remains the preferred mode of screening, every 1–3 years while on therapy.

Additional soft-tissue calcifications may occur. Severe hyperparathyroidism in XLH has been reported with myocardial and aortic valve calcifications [110]. Finally, calcifications of entheses and ocular calcifications are described. XLH patients typically develop enthesopathy, which becomes clinically evident by young adulthood in most patients [67, 111]. The mechanism of enthesopathy is not understood currently. Our recent analysis of a large XLH cohort found that the extent of enthesopathy in patients with XLH was not related to exposure to conventional therapy, either in adulthood or over the entire life span [102]. Other factors associated with the extent of enthesopathy included male sex, BMI, and age. These studies suggest that conventional therapy neither prevents nor promotes enthesopathy, so that treatment of adults aimed at healing osteomalacia is probably safe in regard to this complication. Recent studies in the Hyp mouse have suggested that enthesopathy might be mediated through FGF23 excess, since sites of enthesopathy express the necessary FGF receptors and the cofactor klotho [67].

Hypervitaminosis D is manifested by hypercalciuria and/or hypercalcemia. This complication was frequently encountered with high-dose vitamin D therapy for XLH and when monitoring of serum and urine biochemistries was performed infrequently. The newer 1α-hydroxylated vitamin D metabolites are more polar and are not stored in fat, and due to shorter half-life, toxic biochemical effects improve more rapidly upon withdrawal

than with native vitamin D. Unrecognized vitamin D intoxication has resulted in death in XLH but has not been reported in many years. Monitoring urinary calcium excretion and serum calcium and creatinine are critical to safely managing this disorder. Hypercalcemia or excessive urinary calcium may be a limiting factor, necessitating a decreased calcitriol dose.

Even though FGF23 is the mediator of hypophosphatemia in XLH, its measurement is not part of the standard clinical assessments. Indeed several reports indicate that current therapy with calcitriol and phosphate may increase FGF23 concentrations [112–114], a finding recapitulated in the mouse model for XLH [115, 116]. The clinical relevance of this finding is uncertain, but these findings indicate that the current therapy for the disorder may worsen certain aspects of the biochemical phenotype and underscore the need for improved therapeutic approaches in this disease.

It has been previously established that Chiari I malformations are present in a significant percentage of patients with XLH (up to 44% in one study) [66, 117, 118] and that craniosynostosis may also occur [66]. Moreover, a recent preliminary communication has confirmed the relatively frequent occurrence of Chiari I malformation in a childhood cohort of XLH and furthermore describes the unexpected findings of syringomyelia in children as young as 5 years of age. These investigators suggested that neurologic/neurosurgical referral and/or magnetic resonance imaging of the brain and spinal column may be important in children with persistent and unexplained headache or related neurological symptoms.

23.5.2.4 Other FGF23-Mediated Phosphopenic Rickets

Of the FGF23-mediated causes of hypophosphatemic rickets, XLH is by far the most common. However, hypophosphatemic rickets from ADHR, ARHR, fibrous dysplasia, and TIO are typically treated using similar medical strategies to XLH. ADHR, TIO, and FD may all present with new-onset hypophosphatemic rickets/osteomalacia in later childhood or adulthood. Proper treatment of these requires distinguishing the diagnosis. ADHR can have a late presentation due to waxing and waning of FGF23 concentrations [32] and can be distinguished from other causes by detection of mutations in FGF23. FD lesions can be identified using plain radiographs or ^{99}technetium-DMP scintigraphy of the skeleton. FD may be the initial manifestation of McCune-Albright syndrome, and other related endocrine hyperfunction phenomena, such as hyperthyroidism and precocious puberty, should be considered. TIO may be cured if the causative tumor is removed. Consequently, because of implications for surgical treatment, patients with apparent sporadic hypophosphatemic rickets require serious consideration of this diagnosis. In this setting, mutational analysis might be helpful, prompting evaluation for a tumor locus in the absence of an identified mutation or features of heritable disease.

23.5.2.5 Non-FGF23-Mediated Phosphopenic Rickets

Hereditary hypophosphatemic rickets with hypercalciuria (HHRH) is generally managed by the administration of phosphate salts alone, since $1,25(OH)_2D$ is typically elevated. Treatment with phosphate decreases the elevated circulating $1,25(OH)_2D$ levels, with a concomitant reduction in intestinal calcium absorption, and the resultant hypercalciuria while providing phosphate to enhance skeletal mineralization. Treatment with phosphate alone in this condition also serves as a "calcium binder" to further decrease intestinal calcium absorption. Circulating PTH levels are usually appropriately low to normal in HHRH. The skeletal disease is complex, involving both osteomalacia and osteoporotic defects. Nephrocalcinosis and nephrolithiasis may occur. To further decrease the risk of renal complications, generous oral hydration (so that urine is more dilute) is recommended. A high-sodium diet may further enhance renal calcium excretion and should be avoided. Reported long-term observations of this disease are extremely limited; we are aware of at least two patients that have had continued or progressive nephrolithiasis in their adult years and associated with poor adherence to phosphate administration.

Finally, in phosphopenic rickets secondary to tubulopathies, Fanconi syndrome, or other systemic diseases, specific treatment of the underlying disease is important. Some of these conditions are complicated by wasting of both calcium and phosphate, as well as other electrolytes, and cautious repletion may still be necessary.

23.5.3 Psychosocial Considerations

Nutritional rickets is an easily treated disorder and if diagnosed in a reasonable time frame is completely reversible before long-standing skeletal damage results. It is important to point out to families that remodeling of the skeleton may require several years, while biochemical changes and acute remodeling of the growth plate occur within weeks to months of the onset of treatment. Many children are initially misdiagnosed with muscular dystrophy, cerebral palsy, or skeletal dysplasia, due to the common misconception that nutritional rickets no longer occurs in our society.

In contrast to the rapid and complete recovery with treatment of nutritional rickets, inherited forms usually require long-term treatment. 1α-hydroxylase deficiency usually completely responds to medical therapy, as do many cases of hereditary vitamin D resistance, although lifelong therapy is usually required in order to maintain a normal skeleton and normocalcemia. Since some cases of hereditary vitamin D resistance may be unresponsive to even high-dose $1,25(OH)_2D_3$ and may require intermittent or continuous intravenous calcium therapy, management should always involve a center with considerable experience in the treatment of metabolic bone disease.

XLH is a particularly frustrating disorder to manage over the long term, as current therapy does not correct the underlying defect, and skeletal outcomes are not completely corrected by treatment. Varying degrees of bony deformities from mild to severe may persist, including leg bowing, and surgical intervention is often required to straighten legs. Short stature often persists into adulthood. A number of debilitating long-term complications may occur, including arthritis, spinal stenosis, hearing difficulties, impaired fracture healing, and chronic bone pain. Such complications over years have resulted in considerable disability and limited capacity for employment in some individuals. Those in earlier generations where medical therapy has been limited, or in social situations where compliance with treatment has been poor, have generally been associated with worse outcomes. Psychosocial support is most important in these situations as to avoid depression and other problems such as substance abuse. However it should be noted that clinical disease severity varies quite widely in XLH, with some adults demonstrating only short stature, with minimal bowing of lower extremities. Continued research into therapeutic improvements holds some hope that patients with XLH will have better clinical outcomes as a group in the future.

Case Study

A 16-month-old girl presented with distal tibia and fibula fractures after falling down stairs. Radiographs showed classical rachitic features. Past medical history was notable for cesarean delivery, performed because of a large congenital omphalocele requiring surgical repair. Her course was complicated by dysphagia, gastrointestinal reflux, and intestinal dysmotility, requiring intensive care until she was 7 months of age. Initially a gastric tube and subsequently a gastrojejunal tube were used for feeding; medications included a proton pump inhibitor and erythromycin. She was fed breast milk until age 7 months, when she was transitioned to an elemental, amino acid-based infant formula. No other supplementation was used, and there was no family history of bone or mineral disorders. At age 10 months, her height and weight were concordant, both between 50th and 75th percentiles for age, but mild bowing of her legs had been noted.

Comment: The differential diagnosis would favor vitamin D deficiency as the most likely cause of rickets, particularly in view of the chronic gastrointestinal difficulties described. Nevertheless, other forms of rachitic disease should be considered, even in the absence of a family history of heritable bone disease.

A diagnostic evaluation was performed yielding the following results:

Serum biochemistry	Value	Reference range
Phosphorus	2.5 mg/dl	3.9–6.5 mg/dl
Calcium	10.2 mg/dl	8.5–10.6 mg/dl
Alkaline phosphatase	1139 unit/L	65–380 unit/L
Creatinine	0.3 mg/dl	0.3–0.6 mg/dl
25OH vitamin D	39 ng/ml	20–50 ng/ml
PTH	10 pg/ml	10–65 pg/ml
$1,25(OH)_2D$	356 pg/ml	24–86 pg/ml

Interpretation: The elevated alkaline phosphatase was consistent with rickets/osteomalacia, but the 25OHD and calcium were normal. This combination of findings can sometimes occur in a vitamin D-deficient patient if treatment with vitamin D has begun before blood sampling is undertaken, but this was not the case for this patient, and therefore typical nutritional rickets did not appear to be the cause of these abnormalities. The prominent abnormality in this evaluation, the low blood phosphate level, should prompt a thorough evaluation of renal phosphate handling. A follow-up test revealed a persistently low serum phosphorus and a urine phosphorus concentration below the limit of detection, consistent with appropriate renal phosphate conservation (TmP/GFR is not determinable when the urine phosphorus is not measurable). The $1,25(OH)_2D$ was markedly elevated, an appropriate response to hypophosphatemia. These findings exclude the excess renal phosphate losses often seen in several forms of inherited hypophosphatemia but rather point to impaired intake or gastrointestinal absorption of phosphorus. Her dietary intake was reviewed and she was not taking any phosphate-binding medications. Although not a common cause of hypophosphatemia, we have recently encountered apparent impaired gastrointestinal absorption of phosphate in children with complex gastrointestinal issues, who were receiving amino acid-based elemental infant formula.

Phosphorus supplementation is an appropriate therapy in this setting; however, the necessity for vitamin D or calcium supplementation may arise acutely. Patients should be closely monitored for both hypocalcemia and hyperphosphatemia during initiation of phosphorus supplementation. This patient began phosphorus supplementation and was noted to be hyperphosphatemic within 2 days (serum phosphorus, 10.3 mg/dl). As is often the case with an abrupt rise in serum phosphorus, hypocalcemia was precipitated (serum calcium, 7 mg/dl). The dose of phosphate was therefore decreased and calcium supplementation begun with eventual correction of these values. Within 3 months of beginning phosphate supplementation, follow-up radiographs revealed complete healing of the growth plate abnormalities. Subsequently she underwent transition to an alternative amino acid-based formula and was able to discontinue phosphate supplementation entirely. Biochemical parameters remained normal 3 months afterward.

23.6 Summary

Rickets, due to inherited, nutritional, and other acquired causes, remains a significant problem across the globe. Both general pediatricians and pediatric endocrinologists need to be able to recognize and diagnose the major and most common causes of rickets. While management of nutritional rickets is relatively straightforward, management of rickets due to abnormalities in vitamin D or phosphate metabolism is sufficiently complex as to benefit from the assistance of a metabolic bone specialist.

Review Questions

1. What is the most common etiology of rickets?
   A. X-linked hypophosphatemia
   B. Impaired metabolism of vitamin D
   C. Vitamin D deficiency
   D. Fanconi syndrome

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2. Other than hypophosphatemia, what biochemical findings at diagnosis suggest FGF23-mediated hypophosphatemic rickets?
   A. High $1,25(OH)_2D$, high calcium, low or normal PTH, high alkaline phosphatase
   B. Low $1,25(OH)_2D$, normal calcium, normal or high PTH, high alkaline phosphatase
   C. High $1,25(OH)_2D$, high calcium, low PTH, low alkaline phosphatase
   D. Low $1,25(OH)_2D$, low calcium, high PTH, high alkaline phosphatase
3. Nephrocalcinosis is a potential complication of:
   A. Overtreatment with vitamin D for nutritional rickets
   B. NaPi2c mutations causing HHRH
   C. Treatment of XLH with calcitriol and phosphate
   D. All of the above

Answers

1. C
2. B
3. D

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Osteoporosis: Diagnosis and Management

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Key Points

- Osteoporosis in childhood can be associated with serious morbidity including premature loss of ambulation in those with mobility disorders, chronic back pain, and deformity from vertebral fractures and long bone deformity limiting functional mobility; mortality from fat embolism syndrome has also been described following a low-trauma femur fracture.
- Advanced osteoporosis presentations are no longer acceptable in clinical practice; instead, bone health monitoring should be carried out in those with clinically significant risk factors in order to identify the earliest signs of bone fragility (which are often vertebral fractures).
- Osteoporosis treatment is divided into four phases: stabilization, maintenance, treatment discontinuation and posttreatment monitoring. Intravenous bisphosphonate therapy remains the mainstay of osteoporosis therapy, with newer anti-resorption and anabolic therapies on the horizon for children with primary and secondary osteoporotic conditions.
- Osteoporosis therapy should be undertaken by clinicians with sufficient experience in the safe and effective administration of bone-targeted drugs.

24.1 Introduction

Once considered a disease of the aging, osteoporosis is now recognized as an important facet of clinical care in children with genetic disorders predisposing to bone fragility and in children with serious acute and chronic illnesses. At the same time, approaches to the management of osteoporosis during the pediatric years are complicated by a number of factors, including the impact of variable growth rates and tempos of puberty on size-dependent bone mineral density (BMD) testing, distinguishing pathological fractures from those sustained during the course of normal childhood development, and the fact that informative, well-designed intervention trials are themselves a hurdle due to limitations such as small sample sizes in pediatric compared to adult trials.

While many principles from the adult osteoporosis literature can be adapted to children, the development of the mature skeleton is nevertheless a complex, multi-decade process that gives rise to unique considerations. Some of these differences have been unearthed through long-term natural history studies using standard, widely available evaluative tools, while others have been demonstrated through more recent developments such as peripheral quantitative computed tomography (pQCT) and trans-iliac bone histomorphometry. Knowledge of pediatric-specific principles and their biological basis is essential for forming logical management decisions in the young.

The purpose of this chapter is to review evidence that shapes the current approach to diagnosis, monitoring, and management of osteoporosis in childhood, with particular emphasis on the key biological principles that are pivotal to the overall approach and on the main questions with which clinicians struggle on a daily basis. The scope of this article spans the review of specific disorders and risk factors associated with osteoporosis in childhood, the clinical manifestations of osteoporosis, issues with respect to the definition and the diagnosis, and recommendations for monitoring and prevention of osteoporosis in at-risk children. Finally, this article discusses when a child is a candidate for osteoporosis drug therapy, which agents and doses should be prescribed, the length of therapy, how the response to therapy should be evaluated, and side effects. With this information, the bone health clinician should be poised to identify which children should be targeted for osteoporosis therapy and the clinical outcomes that reflect safety and efficacy.

24.2 The Etiology and Mechanisms of Childhood Osteoporosis

As highlighted in recent reviews [1–5], childhood osteoporosis is typically divided into primary and secondary etiologies, with osteogenesis imperfecta (OI) representing the prototypical primary osteoporosis of childhood. There is a growing list of secondary osteoporotic conditions of childhood (i.e., osteoporosis caused by underlying diseases and/or their treatment), with most falling into two broad categories: glucocorticoid (GC)-treated diseases and disorders with compromised mobility. A list of the most frequent causes of primary bone fragility disorders (and their related genes, proteins, and phenotypic features) is provided in Table 24.1. A list of the secondary osteoporotic conditions of childhood is provided in Table 24.2.

24.2.1 Primary Osteoporosis

Among the most exciting recent developments in the pediatric bone health field has been discovery of genes implicated in heritable bone fragility disorders. While the phenotypic heterogeneity in congenital bone fragility has been known for years [6], the spectrum of the genetic basis has only recently come to the fore. Most cases of congenital bone fragility are still due to mutations in the coding regions of the type I collagen genes (*COL1A1* and *COL1A2*, classically referred to as OI types I, II, III, and IV based on disease severity); however, over a dozen additional genetic causes have been described with novel pathobiology and often discrete clinical features [7, 8] (Table 24.1). In many cases, heritable bone fragility is suggested by the family history or typical physical findings (blue sclerae, dentinogenesis imperfecta). However, these stigmata are not universal even in the presence of type I collagen mutations [9]. In practical terms, the diagnosis of OI remains a possibility in any child with recurrent fractures once a secondary cause has been ruled out (Fig. 24.1).

Table 24.1 Genetic causes and clinical features of bone fragility in childhood

Inheritance and pathogenesis	Diagnosis Gene Protein	Clinical features
A. Causes of bone fragility due to a type I collagenopathy		
Autosomal dominant		
1. Nonsense or frameshift mutations causing premature termination of the COL1A1 coding sequence (also called haploinsufficiency; typically associated with a mild phenotype) 2. Glycine missense mutations in COL1A1 or COL1A2 causing type I collagen structural defects (mild to severe phenotypes)	*Diagnosis*: OI *Genes*: *COL1A, COL1A2* *Protein*: alpha 1 and 2 chains of type I collagen	Variable severity (mild to perinatal lethal) and variable clinical features. The following may be present: gray or blue sclerae, dentinogenesis imperfecta, scoliosis, triangular facies, limb deformity, wormian bones
Autosomal recessive		
1. Mutations in chaperone complexes involved in the initiation of type I collagen chain recognition and helical folding	*Diagnosis*: OI *Gene*: *CRTAP* *Protein*: Cartilage-associated protein	Moderate, severe or perinatal lethal, rhizomelia, normal sclerae, coxa vara, early lower limb deformity
	Diagnosis: OI *Gene*: *LEPRE1* *Protein*: Prolyl-3-hydroxlase 1 (P3H1)	Perinatal lethal or severe, white sclerae, bulbous metaphyses, severe growth restriction
	Diagnosis: OI *Gene*: *PPIB* *Protein*: Cyclophilin B (CyPB)	Moderate, severe or perinatal lethal, growth failure, normal sclerae and teeth
2. Mutations in genes which encode proteins involved in the late stage of type I procollagen quality control, directing final folding and transit from the endoplasmic reticulum to the Golgi	*Diagnosis*: OI *Gene*: *SERPINH1* *Protein*: Heat-shock protein 47 (HSP47)	Severe, triangular facies, blue sclerae, early leg deformity, dentinogenesis imperfecta
	Diagnosis: OI *Gene*: *FKBP10* *Protein*: FK506 binding protein (FKBP65)	Moderate to severe, vertebral fractures, variable dentinogenesis imperfecta, and joint contractures

Table 24.1 (continued)

Inheritance and pathogenesis	Diagnosis Gene Protein	Clinical features
3. Mutations which interfere with late stage type I collagen modification and cross-link formation	*Diagnosis*: OI *Gene*: *SPARC (osteonectin)* *Protein*: Secreted protein, acidic and rich in cysteine (SPARC)	Moderate to severe, vertebral fractures, kyphoscoliosis, white sclerae, no dentinogenesis imperfecta, hypotonia, joint hyperlaxity
	Diagnosis: Bruck syndrome *Gene*: *PLOD2* *Protein*: Lysyl hydroxylase 2 (LH2)	Moderate to severe, vertebral fractures, contractures, normal teeth
4. Mutations which inhibit type I collagen c-propeptide cleavage	*Diagnosis*: OI *Gene*: *BMP1* *Protein*: Bone morphogenetic protein 1 (BMP1)	Moderate to severe, vertebral, fractures, normal teeth, variable sclerae, hypotonia
B. Causes of bone fragility due to mutations in genes unlinked to type I collagen		
Mutations in genes involved in bone cell formation, differentiation, and mineralization		
1. *Autosomal dominant*	*Diagnosis*: OI *Gene*: *IFITM5* *Protein*: Bone-restricted Ifitm-like (BRIL)	Moderate to severe, hypertrophic callus, calcification of the interosseous membrane of the forearm and leg, white sclerae, lack of wormian bones
2. *Autosomal recessive*	*Diagnosis*: OI *Gene*: *SP7 (Osterix)* *Protein*: Transcription factor Sp7 (SP7/Osterix)	Moderate to severe, delayed dental eruption, no dentinogenesis imperfecta, normal hearing, and sclerae
	Diagnosis: OI *Gene*: *SERPINF1* *Protein*: Pigment-epithelium derived factor (PEDF)	Moderate to severe, normal sclerae and teeth, limb deformity, osteomalacia with looser's zones, alkaline phosphatase may be elevated
	Diagnosis: OI *Gene*:*TMEM38B* *Protein*: Transmembrane protein 38B (TMEM38B)	Moderate to severe, normal teeth, sclerae, and hearing
	Diagnosis: OI *Gene*: *WNT1* (heterozygotes have a mild phenotype) *Protein*: WNT1	Moderate to severe, vertebral fractures, short stature, blue sclerae in some patients, normal teeth and hearing
	Diagnosis: OI *Gene:CREB3L1* (heterozygotes have a mild phenotype) *Protein*: Old astrocyte specifically induced substance (OASIS)	Perinatal lethal, tubular bones with accordion-like broadened appearance, beaded ribs, blue sclerae

(continued)

Table 24.1 (continued)

Inheritance and pathogenesis	Diagnosis Gene Protein	Clinical features
C. Causes of bone fragility associated with specific, named diseases		
1. *Autosomal dominant*	*Diagnosis*: Cole-carpenter syndrome *Gene*: *P4HB* *Protein*: Protein disulfide isomerase (PDI)	Craniosynostosis, ocular proptosis, hydrocephalus, distinctive facial features, blue sclerae, popcorn epiphyses of the lower extremities
	Diagnosis: Ehlers-Danlos syndrome *Gene*: *COL3A1* *Protein*: Type III procollagen	Fragility of connective tissues, scoliosis, loose joints and skin, easy bruising, "cigarette-paper" scars, fragile blood vessels and body tissues with arterial and gastrointestinal rupture
	Diagnosis: Marfan syndrome *Gene*: *FBN1* *Protein*: Fibrillin-1	Tall stature, long limbs and digits, joint laxity, scoliosis, ocular and cardiovascular abnormalities
2. *Autosomal recessive*	*Diagnosis*: Homocystinuria *Gene*: *CBS* *Protein*: Cystathionine beta-synthase (CBS)	Marfan-like features, myopia, ectopia lentis, thromboembolic events
	Diagnosis: Osteoporosis-pseudoglioma syndrome *Gene*: *LRP5* (heterozygotes have a mild bone fragility phenotype with normal vision) *Protein*: LDL receptor related protein 5 (LRP5)	Vertebral fractures, scoliosis, short stature and limb deformities, blindness due to ocular pseudoglioma
	Diagnosis: Spondylo-ocular syndrome *Gene*: *XYLT2* *Protein*: Xylosyltransferase 2 (XylT2)	Vertebral fractures (marked platyspondyly with fish bone appearance), enlarged intervertebral spaces, normal height with disproportionate short trunk, thoracic kyphosis, and reduced lumbar lordosis, loss of vision due to retinal detachment, sensorineural hearing loss, and cardiac septal defects

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24.2.2 Secondary Osteoporosis

Advances in pediatric care have led to significant improvements in cure rates for acute disorders such as childhood leukemia [10] and in longevity for chronic disabling conditions such as Duchenne muscular dystrophy (DMD) [11]. With improved outlooks for such children (Table 24.2), there is increasing focus on long-term sequelae and quality of life. Despite advances in chemotherapy and disease-modifying interventions, GC therapy remains the mainstay of treatment for many serious illnesses in the first few years of the illness for disorders, such as leukemia and rheumatic conditions [12, 13], and for decades in boys with DMD [14]. Recently, the use of GC-sparing biological agents has led to improved health outcomes for children with

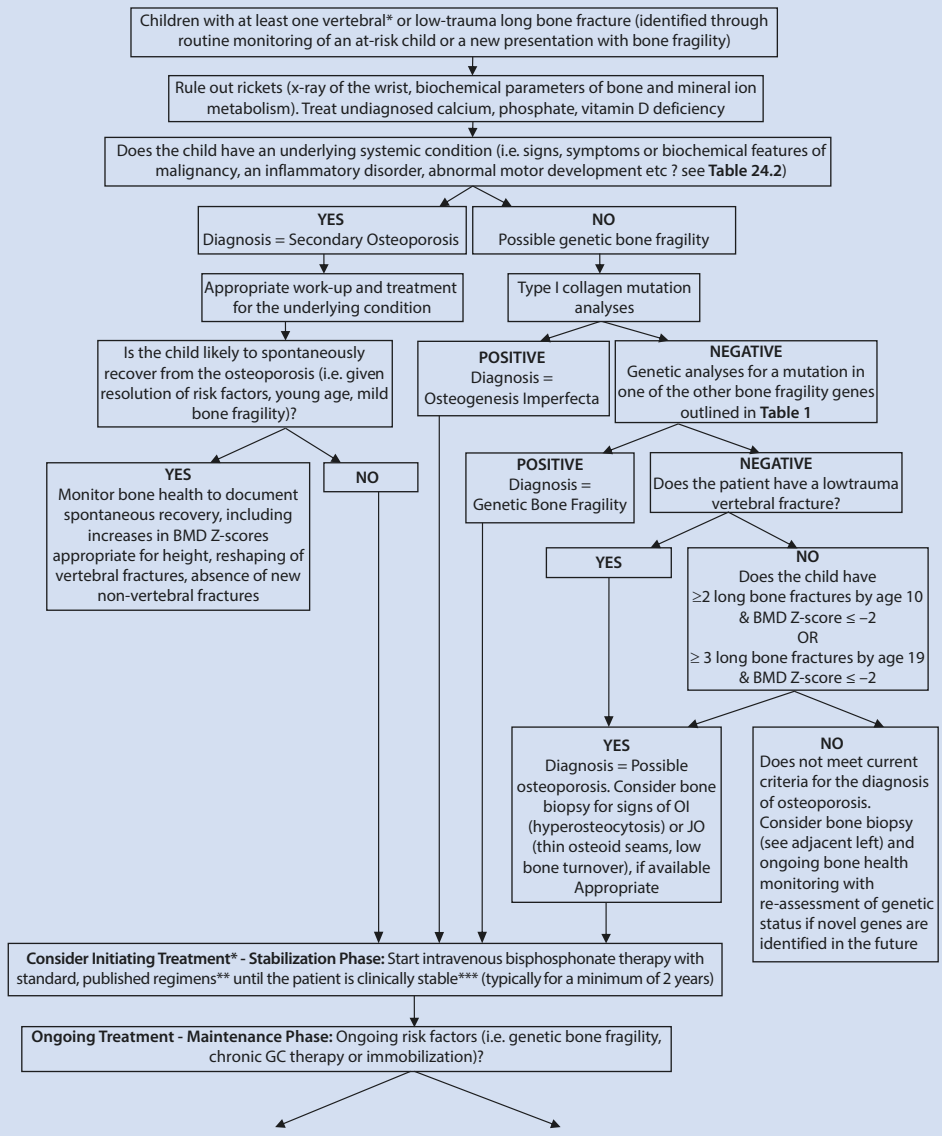
Table 24.2 Disorders linked to secondary osteoporosis in childhood

Chronic illness	**Iatrogenic disorders**
(a) Malignancy (leukemia, lymphoma)	(a) Glucocorticoids
(b) Rheumatologic disorders	(b) Methotrexate
(c) Anorexia nervosa	(c) Cyclosporine
(d) Cystic fibrosis	(d) Heparin
(e) Inflammatory bowel disease	(e) Radiotherapy
(f) Renal disease	(f) GnRH agonist
(g) Transplantation	(g) Medroxyprogesterone acetate (long-term use)[a]
(h) Other: Primary biliary cirrhosis, cyanotic congenital heart disease, thalassemia, malabsorption syndromes, celiac disease, epidermolysis bullosa	(h) L-Thyroxine suppressive therapy
Neuromuscular disorders	(i) Anti-convulsants
(a) Cerebral palsy	**Inborn errors of metabolism**
(b) Rett syndrome	(a) Lysinuric protein intolerance
(c) Duchenne muscular dystrophy	(b) Glycogen storage disease
(d) Spina bifida	(c) Galactosemia
(e) Spinal muscular atrophy	(d) Gaucher disease
Endocrine and reproductive disorders	(e) Homocystinuria
(a) Disorders of puberty	
(b) Turner syndrome	
(c) Growth hormone deficiency	
(d) Hyperthyroidism	
(e) Hyperprolactinaemia	
(f) Athletic amenorrhea	
(g) Cushing syndrome	
(h) Type 1 diabetes	

Ward et al. [5]. Reprinted with permission from Springer
[a]Long-term use (>10 years) has been associated with reductions in BMD among adult women; see text

Crohn's disease [15] and juvenile arthritis [16, 17]; not surprisingly, evidence for a positive effect of these agents on skeletal health has been demonstrated in a number of contemporary studies [16, 18–20].

A recent census of our bone health clinic (carried out in a general, tertiary pediatric hospital) showed that out of 89 patients with chronic illnesses and a history of low-trauma fractures treated with osteoporosis therapy, 40% had GC-naïve neuromuscular disorders (cerebral palsy, congenital myopathy), 27% had GC-treated DMD, 24% had other GC-treated disorders (Crohn's disease, rheumatic disorders myasthenia gravis), and 9% had leukemia or other cancers. This census provides insight into the systemic illness groups likely to present to a pediatric bone health clinic with low-trauma fractures requiring osteoporosis intervention.



* **Typical treatment indications:** Low-trauma long bone or vertebral fractures. Additional treatment considerations include the impact of the fractures on quality of life and lack of potential for spontaneous (i.e. medication-unassisted) recovery due to persistent osteoporosis risk factors

** **IV bisphosphonate starting doses (see text and Table 24.4 for details):** Pamidronate *maximum* 9 mg/kg/year in divided doses, Zoledronic acid *maximum* 0.1 mg/kg/year in divided doses, or Neridronate *maximum* 6 mg/kg/year in divided doses. See text for use of and titration to lower doses

*** **Clinically stable includes:**

- Absence of new VF in previously normal vertebral bodies and absence of further loss of vertebral height at sites of previous fractures
- Reshaping of vertebral fractures
- Absence of new non-vertebral fractures, bone and back pain
- Improved mobility, increases in spine BMD Z-score appropriate for height

Abbreviations: *BMD* bone mineral density, *GC* glucocorticoid, *OI* osteogenesis imperfecta, *JO* juvenile osteoporosis, *VF* vertebral fractures, *non-VF* non-vertebral fractures

Fig. 24.1 Algorithm of the approach to the diagnosis and treatment of children with fractures due to osteoporosis (From: Ward et al. [5]. Reprinted with permission from Springer)

24.3 Clinical Presentations and Predictors of Osteoporotic Fractures

24.3.1 Vertebral Fractures

A number of studies have highlighted that vertebral fractures (VF) are an important yet underappreciated manifestation of osteoporosis in children. This is particularly true in children with GC-treated disorders given the predilection of GC therapy to adversely impact the trabecular-rich spine [21, 22]. In GC-treated illnesses such as rheumatic disorders, nephrotic syndrome, leukemia, and DMD, the prevalence of VF ranges from 7% to 32% [22–25] and the 12-month incidence from 6% to 16% [26–28] depending upon the underlying disease. The peak annual incidence in children with GC-treated rheumatic disorders and leukemia occurs at 1 year, at the time during which annual GC exposure is highest for most patients [12, 13]. At the same time, children with chronic diseases who are GC naïve are not exempt from spine fragility, since vertebral collapse has been shown to occur in 25% of children with motor disabilities in the absence of GC therapy [19].

VF often go undetected in children for two main reasons. First, VF can be asymptomatic [23–28], despite moderate to severe collapse [12, 23]. Secondly, routine surveillance with intermittent spine x-rays has not historically been signaled an important component of osteoporosis monitoring. However, a recent position statement by the International Society for Clinical Densitometry (ISCD) proposed that monitoring beyond BMD is needed in at-risk children, since the diagnosis of osteoporosis in children with at least one VF no longer requires BMD criteria [30]; furthermore, the position statement acknowledges that BMD Z-scores above −2 standard deviations (SD) do not preclude increased vertebral and non-VF risks.

24.3.2 Non-vertebral Fractures

Low-trauma non-VF in childhood are observed most frequently at the femur, tibia, forearm, humerus, feet, and ankles [22, 31, 32]. Long bone fractures are the most frequent and disabling of the non-VF in childhood, while hip fractures occur rarely and should prompt consideration of serious underlying diseases such as childhood leukemia [33]. Looser zones, also known as "insufficiency fractures," may be mistaken for osteoporotic fractures; however, they represent the distinctly different process of osteomalacia, defined histomorphometrically as an increase in osteoid thickness associated with prolongation of the mineralization lag time. Looser zones appear as incomplete cracks in the cortices of the ribs, scapulae, medial shafts of long bones, and pubic rami. In such cases, the patient requires an assessment for a disorder of calcium and/or phosphate metabolism including a hand x-ray (to rule out rickets if the growth plate is still active) and biochemical parameters of bone and mineral ion metabolism (◘ Fig. 24.1).

24.3.3 The Frequency and Clinical Predictors of Fractures in At-Risk Children

In recent years, there has been an effort to delineate disease-specific risk factors for osteoporosis through natural history studies, by assessing the precise relationship between various illness-related factors and fractures, as well as the relationship between measurable indicators of bone health and fractures (such as BMD and back pain; see ◘ Table 24.3). These studies have provided robust results that fine-tune the clinician's ability to identify the at-risk child.

24.3.3.1 Vertebral Fractures

As shown in ◘ Table 24.3, a number of studies have been sufficiently powered to assess clinical predictors of prevalent or incident (new) VF in univariate or multivariable models. Studies which show significant differences in relevant clinical parameters between those with and without VF have also been included in ◘ Table 24.3. Most studies have been retrospective or cross-sectional; relatively few studies have assessed the frequency of incident VF in relation to the evolving clinical trajectory of the child.

From these studies, a number of clinically useful themes have emerged. First, GC exposure is a consistent predictor of both prevalent and incident VF, an observation that is not surprising given clinical experience and the known osteotoxicity of GC therapy. Both cumulative and average daily dose predict VF in a number of different diseases as outlined in ◘ Table 24.3, as well as GC dose intensity ("pulse therapy") in children with leukemia

Table 24.3 Risk factors for fractures in children with specific disorders

Author	Disease	# of patients	Study design	Fracture location (with assessment method for vertebral Fractures)[a]	Fracture prevalence and/or incidence (%)	Clinical predictors of prevalent or incident VF from univariate or multivariable models (with effect size and 95% CI) or from statistical tests comparing children with and without fractures
Predictors of prevalent fractures (vertebral and non-vertebral fractures combined)						
Henderson (2010)	Cerebral palsy or muscular dystrophy	619	Cross-sectional	NR	Prevalence 27% (at an average age of 11.8 yrs)	↓Distal femur BMD (proximal to growth plate) Z-score: RR = 1.09 (1.04, 1.13)[b] ↓Distal femur BMD (transition from metaphysis to diaphysis) Z-score: RR = 1.06 (1.02, 1.10)[b] ↓Distal femur BMD (diaphyseal cortical bone) Z-score: RR = 1.15 (1.09, 1.22)[b]
Fung (2008)	Thalassemia or treated sickle cell disease	136	Prospective, natural history comparative study	Upper and lower extremities and other sites	Prevalence 17% (in patients 12–18 yrs. of age)	Thalassemia vs sickle cell disease: OR = 2.3 (1.2, 4.6)[b, c] Male: OR = 2.6 (1.5, 4.5)[b, c] ↑Age: OR = 1.03 1.03,1.08)[b, c]
Predictors of prevalent fractures (non-vertebral fracture only)						
Dosa (2007)	Spina bifida	221	Historical cross-sectional	Upper and lower extremity and other site	Prevalence 20%[c] (in patients 2–58 yrs. of age) Annual incidence 2.6% (in patients 2–18 yrs. of age)	Predictors of prevalent and incident fractures combined: ↑Spine defect level: RR = 1.65 (1.09, 2.50)[b, c] ↑Age: RR = 1.03 (1.00,1.07)[b, c]
Predictors of prevalent fractures (vertebral fracture only)						
Nakhla (2009)	Rheumatic diseases[d]	90	Cross-sectional	Vertebrae Lateral spine x-ray[a] [55]	Prevalence 19% (at a median age of 13.1 yrs)	Male: OR = 6.04 (2.85, 12.81)[b] ↑Cumulative GC (g/kg): OR = 4.50 (1.42, 14.28)[b] ↑BMI Z-score: OR = 1.49 (1.05, 2.09)[b]
Valta (2009)	Renal transplant[d]	106	Cross-sectional	Vertebrae Dual-energy x-ray absorptiometry and lateral spine x-ray[a] [176]	Prevalence 8% (5.1 yrs. after transplant)	↑Age at the time of study ↑time since transplant

Valta (2008)	Liver transplant[d]	40	Cross-sectional	Vertebrae Dual-energy x-ray absorptiometry and lateral spine x-ray[a] [176]	Prevalence 18% (7 yrs. after transplant)	↑Age at transplant Recently transplanted ↑BMI ↑Whole body fat percentage ↓Cumulative weight-adjusted GC dose ↓LSBMD Z-score ↓Proximal femur BMD Z-score ↓TB BMD Z-score
Helenius (2006)	Solid organ transplant[d]	40	Cross-sectional	Vertebrae Dual-energy x-ray absorptiometry and lateral spine x-ray[a] [176]	Prevalence 35% (11.2 yrs. after transplant)	Male Treated for acute rejection
Halton (2009)	Leukemia[d]	186	Cross-sectional	Vertebrae Lateral spine x-ray[a] [55]	Prevalence 16% (at a median of 18 days from GC initiation)	Back pain: OR = 4.7 (1.5, 14.5)[b] ↓% cortical area Z-score: OR = 2.0 (1.0, 3.2)[b] ↓LS BMD Z-score: OR = 1.8 (1.1, 2.9)[b]
King (2007)	Duchenne muscular dystrophy[d]	143	Retrospective chart review	Vertebrae Spine x-ray Method not specified	Prevalence 32% (mean duration of GC therapy 8 years)	GC treatment ≥1 year
Feber (2012)	Nephrotic syndrome[d]	80	Cross-sectional	Vertebrae Lateral spine x-ray[a] [55]	Prevalence 8% (within the first month following GC initiation)	Vitamin D daily intake <50% DRI
Ben amor (2013)	Osteogenesis imperfecta	58	Cross-sectional	Vertebrae Lateral spine x-ray[a] [55]	Prevalence 71% (at an average age of 7.4 yrs)	↓LSBMD Z-score: OR = 0.4 (0.2, 0.9)[b] Male: OR = 6.6 (1.5, 28.3)[b]
Engkakul (2013)	Thalassemia syndromes	150	Retrospective chart review and cross-sectional	Vertebrae Lateral spine x-ray[a] [55]	Prevalence 13%[c] (at a median age of 15.7 yrs)	Severe thalassemia: OR = 5.7 (2.0, 16.8)[b] Age (20 yrs. or older): OR = 5.0 (1.7, 14.0)[b]
Mayran-paa (2012)	Recurrent fractures	66	Prospective, observational	Vertebrae Lateral spine x-ray[a] [176]	Prevalence 29% (at an average age of 10.7 yrs)	↓Serum 250HD Fewer long bone fractures per child ↓LSBMD Z-score

(continued)

Table 24.3 (continued)

Author	Disease	# of patients	Study design	Fracture location (with assessment method for vertebral Fractures)[a]	Fracture prevalence and/or incidence (%)	Clinical predictors of prevalent or incident VF from univariate or multivariable models (with effect size and 95% CI) or from statistical tests comparing children with and without fractures
Predictors of incident fractures (vertebral and non-vertebral fractures combined)						
Helenius (2006)	Solid organ transplant[d]	196	Retrospective chart review plus 10 years prospective observation after transplant	Upper and lower extremity, vertebrae, and other site Dual-energy x-ray absorptiometry and lateral spine x-ray[a] [176]	Incidence 9.2 per 100 person-years (on average 9.2 yrs. from transplant) Cumulative incidence 40%	Sex (male vs. female): HR = 2.15 (1.22, 3.81)[b] Age at the time of transplant (5–12 vs 0–4 yrs.: HR = 1.80 (1.01, 3.20)[b] Age at the time of transplant (13–20 vs 0–4 yrs.: HR = 2.02 (1.07, 3.83)[b] Transplant organ (liver vs. kidney): HR = 1.78 (1.01, 3.14)[b] Transplant organ (heart vs. kidney): HR = 1.90 (0.93, 3.92)[b]
Predictors of incident fractures (non-vertebral fracture only)						
Helenius (2006)	Solid organ transplant[d]	196	Retrospective chart review plus 10 years prospective observation after transplant	Upper and lower extremity, and other site Dual-energy x-ray absorptiometry and lateral spine x-ray[a] [176]	Incidence 3.8 per 100 person-years (on average 9.2 yrs. from transplant) Cumulative incidence 27%	Fracture before transplant (yes vs. no): RR = 4.61 (1.12, 18.90) Sex (male vs. female): RR = 2.14 (1.17, 3.93) Age at the time of transplant (5–12 vs 0–4 yrs.: RR = 1.95 (0.98, 3.89) Age at the time of transplant (13–20 vs 0–4 yrs.: RR = 2.24 (1.01, 5.00)
Predictors of incident fractures (vertebral fracture only)						
Cummings (2015)	Leukemia[d]	186	48 months prospective, observational	Vertebrae Lateral spine x-ray[a] [55]	Incidence 8.7 per 100 person-years (in the first 4 years after diagnosis) Cumulative incidence 26.4%	Prevalent VF (mild vs none): HR = 4.2 (1.9, 9.6)[b] Prevalent VF (moderate /severe vs none): HR = 6.2 (3.4, 11.4)[b] ↑ Average daily GC (10 mg/m2): HR = 5.9 (3.0, 11.8)[b] ↓ LSBMD Z-score at the time of VF assessment: HR = 1.6 (1.2, 2.2)[b] ↓ Age: HR = 1.1 (1.0, 2.2)[b] ↑ Recent[e] average daily GC (10 mg/m2): HR = 5.1 (2.8, 9.5)[b] ↑ Recent[e] GC dose intensity (10 mg/m^2): HR = 1.2 (1.1, 1.4)[b]

Alos (2012)	Leukemia[d]	155	12 months prospective, observational	Vertebrae Lateral spine x-ray[a] [55]	Incidence 16% (at 12 months following diagnosis)	Prevalent VF (yes vs. no): OR = 7.30 (2.30, 23.14)[b] Prevalent VF (mild vs none): OR = 7.6 (1.8, 31.8)[b] Prevalent VF (moderate/severe vs none): OR = 7.0 (1.6, 30.2)[b] ↓LS BMD Z-score: OR = 1.8 (1.2, 2.7)[b]
LeBlanc (2015)	Rheumatic Diseases[d]	134	36 months prospective, observational	Vertebrae Lateral spine x-ray[a] [55]	Incidence 4.4 per 100 person-years (in the 3 years following GC initiation) Cumulative Incidence12.4%	↑ Average daily GC dose (0.5 mg/kg): HR = 2.0 (1.1, 3.5)[b] ↑VAS score, baseline to 12 months: HR = 1.4 (1.1, 1.7)[b] ↑BMI Z-score in the first 6 months preceding each annual VF assessment: HR = 3.2 (1.6, 6.5)[b] ↓LSBMD Z-score, baseline to 6 months: HR = 3.0 (1.1, 1.8)[b] ↑Duration (month) of GC therapy in the preceding 12 months of each VF assessment: HR = 1.2 (1.1, 1.4)[b]
Rodd (2012)	Rheumatic[d] diseases	117	12 months prospective, observational	Vertebrae Lateral spine x-ray[a] [55]	Incidence 5% (at 12 months following GC initiation)	↑BMI Z-score, study entry to 6 months ↑Weight Z-score, study entry to 6 months ↓LSBMD Z-score, study entry to 6 months LSBMD Z-score < −2.0 at 12 months ↑Cumulative GC ↑Average daily GC
Helenius (2006)	Solid organ transplant[d]	196	Retrospective chart review plus 10 yrs. prospective observation after transplant	Vertebrae Dual-energy x-ray absorptiometry and lateral spine x-ray[a] [176]	Incidence 5.7 per 100 person-years (on average 9.2 yrs. after transplant) Cumulative incidence 18%	BMI ≥19 kg/m^2 at transplant: RR = 4.30 (1.26, 9.97) Age at the time of transplant (5–12 vs 0–4 years): RR = 2.32 (1.07, 5.05) Age at the time of transplant (13–20 vs 0–4 years): RR = 4.16 (1.60, 10.81)
Vautour (2004)	Renal transplant[d]	86	Retrospective chart review plus 15 yrs. prospective follow-up	Vertebrae Lateral spine x-ray[a] [57]	Cumulative incidence 20% [c] (in the 15 yrs. following transplant)	↑Age: HR = 1.8 (1.2, 2.7)[b] Prior diagnosis of osteoporosis: HR = 9.5 (2.6, 35)[b]

Studies were included in the table if prevalence or incidence data were available and risk factors for prevalent or incident fractures were described. Studies which show significant differences in relevant clinical parameters between those with and without fractures were also included

Abbreviations: *BMI* body mass index, *CI* confidence interval, *DRI* dietary reference intake, *GC* glucocorticoid(s), *HR* hazard ratio, *LSBMD* lumbar spine bone mineral density, *OR* odds ratio, *yrs*. years, *TB BMD* total body BMD, *NR* not reported

[a]Assessment methods for vertebral fractures reported in these studies (see references Genant Semi-Quantitative Method; Method reported by Makitie; Method reported by Eastell – 1 study)

[b]Magnitude of association was obtained from multivariable models

[c]This result was based on a cohort with both children and adults

[d]Steroid-treated disease

[e]Recent: 12 months preceding VF assessment

[12]. Secondly, leukemia studies have shown that prevalent VF around the time of GC initiation are highly predictive of future fractures, a phenomenon known in adults as "the VF cascade" [12, 26]. In fact, even mild (grade 1) VF independently predict future fractures, highlighting the importance of identifying early signs of vertebral collapse [12, 26]. While back pain predicted prevalent VF in two studies of children with GC-treated leukemia and rheumatic disorders [23, 25], pain did not predict new VF [12, 13]. The message arising from these data is that a lack of back pain does not rule out the presence of VF in at-risk children.

The fact that prevalent VF around the time of GC initiation predict future VF draws attention to the clinical importance of understanding the skeletal phenotype early in the child's disease course. In children with GC-treated rheumatic disorders, other discrete clinical features in the first year were also independent predictors of future VF, including increases in disease activity scores in the first 12 months of GC therapy as well as increases in body mass index and decreases in lumbar spine (LS) BMD Z-scores, both in the first 6 months of GC therapy [13]. In children with solid organ transplantation, older age was another consistent predictor of increased VF risk [34–37].

24.3.3.2 Non-vertebral Fractures

Predictors of non-VF fractures in children with chronic illnesses are also outlined in Table 24.3, most of which are cross-sectional or retrospective. Loss of ambulation, anticonvulsant medication, and reductions in BMD at various skeletal sites are among the most consistent predictors of non-VF in this setting. An important observation making use of lateral distal femur BMD, a frequent site of fracture in children with neuromuscular disorders, is that every 1 SD reduction in BMD Z-score at this site was associated with a 15% increase in lower extremity fractures [38].

24.4 Outcomes and Complications: Morbidity, Mortality, and Recovery

The clinical consequences of osteoporosis arise from the fractures themselves or short- and long-term consequences of bone fragility. Fractures cause pain, and anyone who has sustained a fracture will attest to the clinically significant nature of such pain. Lower extremity fractures invariably compromise mobility in the short-term; however, premature loss of ambulation is seen in disease groups with tenuous ambulation to begin with such as cerebral palsy and DMD. Fractures can also cause deformity of the spine and extremities, both of which can lead to functional impairment. In such cases, surgical intervention may be needed to restore functional abilities. In adults, mortality has long been linked to hip and spine fractures [39]; whether these associations are true in children remains unclear. However, fat embolism syndrome following long bone fractures has been described in pediatric DMD [40, 41], while another study suggested that bisphosphonate therapy for osteoporosis was linked to survival [42].

The pediatric skeleton is a dynamic structure with the distinct capability to not only reclaim BMD lost during transient bone health insults, but to reshape fractured bone (including vertebral bodies) through the process of skeletal modeling. Both indices are important measures of recovery in children, either spontaneously or following osteoporosis therapy (i.e., bisphosphonate treatment). Since vertebral body reshaping appears to be growth-mediated, as it has never been unequivocally reported in adults [43], we postulate that bisphosphonate therapy does not directly bring about reshaping but rather has a permissive effect by enhancing BMD in order to prevent further collapse [44].

The disease that has been best-studied for signs of recovery from skeletal insult in the absence of osteoporosis therapy is acute lymphoblastic leukemia (ALL). This is unsurprising, since ALL represents a transient threat to bone health in the majority of patients undergoing current treatment strategies. Mostoufi-Moab [45] assessed children by tibia pQCT and found that trabecular and cortical BMD Z-scores were low compared to healthy controls within 2 years post-chemotherapy completion, but that significant improvements (on average 0.5 SD) were evident a year later. Cortical dimensions also increased, followed by increases in cortical BMD. Other studies have also shown recovery in bone mass and density in the years following chemotherapy [46, 47]. Lack of BMD restitution is linked to craniospinal radiation, particularly at doses $\geq$ 24 Gy [47]. However, it should be noted that the lower spine BMD among those with radiation exposure appears to arise in part from hormone deficiency-related short stature. Other recognized risk factors

for incomplete BMD recovery in ALL include untreated hypogonadism, vitamin D deficiency, hypophosphatemia, low IGF-binding protein-3, and reduced physical activity [48].

The fact that reshaping can occur during leukemia chemotherapy (i.e., during high-dose GC treatment) is hypothesized to result from the saltatory pattern of GC exposure with current treatment protocols (Fig. 24.2a). Vertebral body reshaping has also been observed in our clinic among children with rheumatic disorders post-GC cessation, though not previously reported (Fig. 24.2b). On the other hand, older children who have insufficient residual growth potential can be left with permanent vertebral deformity after vertebral collapse (Fig. 24.2c). The long-term consequences of permanent deformity remain unstudied; however, reports in adults indicate reduced quality of life due to pain and functional limitation [49, 50]; whether the same is true in later stages of life following permanent vertebral deformity sustained during childhood warrants further study.

To understand the vertebral body reshaping phenomenon further, the Canadian *ST*eroid-Induced *O*steoporosis in the *P*ediatric *P*opulation (*STOPP*) Consortium has studied determinants of complete versus incomplete reshaping in bisphosphonate-naïve ALL (quantified by a decrease in a positive spinal deformity index (SDI) [51] by 100% in the 6 years following diagnosis). Preliminary analyses suggest that many children reshape following VF in ALL but those with moderate or severe vertebral collapse and those who are older children appear to reshape less frequently. The next question is whether children with VF and persistent bone health threats in the context of other diseases such as GC-treated DMD can undergo vertebral body reshaping without bisphosphonate therapy. To date, there are no published reports to suggest such they do, a fact that is concordant with our own clinical experience.

24.5 The Definition of Osteoporosis and Diagnostic Evaluation in At-Risk Children

24.5.1 Bone Health Monitoring: Goals and Candidates

The ultimate goal of monitoring is to identify high-risk patients for intervention that will prevent the first fracture. However, lack of available data to support such primary prevention has led to monitoring that identifies early rather than late signs of osteoporosis, followed by bone-active treatment in those with limited potential for spontaneous recovery (including limited potential to undergo vertebral body reshaping). This is in line with a secondary prevention approach, which seeks to mitigate the progression of the osteoporosis following identification in its earlier stages.

Two important observations have shifted monitoring away from a BMD-centric to a more functional approach: (1) the use of a BMD Z-score threshold to identify a child who is at risk is problematic due to variability in the Z-scores generated by the different available normative databases [52–54] and (2) asymptomatic VF can occur at BMD Z-scores > −2, thereby requiring imaging surveillance for VF detection. Other functional outcomes should also be tracked during monitoring including any history of non-VF, growth, pubertal status, mobility, pain, muscle strength, and the potential for spontaneous recovery (vertebral body reshaping and bone density restitution). BMD remains a vital part of the bone health monitoring approach but as an adjuvant tool to chart the child's BMD trajectory, thereby signaling a child who is losing ground and thereby at increased risk for fractures or who is showing signs of recovery following a transient bone health threat (potentially obviating the need for osteoporosis treatment).

Patients expected to be GC-treated for ≥3 months should be considered for a baseline spine radiograph (or high-quality dual-energy x-ray absorptiometry (DXA)-based VF assessment (VFA), if available) at the time of GC initiation. GC therapy for ≥3 months is the recommended cutoff since the earliest incident VF reported after GC initiation in children is at 4 months [28]. Children meeting the criteria for baseline spine imaging should also undergo a follow-up radiograph at 12 months, since this is the time point with the highest annual incidence of VF in GC-treated children [12, 28]. Imaging for VF is advised every 1–2 years thereafter for those with ongoing GC exposure. The predictors of VF outlined in Table 24.3 can facilitate the decision around frequency of VF follow-up assessments beyond 12 months in the various disease groups.

Among children with other risk factors for bone fragility apart from GC exposure

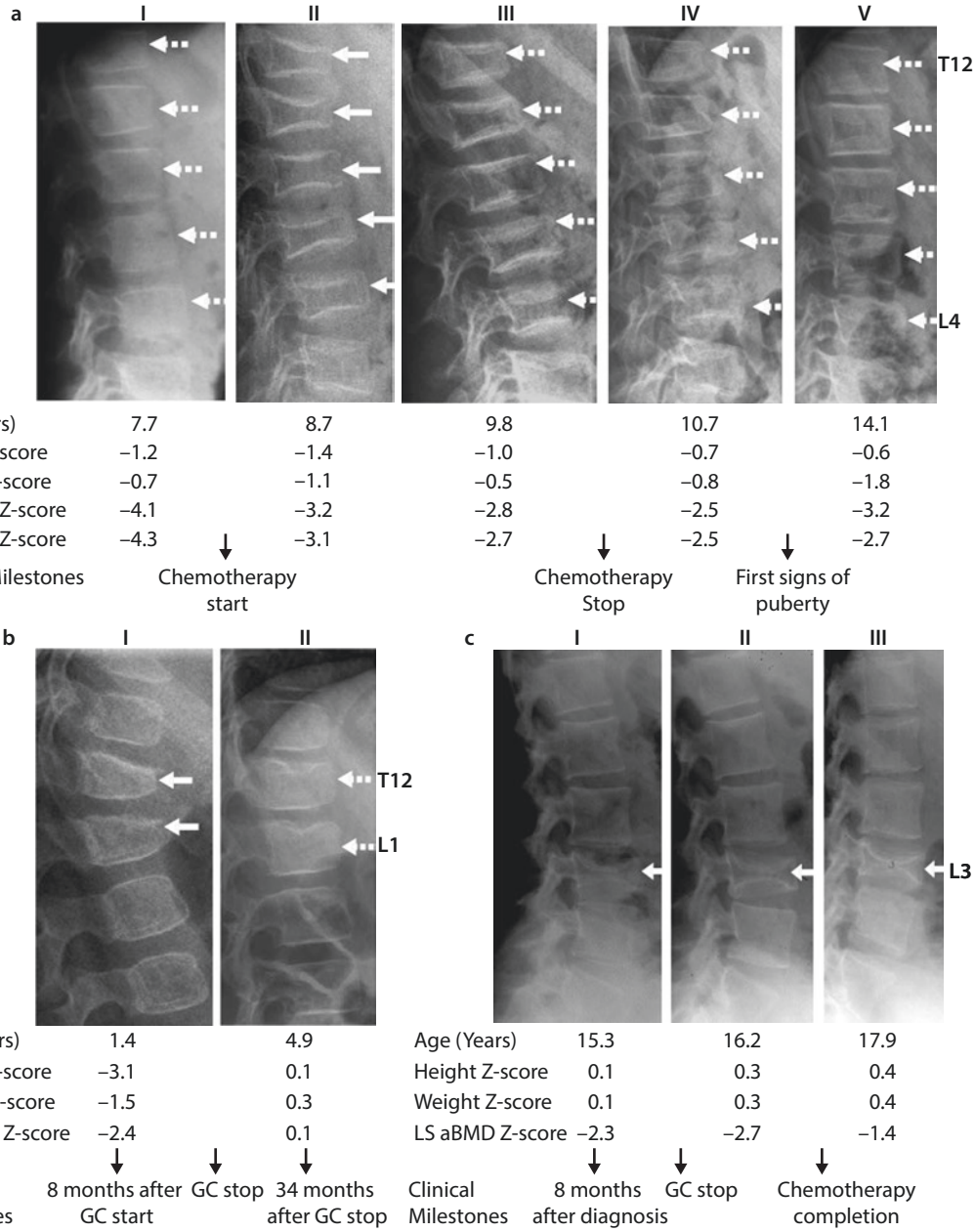


Fig. 24.2 **a** (I) Lateral spine radiographs in a 7.7-year-old girl at diagnosis with pre-B acute lymphoblastic leukemia showing a normal spine radiograph. (II) Vertebral fractures after 1 year of chemotherapy are as follows: grade 3 (severe) wedge fractures at T12 and L1, grade 2 (moderate) biconcave fracture at L2, and grade 3 (severe) biconcave vertebral fractures at L3 and L4. (III–V) These panels show stages in vertebral body reshaping with a "bone within bone" appearance during and after chemotherapy, in the absence of bone-specific (bisphosphonate) therapy. **b** (I) Lateral spine radiographs showing vertebral fractures in a toddler with systemic-onset juvenile idiopathic arthritis. Grade 2 vertebral fractures at T12 and L1 on GC therapy at 1.4 years of age. (II) At 4.9 years of age, she has almost complete recovery of vertebral height ratios with the typical "bone within bone" appearance, in the absence of bone-specific (bisphosphonate) therapy. **c** (I) Lateral spine radiographs showing a grade 3 (severe) fracture at L3 in a 15.3-year-old girl with pre-B acute lymphoblastic leukemia 3 months after diagnosis. At diagnosis, she had already attained final adult height. (II, III) Lack of reshaping due to fused epiphyses and absence of endochondral bone formation (From: Ward et al. [5]. Reprinted with permission from Springer)

(Tables 24.1, 24.2, and 24.3), the same principles apply; that is, the patient should be assessed for both non-VF *and* VF since GC-naïve children with mobility issues and genetic bone fragility can also develop VF [7, 29]. In youth with impaired mobility due to cerebral palsy and congenital myopathies, a spine radiograph is recommended at the latest by about 6 years of age and then at intervals thereafter until the end of growth or sooner in the presence of back pain. Monitoring is recommended to start by this time since treatment should be initiated *before* there is insufficient residual growth potential for vertebral body reshaping.

Since BMD is useful as a serial measurement to assist the clinician in understanding the child's overall bone health trajectory and in making logical decisions about the need for ongoing monitoring, discharge from bone health care, or intervention, it is recommended that a BMD assessment be carried out at least as frequently as spine radiographs according to the above guidelines, with assessments every 6 months in those children at greatest risk [4, 30].

24.5.2 Axial Skeletal Health: Vertebral Fracture Detection Methods and Imaging Modalities

The most widely used tool for the assessment of VF in both children and adults is the Genant semiquantitative method [55, 56]. According to the Genant method, the definition of a VF is ≥ 20% loss in vertebral height ratio regardless the VF morphology (wedge, bi- or mono-concave, or crush). VF are subjectively graded by trained readers according to the magnitude of the reduction in vertebral body height ratios, without direct measurement. Vertebral height ratios are generated when the anterior vertebral height is compared with the posterior height (for an anterior wedge fracture), middle height to the posterior height (bi- or mono-concave fracture), and posterior height to the posterior height of adjacent vertebral bodies (crush fracture). The Genant scores correspond to the following reductions in height ratios: grade 0 (normal), <20%; grade 1 fracture (mild), ≥20% to 25%; grade 2 fracture (moderate), >25% to 40%; and grade 3 fracture (severe), >40%. Overall, the Genant semiquantitative method is preferred over quantitative (6-point) vertebral morphometry [57], since it is faster, and takes into consideration the expertise of an experienced reader. In addition, it quantifies the severity of VF (an important predictor of the lack of potential for spontaneous vertebral body reshaping following VF in children).

Furthermore, the Genant scoring system permits calculation of the SDI, the sum of the Genant grades along the length of the spine [51]. The SDI is a global index of spine morbidity that is useful clinically and can be used as a continuous outcome variable in research studies [58]. The kappa statistics for intra- and interobserver agreement are similar for children compared to adults using the Genant semiquantitative method [55, 59, 60].

A number of recent studies have provided validity for the Genant approach in children. First, Genant-defined VF show a bimodal distribution from T4 to L4 similar to the known distribution in adults [61–64], with a predilection for the mid-thoracic region (T5 to T8, the site of the natural kyphosis) and the thoracolumbar junction (the site of transition to the natural lordosis) [23, 64]. Secondly, biologically relevant clinical predictors of Genant-defined VF have been identified including back pain, low LS BMD Z-scores, longitudinal declines in LS BMD Z-scores, and GC exposure [13, 23, 26]. One of the most important observations to assert the validity in children is that both mild and moderate-severe Genant-defined VF at leukemia diagnosis are robust clinical predictors of new VF over the next 3 years [12, 26].

To date, the most common imaging tool for VF detection in childhood is lateral thoracolumbar spine radiographs. In view of the high radiation exposure from spine radiographs but nevertheless critical need for VF assessments as part of bone health evaluations, non-radiographic imaging techniques have been developed which use the scoring methods described above. The use of DXA to diagnose VF is called VF assessment (VFA), with images captured on a lateral spine view. VFA is attractive as an assessment tool given its minimal radiation and the fact that fan-beam technology facilitates capture of the entire spine on a single image without divergent beam issues due to parallax. Newer DXA machines have a rotating

"c-arm" which obviates the need to reposition the patient from the supine to lateral position. Image quality varies significantly depending on the densitometer [65]. Using a Hologic Discovery A machine, Mayranpaa et al. [66] showed low diagnostic accuracy for VFA compared to lateral spine radiographs and poor visibility in children. Pediatric studies on newer DXA machines are presently underway and preliminary data are promising.

24.5.3 Axial Skeletal Health: Trans-iliac Bone Biopsies

Iliac crest bone biopsies with tetracycline labeling provide unique diagnostic information about static and dynamic bone properties that cannot be obtained by any other means (i.e., osteoid thickness, bone formation rate, mineralization lag time, and other bone formation and resorption indices) [67]. In practical terms, biopsies are useful in establishing the cause of osteoporosis in special cases such as a child with unexplained bone fragility and negative genetic studies. Idiopathic juvenile osteoporosis has a characteristic histomorphometric appearance – low bone turnover and thin osteoid seams – but clinically may be difficult to distinguish from other forms of osteoporosis such as non-deforming OI without blue sclerae, wormian bones, or a family history [68, 69]. Similarly, patients with OI typically have a histological hallmark (hyperosteocytosis) that is helpful diagnostically in rare cases when studies are falsely negative [68, 69]. At the same time, few clinicians are trained in this technique, and so overall, it is a rarely used tool aside from highly specialized clinics.

24.5.4 Axial and Appendicular Skeletal Health: Dual-Energy X-Ray Absorptiometry

DXA is the most commonly used and widely available technique to measure bone mass and density in children, since it is highly reproducible and inexpensive and confers low radiation exposure. LS and total body less head are the preferred measuring sites [70]; recently, lateral distal femur BMD Z-scores have also been useful in children with neuromuscular disorders who prefer to position on their side [38, 71] (Table 24.3). BMD raw values are converted to age- and sex-specific SD scores (Z-scores) and require additional interpretation in view of body size, ethnicity, and pubertal staging or skeletal maturity (the latter, by bone age) [72]. Since BMD can be underestimated in children with familial short stature, and children with chronic illnesses may be transiently or permanently short due to the effects of the disease/treatment on linear growth and puberty, adjustment for bone size using a technique such as derivation of bone mineral apparent density (BMAD, in g/cm^3) [73] or height Z-score-corrected BMD Z-scores [74] is required to avoid underestimation of BMD parameters. BMAD has the advantage that it has been tested for its ability to accurately predict VF [75], whereas height Z-score-corrected BMD Z-scores have not. Lateral distal femur BMD Z-scores predicted non-VF in children with neuromuscular disorders [38] and furthermore, this assessment method is taken at a clinically relevant site, since children with neuromuscular disorders often fracture at this location. Despite challenges in BMD interpretation due to variable growth rates and timing and tempos of puberty, numerous studies (Table 24.3) confirm an inverse relationship between BMD and fracture rates, and serial measurements provide additional information about the child's overall bone health trajectory that can inform whether there is a need for ongoing bone health monitoring.

24.5.5 Appendicular Skeletal Health: Peripheral Quantitative Computed Tomography

pQCT at the radius and tibia provides information that cannot be obtained by DXA about musculoskeletal geometry as well as "true" (volumetric) cortical and trabecular BMD. For example, in children with cerebral palsy, it has been shown that smaller bone and cortical cross-sectional area are the main structural defect, rather than lower cortical BMD [76]; pQCT studies have also shown that cortical thickness and not density is the main parameter impacted by growth hormone deficiency and treatment [77]. pQCT is particularly useful when DXA studies are precluded due to spine deformity, hip and knee contractures, or metallic hardware. The newest technique, high-resolution

pQCT, has the spatial resolution to measure trabecular geometry and microarchitecture. At the moment, pQCT and high-resolution pQCT are research tools in most centers.

24.5.6 Bone Turnover Markers

Bone turnover markers (BTM) are often measured in children undergoing a bone health assessment or while on osteoporosis therapy. Recently, two markers have been recommended by the International Osteoporosis Foundation and the International Federation of Clinical Chemistry and Laboratory Medicine [78]: serum procollagen type I N-terminal propeptide (PINP, a marker of bone formation) and serum collagen type I cross-linked C-telopeptide (CTx, a marker of bone resorption), both of which have been studied in healthy children in order to generate reference data [79–82]. These analytes were chosen because of their specificity to bone and relationship to relevant outcomes in adult clinical studies as well as their stability, wide availability, and ease of analysis and procurement.

BTM are influenced by several factors that lead to high intra- and interindividual variability, including age/pubertal stage, gender, time of day, food intake, physical activity, recent fractures, serum 25-hydroxyvitamin D status, assay methods, and sample transport and storage conditions. One of the main factors that have limited their use in children, particularly for those with chronic illness and growth delay, is that BTM are largely a reflection of linear growth and not bone turnover per se. In children, the only available method to determine the bone turnover status with certainty is to directly measure bone formation and resorption on trabecular surfaces via trans-iliac bone biopsy; however, this tool is not in widespread clinical use.

In children, BTM provide some insight into general diagnostic categories; for example, urinary NTx levels are high pre-bisphosphonate treatment in children over 3 years of age with OI [83] and correlate with an increased trabecular bone formation rate on trans-iliac biopsies [84]. Low BTM and trabecular bone formation are frequently observed in chronic illness osteoporosis both before [44, 85] and after years [44] of GC therapy. *LRP5* mutations causing juvenile osteoporosis are also characterized by low BTM and trabecular bone formation [86, 87]. On the other hand, brisk increases in BTM can signal recovery from growth failure and bone mass deficits as observed in children undergoing effective treatment for Crohn's disease [16]. A low alkaline phosphatase can separate patients with OI from those with hypophosphatasia – an important distinction since bisphosphonates are contraindicated in hypophosphatasia and furthermore, a life-saving medical therapy is now available to treat the severe infantile form [88].

To date, there are no studies in childhood which have assessed fracture risk reduction or frequency of adverse effects according to thresholds of bone turnover marker reduction with bisphosphonate therapy. At the present time, BTM during pediatric osteoporosis therapy serve to document that the drug is exerting the anticipated biological effect and provide a measure of compliance.

24.5.7 The Definition and Diagnosis of Osteoporosis in Children

The definition and diagnosis of osteoporosis in children has been fraught with challenges and controversy over the years, following the widespread availability of BMD by DXA that led to zealous testing in myriad pediatric populations. In recent years, there has been a move away from a BMD-centric diagnostic focus to a more functional approach. While the clinical relevance of BMD testing has been clearly affirmed by numerous studies showing consistent, inverse relationships between BMD Z-scores and low-trauma fractures in children (Table 24.3), the proportion of children assigned a BMD Z-score ≤ -2.0 varies considerably depending on the BMD normative database that is used to generate the Z-scores [52–54]. Specifically, the Canadian STOPP Consortium reported the magnitude of the disparity in LS BMD Z-scores generated by normative databases from both Hologic and Lunar machines in children with ALL at diagnosis [54], showing a difference of as much as 2.0 SD depending upon which database was used to generate the Z-scores. Secondly, the Consortium reported that 48% of children with VF at the time of leukemia diagnosis had BMD Z-scores > -2.0.

These disparate results in BMD Z-scores depending on the reference data that is used plus

the fact that VF can occur above the −2.0 threshold suggested that the use of a LS BMD Z-score cutoff as part of the definition of osteoporosis in children with VF was not valid [54]. This view has been underscored by the ISCD in an updated (2013) position statement [30] which notes a BMD Z-score threshold of ≤ −2.0 is no longer required to diagnose osteoporosis in a child with a VF; in fact, there are no longer BMD Z-score requirements at all in the setting of a low-trauma VF. In the 2013 ISCD recommendation, the use of a BMD Z-score threshold (−2.0 or worse) has been retained to denote osteoporosis in children with long bone fractures, provided such children also have a clinically significant fracture history defined as ≥ 2 long bone fractures by age 10 and ≥ 3 long bone fractures by age 18 [30]. At the same time, the 2013 ISCD position statement notes that a BMD (or bone mineral content) Z-score > −2.0 does not preclude an increased fracture risk of long bone fractures. This caveat is affirmed by Henderson et al.'s report that up to about 15% of children with neuromuscular disorders and lower limb fractures had lateral distal femur BMD Z-scores > −2.0 [38]; similar observations have been made in adolescents with anorexia nervosa [89].

Despite the disparity in LS BMD Z-score generated by different normative databases, Ma et al. [54] showed in children with ALL at diagnosis that the relationships between LS BMD Z-scores and VF are consistent regardless of the reference databases that are used to generate the Z-scores. This is not surprising, since the available reference databases are all highly correlated with one another (with *r* value ranges from 0.85 to 0.99) [54]. These findings suggest that while the use of a LS BMD Z-score threshold is not valid for the diagnosis of osteoporosis in children with VF, and that this is likely also true in relation to other BMD sites in children with extremity fractures [38], the use of LS BMD Z-scores as a continuous variable risk factor for VF in clinical research studies nevertheless remains valid.

Where does this leave the clinician in the pivotal decision to label a child with osteoporosis? On balance, current evidence puts the weight of the diagnosis on the fracture history. Among children with risk factors for osteoporosis, a low-trauma fracture is usually apparent (falling from a wheelchair, sustaining a fracture during a seizure); in such cases, a size-corrected BMD Z-score > −2.0 should not deter the clinician from the osteoporosis diagnostic label.

On the other hand, in the case of an otherwise healthy child with recurrent fractures but absence of risk factors, stigmata of OI, or a genetically confirmed family history of osteoporosis, it is incumbent upon the clinician to find evidence of additional features to support the diagnosis of osteoporosis (Fig. 24.1). A VF without a history of trauma is highly suggestive of an underlying bone fragility condition, and the lower the BMD, the more likely an osteoporotic phenotype (although a normal BMD does not categorically rule out osteoporosis as discussed). Genetic testing is indicated in such children, since even children with type I collagen mutations can lack typical stigmata. Overall, about 7% of patients with a mutation in the type I collagen genes will be without either blue sclerae or dentinogenesis imperfecta [Frank Rauch, personal communication].

Since over a dozen genes have now been implicated in OI or "OI-like" bone fragility (Table 24.1), questions have been raised about the best way to describe the various forms of mild, moderate, and severe genetic forms of osteoporosis. While some reports retain the original OI subtype nomenclature [90] (i.e., types I to XVI, expanding on the initial classification proposed by Sillence [91]), recently it has been proposed that congenital bone fragility should be described according to the implicated gene and that the term OI should be reserved for genetic forms which involve type I collagen pathobiology [92]. This approaches simplifies the diagnosis of genetic bone fragility for the clinician, clustering diagnoses into broad categories based on known genetic underpinnings (see Table 24.1 for phenotypic characteristics associated with each). Figure 24.1 provides an overview of the approach to the diagnosis of osteoporosis in children. It should be remembered that a young child with unexplained fractures, lack of evidence for a secondary cause of osteoporosis, and normal genetic studies may be the victim of non-accidental trauma.

24.6 Treatment

24.6.1 General Measures for Optimization of Bone Health

First-line measures to optimize bone health fall into three main categories: nutrition (calcium, vitamin D, protein, potassium, magnesium, copper,

iron, fluoride, zinc, and vitamins A, C, and K), physical activity, and treatment of the underlying condition and associated comorbidities; these have been recently reviewed extensively elsewhere [1, 2, 5, 93–99] and will not be reiterated here with the exception of vitamin D status given its tendency to be entrenched in controversy, making it difficult for the clinician to see the forest for the trees.

The recommended intake of vitamin D is a minimum of 600 IU/day [100], although higher doses are often required to meet target levels, particularly in those with malabsorption, obesity, and darker skin [100]. Adequate total body vitamin D stores have been defined at a serum 25-hydroxyvitamin D level ≥ 50 nmol/L (20 ng/mL) [100, 101] or ≥ 75 nmol/L (30 ng/mL) [102], mostly based on adult studies. In children, the optimal serum 25OHD threshold remains under debate. A meta-analysis showed a lack of significant effect of vitamin D supplementation and 25OHD levels ≥ 50 nmol/L on BMD in healthy youth [103], a bone histomorphometric study in children with OI failed to show an association between serum 25OHD levels and bone mineralization or bone mass [104], and calcium plus vitamin D supplementation had no effect on spine BMD in children with inflammatory bowel disease [105] and leukemia [106]. Overall, the optimal serum 25OHD threshold associated with health benefits across the life cycle remains controversial as discussed in a large contemporary "umbrella" assessment of published systematic reviews and meta-analyses [107]. From a practical perspective, a minimum 25-hydroxyvitamin D level of 50 nmol/L (20 ng/mL) is recommended in youth through diet and/or supplementation, with measurement of 25-hydroxyvitamin D in high risk populations ideally at the end of winter in order to determine compliance with and efficacy of prescribed doses at the time of the nadir.

For children with chronic illnesses, adequate treatment of the underlying illness is the mainstay of osteoporosis prevention and treatment. The situation is complicated by the fact that some of the standard therapies are osteotoxic, including GC, high-dose methotrexate in the cancer setting [108], calcineurin inhibitors [109], hepatic microsomal enzyme-inducing anti-epileptics increasing catabolism of 25-hydroxyvitamin D, and long-term use of anticoagulants [110] and medroxyprogesterone [111]. Wherever possible, these agents should be used sparingly in children with risk factors for osteoporosis, a principle that is not always practical given, for example, the need for GC therapy to treat systemic inflammatory diseases and leukemia and to slow the progression of the myopathy in DMD.

Identification of endocrine comorbidities is also appropriate, including treatment of delayed puberty, growth hormone deficiency, hyperthyroidism, and diabetes. Growth hormone therapy increases areal BMD even after final adult height attainment and should be continued through adulthood in those with low size-adjusted BMD or fractures [112]. A word of caution in the use of growth hormone to treat GC-induced growth failure in DMD – in addition to a paucity of data to support the safety and efficacy of this approach, one of the current hypotheses is that short stature may be beneficial to muscle strength in DMD since stresses on the sarcolemma are higher with increases in the size of the muscle fiber [113]. A comprehensive review of the management of specific chronic conditions such as anorexia nervosa is beyond the scope of this chapter and is discussed elsewhere (▶ Chap. 12).

24.6.2 Drug Therapy: Candidates for Medical Intervention and Timing of Treatment Initiation

When to initiate medical treatment is a frequently posed question by clinicians. To date, intervention studies in children have largely been limited to case series and small observational or case-control studies, given the relative paucity of patients with various diseases at any one medical center and the challenges in securing funding for large, multicenter drug trials in the young. The absence of treatment trials targeting prevention of first-ever fractures in children has led to a conservative approach overall, with therapy typically reserved for children with overt bone fragility. Among those with chronic illness and osteoporosis, there is an additional consideration – not every child with symptomatic osteoporotic fractures and chronic illness requires osteoporosis therapy given the potential for spontaneous (medication unassisted) recovery if risk factors are transient, including reshaping of previously fractured vertebral bodies. The potential for spontaneous recovery in children

with transient risk factors demands controlled trials in this setting.

Where primary prevention with drug therapy prior to the first fracture is concerned, at the present time, there is insufficient data to recommend osteoporosis therapy other than the general measures discussed previously. In the future, primary prevention drug trials should target priority disease groups including the progressive neuromuscular disorders like GC-treated DMD. Here, there is an urgent need for well-designed trials on sufficient numbers of patients to effectively assess functional outcomes including fractures, pain, and mobility when treatment is started before the first fracture.

Since there are insufficient data to recommend drug therapy for the primary prevention of osteoporotic fractures in children with any condition at the present time, careful monitoring in at-risk children to identify those with early signs of bone fragility, particularly in those with limited potential for spontaneous recovery, is indicated. Such an approach follows the principles of secondary prevention – to mitigate osteoporosis progression and foster recovery in those with earlier (rather than later) signs of osteoporosis. Given the knowledge that has emerged about the clinical populations at risk for osteoporosis and the disease-specific predictors of fractures, it is no longer appropriate for children to present to medical attention with, for example, back pain due to advanced vertebral collapse necessitating "rescue therapy." Rather, pediatric programs should be established to effectively monitor at-risk children in order to identify earlier stages of vertebral collapse, followed by an assessment of the child's potential for medication-unassisted recovery versus need for osteoporosis treatment. A monitoring program also provides the clinician with an opportunity to identify and treat vitamin D, mineral, and hormonal deficiencies, to encourage a healthy weight, to promote physical activity within the limits of the child's underlying condition, and to encourage compliance with treatment of the underlying condition [16, 114].

Bisphosphonate therapy is typically reserved for children with a history of low-trauma fractures but also limited potential for spontaneous (i.e., medication-unassisted) recovery due to permanent or persistent osteoporosis risk factors (Fig. 24.1). Low-trauma long bone fractures and symptomatic VF (or asymptomatic VF that are moderate or severe) are the most frequent indications for treatment. Extremity fractures at sites other than long bones (such as hands and feet, fingers and toes) do not usually warrant treatment. Studies are currently underway to evaluate the safety and efficacy of treating mild (Genant grade 1) asymptomatic or minimally symptomatic VF in pediatric osteoporosis; for now it is recommended that such fractures be closely monitored for symptomatology and/or progressive vertebral height loss that would prompt treatment.

After determining the child's vertebral and long bone fractures status, the clinician assesses the potential for medication-unassisted recovery in view of the osteoporosis severity (including degree of vertebral collapse), residual growth potential, and whether risk factors are persistent or resolving. In the face of resolving risk factors at a young age (such as withdrawal of GC therapy in a pre-pubertal child), a conservative approach can often be taken that involves monitoring to document the child's anticipated recovery. In contrast, children who are peri-pubertal or older as well as younger children with ongoing risk factors or heritable forms of osteoporosis will have less potential for spontaneous reshaping of vertebral bodies and reclamation of BMD – such children are optimal candidates for osteoporosis therapy. Of course, symptomatic osteoporosis (such as pain from VF limiting the child's quality of life) is itself an indication for treatment; in such cases, osteoporosis therapy is recommended to relieve pain and allow the child to regain quality of life regardless of the child's potential for spontaneous recovery in the future.

Following these steps facilitates the decision to start treatment in a child with a clear diagnosis of primary or secondary osteoporosis. As shown in Fig. 24.1, a frequent conundrum is whether to start treatment without a specific underlying diagnosis – a scenario referred to as "low-trauma, recurrent (usually extremity) fractures in otherwise healthy children." In such cases, the clinician needs to make every effort to unearth a known cause, including the now expanded etiologies of heritable bone fragility outlined in Table 24.1 or chronic illnesses with insidious onset (such as Crohn's or rheumatic diseases) outlined in Table 24.2. A low-trauma VF in this setting is highly suggestive of a bone fragility condition. When genetic and chronic illness evaluations are

negative, a trans-iliac bone biopsy can also provide important clues although it is less readily available. When no specific diagnosis is forthcoming despite a comprehensive evaluation, the criteria to label a child with osteoporosis provided in the most recent ISCD position statement support the decision to initiate osteoporosis treatment: ≥ 2 long bone fractures by age 10 or ≥ 3 or more long bone fractures by age 18 *and* a size-corrected BMD or bone mineral content Z-score of −2 [30]. Low-trauma VF may also prompt treatment in these cases.

24.6.3 Bisphosphonate Treatment of Primary and Secondary Osteoporosis in Childhood

Bisphosphonates, synthetic analogues of pyrophosphate, are the most extensively published agents to treat osteoporosis in childhood [115, 116], despite the fact that they remain off-label in most countries. The vast majority of publications describing the effect of bisphosphonate therapy in children are observational, pre-post studies; there are relatively few controlled studies of bisphosphonate therapy in children, and even fewer studies have been sufficiently powered to assess fracture outcomes. The paucity of fracture outcome data in controlled trials reflects a number of considerations when studying children: the relatively small numbers of patients available for study, the historically adult focus of industry-sponsored trials, and the logistical and philosophical challenges enrolling younger patients. The latter issue includes pressure from families and health-care providers alike to treat individual pediatric patients despite insufficient evidence, instead of enrolling children in controlled trials that address uniquely pediatric safety and efficacy issues. Nevertheless, the few controlled studies available in addition to a number of key observational studies provide important and useful information about pediatric patients' responses to bisphosphonate therapy.

24.6.4 Oral Versus Intravenous Bisphosphonate Therapy

The use of oral versus intravenous (IV) bisphosphonate therapy for pediatric osteoporosis has long been debated [117]. Overall, IV pamidronate is the mostly extensively reported agent in children following the inaugural, observational study in the late 1990s which showed improved pain, mobility, and reshaping of vertebral bodies following pamidronate therapy in children with moderate to severe OI [118]. Children were treated with cyclical, IV pamidronate at a dose of 9 mg/kg/year divided every 2–4 months up to 5 years' duration [118]. In recent years, IV zoledronic acid has been introduced given the advantage that it can be given over a shorter period of time and less frequently [44, 119]; zoledronic acid is 100 times more potent than pamidronate [120]. Both agents are nitrogen-containing bisphosphonates that inhibit farnesyl diphosphate synthase and thereby protein prenylation, a process crucial for osteoclast survival. A randomized study comparing the two agents in OI showed that zoledronic acid had similar effects on LS BMD Z-scores and fracture rates over 12 months [119]. Of the oral agents, alendronate and risedronate have been the most extensively studied, with one report confirming that the oral bioavailability of alendronate in children is <1%, similar to adults [121].

Figure 24.3 shows the mean difference in LS areal BMD Z-score change in published, controlled trials of bisphosphonate therapy for the treatment of childhood osteoporosis, with comparison of results in the treatment versus placebo/untreated control groups. As shown in Fig. 24.3, increases in spine BMD Z-scores were a consistent finding in all of the available controlled studies using oral alendronate or risedronate in children with OI; one report showed no effect of oral alendronate in a study of girls with anorexia nervosa [122]. In addition, a controlled study by Gatti et al. in pediatric OI (Table 24.4) showed a significant effect of IV neridronate on the percent change in spine and hip BMD compared to controls after 1 year. Overall, it appears that IV and oral bisphosphonates consistently increase BMD parameters in children, as confirmed in recent Cochrane reviews on the use of bisphosphonates in pediatric secondary osteoporosis [116] and OI [115].

On the other hand, the effects of IV versus oral bisphosphonates on fracture outcomes are less homogeneous, an observation that is evident in Fig. 24.4 (describing the relative risk of fractures in controlled bisphosphonate trials from data on the number of patients with fractures in the two groups) and Fig. 24.5 (showing the incidence rate of fractures in controlled trials from

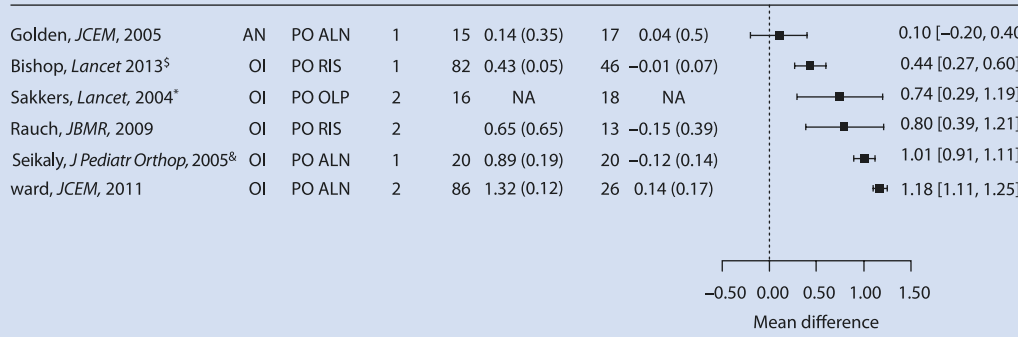


Fig. 24.3 Mean difference in the lumbar spine area. BMD Z-score in published, controlled trials of bisphosphonate therapy for the treatment of children with osteoporosis, with comparison of results for the treatment versus placebo/untreated control groups. Studies were included with the following criteria: (1) at least ten patients per group, (2) prospective design with a placebo or untreated control arms, and (3) available data on either the pre- and post-treatment change in LS BMD Z-score with standard error OR the percent change in LS BMD Z-score. *Details about the magnitude of the mean change in LS BMD Z-score were not reported; however, the effect size with 95% CI was provided. &Seikaly 2005 was a placebo-controlled crossover study design with the results from the first year of the study presented. $Bishop 2013 reported least-squares mean difference. Abbreviations: *ALN* alendronate, *AN* anorexia nervosa, *CF* cystic fibrosis, *GC* glucocorticoids, *IV* intravenous, *yrs*. years, *NER* neridronate, *OI* osteogenesis imperfecta, *OLP* olpadronate, *PO* oral, *Pts* patients, *RIS* risedronate (From: Ward et al. [5]. Reprinted with permission from Springer)

Table 24.4 Bisphosphonate therapy in children: results of prospective controlled trials with at least ten patients per group

Publication, study design, and diagnosis	Number of patients, age (years)	Agent, dose, and route	Main efficacy outcomes	Side effects
Bishop 2013 RCT, double-blind OI (mild to severe) Duration with comparison to control group: 1 year	Treatment group *N* = 94 Age: mean (SD) = 8.9 (3.4) Placebo group *N* = 49 Age: mean(SD) = 8.6 (3.1)	Oral risedronate 2.5 mg/day if weight 10–30 kg; 5 mg/day if weight > 30 kg	See BMD and fracture outcomes in Figs. 24.3 and 24.4 ↓ Urinary NTx/creatinine with risedronate versus placebo	Similar between treatment and placebo groups
Ward (2011) RCT, double-blind OI (mild to severe) Duration: 2 yrs.	Treatment group *N* = 109 Age: mean (SD) = 11.0 (3.6) Placebo group *N* = 30 Age: mean (SD) = 11.1 (4.0)	Oral alendronate 5 mg/day if weight < 40 kg; 10 mg/day if weight ≥ 40 kg	See BMD and fracture outcomes in Figs. 24.3 and 24.4 ↓ In urinary NTx with risedronate versus placebo No differences: Average midline vertebral height, iliac cortical width, bone pain, physical activity	Similar between treatment and placebo groups

Table 24.4 (continued)

Publication, study design, and diagnosis	Number of patients, age (years)	Agent, dose, and route	Main efficacy outcomes	Side effects
Gatti (2005) RCT, unblinded OI (mild to severe) Duration: 1 year	Treatment group *N* = 42 Age: mean (SD) = 9.0 (2.3) Untreated control group *N* = 22 Age: mean (SD) = 8.6 (2.4)	IV neridronate 2 mg/kg every 3 months Intravenous	See fracture outcomes in Figs. 24.4 and 24.5 Significant differences compared to untreated controls: ↑ Spine and hip BMD ↑ Height and DXA-derived LS projected area ↓ Total number of fractures Nonsignificant differences compared to untreated controls: Number of patients with non-vertebral fractures	Flulike symptoms; 10/42 in the neridronate group; 0/22 in the untreated control group
Sakkers (2004) RCT, double-blind OI (mild to severe) Duration: 2 years	Treatment group *N* = 16 Age: mean (SD) = 1.0 (3.1) Placebo group *N* = 18 Age: mean (SD) = 10.7 (3.9)	Oral olpadronate 10 mg/m^2 daily	See Figure BMD and fracture outcomes in Figs. 24.3, 24.4, and 24.5 Significant differences compared to placebo: ↓ Relative risk of long bone fractures ↑ Spine BMC Nonsignificant differences compared to placebo: Mobility, self-care, muscle strength, anthropometry, height of the vertebral bodies, urinary bone resorption markers	Not reported
Rauch (2009) RCT, double-blind OI type I Duration: 2 years	Treatment group *N* = 13 Age: mean (SD) = 11.7 (3.6) Placebo group *N* = 13 Age: mean (SD) = 11.9 (4.0)	Treatment group Oral risedronate 15 mg/wk. if weight < 40 kg; 30 mg/wk. if weight > 40 kg	See BMD and fracture outcomes in Figs. 24.3, 24.4, and 24.5 Significant differences compared to placebo: ↓ Serum NTx Nonsignificant differences: BMC/BMD at the radial metaphysis and diaphysis, hip, and total body; trans-iliac cortical width, trabecular bone volume, bone turnover; vertebral height; second metacarpal cortical width, grip strength, bone pain	Similar between treatment and placebo groups
Seikaly (2005) RCT with double-blind crossover design OI (mild to severe) Duration: 1 year treatment then crossover to placebo OR 1 year placebo then crossover to treatment	Treatment group *N* = 20 Age: mean (SD) = 9.8 (1.06) Placebo group Crossover design, therefore same patients as in the treatment group	Treatment group Oral alendronate 5 mg/day if weight < 30 kg; 10 mg/day if weight > 30 kg	See BMD and fracture outcomes in Figs. 24.3 and 24.5 Significant differences compared to placebo: ↑ Improved QOL scores, except for mobility ↑ Height Z-score ↓ Urinary NTx Non-significant differences compared to placebo: Serum calcium, osteocalcin, PTH, 1,25 $(OH)_2$ vitamin D_3, urinary hydroxyproline	Alendronate group: 2/20 had mild gastrointestinal discomfort; 0/20 in the placebo group

(continued)

Table 24.4 (continued)

Publication, study design, and diagnosis	Number of patients, age (years)	Agent, dose, and route	Main efficacy outcomes	Side effects
Bianchi (2013) RCT, double-blind Cystic fibrosis Duration: 1 year	Treatment group: *N* = 65 Age: mean (SD) = 13.5 (5.3) Placebo group: *N* = 63 Age: mean (SD) = 13.2 (5.1)	Treatment group: Oral alendronate 5 mg/day if weight ≤ 25 kg or 10 mg/day if weight > 25 kg	See fracture outcomes in Figs. 24.4 and 24.5 Significant differences compared to placebo: ↑ LS BMAD ↑ Proportion of patients who attained a normal-for-age bone BMAD Z-score ↓ Serum CTx and urinary NTx ↓ Serum bone-specific alkaline phosphatase Non-significant differences compared to placebo: Serum osteocalcin, PTH	Similar between treatment and placebo groups
Golden (2005) RCT, double-blind Anorexia nervosa Duration: 1 year	Treatment group: *N* = 15 Age: mean (SD) = 16.9 (1.6) Placebo group: *N* = 17 Age: mean (SD) = 16.9 (2.2)	Treatment group: Oral alendronate 10 mg/day	See BMD and fracture outcomes in Figs. 24.3 and 24.4 Significant differences compared to placebo: Significant differences compared to placebo: ↑ Femoral neck vBMD Non-significant differences compared to placebo: Femoral neck and LS areal BMD, bone-specific alkaline phosphatase, urinary deoxypyridinoline	Placebo group: 1 patient discontinued the medication because of dyspepsia Adverse events otherwise similar between groups
Rudge (2005) RCT, double-blind Chronic illness treated with GC therapy Duration: 1 year	Treatment group: *N* = 11 Age: median (min, max) = 8.7 years (6.3, 14.5) Placebo group: *N* = 11 Age: median years (min, max) = 8.0 (4.3, 17.2)	Treatment group: Oral alendronate 1–2 mg/kg once-weekly	See fracture outcomes in Figs. 24.4 and 24.5 BMD: Comparisons between groups not reported Alendronate group: ↑ LS vBMD compared to baseline Placebo group: No change in LS vBMD compared to baseline Non-significant differences compared to placebo: Alkaline phosphatase	No major adverse events in either treatment or placebo group

From: Ward et al. [5]. Reprinted with permission from Springer
Studies were included with the following criteria: (1) prospective comparison of drug versus placebo or untreated controls, (2) at least ten patients per group, (3) outcomes that were compared between, and not just within, treatment and control groups
Abbreviations: *BMC* bone mineral content, *BMD* bone mineral density, *BMAD* bone mineral apparent density, *vBMD* volumetric bone mineral density, *CTx* serum c-telopeptide of type I collagen, *GC* glucocorticoid, *LS* lumbar spine, *NTx* urinary n-telopeptide of type I collagen, *OI* osteogenesis imperfecta, *PTH* parathyroid hormone

data on the number of fracture events in each group). Of the nine studies which permitted calculation of the relative risk of non-VF, only one by Bishop et al. [123] using risedronate in pediatric OI showed a decrease in non-VF risk. The other studies in Fig. 24.4 [122–129] found no significant differences compared to placebo or untreated controls in the relative risks of non-VF after oral alendronate, oral olpadronate, and IV neridronate. At the same time, Fig. 24.4 highlights that

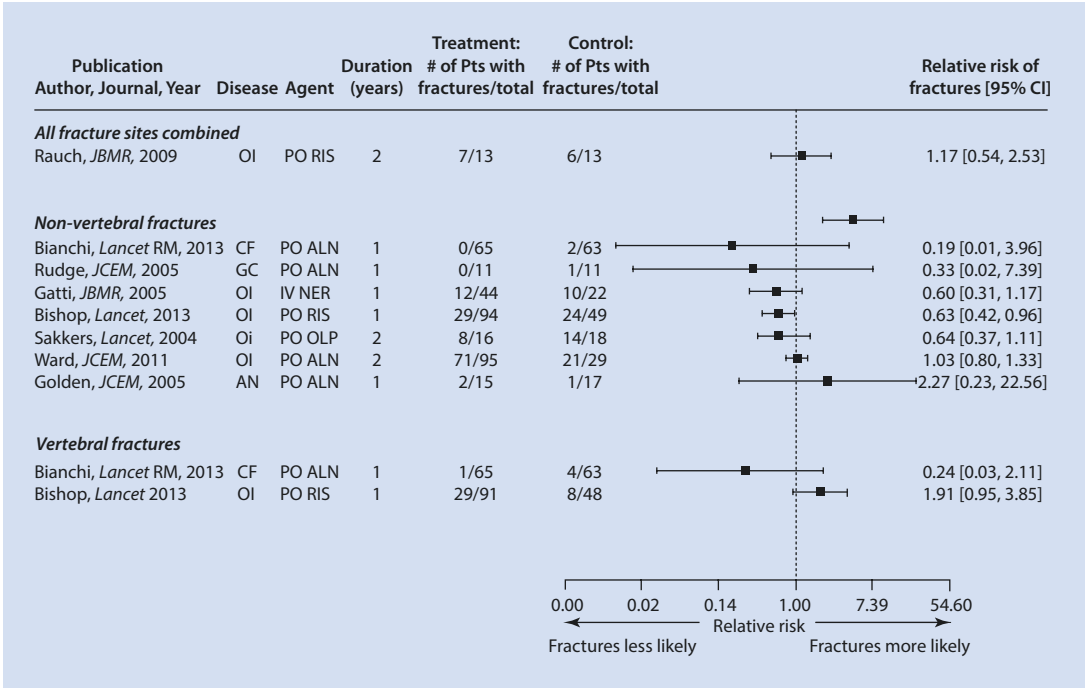


Fig. 24.4 Relative risk of vertebral and non-vertebral fractures in published, controlled trials of intravenous or oral bisphosphonate therapy for the treatment of children with osteoporosis, with comparison of the number of children with fractures in the treatment versus placebo/untreated groups. Studies were included in the figure if they met the following criteria: (1) at least ten patients per group, (2) prospective design with a placebo or untreated control arm, and (3) available data on the number of patients with fractures in each group. Abbreviations: *ALN* alendronate, *AN* anorexia nervosa, *CF* cystic fibrosis, *GC* glucocorticoid-treated, *IV* intravenous, *yrs*. years, *NER* neridronate, *OI* osteogenesis imperfecta, *OLP* olpadronate, *PO* oral, *Pts* patients, *RIS* risedronate (From: Ward et al. [5]. Reprinted with permission from Springer)

the direction of effects for non-VF risks in the nonsignificant studies was favorable for treatment in all but one study [122]. Figure 24.5 shows the incidence rate ratio of fractures using the number of fracture events in the two groups (a more powerful calculation since there are typically more fracture events than patients with at least one fracture). Two studies with nonsignificant results for the relative risk of non-VF had positive results when the incidence rate ratio was calculated [127, 128]. Most of the nonsignificant estimates in Figs. 24.4 and 24.5 had extremely wide confidence intervals but directions of effect in favor of treatment, suggesting that sample sizes were likely inadequate to show differences in fracture rates between the two groups.

So how do we adjudicate whether oral or IV bisphosphonate therapy is more efficacious in the presence of such little controlled data and inadequate sample sizes to determine the effects on fractures? The answer appears to lie in the VF and vertebral body reshaping data. Based on observational studies, it is expected that fractured vertebral bodies will undergo reshaping with bisphosphonate therapy [44, 58, 130, 131], thereby providing a key index of benefit. The controlled trials to date which quantified vertebral body height clearly showed increases in those receiving IV bisphosphonate therapy [127, 132, 133], whereas none of the controlled oral bisphosphonate studies in which it was measured showed a positive effect on vertebral height [124, 128, 134]. Furthermore, in a large randomized trial of daily oral alendronate for moderate and severe pediatric OI [129], there was no effect of alendronate on the cortical width of trans-iliac specimens. In contrast, this is a key structural index derived from a precise measurement which has shown a positive response in OI to IV bisphosphonate therapy [84]. Another compelling observation that supports IV over oral therapy is from a controlled OI trial [124], where risedronate did not

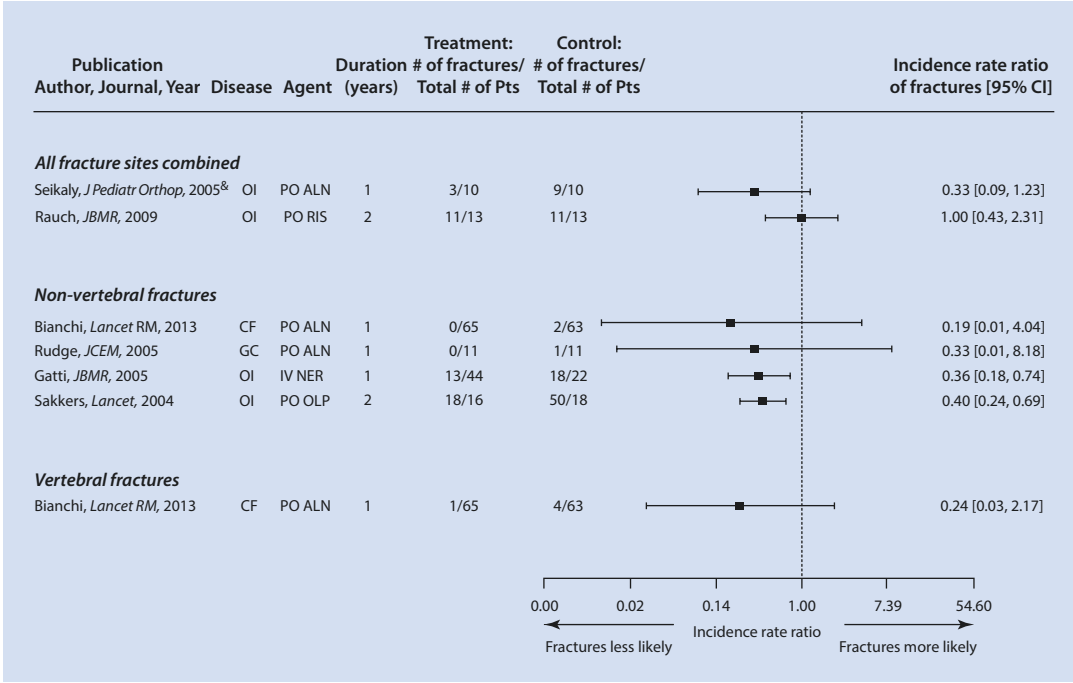


Fig. 24.5 The incidence rate ratio in published, controlled trials of intravenous or oral bisphosphonate therapy for the treatment of children with osteoporosis with comparison of the number of fracture events in the treatment versus placebo/untreated control groups. Studies were included with the following criteria: (1) at least ten patients per group, (2) prospective design with a placebo or untreated control arm, and (3) data available on the number of fractures in each intervention group. &Seikaly 2005 was a placebo controlled crossover study design with the results from the first year of the study presented. Abbreviations: *ALN* alendronate, *CF* cystic fibrosis, *GC* glucocorticoid-treated, *IV* intravenous, *yrs*. years, *NER* neridronate, *OI* osteogenesis imperfecta, *OLP* olpadronate, *PO* oral, Pts patients, RIS risedronate (From: Ward et al. [5]. Reprinted with permission from Springer)

lead to an increase in the trabecular volumetric BMD at the distal radius compared to placebo; on the other hand, IV therapy caused significant increases in BMD at this site [135]. Overall, these data support the use of IV instead of oral bisphosphonate therapy first-line. At the same time, Figs. 24.4 and 24.5 underscore the need for controlled trials of osteoporosis therapies, especially in the secondary osteoporoses where there are only three controlled trials published to date and none sufficiently powered to address any fracture outcomes.

24.6.5 Monitoring the Efficacy of Bisphosphonate Treatment

Gauging the efficacy of bisphosphonate therapy rests on a number of clinical parameters, most of which are focused on the functional musculoskeletal health of the child. One of the main goals of therapy is remittance of back and bone pain which typically occurs within 2–6 weeks following IV bisphosphate therapy [44, 118]. In a child with VF, follow-up spine radiographs should be carried out in order to evaluate a number of efficacy parameters as outlined in Fig. 24.1.

In addition, the history of new non-VF should be recorded, along with details about the site of fracture, degree of trauma associated with the injury, need for surgical management, impact to quality of life, and duration of healing. Improvements in energy level [118], mobility, and muscle strength [136] are also monitored. BMD parameters are tracked as a measure of efficacy following initiation of bisphosphonate therapy; however, there are no studies which have addressed which BMD increment or cutoff is associated with a clinically acceptable decrease in fracture rates post-treatment initiation. In the absence of such data, it is advised that the areal BMD Z-score

should stabilize (if previously on the decline) or increase beyond the precision of the measurement and furthermore, the areal BMD Z-score will approximate the patient's height Z-score. Another approach is to aim for a BMD Z-score > −2 SD [58].

24.6.6 Bisphosphonate Dose Adjustments, Duration of Treatment, and the Effect of Treatment Discontinuation

The most frequently prescribed IV bisphosphonate regimen is cyclical IV pamidronate (maximum dose 9 mg/kg/year for children ≥3 years, 3 mg/kg divided equally over 3 days given every 4 months) [6, 83, 116, 118, 137]. Due to high bone turnover in younger children, pamidronate is dosed more frequently (2.25 mg/kg divided equally over 3 days, every 3 months for children 2–3 years of age, and 1.5 mg/kg divided equally over 3 days, every 2 months to children <2 years of age). Zoledronic acid is increasingly used in clinical care due to its ease of less frequent dosing intervals and shorter infusion time compared to pamidronate (maximum dose 0.1 mg/kg/year given as two equal doses (0.05 mg/kg) every 6 months in children ≥ 2 years and 0.025 mg/kg every 3 months in children <2 years) [119, 138, 139]. Some investigators have favored a lower annual starting dose (such as a single-day pamidronate infusion 1 mg/kg every 3 months, 4 mg/kg/year) [140, 141]. Apart from these regimens, other IV doses and intervals have also been reported (Table 24.4) though none has gone head to head in controlled, comparative trials, the exception being pamidronate versus zoledronic acid which showed similar effects on BMD and fracture rates in OI [119]. With such little controlled comparative data, it is impossible to state which IV agents and regimens achieve the best results for mitigating fractures and pain and improving overall function. Regardless, bisphosphonate therapy should only be administered by clinicians with the appropriate expertise and infrastructure to support peri-infusion care, and the maximum, published annual doses should not be exceeded so as to avoid iatrogenic osteopetrosis arising from toxic doses [142].

The approach to dose adjustments and the duration of bisphosphonate therapy are also questions frequently posed by pediatricians. A number of key observations unique to children have influenced practice in this regard. The first observation has led to continuing bisphosphonate therapy until final height attainment in those with permanent or persistent risk factors, as follows. Among children with open epiphysis and ongoing endochondral bone formation, following treatment discontinuation the newly formed bone adjacent to the growth plate will be "treatment naïve" and thereby low density, creating a stress riser between high (previously treated) and low (untreated) density bone [135]. Not surprisingly, metaphyseal fractures have occurred post-bisphosphonate discontinuation in children with OI (i.e., persistent risk factors for low bone density) at the interface between the treated and untreated bone [143]. In fact, metaphyseal fractures have even occurred *during* intermittent IV bisphosphonate therapy at the interface between the dense metaphyseal lines created at the time of therapy and the (2 mm) adjacent treatment-naïve bone [144]. This latter report raises the question whether IV bisphosphonates should be administered with as short an infusion interval as possible, a line of thinking that is challenged by the demands on the patient from frequent infusions.

Further support for continuation of therapy to final height in those with persistent or permanent risk factors arises from a study by Rauch et al. [143]. These investigators showed using pQCT that there were significant declines in trabecular BMC Z-scores at the distal radius following pamidronate discontinuation in children with OI who were still growing. On the other hand, discontinuation after epiphyseal fusion was associated with more stable BMD Z-scores 2 years later. Balancing these observations with the lingering concern about over-suppression with longer-term therapy, the current recommended approach is to treat patients initially with a higher-dose regimen until the patient is clinically stable (Fig. 24.1). Usually this equates to a minimum of 2 years, the time point at which the maximum benefit from bisphosphonate therapy has been observed in children with OI [84]. Once the patient is clinically stable, a lower (half-dose or less) [58, 145] maintenance protocol is given until the patient attains final adult height, at which time treatment can be discontinued if the patient is stable [58]. The goal of the maintenance phase of therapy in

children with permanent or persistent risk factors is to preserve the gains realized during high-dose therapy while avoiding overtreatment [58, 145]. To this end, the dose of IV bisphosphonate therapy in the maintenance phase may require further downward titration to avoid unnecessarily high BMD Z-scores – this can be achieved by decreasing the dose or by increasing the interval between infusions. Palomo et al. [58] recently reported that long-term (at least 6 years) bisphosphonate therapy with downward dose titration in pediatric OI led to higher BMD Z-scores compared to historical controls and to vertebral body reshaping, although it was notable that non-VF rates were still high and most patients developed scoliosis. An outstanding question about the duration of therapy in those who stop around the time of adult height attainment but have persistent risk factors for fractures (e.g., OI, ongoing GC exposure) is whether they will require reintroduction of bisphosphonate therapy in the adult years and, if so, at what time point.

In children with resolution of risk factors during growth (i.e., cessation of GC therapy, resolution of inflammation, recuperation of mobility), discontinuation of therapy can be considered once the child has been fracture-free (VF and non-VF) for at least 6–12 months, previously fractured vertebral bodies have stabilized or undergone reshaping, and BMD Z-scores are appropriate for height. Reintroduction of therapy may be required during growth if the prior risk factors for osteoporosis recur and patients once again meet the criteria for treatment initiation.

24.6.7 Bisphosphonate Therapy Side Effects and Contraindications

24.6.7.1 Short-Term

The most frequent side effects of bisphosphonate therapy, reported with both oral and IV treatment [118, 121, 127], are collectively referred to as "the acute phase reaction" and include fever, malaise, back and bone pain, nausea, and vomiting. These symptoms usually begin 24–72 h following the initial dose, remit over a few days, typically do not occur with subsequent infusions or oral doses, and are effectively managed with anti-inflammatory and antiemetic medications. Asymptomatic hypocalcemia is frequent even with repeat infusions (though most marked with the first), reaching a nadir usually 1–3 days post-infusion [83]. The frequency of first-dose hypocalcemia appears to be mitigated by reducing the initial dose [130], a practice that is now in widespread use. Interestingly, a lower dose with the first infusion does not appear to mitigate the frequency of acute phase side effects [130]. Symptoms have been reported in up to 30% of children with first-infusion hypocalcemia [44, 130]. This has led to the widespread practice of prescribing calcium supplementation at published doses [100] for 5–10 days following the first bisphosphonate infusion, as well as ensuring vitamin D adequacy pre- and posttreatment. Children at risk for either hypocalcemia or its consequences (i.e., children with hypoparathyroidism or seizure disorders) may require even more aggressive hypocalcemia prevention such as an active form of vitamin D. Untreated hypocalcemia, hypophosphatemia, vitamin D deficiency, and rickets/osteomalacia are contraindications to bisphosphonate therapy. In these cases, the underlying vitamin D and/or mineral ion deficiency must be adequately treated before bisphosphonate therapy is administered (i.e., 25-hydroxyvitamin D level $\geq$ 50 nmol/L (20 ng/mL) and calcium intake sufficient for age).

The more serious acute side effects associated with bisphosphonate therapy in adults (such as uveitis, thrombocytopenia, and mucosal ulcerations with oral agents) are rare in children. Furthermore, a recent review of bisphosphonates in adults concluded that there is no link between bisphosphonates and atrial fibrillation, while the association between oral agents and esophageal cancer remains inconclusive [146]. In any patient with poor renal function (estimated glomerular filtration rate < 35 ml/min), bisphosphonates are contraindicated. Recently, the US Food and Drug Administration updated the label for zoledronic acid, stating it is also contraindicated in patients with acute renal impairment and that patients should be screened for renal insufficiency prior to

initiating treatment. To this end, it should be noted that serum creatinine may not be a reliable marker of renal function in those with myopathies such as DMD, raising the need for other measures such as cystatin C to ensure adequate renal function prior to each zoledronic acid infusion. In our center, we also verify normal renal function prior to all pamidronate infusions.

24.6.7.2 Long-Term

Concern about the effects of bisphosphonates on linear growth have ultimately been quelled by studies which confirm expected growth rates in children with bisphosphonate-treated OI [137] and osteoporosis [147]; there are even reports of improved growth with long-term bisphosphonate therapy [58], likely attributable to a positive effect on vertebral height. On the other hand, chronic bone turnover suppression has two rare but serious sequelae in adults: osteonecrosis of the jaw (ONJ) and atypical subtrochanteric or metaphyseal "fatigue" fractures (AFF). Both are proposed to arise from accumulated microdamage due to suppressed osteoclast activity. ONJ is defined as exposed bone in the maxillofacial area that does not heal within 8 weeks following identification by a health-care provider, in the absence of radiation therapy [148]. In children, there are no reports of ONJ despite three studies which examined over 350 bisphosphonate-treated children with OI following dental procedures [149–151]. Despite the lack of reported ONJ in children to date, one position statement has nevertheless recommended to safeguard the bisphosphonate-treated child's oral health by referral to a dentist prior to bisphosphonate initiation, completion of necessary invasive dental procedures prior to treatment initiation, regular dental evaluations by a dentist during treatment, and good daily oral hygiene [152].

AFF are also rare in adults, and while there is no direct causal link between bisphosphonates and AFF, the number of case series and cohort analyses suggesting an association is increasing, as summarized in a recent report [146]. These fractures are located in the subtrochanteric region or femoral shaft, arise from minimal or no trauma, and are characterized by transverse or short oblique fracture lines without comminution and a medial spike when the fracture is complete [153]. They are often bilateral (in up to two-thirds of cases) and may be associated with prodromal thigh pain. In the pediatric setting, Hegazy et al. [154] reported unusual femur stress fractures in children with OI and intramedullary rods on long-term bisphosphonate therapy (6–11 years); two patients had a "drug holiday" of 18–24 months prior to the femoral fractures. Of 72 children on IV pamidronate therapy, 18 had femur fractures and of these, 6/72 met the adult criteria for AFF (8%). All children had intramedullary rodding, none of the fractures were displaced, and all were treated successfully with protected weight-bearing and a hiatus from bisphosphonate therapy. While the duration of bisphosphonate therapy in those with AFF was reported in this study [154], there was no record of the frequency of such fractures in bisphosphonate-naïve children, nor the approach to pamidronate dosing (starting dose, maximum dose, dose titration and total cumulative pamidronate dose). As such, it is difficult to know whether these results are generalizable to other centers; nevertheless, the observation is call for concern and underscores the need for clinicians to report similar observations. Whether downward dose titration with long-term therapy such as currently practiced can obviate AFF remains unknown. Similarly, the benefits and risks of drug holidays in children with permanent or persistent bone health threats needing long-term therapy remain unexplored. Although rare, AFF have led adult care providers to consider drug holidays in those with a low risk of first-ever fractures and in those with a moderate risk who are clinically well after 3–5 years of therapy [146]. High-risk adult patients – those with a history of bone fragility or a T score ≤ -2 SD – are not considered candidates for drug holidays [146].

Delayed osteotomy but not fracture healing has been shown in children with bisphosphonate-treated OI and intramedullary rods; higher mobility scores was the only positive predictor of delayed healing that was identified [155]. This observation has led to withholding bisphosphonate therapy in the week leading up to surgery, and withholding therapy following intramedul-

lary rodding until adequate fracture healing has been documented on x-ray, usually about 4 months. Surgical management has also switched to the use of an osteotome instead of a power saw. With these changes to medical and surgical management, a recent study has reported a significant reduction in the frequency of delayed osteotomy healing [156].

Since the skeleton acts as an endogenous reservoir of bisphosphonates that theoretically can be mobilized in subsequent years, concern has been raised about the safety of preconceptual use. Despite this theoretical concern, there have been no human reports to date of a significant adverse effect of bisphosphonates when administered either preconception or during pregnancy. This appears to stem from the fact that the amount of bisphosphonate mobilized from the skeleton in subsequent years is clinically insignificant. For example, data from Papapoulos [157] shows that 4–10 years after daily oral pamidronate administration to children with osteoporosis, a maximum of 0.13 mg/kg/year is excreted in the urine (less than 0.02% of the annual dose). The fact that the amount released from the skeleton is clinically insignificant is supported by numerous human reports. Reviews of women or girls who have received bisphosphonates preconception or during pregnancy reported an absence of skeletal abnormalities or congenital malformations in the infants, apart from marginal decreases in gestational age, weight, and transient, asymptomatic hypocalcemia [158–161]. While these data are reassuring, clinicians should ensure that menstruating females have negative pregnancy tests prior to each infusion and/or they are using a medically approved form of contraception if sexually active.

24.7 Novel Therapies

A number of important signaling pathways that modulate bone mass have led to novel drug developments in recent years. RANKL is an essential mediator of osteoclast formation, function, and survival [162], and both preclinical and clinical data suggest that inhibition of RANKL is a viable strategy for the treatment of osteoporosis [163]. Denosumab is a human, monoclonal antibody administered subcutaneously that targets RANKL to prevent the activation of RANK, thus inhibiting bone resorption and increasing bone strength at both trabecular and cortical sites without directly interacting with bone surfaces [164]. A large study on close to 8000 women with postmenopausal osteoporosis (the FREEDOM trial) showed that denosumab 60 mg every 6 months reduced vertebral, non-vertebral, and hip fracture risk without an increased risk of side effects compared to placebo [165]. Given its convenient route of administration, favorable safety profile and proven efficacy in adults, denosumab now merits exploration in children. To date, its use on compassionate grounds has been reported in a few children with osteoporosis due to OI (type VI, a subtype which is not as responsive to IV bisphosphonate therapy as other OI forms) [166] and in children with giant cell tumors [167], aneurysmal bone cysts [168], and fibrous dysplasia [169]. Importantly, there is no evidence to date of an adverse effect of denosumab on human growth plate activity [170].

Sclerostin, the product of the *SOST* gene, binds to LRP5/6 receptors and is a powerful inhibitor of the canonical Wnt signaling pathway that results in decreased bone formation. In a mouse model of moderate-severe OI, anti-sclerostin antibody resulted in improved bone mass and reduced long bone fragility [73]; emerging studies in humans show similar promise [171, 172]. Not surprisingly, sclerostin levels are elevated in patients with bone loss due to immobilization disorders, a clinical setting that may benefit from sclerostin suppression. Another novel agent, odanacatib, is a potent and selective inhibitor of cathepsin K (Cat K), which suppresses CatK-mediated bone resorption, but it does not suppress bone formation to the same extent as bisphosphonate therapy [173]. This oral agent is interesting in the chronic illness osteoporosis and GC-induced bone fragility settings where bone turnover is typically low [44, 85]; however, clinical trials in adults have shown an increased risk of atrial fibrillation and stroke and have subsequently been halted [174]. Finally, excessive transforming growth factor-β (TGF-β) signaling has been implicated in the pathogenesis of both *CRTAP* recessive and type I collagen dominant OI; anti-TGF-antibody rescues the phenotype in both forms of the disease, garnering interest in other high bone turnover osteoporotic states.

Case Studies

The following two cases highlight disparate clinical trajectories depending on whether osteoporosis is diagnosed and treated in its earlier versus later stages. In the first case, a 14-year-old boy presented to a bone health clinic with back pain following a history of GC therapy for 8 years due to severe asthma. A spine x-ray (Fig. 24.6a) confirmed multiple grade 3 (severe) vertebral fractures. Intravenous bisphosphonate therapy was initiated which resulted in significant back pain relief; however, the patient was close to final adult height attainment at the time of presentation; therefore, there was insufficient time to completely reshape vertebral bodies resulting in permanent vertebral deformity. At the same time, the signs of early vertebral collapse were evident on chest x-rays carried out years before to assess his respiratory status. This case highlights the lack of routine spine health surveillance in a child with a chronic, GC-treated illness that resulted in life-long spine deformity. As discussed previously in this chapter, adults with permanent vertebral deformity have been shown to suffer from chronic back pain and functional limitation, raising the importance of early identification and treatment of spine collapse in those with risk factors through periodic spine radiographs.

In contrast, an 8-year-old boy with DMD was referred to the pediatric bone health clinic for a bone health evaluation shortly after starting deflazacort 0.9 mg/kg/day. At 10 years of age, he showed early signs of vertebral collapse (Fig. 24.6b, Genant grade 1 VF at T9 and T10) and had minimal back pain elicited on direct questioning. He was started immediately on intravenous zoledronic acid therapy as per the dosing guidelines in Fig. 24.1; 7 years later, despite continued high-dose GC therapy, his vertebral fractures have undergone reshaping, and there were no new vertebral fractures nor back pain. This case highlights the success of early monitoring and intervention, which in this case allowed the patient to remain on GC therapy without any further signs of osteotoxicity.

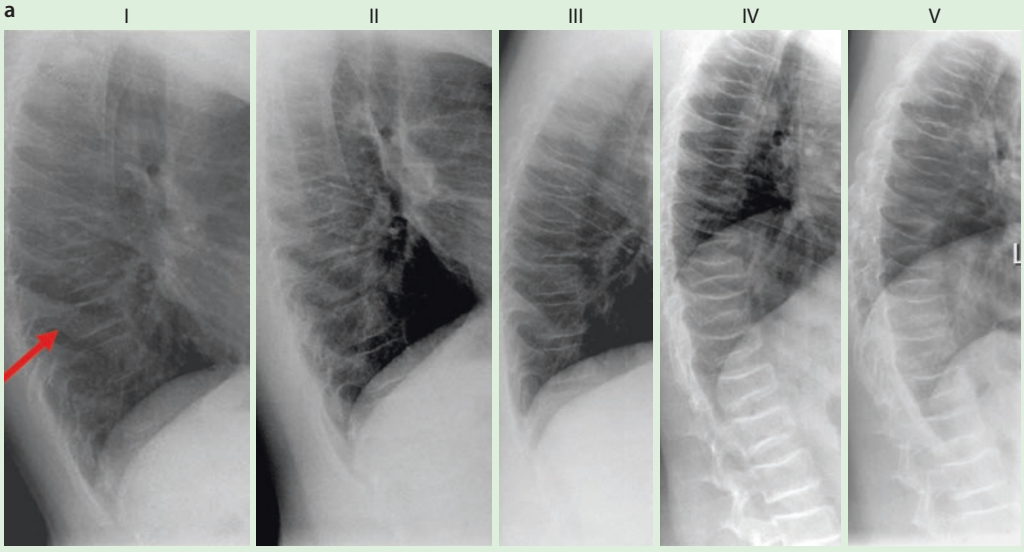


Fig. 24.6 **a** (I) 10-year-old boy on high-dose GC therapy for asthma since the age of 4 years. Early signs of vertebral collapse on a radiograph taken to assess his respiratory status at 10 years of age. No treatment or bone-specific monitoring was initiated. (II to IV) No significant deterioration in vertebral collapse despite ongoing GC therapy. (V) Presented with back pain at age 14 years due to multiple severe vertebral fractures. After 1 year of intravenous bisphosphonate therapy, back pain was improved but permanent vertebral deformity was presented due to final adult height attainment in the interim. **b** (I): 10-year-old boy with GC-treated DMD and back pain. X-ray shows early signs of vertebral fractures at T9 and 10 (Genant Grade 1 (mild) VF). (II) Lateral spine radiograph following 7 years of treatment with intravenous bisphosphonate therapy. Back pain has gone and vertebral bodies are dense and have undergone reshaping

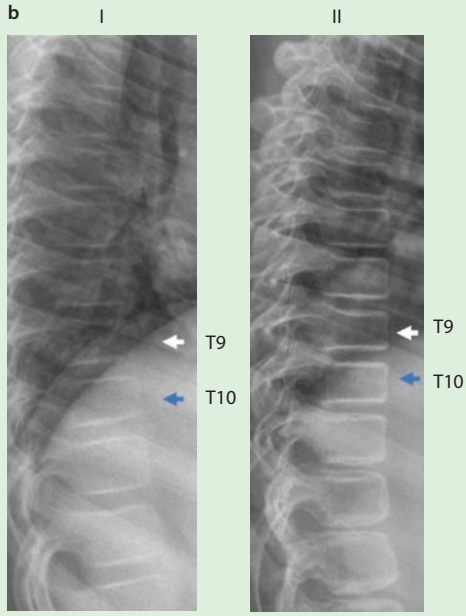


Fig. 24.6 (continued)

24.8 Summary and Future Directions

There have been significant advances in the pediatric osteoporosis field over the past decade following identification of numerous heritable bone fragility genes, including those associated with phenotypes that lack the classic features of OI. In children with chronic illness, we now better understand the frequency of fractures and time points at which they are most likely to occur, as well as clinical predictors of fractures and potential for spontaneous recovery. This knowledge has facilitated the development of logical osteoporosis monitoring strategies in children with chronic illnesses and improved our understanding about best candidates for osteoporosis therapy. Advances in our understanding of the pathobiology of osteoporosis have led to recently discovered novel drug therapies which hold promise for children with both high and low bone turnover states; their efficacies and safety now merit testing in well-designed trials. As pediatric researchers go forward, there is a need for greater consensus on the methods and clinical outcomes for reporting treatment trials so that data across studies can be better aggregated in order to draw overall conclusions. Importantly, children are not small adults and this is particularly true in the study of skeletal disorders, where bone growth and modeling distinguish the pediatric skeleton from that of the more staid adult situation. A classic example is that vertebral bodies can undergo medication assisted *and* unassisted reshaping following fractures; at the same time, declines in BMD can be profound when treatment is stopped while the child is still growing. As well, pediatricians, funding agencies, and policy makers need to consider the challenges to clinical care that are created by lack of controlled clinical trials that address these issues [175]. Overall, optimal bone strength across the life span rests on outcomes which take place during the time when the skeleton is under construction – the growing years. This reminds us that it is incumbent upon the pediatric bone health communities to champion the diagnosis, treatment, and study of osteoporosis in childhood.

Review Questions

1. What are the two main risk factors for osteoporosis in children with chronic illnesses?
2. What is the definition of osteoporosis in children?
3. What is the preferred first-line therapy to treat osteoporosis – oral or IV bisphosphonate drugs?
4. Name at least one absolute contraindication to zoledronic acid therapy.
5. What are the clinical goals of treatment efficacy in those prescribed bisphosphonate therapy? Name at least five.
6. What are the most common side effects of intravenous bisphosphonate therapy? How are these monitored and treated?

Answers

1. Glucocorticoid therapy and impaired mobility.
2. The definition of osteoporosis in children is based on the presence of a clinically significant fracture history: either a low-trauma vertebral fracture at any age or a low-trauma long bone fractures (≥2 or more long bone fractures by age 10 and ≥ 3 long bone fractures by age 19). In the presence of long bone fractures but

absent vertebral fractures, a size-corrected BMD or BMC Z-score ≤ 2.0 is recommended; however, a BMD or BMC Z-score > 2.0 does not preclude an increases fracture risk [30].

3. IV bisphosphonates.
4. Glomerular filtration rate < 35 ml/min or acute renal impairment.
5. Absence of new VF, absence of new long bone fractures, absence of deterioration of existing VF, reshaping of VF (restoration of vertebral dimensions), improved mobility, resolution of back pain due to VF, improved quality of life.
6. First infusion side effects (fever, malaise, bone pain, nausea, vomiting – monitored clinically and treated with anti-inflammatory medications, antipyretics, and anti-nausea agents), hypocalcemia (prevented by ensuring calcium and vitamin D sufficiency pretreatment (documented with serum levels)), treated with calcium and vitamin D supplementation and occasionally 1,25-dihydroxyvitamin D_3 therapy.

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Conflict of Interest

LMW has been a consultant to Novartis, Amgen, and Alexion Pharmaceuticals and has participated in clinical trials sponsored by Novartis and Amgen. JM has no conflicts of interest to disclose.

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Reproductive Disorders and Contraception

Delayed Puberty and Hypogonadism

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Key Points

- Delayed puberty is defined as the absence of any sign of puberty in a child at a chronologic age 2 standard deviations above the mean age of pubertal development for a given population.
- Causes of delayed puberty include variants of normal puberty timing, functional disorders leading to transient hypogonadism, hypogonadotropic hypogonadism, and hypergonadotropic hypogonadism.
- Constitutional delay of growth and puberty does not require treatment; however, short-term low-dose sex hormone administration may be considered.
- In most cases, the diagnosis of idiopathic hypogonadotropic hypogonadism should be delayed until 18 years of age in the setting of absent pubertal development.

25.1 Introduction and Background

Following the mini-puberty of the fetal and neonatal period, the hypothalamic-pituitary-gonadal (HPG) axis undergoes a period of quiescence in childhood and is subsequently reactivated at the onset of puberty. This reactivation is due to a complex interplay of genetic, environmental, and nutritional factors. Puberty is initiated by the release of tonic inhibition of pulsatile secretion of gonadotropin-releasing hormone (GnRH) from the hypothalamus in the setting of increased stimulation of GnRH secretion. Kisspeptin is a principal regulator of the secretion of GnRH. Following paracrine stimulatory and inhibitory inputs from neurokinin B and dynorphin (KNDy neuropeptides), kisspeptin signals directly to GnRH neurons to control pulsatile GnRH release. These pulses cause release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the pituitary gland. These pituitary gonadotropins then circulate to the gonads and stimulate production of sex steroids, predominantly estrogen from the ovary and testosterone from the testes [1].

Delayed puberty is defined as the absence of any sign of puberty in a child at a chronologic age 2 standard deviations above the mean age of pubertal development for a given population. This is defined as a testicular volume remaining less than 4 mL at 14 years in a boy or absence of any breast development at 13 years in a girl [2, 3]. In addition, pathologic abnormalities may be associated with abnormal progression through puberty once initial pubertal changes have begun and are worthy of further evaluation. In boys, a period of 3.2 ± 1.8 (mean ± SD) years is necessary to achieve adult testicular volume after the onset of puberty. In girls, the period from breast budding to menarche is 2.4 ± 1.1 (mean ± SD) years [4]. Therefore, evaluation is warranted if more than 4–5 years has elapsed from the onset of puberty to adult testicular size in boys or menarche in girls. Typically further evaluation is pursued in girls in the absence of menarche by age 16 or after 3 years from the onset of breast bud development. Further evaluation is necessary to determine the etiology of pubertal delay and for determination of necessary therapy. Clinical and laboratory assessment is aimed at differentiating a lag in normal pubertal development from abnormalities in need of further investigation and/or therapy.

25.2 Etiology

The differential diagnosis of pubertal delay is extensive but can most easily be divided into four categories. The first two categories represent transient forms of hypogonadotropic hypogonadism including constitutional delay of growth and puberty, which is a variant of normal pubertal timing, and functional disorders leading to delayed puberty. The other two categories refer to typically permanent forms of hypogonadism including hypogonadotropic (central) hypogonadism, in which hypothalamic or pituitary failure results in deficiency of circulating gonadotropins and hypergonadotropic (peripheral) hypogonadism, which results from primary gonadal failure, with subsequent lack of negative feedback of sex steroids at the hypothalamic and pituitary levels resulting in elevated serum gonadotropin levels. Table 25.1 lists the various etiologies of hypogonadism resulting in delay, failure, or arrest of pubertal development.

Table 25.1 Etiologies of delay or failure of pubertal development

Variant of normal puberty timing	*Hypogonadotropic hypogonadism*	*Hypergonadotropic hypogonadism*
Constitutional delay of growth and/or puberty Sporadic Familial	Congenital Monogenic mutations Kallmann syndrome (e.g., *KAL1*) Normosmic idiopathic hypogonadotropic hypogonadism (e.g., *GNRHR*) Gonadotropin gene mutations (*LHβ*, *FSHβ*) Multiple pituitary hormone deficiencies Idiopathic Genetic (e.g., *PROP-1)* Adrenal hypoplasia congenital (*DAX1*) Obesity-related (*LEP*, *LEPR*, *PCSK1*) Syndromic (Prader-Willi, CHARGE, Lawrence-moon, Bardet-Biedl syndromes) Acquired Suprasellar tumors (e.g., craniopharyngiomas) Infiltrative/inflammatory (e.g., histiocytosis X) Effects of radiotherapy Effects of surgeryCranial trauma	Congenital Males Klinefelter syndrome Pure gonadal dysgenesis Defects in steroidogenesis Leydig cell hypoplasia (*LHCGR)* Androgen insensitivity syndromes Testicular regression syndrome or cryptorchidism Noonan syndrome Females Turner syndrome Pure gonadal dysgenesis Gonadotropin receptor mutations (*LHCGR*, *FSHR*) Androgen insensitivity syndromes Estrogen receptor-alpha mutation (*ESR1)* Noonan syndrome Acquired Males Surgical or traumatic castration Bilateral orchitis Chemotherapy, radiotherapy effects Hemochromatosis Females Surgical or traumatic castration Premature idiopathic ovarian failure Autoimmune ovarian failure Fragile X (*FMR1*) premutation carrier Chemotherapy, radiotherapy effects Galactosemia
Functional and systemic disorders Nutritional disorders Malnutrition Anorexia nervosa Excessive energy expenditure, exercise Psychological or emotional stress Chronic illness (gastrointestinal disease, renal failure, hepatic disease, hematologic abnormalities, malignancy, pulmonary disease, etc.) Endocrinopathies Diabetes mellitus Growth hormone deficiency Hypothyroidism Hyperprolactinemia Glucocorticoid excess Medication or drug effect (i.e., anabolic steroids, opiates)		

25.2.1 Variant of Normal Puberty Timing

Pubertal onset at the extreme of the distribution of normal pubertal timing, as opposed to overt pathology, accounts for the majority of cases of delayed puberty (65% of males and 30% of females) in both genders. Despite its high frequency, constitutional delay of growth and puberty remains a diagnosis of exclusion. While many alternate causes of delayed puberty due to organic conditions may be evident from the history, physical examination, and biochemical evaluation, differentiating constitutional delay of puberty from idiopathic hypogonadotropic hypogonadism can be challenging [5].

25.2.1.1 Constitutional Delay of Puberty

The most common cause of delay is constitutional delay of growth and puberty, which is a transient form of hypogonadotropic hypogonadism. These children represent the extreme of the normal physiologic variations in the timing of the onset of puberty, on average 3 years delayed in boys and 2.5 years in girls. Constitutional delay of growth and puberty occurs twice as frequently in boys compared to girls. This may partly be explained by a higher degree of social pressure placed on boys with delayed development and, subsequently, a higher frequency of referral for evaluation. Frequently, there is a family history of late menarche in the mother or sisters or a delayed growth spurt in the father, suggesting

an autosomal dominant inheritance pattern. Boys and girls with constitutional delay frequently have a positive family history of delay (50–70%) with both parents contributing to this genetic predisposition [6, 7]. However, sporadic cases are also seen, and a lack of family history does not exclude this diagnosis. Children with constitutional delay have a longer duration of the childhood phase of growth, delayed skeletal maturation, shorter interval from pubertal onset to pubertal growth velocity, and delayed yet attenuated pubertal growth spurt. Height and growth velocity are appropriate when evaluated in the context of bone age. The adult height is often shorter than predicted by the midparental height or predicted height from a bone age, on average 1.85 standard deviations below the mean. In addition, familial short stature co-occurs in 40% of cases [8]. Ongoing concerns about compromised bone mineral density and fertility warrant the need for future studies [9].

25.2.2 Functional and Systemic Disorders Leading to Delayed Pubertal Onset

25.2.2.1 Functional Disorders

Patients with nutritional disorders, including anorexia nervosa, may present with delays in growth and/or pubertal development [10]. In the case of anorexia nervosa, the cause is most likely both lack of energy intake and a central nervous system effect altering neuroendocrine control of gonadotropins [11, 12]. Excessive energy expenditure, such as that seen in young gymnasts and long-distance runners, causes pubertal delay by similar mechanisms [13, 14]. Psychological and emotional stress can also lead to delayed pubertal onset.

25.2.2.2 Chronic Systemic Disease

A variety of chronic diseases are associated with delayed growth and puberty and may be diagnosed in the context of endocrinologic evaluation of an otherwise asymptomatic child. Gastrointestinal disorders such as inflammatory bowel disease or celiac disease, as well as chronic renal failure, cardiac disease, and other severe chronic illnesses are causes of pubertal delay. These disorders are usually associated with impaired availability or utilization of fuels, although clinical evidence of malnutrition may be absent.

25.2.2.3 Endocrinopathies

Other endocrinopathies can also be associated with delays of puberty and growth. Isolated growth hormone deficiency is an important differential diagnosis in children with constitutional delay of growth and puberty, because both present with decreased growth velocity for chronologic age and bone age retardation. Furthermore, growth delay in children with constitutional delay may develop early and therefore make the distinction between these two entities more difficult. Delay or arrest in pubertal development may also be caused by acquired hypothyroidism and hyperprolactinemia. Type 1 diabetes mellitus may be associated with delays in pubertal development, even with optimal glycemic control [18]. Cushing's syndrome may cause delayed growth and puberty, though it may also result in premature development of sexual hair.

25.2.2.4 Drug Effects

A history of the use of medications, recreational drugs, and supplements must be included in the evaluation. Anabolic steroid, opiate, and marijuana use are well-known causes of hypogonadism. However hidden sources of steroids or opiates may be found in easily obtained supplements, such as Kratom, or *Mitragyna speciosa*, a plant in the coffee family, which is believed to bind to the mu-opioid receptor and can result in hypogonadism [15–17].

25.2.3 Hypogonadotropic Hypogonadism

Disorders in this category are characterized by low or low-normal circulating levels of the pituitary gonadotropins, LH and FSH. This may be the result of genetic defects altering hypothalamic and/or pituitary development, defects in hormonal synthesis or action, or acquired due to intracranial disease or trauma.

25.2.3.1 Congenital Hypogonadotropic Hypogonadism

Defects in the development of GnRH neurons, impaired GnRH secretion, and GnRH resistance are causes of idiopathic hypogonadotropic hypogonadism (IHH). To date, 25 genes have been described in association with IHH. These single-gene defects account for approximately 35–45%

of all cases of IHH [19]. Identification of these genes has been difficult due to the rarity disease, small kindreds due to reproductive abnormalities, incomplete penetrance and variable expressivity affecting phenotype, and overlapping phenotypes [20, 21]. IHH is frequently divided into two categories: IHH with anosmia (Kallmann syndrome) and normosmic idiopathic hypogonadotropic hypogonadism (nIHH). Kallmann syndrome results when embryonic aplasia of GnRH neurons occurs in the context of developmental defects of the olfactory bulb. However, genes involved in GnRH neuronal migration associated with Kallmann syndrome may also present with normosmic IHH [22, 23].

25.2.3.2 Kallmann Syndrome

Kallmann syndrome is a well-recognized form of hypogonadotropic hypogonadism consisting of gonadotropin deficiency accompanied by anosmia or hyposmia, found in approximately 50% of cases of IHH [22]. It is frequently transmitted as an X-linked recessive trait but may less commonly also have autosomal dominant or recessive inheritance patterns. The X-linked form of Kallmann syndrome has been associated with defects in the *KAL1* gene, located on the pseudoautosomal region of Xp [20]. The protein product of the *KAL1* gene, anosmin-1, has neural cell adhesion molecule properties and provides scaffolding for GnRH neuron and olfactory nerve migration across the cribriform plate to their appropriate synapses [24]. In the absence of anosmin-1, GnRH and olfactory fibers do not synapse properly, producing GnRH deficiency and anosmia. However, *KAL1* gene mutations account for only about 10–20% of individuals with KS. Autosomal dominant forms of Kallmann syndrome can occur with mutations in *FGF8*, *FGFR1*, *SEMA3A*, *HS6ST1*, *FGF17*, *IL17RD*, *DUSP6*, *SPRY4*, and *FLRT3*. Autosomal recessive forms of Kallmann syndrome also have been reported in conjunction with mutations in *PROK2*, *PROKR2*, *CHD7*, and *WDR11*. These collectively account for 25–30% of KS [25]. The cause in the remainder of cases remains unclear. Individuals with Kallmann syndrome may also have a variety of other associated anomalies which may direct genetic evaluation such as unilateral renal agenesis in *KAL1* mutations and dental agenesis or digital bone abnormalities in *FGF8* or *FGFR1* mutations.

25.2.3.3 Normosmic Idiopathic Hypogonadotropic Hypogonadism

Mutations in some genes (e.g., *FGFR1*, *PROK2*, *PROK2R*, *CHD7*, and *FGF8*) overlap in causing Kallmann syndrome and normosmic IHH. The reasons for the variation in phenotype remain unclear but imply not only a role in migration but potentially the secretion of GnRH. Autosomal recessive forms of nIHH have been associated with mutations in *GNRH1*, *GNRHR*, *TAC3*, *TAC3R*, *KISS1*, and *KISS1R* (*GPR54*), all of which cause variations in GnRH secretion or response [26, 27]. Mutations in *GNRHR* are the most common cause of normosmic IHH. Identified mutations in these genes account for approximately 50% of cases of nIHH [20]. Digenic cases of IHH have been reported with individuals having mutations in two or more genes known to cause GnRH deficiency.

25.2.3.4 Other Inherited Forms of Hypogonadotropic Hypogonadism

A host of disorders causing IHH associated with neurodegenerative syndromes have now been described, such as Gordon Holmes syndrome, which is associated with cerebellar ataxia and atrophy. Inactivating mutations in *PNPLA6*, *RNF216*, *OTUD4*, and *STUB1* have been associated with this syndrome. Other mutations associated with neurodegenerative syndromes and IHH include mutations in *POLR3A*, *POLR3B*, *RAB3GAP1*, *RAB3GAP2*, *RAB18*, and *TBC1D20* [28]. Leptin deficiency and leptin receptor defects have also been associated with hypogonadism. Patients with defects in leptin production (*LEP*) or action (*LEPR*) typically have extreme obesity and hyperinsulinemia, in addition to hypogonadism [29]. Deficiency in proprotein convertase 1, a rare form of monogenic obesity, can also lead to hypogonadotropic hypogonadism due to mutations in *PCSK1* [30].

Several patients with combined pituitary hormone deficiency, characterized by deficiencies in growth hormone, TSH, prolactin, and gonadotropins, have been shown to have mutations in the *PROP1* gene [31, 32]. *PROP1* is a transcription factor necessary for differentiation of multiple pituitary cell lineages, and its absence results in lack of proper pituitary cellular development. Mutations

in *LHX3*, *HESX1*, and *SOX2*, other genes important in pituitary development and function, are also associated with IHH in the context of combined pituitary hormone deficiencies [33].

DAX-1 is a nuclear receptor important for adrenal development and development of the pituitary gonadotroph. Mutations in the DAX-1 gene (*NR0B1*) are associated with IHH and adrenal hypoplasia congenital (AHC), an X-linked form of adrenal insufficiency due to lack of proper adrenal development [34]. While males are most frequently affected due to the X-linked inheritance pattern, a female patient with IHH, but not AHC, has been identified in a family of males with AHC and IHH and was found to have an AHC gene mutation [35].

Defects in the genes coding for the subunits of pituitary gonadotropins have been isolated in cases of delayed puberty. Pituitary glycoprotein hormones consist of a common α-subunit encoded by a single gene and a β-subunit that is specific for LH, FSH, hCG, and TSH. No α-subunit mutations have been described in humans. However, inactivating β-subunit mutations have been identified in both *LHB* and *FSHB*. In boys, mutations in *LHB* (fertile eunuch syndrome) lead to absent pubertal development with normal testicular size and viable spermatogenesis. Weiss et al. described a male patient who presented at age 17 with delayed puberty, elevated serum LH levels, but low FSH and testosterone levels. He was found to have an autosomal recessive defect in *LHB* that produced LH that was immunoactive and therefore measurable by current assays, but had decreased bioactivity, resulting in delayed puberty [36]. Girls typically present with normal pubertal development but may have secondary amenorrhea, infertility, and multicystic ovaries [37].

Inactivating mutations have also been identified in the FSHβ subunit. Boys typically undergo normal pubertal development although they have azoospermia, whereas girls present with delayed puberty and primary amenorrhea. Matthews et al. and Layman et al. each described young women with no evidence of thelarche, undetectable FSH levels, and elevated LH. Both young women had no FSH response to GnRH stimulation but had an exaggerated rise in LH to menopausal levels [38, 39]. In females, FSH deficiency is expected to produce delayed puberty as a result of lack of follicular development, estradiol production, and maturation of oocytes, as has been described. However, in males, as LH stimulation is responsible for testosterone production, one would predict normal pubertal development, but azoospermia or oligospermia due to lack of FSH stimulation. However, one case of delayed puberty in a boy with an *FSHB* mutation has been described [40].

25.2.3.5 Associated Syndromes

Hypogonadotropic hypogonadism is also associated with other more complex syndromes. A recent report found a mutation in the *CHD7* gene in an individual with delayed puberty associated with CHARGE syndrome [41]. Hypogonadotropic hypogonadism is also seen in Prader-Willi, Bardet-Biedl, and Laurence-Moon syndromes. The etiology of gonadotropin deficiency in these syndromes remains unclear, though further investigation of the genetic causes of the underlying syndrome may provide important insights into the regulation of this complex system.

25.2.3.6 Acquired Causes of Hypogonadotropic Hypogonadism

Intracranial disease processes, or therapy designed to treat such diseases, are well-known acquired causes of hypogonadotropic hypogonadism. Histiocytosis X may be associated with HH, depending on the extent of pituitary involvement. Other infiltrative and inflammatory processes such as sarcoidosis or hemochromatosis may also be involved. Suprasellar tumors, such as craniopharyngiomas, frequently involve the pituitary and/or hypothalamus. Gonadotropin deficiency may be caused by tumor invasion or by surgical removal of the tumor with subsequent damage to the pituitary or hypothalamus. Radiation therapy for any intracranial tumor may cause hypogonadism, depending on the field involved and the dose of radiation received by the hypothalamus and pituitary. The exact dose required to cause hypothalamic or pituitary dysfunction is unclear. However, hypothalamic GnRH neurons and the pituitary gonadotrophs appear to be less sensitive to radiation effects than somatotrophs, making gonadotropin deficiency unusual in the absence of growth hormone deficiency [42]. Cranial trauma causing hypothalamic or pituitary damage, as well as infection involving these areas of the brain are associated with gonadotropin deficiency.

25.2.4 Hypergonadotropic Hypogonadism

Disorders in this category are characterized by elevated gonadotropin levels. The hypothalamic-pituitary-gonadal axis is activated with the release of GnRH in a pulsatile manner from the hypothalamus, subsequent increases in LH and FSH, and circulation of these hormones to the gonads. If the gonads cannot properly respond by producing estrogen or testosterone, there is a failure in the normal feedback to the hypothalamus and pituitary with a compensatory increase in gonadotropin levels above the normal range. Patients present with lack of pubertal development, low serum testosterone or estrogen levels, and elevations of LH and FSH.

25.2.4.1 Klinefelter Syndrome

Klinefelter syndrome is the most frequent form of hypogonadism in males with an incidence of approximately 1:500–1000 males. The pubertal delay in this syndrome is caused by seminiferous tubule dysgenesis. These children typically have a karyotype of 47,XXY or its variants, including mosaicism, 48,XXXY, and 49,XXXY. These patients usually enter into puberty at an average age but do not appropriately progress. Typical phenotypic characteristics include tall stature with long legs and arms, gynecomastia, small, firm testes, borderline IQ, and poor social adaptation. Physical examination typically reveals the so-called "eunuchoid" proportions with an upper-to-lower segment ratio of less than one, demonstrating increased long bone growth. Some children with Klinefelter syndrome may have micropenis, hypospadias, and/or cryptorchidism [43, 44].

25.2.4.2 Turner Syndrome

Turner syndrome, characterized by a karyotype of XO or its mosaics, is the most common cause of hypergonadotropic hypogonadism in females. The syndrome is characterized by 45,X or mosaic karyotype, short stature, webbed neck, low posterior hairline, hypertelorism, and left-sided cardiac defects. However, all of these features need not be present. Ovarian function varies in girls with Turner syndrome, with variable progression through puberty, with a small proportion of patients reaching menarche. In girls presenting with short stature and pubertal delay, possible phenotypic features of Turner syndrome should be evaluated and a karyotype considered [45].

25.2.4.3 Gonadal Dysgenesis

In phenotypic females, pure gonadal dysgenesis is defined by the complete or nearly complete absence of ovarian tissue. Genotype in these girls may be 46,XX, 46,XY, or 45,XO/46,XY mosaic. In cases of a 46,XY karyotype, genetic studies have shown microdeletions on either the Y or the X chromosome [46, 47]. Girls with pure gonadal dysgenesis may have complete lack of pubertal development, or some degree of breast development, but remain amenorrheic. Gonadal dysgenesis is also associated with Denys-Drash syndrome (nephropathy, Wilms tumor, and genital abnormalities) and the WAGR complex (Wilms tumor, aniridia, genital abnormalities, and mental retardation).

25.2.4.4 Gonadotropin Receptor Mutation

Several patients with Leydig cell hypoplasia or aplasia have been shown to have inactivating mutations in the luteinizing hormone receptor gene *LHCGR*. The degree of masculinization is variable, depending on the particular mutation, ranging from microphallus to genital ambiguity. Testes are small to normal size; FSH levels are normal, with low serum testosterone, but LH levels are elevated, implying resistance. Women identified with LH receptor defects appear to present with amenorrhea and partial ovarian failure characterized by defective folliculogenesis, anovulation, absence of a luteal phase, delayed or incomplete feminization at puberty, and infertility [48–51].

Inactivating mutations in the FSH receptor gene (*FSHR*) leads to normal pubertal progression in males with normal or small testicular size and impaired fertility. Girls may have primary or secondary amenorrhea, infertility, and premature ovarian failure. These patients are clinically similar to patients with pure ovarian dysgenesis, presenting with variable development of secondary sexual characteristics, primary or early secondary amenorrhea, and high serum FSH and LH levels. However, women with FSH receptor mutations frequently have ovarian follicles on ultrasound examination, possibly due to residual receptor activity [52, 53].

25.2.4.5 Defects in Steroidogenesis

Inborn errors of enzymes required in the biosynthetic pathway of testosterone can result in incomplete male sexual differentiation and incomplete progression through puberty. Five enzymes are necessary for testosterone production, three of which are common to the pathway of cortisol production as well. The phenotypic presentation of patients with these defects varies with the location of the enzyme defect in the pathway of steroid production.

Congenital lipoid adrenal hyperplasia is due to deficiency in either the steroidogenic acute regulatory (StAR) protein or cholesterol side-chain cleavage deficiency (P450scc). Affected 46, XY subjects do not show pubertal development, whereas affected 46, XX subjects may undergo breast development at the usual age of puberty and have cyclical vaginal bleeding (at least for some time) [54]. This is because lipid accumulation in the ovaries in patients with STAR defects begins at puberty and is minimal earlier when the ovaries are not active. 3β-hydroxysteroid dehydrogenase deficiency presents with adrenal insufficiency with salt-losing and abnormal sexual differentiation. Males have genital ambiguity, and females may be normal or have moderate clitoromegaly. As this enzyme complex is common to synthesis of all active steroid hormones, puberty is incomplete in both sexes. 17α-hydroxylase is essential for biosynthesis of androgens, glucocorticoids, and estrogens. Deficiency causes lack of virilization in genetic males, with elevated LH and FSH and low testosterone levels in puberty [17, 20]. Desmolase deficiency impairs production of androgens and estrogens with normal cortisol and aldosterone. Genetic males with this deficiency may present with incomplete virilization, or as phenotypic females, with absent uterus and lack of pubertal virilization. In 17β-hydroxysteroid dehydrogenase type III deficiency, affected genetic males present with female external genitalia and moderate labioscrotal fusion. At puberty, breast development is associated with acne, hirsutism, voice deepening, and amenorrhea, and the virilization is consequent to the conversion of androstenedione to testosterone by extra-gonadal 17 β-HSD isoforms. These patients have elevated plasma androstenedione and estrone levels with low or normal testosterone [55, 56].

5α-reductase enzyme activity is necessary for the conversion of testosterone to dihydrotestosterone (DHT). DHT is necessary for masculinization of the fetal external genitalia. Autosomal recessive defects in 5α-reductase type II result in female external genitalia with male internal genital structures and a urogenital sinus with a perineal opening. Partial virilization may be seen at puberty due to increases in testosterone production and residual enzymatic activity or from the action of 5α-reductase type I in peripheral tissue. Females with aromatase deficiency, the enzyme responsible for the conversion of testosterone to estradiol, are typically detected in the newborn period and later have absent pubertal development with progressive virilization [57].

25.2.4.6 Estrogen Receptor α Mutation

One woman and an unrelated man have been described with estrogen resistance due to estrogen receptor α mutations. They have a similar phenotype to those with aromatase deficiency but biochemically have very elevated estradiol levels [58, 59].

25.2.4.7 Androgen Insensitivity Syndrome

Androgen insensitivity syndrome (AIS) is a heterogeneous disorder caused by mutations in the androgen receptor gene. Phenotype in affected patients varies greatly from normal female (complete androgen insensitivity syndrome (CAIS) to ambiguous forms more closely resembling male (partial androgen insensitivity (PAIS)). This variation is dependent on the location and extent of mutation and the subsequent activity of the androgen receptor. Karyotype in these patients is 46,XY, and under the influence of anti-Müllerian hormone (AMH), they have male internal structures. However, they fail to develop male external genitalia that normally results from androgen effects in embryonic development. Phenotypic females usually enter puberty with breast development as a result of aromatization of testosterone to estrogen, but there is little or no body hair development. Primary amenorrhea may be the first manifestation of this condition, particularly for complete androgen insensitivity. Phenotypic males will have variable progression through puberty depending on the activity of the androgen receptor [60–62].

25.2.4.8 1Testicular Regression Syndrome (Vanishing Testes Syndrome)

Bilateral anorchia is found in approximately 1 in 20,000 males. Their external genitalia are normal, implying normal testicular function during the first 14–16 weeks of embryonic development. However, at birth, no testicular tissue is present, resulting in the term "vanishing testes." These patients are born with cryptorchidism and may fail to develop secondary sexual characteristics. Unlike males with abdominal cryptorchidism, these patients have no increase in testosterone levels following human chorionic gonadotropin administration, and AMH levels are undetectable [63].

25.2.4.9 Noonan Syndrome

Noonan syndrome shares many phenotypic features with Turner syndrome including short stature, webbed neck, low posterior hairline, hypertelorism, and hypogonadism. However, patients with Noonan syndrome have a normal 46,XX or XY karyotype as this is an autosomal dominant disorder. Females with Noonan syndrome typically undergo normal pubertal development and have normal ovarian function but can also have delayed pubertal development. Male patients, in contrast, typically have undescended testes and abnormal Leydig cell function, causing hypergonadotropic hypogonadism. Noonan syndrome is caused by mutations on the long arm of chromosome 12 and is inherited in an autosomal dominant manner [64, 65].

25.2.4.10 Acquired Causes of Gonadal Failure

Bilateral testicular torsion, surgical castration, and severe trauma to the scrotum and testes are known causes of hypergonadotropic hypogonadism in males. Bilateral orchitis (e.g., mumps) is also an unusual but known cause of gonadal failure. Exposure to chemotherapeutic agents may cause gonadal failure but is more likely to affect Sertoli cell development and cause infertility, rather than pubertal delay [66]. Inclusion of the testes in the direct field of radiation usually causes testicular failure. Exposure to total body radiation associated with bone marrow transplantation raises concern for possible gonadal failure, though approximately half of patients have normal pubertal development with fractionated regimens [67]. Hemochromatosis typically causes iron accumulation in the pituitary gland but may also affect the testes.

Autoimmune ovarian failure is seen in girls, either as an isolated autoimmune phenomenon or, more frequently, in association with polyglandular autoimmune failure. The presence of a Fragile X premutation carrier, associated with mutations in *FMR1*, should be considered if antibody testing for autoimmune POI is negative [68]. Idiopathic premature ovarian failure is an infrequent cause. As with boys, ovarian failure may be consequent to chemotherapy or radiation therapy, as well as surgical or traumatic injury to the ovary. Galactosemia is a rare cause of hypergonadotropic hypogonadism in females [69].

25.3 Clinical Presentation

25.3.1 History

A thorough medical history and family history are essential in the evaluation of pubertal delay. The hallmark of pubertal onset in girls is breast bud development and in boys is testicular enlargement; the latter may make the exact timing of pubertal onset challenging to assess given its subtlety. The history should include the presence or absence of secondary sexual characteristics including breast development, acne, changes in skin, axillary and pubic hair, apocrine body, growth acceleration, distribution of fat to the thighs and hips, leukorrhea, and vaginal bleeding. In boys, this includes penile and testicular enlargement, axillary and pubic hair, apocrine body odor, acne, facial hair, Adam's apple protrusion, deepening of the voice, erections, changes in body composition, and growth acceleration. The progression and maintenance of these signs should be explored as hypogonadism may develop after pubertal onset, leading to stalled puberty. Differentiating signs of pubertal onset from adrenarche may be important in children with constitutional delay of growth and puberty as they often have concomitant delay in adrenarche, while those with IHH may have isolated pubertal delay.

The review of systems must probe for historical details that are consistent with systemic disease or

chronic disorders, including other endocrinopathies, associated with pubertal delay. Hyposmia or anosmia should be assessed, such as being able to smell what is being cooked for a meal, as formal olfactory testing is typically not performed during the physical exam. A family history of delayed puberty is commonly seen in those with constitutional delay of growth and puberty, but a lack of such history does not exclude the diagnosis of IHH. A family history of significant pubertal delay, treatment for such delay, or a history of infertility may point to an underlying genetic abnormality. A family history of any autoimmune disorders should also be acquired.

Review of previous growth data, including weight for height, is essential. The pubertal growth spurt occurs in the early stages of puberty in girls (peaking at Tanner stage III) but at the later stages of puberty in boys (peaking at Tanner IV–V) with a peak pubertal growth velocity of 8–12 cm/year in girls and 10–14 cm/year in boys. Parents may note an increase in shoe size as hands and feet are the first to increase during a growth spurt. Interpreting the height and growth pattern in the context of midparental height is important. A discrepancy of more than two standard deviations (10 cm or 4 in.) from midparental height may indicate underlying pathology. Parental heights may need to be measured in clinic for accurate assessment. Plotting growth data on a longitudinal growth curve, such as the Bayer and Bayley, is useful to demonstrate a pattern of growth consistent with constitutional delay [70]. These children frequently have relatively slow growth during childhood. Low weight for height increases the suspicion for nutritional disorders or underlying gastrointestinal disease. Patients with hypothyroidism, glucocorticoid excess, or Prader-Willi syndrome may have slight or significantly increased weight for height.

25.3.2 Physical Examination

Physical examination should include a careful evaluation of pubertal staging which may note the presence of breast development or testicular enlargement not previously detected by the child, parents, or other medical providers. In girls, breast development begins with formation of breast buds. This development is frequently unilateral for several months. Enlargement of the areolar diameter usually accompanies breast budding. Development of axillary and pubic hair may or may not accompany the onset of puberty, as androgens are mainly produced by the adrenal gland, which is under separate control. Under the influence of estrogen, the vaginal mucosa changes from a reddish tint to pink, and a whitish vaginal discharge may be seen. The average interval from breast budding to menarche is 2 years.

The increase in testicular size is usually the first sign of puberty in boys. In general, testicular size greater than or equal to 4 mL in volume or a longitudinal measurement greater than 2.5 cm is consistent with the onset of pubertal development. Penile length is not routinely assessed; however, penile width is easily obtained. Scrotal skin also changes in texture and reddens in early puberty. Pubic hair development usually correlates with genital development in boys, as both are under androgen control, but pubertal stage is best assessed by evaluating these factors separately [71]. Gynecomastia is a common finding in midpuberty but may also be associated with Klinefelter syndrome or partial androgen insensitivity.

In addition to height and weight, arm span and lower segment measurements should be performed. The lower segment measurement is the distance from the pubic symphysis to the floor when standing. An upper-to-lower segment ratio can be determined by subtracting the lower segment measurement from the standing height (upper segment) and evaluating the ratio. During normal pubertal development, this ratio changes from greater than 1 prepubertally (with torso length greater than leg length) to slightly less than or equal to one with increased long bone growth at puberty. Children with delayed puberty will have an increased upper-to-lower ratio for age and an arm span exceeding height sometimes by more than 5 cm due to delayed epiphyseal closure. In patients with Klinefelter syndrome, the upper-to-lower segment ratio is low due to long bone growth but without significant signs of pubertal development.

A careful neurological evaluation is particularly important in the evaluation of delayed puberty. Neurologic deficits may indicate the presence of central nervous system disease. Synkinesia, or mirror movements of the hands, should raise suspicion for Kallmann syndrome. Physical abnormalities suggestive of genetic syndromes such as Turner or Klinefelter syndromes as described above should be specifically evalu-

ated, as well as findings that suggest underlying chronic illness or endocrinopathy.

25.4 Diagnostic Evaluation

In patients with findings suspicious of underlying chronic disease, either by history or physical examination, individual evaluation, aimed at the suspected diagnosis, should be undertaken. This may include an erythrocyte sedimentation rate for evaluation of inflammatory disease, a complete blood cell count, electrolytes, renal or liver panel, or gastrointestinal studies to rule out systemic conditions, and exclusion of endocrinopathies such as thyroid disease or hyperprolactinemia.

Bone age evaluation is frequently helpful in the assessment of delayed puberty; however, a delayed bone age does not confirm a diagnosis of constitutional delay of growth and puberty. Skeletal age more closely correlates with sexual development than with chronologic age. A skeletal age of 10 in girls and 12.5 in boys usually correlates with the onset of pubertal development [72, 73]. If bone age is appropriate for chronologic age, further evaluation for the etiology of pubertal delay is appropriate. If a patient's bone age is significantly delayed, pubertal delay may be caused by underlying chronic disease or endocrinopathy, and further diagnostic evaluation is indicated. In constitutional delay, bone age is usually comparable to height age, and observation may be appropriate.

In patients who are apparently healthy and have no indications for etiology of delay, most authors recommend initial assessment of serum LH and FSH levels obtained in the morning. If LH and FSH are elevated, demonstrating hypergonadotropic hypogonadism, the etiology for gonadal failure should be further investigated based on the differential diagnosis in Table 25.1. However, normal or low gonadotropin levels may represent constitutional delay or hypogonadotropic hypogonadism. This distinction may be quite difficult without obvious clinical signs associated with true hypogonadotropic hypogonadism.

Assessment of estradiol levels is infrequently helpful in early puberty. Measurable levels are reassuring for onset of early puberty, but levels below the limit of many standard assays may also be seen in early puberty. Morning serum testosterone levels may be useful in determination of progression into early puberty. One study determined that an 8 a.m. serum testosterone level greater than 0.7 nmol/L predicted an increase in testicular size to greater than 4 mL within 1 year in 77% in boys and within 15 months in 100% of boys. In boys with levels less than 0.7 nmol/L, only 12.5% of boys progressed to the same point within 1 year [74]. Total testosterone levels may be lower in adolescent boys due to lower levels of sex hormone-binding globulin.

Karyotype determination, while not routinely indicated, should be carried out if physical examination suggests the presence of a genetic syndrome such as Klinefelter or Turner syndrome. It should also be performed in cases of hypergonadotropic hypogonadism to evaluate for gonadal dysgenesis. Cranial magnetic resonance imaging should be performed in patients with hypogonadotropic hypogonadism suspected of having intracranial lesions or defects on the basis of initial history or physical examination. In other patients, such imaging may be considered after further evaluation is completed but need not be performed as part of the initial evaluation. Similarly, in phenotypic males with cryptorchidism or phenotypic females suspected of having androgen insensitivity, pelvic ultrasound may be part of the initial assessment, though this is typically deferred in most cases to a later time. hCG stimulation testing may be performed or AMH levels measured to assess for the presence or function of testicular tissue. In girls with hypergonadotropic hypogonadism, anti-21-hydroxylase antibodies should be sent to screen for autoimmune POI. If negative, testing for *FMR1* premutations may be considered.

Despite promising developments, there is currently no single simple discriminating biochemical test to accurately distinguish CDGP from IHH, with considerable overlap of results among patients with these diagnoses. Studies have explored using basal gonadotropins, nocturnal gonadotropin sampling, GnRH, GnRH analog, and hCG stimulation tests without consistent results. Inhibin B and AMH have also been examined as potential biochemical markers. Despite advances in identifying genes that cause IHH, mutations in known genes account for only 30–40% of cases. Thus, routine genetic testing for diagnostic differentiation between CDPG and IHH is not indicated. Differentiating IHH from constitutional delay of growth and puberty can

be challenging, and the diagnosis of IHH should be delayed until age 18 years if there is clinical uncertainty. Absent, slow pubertal tempo or cessation of pubertal development, bilateral cryptorchidism, small penile size, and anosmia raise the suspicion for IHH [75, 76].

25.5 Outcomes and Possible Complications

Adult height in patients with CDGP can be difficult to predict. Some observational studies suggest that children with CDGP eventually reach their genetic potential [77, 78]. However, other studies have reported that final height is consistent with predicted height based on bone age but shorter than midparental target height [79–81]. Treatment with short-term low-dose sex steroids does not appear to impact adult height in CDGP [78, 82]. While self-esteem was not correlated with adult height, Crowne et al. found that most girls and boys felt that their pubertal delay had an impact on success at school, work, or socially [80, 81]. Some, but not all, studies have shown lower bone mineral density in those with a history of delayed pubertal development, regardless of sex steroid therapy [9].

In those with IHH, fertility requires treatment with gonadotropins or pulsatile GnRH. While not all patients respond to therapy, 90% of women and 50% of men with IHH achieve fertility with pulsatile GnRH therapy with rates slightly lower for gonadotropin therapy [9]. Patients with IHH can undergo a so-called reversal with activation of their HPG axis and normalization of steroidogenesis. This may occur in an estimated 22% of patients with IHH and with varying genotypes and phenotypes. As a result, patient with IHH requires lifelong monitoring [83].

Both chemotherapy and radiation therapy for treatment of childhood cancers are known to cause hypergonadotropic hypogonadism. However, counseling concerning fertility preservation prior to therapy can be difficult due to the need for urgent therapy, parental concerns, and confusion over the responsibility of various providers [84]. As a result, the risks of infertility and the possibility of techniques for preservation may not be fully discussed. Recent articles have advocated for a multidisciplinary approach including oncologists, fertility specialists, ethicists, and mental health providers. Discussions prior to therapy may allow sperm banking or cryopreservation of ovarian tissue as indicated [85, 86].

Fertility preservation and other options for parenting should also be discussed with patients diagnosed with Turner syndrome and Klinefelter syndrome. Girls diagnosed with Turner syndrome at an early age may have ovarian reserve, and cryopreservation of mature oocytes or ovarian tissue may be possible, though there are ethical considerations [87, 88]. In Klinefelter syndrome, there is progressive decline in testicular function. However, spermatozoa can be found by testicular sperm extraction (TESE) in 50% of adults with KS [89]. While prior recommendations had been for sperm retrieval via TESE in the peri-pubertal or adolescent age range, current evidence suggests that there may be no advantage to this practice and argues for retrieval to be delayed until at least after 16 years of age or until adulthood when decisions can be made by the patient and any potential partner [89].

25.6 Treatment

Sex hormone replacement therapy is indicated in children with permanent forms of hypogonadism requiring pubertal induction and may also be considered in those with constitutional delay of growth and puberty for psychosocial well-being [76]. Treatment in children with functional disorders is less clear, and the first-line therapy should be aimed at improving the underlying disease when possible and attainment of a healthy body weight to promote resumption of HPG activity. For children warranting treatment for hypogonadism, testosterone and estrogen replacement in boys and girls, respectively, are first line. In some cases of hypogonadotropic hypogonadism, pulsatile administration of kisspeptin- or gonadotropin-releasing hormone has resulted in induction of puberty [90, 91]; however, these remain experimental. GnRH or gonadotropins are options for stimulation of spermatogenesis or induction of ovulation in infertile adult patients due to hypothalamic etiologies.

25.6.1 Boys

Short-term and low-dose testosterone therapy is utilized for induction of puberty in boys with constitutional delay of puberty and in escalating doses for long-term treatment in permanent forms of hypogonadism. Oral testosterone is not FDA-approved in the United States due to the risk of liver toxicity.

Testosterone esters, administered via injection, are most commonly prescribed. Testosterone enanthate and cypionate have longer duration of action than testosterone propionate and are more commonly prescribed. While typically administered intramuscularly, administering testosterone esters more frequently and at lower doses subcutaneously is also gaining in clinical practice at some centers. Transdermal systems for delivering testosterone include a non-genital patch and gel formulations and solution. The scrotal patch is no longer available in the United States. Gel formulations must be used with care. When applied daily, these transdermal systems result in similar testosterone concentrations to those seen in normal young men in magnitude and diurnal variation; however, use of the patch may be limited by skin irritation. Care must be taken in children trying gel preparations with good handwashing and covering treated areas of the body so as not to transfer testosterone to other individuals. Replacement therapy is typically initiated with testosterone injections, as the very small doses necessary to mimic early pubertal levels may be difficult to establish with the gel or the patch, although recent data suggest that early pubertal levels may be achievable using the patch. Once a patient is on near full replacement doses, he may be switched to the gel or patch if desired. Other available testosterone formulations for which there is little clinical experience in this pediatric population include nasal testosterone spray which must be administered several times per day, buccal tablet, and subcutaneous testosterone pellets.

In boys with probable constitutional delay of growth and puberty, most physicians advocate a period of "watchful waiting"; however, a short course of testosterone therapy may be initiated in some cases, typically after age 14. A low dose of testosterone enanthate or cypionate (50–100 mg given intramuscularly every 4 weeks for 3–6 months) will stimulate linear growth and some secondary sexual characteristics without inappropriately accelerating bone age and compromising adult height [92]. When distinction between constitutional delay and true hypogonadotropic hypogonadism is difficult, a short course of therapy followed by discontinuation and monitoring for 4–6 months for progression of puberty and continued testicular enlargement may be of diagnostic use. Enlargement of testicular volume under testosterone treatment becomes obvious in patients with constitutional delay as opposed to those with hypogonadotropic hypogonadism [93, 94]. In addition, patients with constitutional delay may continue with pubertal progression following a "jump start" with testosterone therapy. However, one 6-month course may not be sufficient, even in patients with true constitutional delay, and sometimes a second such course is administered. Adjunct therapies in CDGP have included growth hormone, aromatase inhibitors, or anabolic steroids such as oxandrolone.

Testosterone esters are also appropriate therapy for permanent hypogonadal states. Various schemes have been proposed, but most authors advocate a starting dose of 50 mg every 4 weeks. When the pubertal growth spurt is well established, the dose should be increased gradually, on average every 6 months, to a full adult dose of 100–200 mg every 2 weeks. When hypogonadism is diagnosed at a prepubertal age due to a known abnormality, testosterone therapy may be started as early as a bone age of 12 years in order to decrease the psychological disturbance associated with delays in pubertal development [95, 96]. Monitoring serum testosterone levels should occur halfway between injections with optimal serum levels in the middle of the therapeutic range.

While transdermal therapies are an appealing alternative to intramuscular injections and have been used for induction of pubertal development, no clear guidelines for dosing have been established and vary based on the type of gel preparation. Some studies have shown that appropriate serum testosterone levels for early puberty can be attained using a transdermal patch (Androderm) of 2.5 mg/day for 8–12 h overnight [98, 99]. More

experience is needed with these preparations. Of note, they may provide a means for slower increases in testosterone levels than achieved using available testosterone esters.

25

25.6.2 Girls

Estrogen replacement induces breast development, growth of the uterus and endometrium, maintenance of skeletal health, and attainment of target adult height in girls with hypogonadism. Similar to therapy with testosterone in boys, lower doses may be administered for constitutional delay of growth, and/or stepwise dose increases may be needed for pubertal induction in those with permanent hypogonadism. There are many methods of pubertal induction, and no optimal method has been identified. However, generally, estrogen replacement alone is initiated at 1/8 to 1/10 of the full adult replacement dose, and progesterone administration begins only after full replacement doses are achieved. Decisions about the timing and progression of therapy should be individualized for each patient based on chronologic age and the psychological issues of developing at a similar time as peers. This is typically initiated at 12–13 years of age.

The use of transdermal 17-beta estradiol for pubertal induction and maintenance is increasing in clinical practice and is now available in formulations that are changed once or twice weekly. If estrogen replacement is given orally, the increasing use of 17-beta estradiol is preferred over conjugated equine or ethinyl estradiol. A starting dose of oral 17-beta estradiol at 0.5 mg daily can be used and gradually increased over an 18–24-months period to a full adult dose of 1–2 mg daily. Alternatively, transdermal 17-beta estradiol matrix patches can be used with a starting dose of 1/4 to a full 25 mcg/24 h and titrate up to a maximum dose of 75–100 mcg/24 h [99]. These patches are commonly cut to provide the lowest dose to patients. Given some controversy about cutting the patches, as this is not supported by the manufacturer, some practitioners prefer to apply higher doses overnight only or cycling doses for limited time periods during a month [100]. Injectable estradiol cypionate is now less commonly used, and limited data are available for other preparations (i.e., gels) in this population. Monitoring of serum estradiol levels is not common practice given limitations of the assay; breast development and growth are typically used as biomarkers of estrogen exposure.

With each of these therapies, a progestogen should be added after 12–24 months of therapy, preferably before spontaneous menstrual bleeding occurs and/or breast development is complete or if the child is at maximum adult replacement dose of estrogen. This can be given as micronized progesterone 200 mg daily or medroxyprogesterone 5–10 mg daily for 7–14 days per month [99]. Dose and duration should be tailored to the individual patient based on occurrence of side effects, such as nausea. After adult doses of estradiol and progestogen are reached, an oral contraceptive pill may be substituted for separate preparations of these compounds. In girl without a uterus, such as in androgen insensitivity or XY gonadal dysgenesis, the same guidelines for estrogen replacement can be used, but there is no need for the addition of progestogen, as the latter is mostly necessary for maintenance of uterine health by inducing cyclic endometrial shedding.

Case Study

H.S. is a 12 years, 7 months old boy referred for evaluation of short stature. He and his parents are concerned that he is small for his age and that this may affect his adult height. Neither he nor his parents report any body odor, acne, and axillary hair or pubic hair development. His birth weight was 7 lbs., 8 oz. at term. He was noted to have a dermoid cyst that was removed, but no other abnormalities. He has no chronic diseases and has had no other surgeries or hospitalization. His review of systems is remarkable for a variable appetite, but both he and his parents report that he smells food cooking in the kitchen without difficulty. He has no hair or skin changes and no heat or cold intolerance. His weight gain is variable depending on his level of activity and his appetite.

Review of his prior growth data shows consistent growth along the third percentile for height and for weight. His

mother is 5′3″ tall and had menarche at age 15. His father is 5′10″ tall and had average puberty. His 13-year-old sister has not yet reached menarche. His paternal grandmother had thyroid disease, but there is no other family history of autoimmunity or endocrinopathy.

On initial physical examination, he was a well-appearing, small, slightly thin boy who appeared younger than his chronologic age. He had no dysmorphic features or midline abnormalities. He had no axillary or pubic hair and no acne. His testes were 3 cc in volume bilaterally. His exam was otherwise unremarkable.

His initial evaluation included normal screening for growth hormone deficiency (IGF-1 normal for pubertal status), thyroid dysfunction, celiac disease, anemia, inflammatory bowel disease, and hyperprolactinemia. Morning gonadotropins were prepubertal, as was his testosterone. His bone age was delayed at 11 years. He was felt to have constitutional delay of growth and puberty, and observation was recommended.

He was next seen at 14 years, 1 month of age. In the interval, he had been well with no major intercurrent illness. His growth velocity had been 4.42 cm/year with a decline in his height percentiles to the first percentile. On physical exam, he had no pubic or axillary hair, but his testes were now 4 cc bilaterally. Laboratory evaluation revealed pubertal LH and FSH and early pubertal testosterone. Bone age had advanced to 12.5 years, appropriate change for the interval. He was felt to be on the cusp of puberty with the start of testicular enlargement.

When seen again at age 14 years, 9 months his growth rate was 4.68 cm/year with maintenance of his height at first percentile, and his physical examination was essentially unchanged. Repeat gonadotropins were unchanged, and his testosterone remained steady in the early pubertal range. As a result of his lack of progressive pubertal development and his desire to enter puberty with his peers, a course of testosterone enanthate, 50 mg IM every 4 weeks, was prescribed.

At 15 years, 2 months of age, after 5 months of testosterone therapy, his growth velocity had increased to 10.4 cm/year, and his testes had increased in size to 6 cc bilaterally. He had sparse pubic and axillary hair and some penile enlargement. His testosterone enanthate was discontinued in favor of continued monitoring. At 15 years, 7 months his testicular volume had continued to increase, along with pubic hair development and penile enlargement. His growth velocity was consistent with mid-puberty at 10 cm/year. At his last follow-up visit at 16 years of age, his testes were 8 cc bilaterally, and his growth rate continued at 10 cm/year. He was very pleased with his growth and progress.

Discussion

Boys are more commonly referred for evaluation of delayed puberty, particularly when associated with short stature. This is likely related to a greater degree of parental and patient concern with adult height. Determining the etiology of this delay can be difficult, unless the history or physical examination reveals abnormalities implying underlying associated genetic syndromes or functional abnormalities. In this case, at presentation, the patient did not meet criteria for a diagnosis of delayed puberty as he was younger than 14 years. However, his pattern of growth at a percentile lower than expected for family and his delayed bone age, along with his normal laboratory screening, were consistent with constitutional delay of growth and puberty.

By age 14 years, 1 month the patient had continued to grow at a prepubertal rate resulting in a decline in growth percentiles, while his peers progressed through their pubertal growth spurt. However, his physical examination implied spontaneous entrance into puberty with testicular enlargement to 4 cc. This was supported by his pubertal gonadotropins and testosterone. However, 8 months later, he had failed to progress through puberty, raising concern. As noted in the chapter, utilization of sex steroid therapy in cases such as this allows appropriate growth and physical development which ease the concerns of the patient and family and can also "jump start" spontaneous pubertal development. In boys, the use of testosterone enanthate or cypionate at a dose of 50 mg IM every 4 weeks for four to six doses can often be sufficient to induce activation of the HPG axis.

Differentiation between CDGP and IHH often requires long-term follow-up. Patients with IHH generally do not continue to progress through puberty following testosterone therapy, though this is not consistent. In this case, the patient progressed spontaneously following short- term administration of testosterone and, at last report, had continued to progress to adult reproductive status. While the incidence of CDGP is higher than that of IHH, making CDGP the more likely diagnosis, long-term follow-up of patients through their teen years is needed for a final diagnosis (Fig. 25.1).

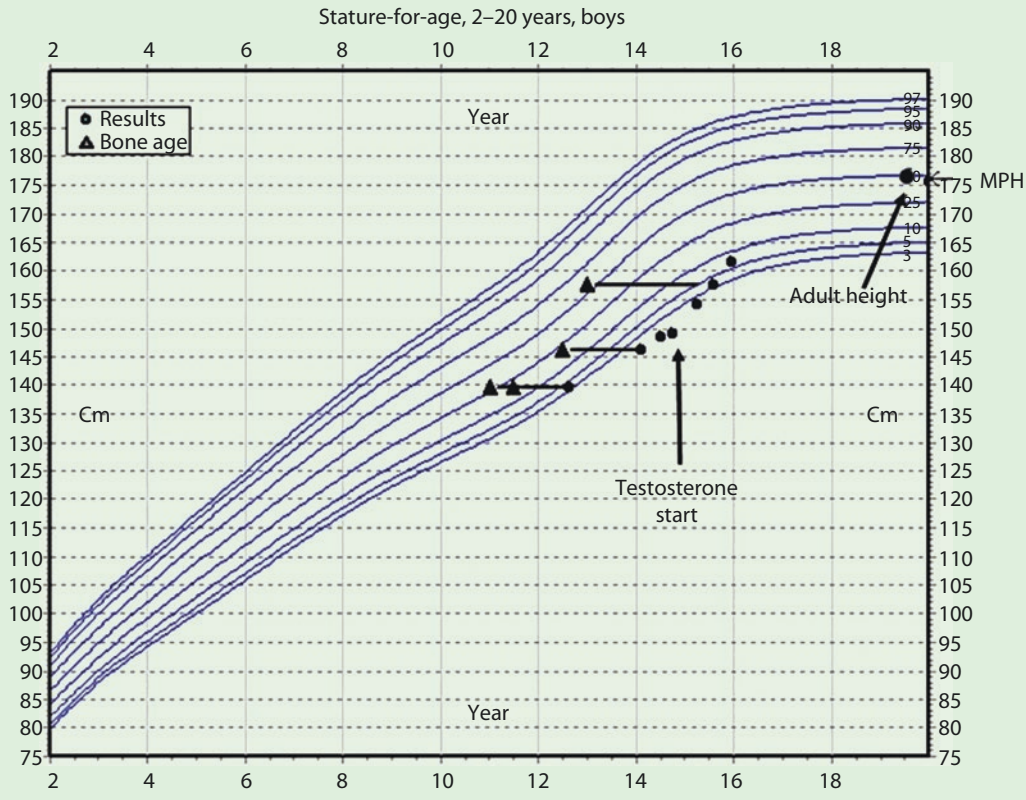

Fig. 25.1 Growth chart for delayed puberty case. MPH = midparental target height

25.7 Summary

The causes of delays in pubertal development can be most easily divided into four categories: (1) variations of normal pubertal timing, (2) functional and systemic disorders, (3) hypogonadotropic hypogonadism, and (4) hypergonadotropic hypogonadism. Although constitutional delay of growth and puberty is the most common cause, evaluation for other possible etiologies is warranted in children with no signs of pubertal development by 14 years of age for boys and 13 years of age for girls. While genetic etiologies for pubertal delay continue to be uncovered, there remain many questions about the control of pubertal development, frequently making definitive diagnosis difficult. Replacement of sex steroids may be indicated, even without a definitive diagnosis, and the "tincture of time" with continued monitoring may be needed to help differentiate constitutional delay of growth and puberty from other permanent forms of hypogonadotropic hypogonadism.

Review Questions

1. FGFR1 mutations are associated with which of the following:
   A. CHARGE syndrome
   B. Combined pituitary hormone deficiency
   C. Kallmann syndrome
   D. Klinefelter syndrome
2. A 14-year-old boy presents for evaluation for pubertal delay. His height is at the 10th percentile for age, declining from 25th percentile at 11 years of age. His weight is at the 20th percentile. On examination, his testes are 4–5 cc

bilaterally, and he has Tanner I pubic hair. The most likely diagnosis for this boy is:

A. Idiopathic hypogonadotropic hypogonadism (IHH)
B. Klinefelter syndrome
C. Growth hormone deficiency
D. Constitutional delay of growth and puberty

3. Each of the following genetic syndromes is associated with hypogonadotropic hypogonadism *except*:

A. Prader-Willi syndrome
B. CHARGE syndrome
C. Noonan syndrome
D. Bardet-Biedl syndrome

Answers

1. C
2. D
3. C

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Precocious Puberty

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Key Points

- Precocious puberty is defined as onset of puberty in a child at a chronologic age 2 standard deviations below the mean age of pubertal onset for a given population.
- Causes of precocious puberty include benign variants (such as premature adrenarche and premature thelarche) and gonadotropin-dependent (central) and gonadotropin-independent (peripheral) causes.
- Benign variants typically do not require treatment; however, associated pathology should be ruled out.
- Gonadotropin-dependent (central) precocious puberty is more likely to be associated with pathological causes in boys than in girls and responds to depot GnRH analog therapy.
- Gonadotropin-independent precocious puberty is always pathological, and treatment is directed at the underlying pathology; however, patients may also require depot GnRH analog therapy if prolonged sex-steroid exposure has led to gonadotropin-dependent precocity.

26.1 Introduction and Normal Development

Precocious puberty has been a focus of interest for both the pediatric endocrinologist and the primary care pediatrician for many years. In the 1980s, the development and application of GnRH agonist (GnRHa) therapy to treat central precocious puberty significantly changed our approach to this disorder. More recently, application of molecular biological techniques has provided us with a better understanding of the intricacies of the regulation of gonadotropin and sex-steroid production characteristic of normal pubertal development and provided us with the tools to elucidate etiologies of previously uncharacterized disorders of precocious puberty.

26.1.1 The Hypothalamic–Pituitary–Gonadal Axis

The onset of puberty requires activation of the hypothalamic–pituitary–gonadal axis. The hypothalamic–pituitary–gonadal axis is functional by mid-gestation [1]. Subsequently, the "minipuberty" of infancy is followed by a period of quiescence through childhood until the onset of puberty. GnRH or gonadotropin-releasing hormone is a decapeptide secreted by neuroendocrine neurons residing in the supraoptic and ventromedial nuclei of the preoptic and medial basal hypothalamus. Their nerve termini are found in the lateral portions of the median eminence adjacent to the pituitary stalk. GnRH secretion by these neurons is coordinated such that, when grown in culture, individual cells exhibit pulsatile secretion that becomes synchronous when the cells are placed in physical proximity to each other. These cells are one of the few cell types that originate outside the central nervous system, in the region of the olfactory placode [2]. During fetal development, they migrate with the olfactory neurons to their final location in the hypothalamus (Fig. 26.1). The initial secretion of GnRH appears unrestrained and occurs between 100 and 150 days of gestation. Pulsatile secretion of GnRH is essential for the activation of pituitary gonadotropes, and pulses need to be of sufficient amplitude and frequency to stimulate release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH). The frequency of GnRH pulses alters the pattern of LH and FSH secretion such that faster frequencies increase gonadotrope release of both LH and FSH, slower pulse frequency increases secretion of FSH relative to LH, and a constant infusion inhibits release of both LH and FSH [3]. Maturation of the negative feedback effect of sex steroids occurs after 150 days of gestation with a progressive decrease in GnRH secretion resulting in low levels of GnRH secretion at term [4, 5]. The GnRH "pulse generator" is highly functional 12 days after birth, presumably secondary to the withdrawal of maternal and placental sex-steroid exposure. This leads to prominent FSH and LH release until approximately 6 months of age in males and 12 months in females. This gonadotropin release in infancy leads to transient increases in sex steroids in infants that can approximate levels seen in mid-puberty [6]. Negative feedback control of FSH and LH secretion becomes highly sensitive to sex steroids by 2 years of age. The role of sex-steroid negative feedback in this period has been supported by the observation of high gonadotropin levels in agonadal infants and those with Turner syndrome [1]. Beyond 3–4 years of age,

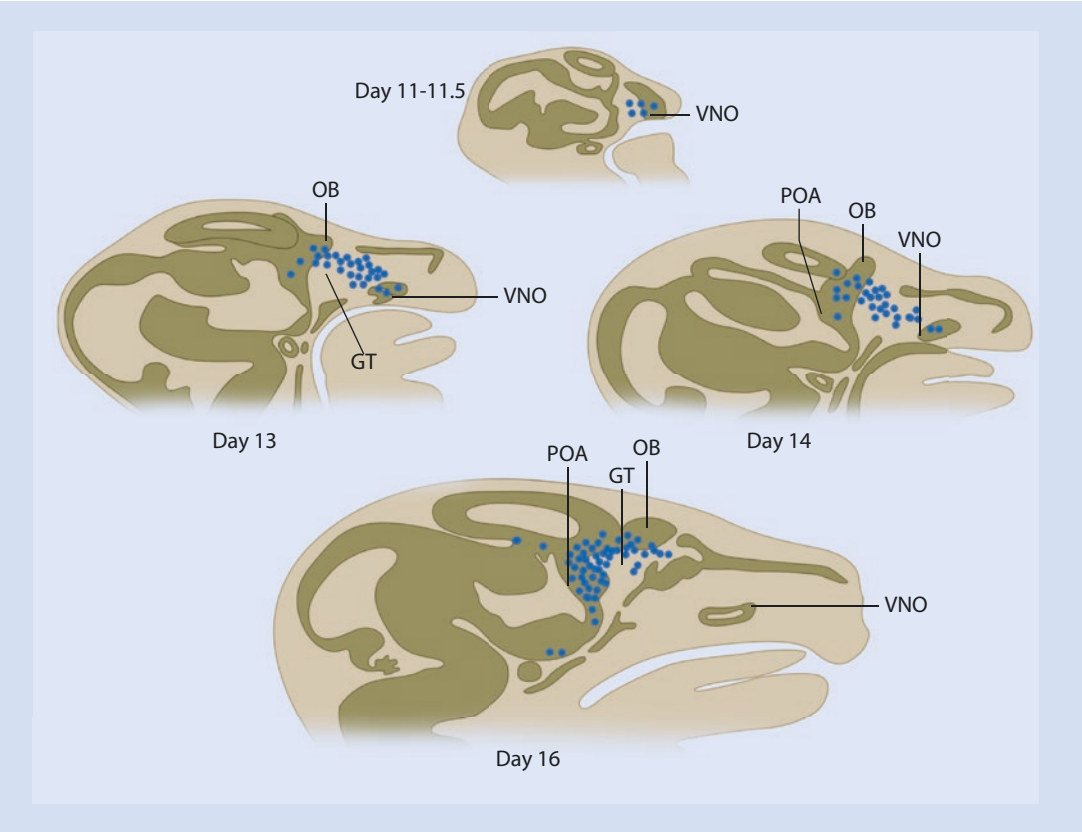

Fig. 26.1 Ontogeny of the GnRH neurosecretory neurons in the mouse. The migratory route of the GnRH neurons is indicated by the *blue dots*. Their progression is illustrated according to embryonic day of development. At days 11–11.5, the neurons are in the area of the vomeronasal organ (VNO) and the medial wall of the olfactory placode. By day 13, cell number has increased and their distribution has extended to the olfactory bulb (OB) and the ganglion terminalis (GT). By day 14, the cells approach the preoptic area (POA) and begin to enter the hypothalamus. By day 16, the migration is nearly complete

until puberty, the mechanism by which GnRH secretion is inhibited is less well understood as LH and FSH secretion is suppressed even in the agonadal individual. In part, this finding has led to the hypothesis that there is an "intrinsic CNS inhibitory mechanism" that prevents secondary sexual development until "disinhibition" occurs at the time of puberty. Studies in nonhuman primates have identified gamma-aminobutyric acid (GABA) as an inhibitory neurotransmitter responsible for restricting GnRH release [7]. Reduction in tonic GABA inhibition appears to allow an increase in the response to other neurotransmitters such as glutamate that stimulate GnRH release, heralding the onset of puberty [8, 9].

At the time of normal onset of puberty, GnRH pulsatile secretion is reestablished, presumably following reduction in GABA inhibition of the pulse generator, interaction of kisspeptin and its receptor GPR54, and decreased negative feedback sensitivity to low levels of sex steroids. This leads to increased GnRH pulsatility (and therefore LH pulsatility) that is initially sleep associated. As puberty progresses, there is an increase in LH pulse amplitude during the daytime as well, leading to sex-steroid production and progressive development of secondary sexual characteristics. By mid- to late puberty, spermatogenesis is established in males and a positive feedback mechanism develops in females resulting in the capacity to induce an estrogen-induced LH surge that leads to ovulation.

26.1.2 Normal Pubertal Development

Normal puberty involves activation of both the hypothalamic–pituitary–gonadal axis (gonadarche)

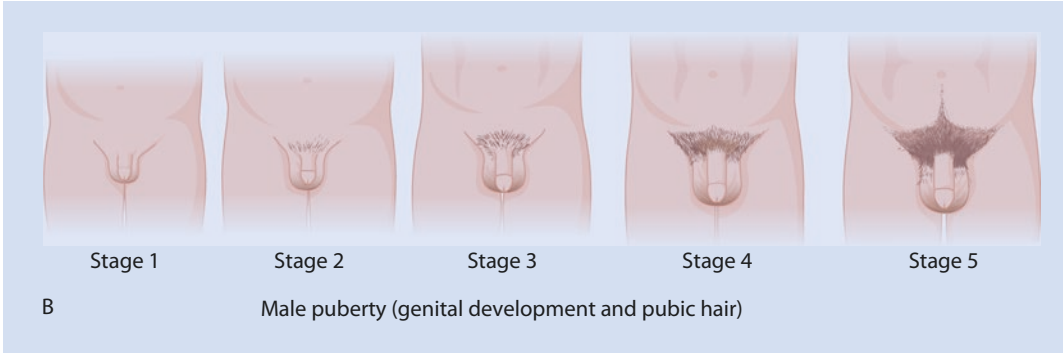

Fig. 26.2 Tanner stages of male genital and pubic hair development according to Marshall and Tanner and Reynolds and Wines

and maturation of the adrenal axis (adrenarche). Adrenarche is associated with an increase of adrenal androgen production that leads to pubarche or the first appearance of pubic hair. This increase in adrenal androgens begins approximately 2 years before elevations of pituitary gonadotropins and gonadal sex steroids [10, 11]. Adrenarche and gonadarche are independent events, as evidenced in agonadal children and those with Turner syndrome who exhibit adrenarche, but not gonadarche. The mechanism by which adrenarche is initiated remains unclear despite various theories and investigations over many years. The 17,20-lyase activity of the P450c17 enzyme is dramatically increased, particularly in the zona reticularis of the adrenal cortex leading to increased dehydroepiandrosterone (DHEA) and androstenedione production. There has been speculation that a pituitary factor is responsible for stimulating the maturation of the zona reticularis; however, there are no definitive data to support this claim [12]. Preliminary evidence has suggested that posttranslational phosphorylation of the P450c17 enzyme may cause the increase in 17,20-lyase activity with consequent increase in adrenal androgen production [13, 14].

Secondary sexual features of puberty in the male are first noted between the ages of 9 and14 years. The first physical sign of puberty is testicular enlargement to greater than 2.5 cm in longest diameter or greater than 3 ml in volume. This is largely due to an increase in Sertoli cell and seminiferous tubular volume with a small contribution by the Leydig cells. Pubic hair and phallic enlargement (first in length and then in width) occur within a few months. Tanner stages of genital and pubic hair development are illustrated in Fig. 26.2. Increasing androgen levels lead to increased oiliness of the hair and skin resulting in acne, adult body odor, deepening of the voice, penile erections, and nocturnal emissions. Normal variations in androgen to estrogen ratios lead to a transient breast budding or gynecomastia in the majority of pubertal boys [15]. In boys, the pubertal growth spurt is evident during Tanner stages III to V and peaks at Tanner stage IV.

Puberty in females is heralded by breast development, or thelarche, between the ages of 8 and 13 years. Breast development may be unilateral for 6 months before development of the contralateral breast. Pubic hair appears within a few months during Tanner stage II breast development with menarche occurring approximately 2 years after the onset of puberty, during Tanner stage IV breast development. Menarche is not a presenting feature of pubertal onset, and its presence should prompt investigation into the possibility of a foreign body or invasive lesion of the vaginal vault, cervix, or uterus. The stages of breast and pubic hair development in girls are summarized in Fig. 26.3. As indicated in Fig. 26.4, in contrast to males, the pubertal growth spurt in females follows shortly after the onset of puberty and peaks at Tanner stage III. This is clearly evident when one surveys the relative tall stature of females as compared to males at approximately 12 years of age.

Additional features of puberty in the female include growth of the body of the uterus and "estrogenization" of the vaginal mucosa as the epithelium is transformed, the vaginal pH drops

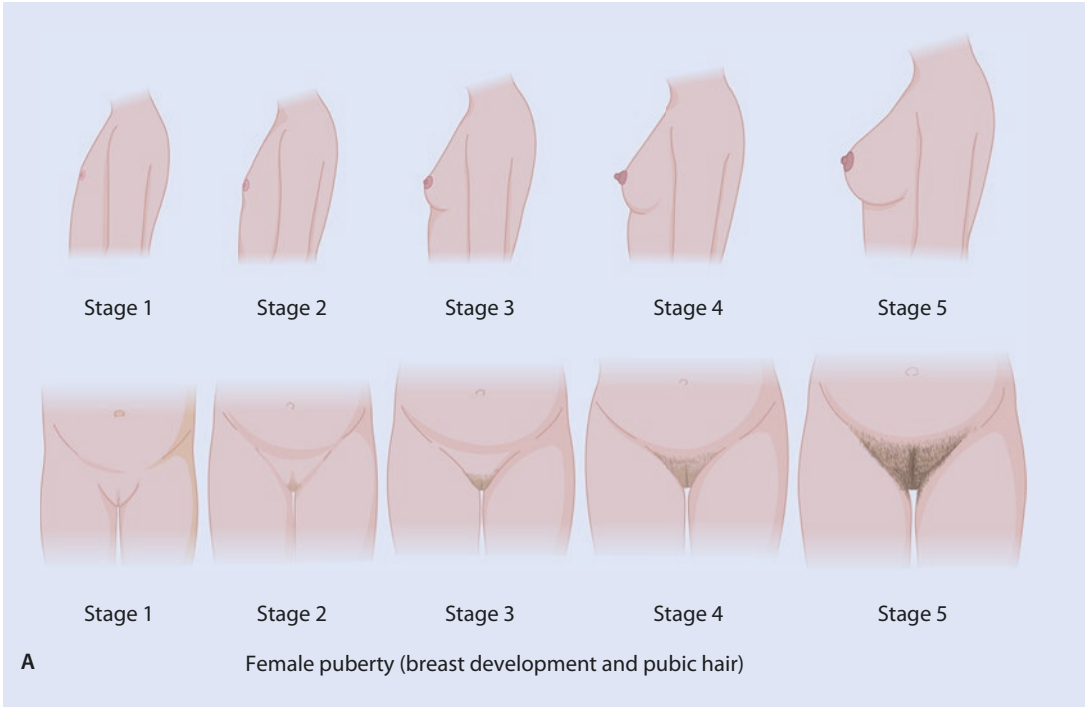

Fig. 26.3 Tanner stages of female breast and pubic hair development according to Marshall and Tanner and Reynolds and Wines

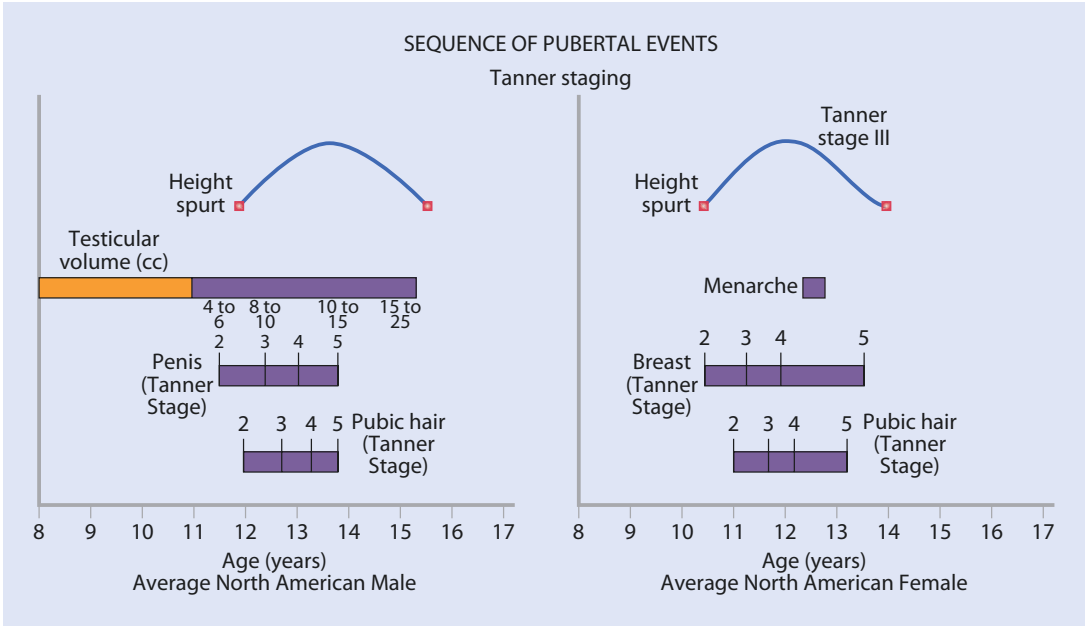

Fig. 26.4 Sequence of pubertal development in males and females. Relative growth velocity, testicular volume or menarche, and Tanner staging are indicated for age (Based on Tanner [179])

(acidifies), and leukorrhea appears. The uterus, as evaluated by pelvic ultrasound, is judged as pubertal in configuration when the length of the body of the uterus is longer than the cervix, with a length > 3.5 cm or a volume greater than 18 mL.

26.2 Precocious Puberty: Definitions

Recent discussions among pediatricians and pediatric endocrinologists have focused on the current definition of precocious puberty in females. In 1997, the Pediatric Research in Office Settings (PROS) Network of the American Academy of Pediatrics reported that the onset of puberty in girls in the USA is occurring earlier than previous studies have documented [16]. The study reported that breast and pubic hair development is occurring 1 year earlier in Caucasian girls and 2 years earlier in African-American girls despite no change in the age of menarche. These findings would indicate that girls are entering puberty at an earlier age but progressing at a slower rate. Further analysis of the data indicated that particularly in white girls, and to a lesser extent in black girls, obesity was associated with early puberty and also with isolated pubic hair development [17]. More recent data from almost 2400 females further demonstrate that adult height in females is associated with age at menarche in both races. The early maturing, more rapidly growing girls are the tallest among their peers early on but become the shortest adults [18]. The PROS data prompted a review of the literature and the issuance of standards of practice by the Drugs and Therapeutics and Executive Committees of the Pediatric Endocrine Society (formerly the Lawson Wilkins Pediatric Endocrine Society) [19] of this body, which concluded that aggressive evaluation and treatment are unlikely to be beneficial in African-American females having onset of puberty after 6 years of age and white females with the onset of puberty after age 7 years. However, many pediatric endocrinologists expressed concern that the adoption of these standards was premature and carried the risk of overlooking pathology.

A subsequent study examined the NHANES III sample [20] and reported that pubertal signs occurred before 8 years of age in <5% of the normal BMI general and non-Hispanic white female population. However, pubertal milestones were reached earlier in normal BMI non-Hispanic black and Mexican American girls, with thelarche occurring before 8 years in 12–19% of these girls. Pubarche was also noted to be occurring earlier in minority groups. The fifth percentile for menarche was 0.8 years earlier for non-Hispanic black compared with non-Hispanic white girls. Thelarche and menarche occurred earlier in those with higher BMI. Another recent study indicated that 10, 23, and 15% of white, African-American, and Hispanic girls, respectively, had breast development of at least Tanner stage II between the ages of 7 and 8 years [21]. Further, the longitudinal National Institute of Child Health and Human Development (NICHD)-sponsored Study of Early Child Care and Youth Development demonstrated an average age at breast Tanner stage II of 9.9 and 9.1 years for white and African-American girls, respectively [22]. Of note, although pubertal onset (Tanner stage II breast development) appears to be occurring earlier than previously reported, the age at Tanner stage III does not appear to have changed significantly over time, and the timing of menarche is only minimally earlier (average between 12 and 12.5 years and just 4 months younger than documented in the mid-twentieth century) [23]. Based on available data, some suggest that premature sexual development in Caucasian girls younger than 8 years and African-American girls younger than 7 years deserves medical evaluation [24], and breast development to Tanner stage III before 8 years of age is clearly early [25].

Data for pubertal onset in boys are to some extent similar to that for girls. Based on earlier studies, pubertal onset at <9 years of age in boys was considered precocious, with a mean age of 11.5 years at pubertal onset. More recently, the average age at genital Tanner stage II was reported to be 10.1 years in whites and 9.1 years in African-Americans by the PROS network, 10.1 years in white boys and 9.5 years in African-Americans in the NHANES III study, and 10.4 years in white boys and 9.6 years in African-Americans in the NICHD study. At this time, however, we continue to use a lower limit of 9 years as indicative of the normal age of pubertal onset for boys with pubertal onset before 9 years being considered precocious.

Precocious puberty is secondary to either central or peripheral mechanisms. Several forms of premature puberty, including premature thelarche and premature pubarche, are considered benign. Premature thelarche is the

early appearance of isolated breast development. Premature pubarche is the early appearance of pubic hair, which is most often secondary to premature adrenarche, an "early awakening" of the adrenal gland. Adrenarche occurs in association with increased adrenal androgen production leading to the appearance of pubic hair and other androgen effects such as acne, body odor, and axillary hair development. Central precocious puberty or gonadotropin-dependent precocious puberty results from the premature activation of the hypothalamic–pituitary–gonadal (HPG) axis leading to premature secondary sexual development that proceeds in a fashion similar to normal pubertal progression. Potential triggers of central precocious puberty are listed below.

In contrast, gonadotropin-independent precocious puberty occurs in the absence of HPG axis activation and may be secondary to numerous etiologies, including those listed below. Because of the significant morbidity associated with many of these conditions, determination of the etiology is essential. In addition, any child with premature sexual development is at risk for psychological and social stresses, including sexual abuse. One of the most significant consequences of untreated, rapidly progressive, central precocious puberty is a compromise in adult height.

Differential Diagnosis of Gonadotropin-Dependent Precocious Puberty

- *Idiopathic*
  - Sporadic
  - Familial
  - Adoption from developing country
  - Following chronic exposure to sex steroids
- *Genetic*
  - Mutations in *MKRN-3, DLK-1, KISS-1, and KISS1R*
- *Central nervous system disorders*
  - Hypothalamic hamartoma
  - Hydrocephalus
  - Congenital anomalies
    - Myelomeningocele
    - Midbrain developmental defects
  - Cysts
    - Arachnoid
    - Glial
    - Pineal
  - Neoplasms
    - Astrocytoma
    - Craniopharyngioma
    - Ependymoma
    - Glioma
    - Neuroblastoma
    - Pinealoma
  - Histiocytosis X
  - Vascular lesion
  - Global CNS injury
  - Cranial irradiation
  - Infection
    - Abscess
    - Encephalitis
    - Meningitis
- *Syndromes*
  - Neurofibromatosis type I
  - Russell–Silver syndrome
  - Williams syndrome
  - Klinefelter syndrome
  - Cohen syndrome
  - Pallister–Hall syndrome
  - Solitary maxillary incisor

Differential Diagnosis of Gonadotropin-Independent Precocious Puberty

- *Autonomous gonadal function*
  - McCune–Albright syndrome
  - Peutz–Jeghers syndrome
  - Familial male limited precocious puberty (testotoxicosis)
  - Ovarian cysts
- *Gonadal tumors*
  - Ovarian
    - Granulosa cell
    - Theca cell
    - Combination
  - Testicular
    - Leydig cell
    - Sertoli cell
- *Exogenous steroid ingestion/exposure*
- *hCG-secreting tumors*[a]
  - Hepatoblastoma
  - Pinealoma
  - Germinoma
  - Thymic tumors
  - Testicular tumors
    - Choriocarcinoma
    - Teratoma
- *Adrenal disorders*
  - Congenital adrenal hyperplasia
  - Adenoma
  - Carcinoma
- Severe primary hypothyroidism

[a]hCG is a gonadotropin; however, for the purposes of classification of causes of precocious puberty, it is generally defined as being a cause of gonadotropin-independent precocious puberty

26.3 Benign Premature Development

Premature thelarche and premature adrenarche may be the consequence of benign, self-limited processes that require no therapeutic intervention. Premature thelarche is limited to modest breast development but may also include estrogenization of the vaginal mucosa and, rarely, vaginal bleeding. Premature adrenarche results in the appearance of pubic and/or axillary hair and occasionally acne and body odor. In general, these conditions are not associated with significant growth acceleration or skeletal maturation. Of note however is the increasing attention to studies that question the benign nature of premature adrenarche [26–31].

26.3.1 Premature Thelarche

Premature thelarche is the isolated premature appearance of breast development in girls that occurs during the first 3–4 years of life [32], with a peak prevalence in the first 2 years [33, 34]. It should be differentiated from neonatal breast hyperplasia which is generally present at birth, is a consequence of gestational hormones, and generally spontaneously resolves within the first few months of life. Growth acceleration, significant bone age maturation, or other signs of precocious puberty do not accompany benign premature thelarche [35]. The breast development may be unilateral or bilateral with a waxing and waning course. Regression often occurs within 18 months. However, it has been suggested that complete regression may only be seen if onset is before 2 years of age [33, 34]. Furthermore, girls with more than Tanner stage II breast development are less likely to have breast tissue regression [36].

The precise etiology of premature thelarche remains unknown. It is postulated that in some girls, the glandular breast tissue is particularly sensitive to low levels of circulating estrogen [37, 38]. It is important to rule out ingestion or exposure to exogenous estrogenic agents such as oral contraceptives or creams in girls with premature thelarche. Environmental pollutants including xenoestrogens, such as plasticizers classified as endocrine disruptors, or phytoestrogens may exhibit estrogenic and antiandrogenic activities and have also been implicated in the etiology of premature breast development [39]. High levels of sex hormone-binding globulin (SHBG) in conjunction with decreased free testosterone can lead to an alteration of the ratio of androgens to estrogens and is another postulated etiology for premature thelarche [40]. Serial and stimulated gonadotropin measurements in these girls reveal elevated FSH levels consistent with those seen in early puberty [41–44]. Premature thelarche thus may be a consequence of early and slowly progressive activation of the hypothalamic–pituitary–ovarian axis and, thus, may represent part of the continuum of central precocious puberty.

Premature thelarche may be difficult to diagnose in obese females. Adipose tissue over the pectoral area may appear much like breast tissue and may be difficult to differentiate from glandular tissue. Palpation of the breast may reveal absence of tissue in the subareolar area, which some clinicians refer to as "the doughnut sign." During initial breast development, glandular tissue first appears in the subareolar region and subsequently extends outward. Ultrasound examination may rarely be useful to make this distinction or to identify a cyst, abscess, or breast tumor [45]. Pelvic ultrasound may reveal small ovarian cysts in children with premature thelarche. However, because this finding is usually seen in normal prepubertal girls, it is not a helpful diagnostic test. On occasion, girls with premature thelarche have a single, large follicular cyst that produces estradiol. Some of these cysts are self-limited and resorb spontaneously. However, girls prone to ovarian cyst development may have cyst recurrence.

Serial observation and reassurance of the family is all that is necessary for a girl with isolated premature thelarche. A bone age radiograph may be indicated to gauge the extent of estrogen exposure. Evidence of advanced skeletal maturation usually suggests more significant pathology than premature thelarche. It should be kept in mind, however, that bone age might be slightly advanced in children with obesity. Although premature thelarche has been viewed as a self-limited variation of normal development, these patients should be followed at 3–6-month intervals, unless the condition resolves. Long-term studies have indicated that as many as 18% of girls with premature thelarche may progress to central precocious puberty despite their typical presentation [32, 34, 43, 46].

26.3.2 Premature Pubarche

Premature pubarche is defined as the appearance of pubic and/or axillary hair before 8 years of age in girls and 9 years of age in boys. The child with premature pubarche typically presents with early appearance of pubic and possibly axillary hair. In addition, children may manifest features of mild hyperandrogenism, including apocrine or adult body odor and comedones. Pubic hair is generally limited to Tanner stage II development with few scattered hyperpigmented curly hairs appearing over the labia majora or perineum in females or at the base of the penis in males. Hirsutism is not a feature of benign premature pubarche. If considerable hirsutism exists, it may be quantified utilizing the Ferriman–Gallwey index (Fig. 26.5).

Premature pubarche is most commonly a consequence of premature adrenarche, which is more common in females and often observed in children with CNS abnormalities and exogenous obesity.

As was noted above, work by Herman-Giddens et al. and the National Heart, Lung, and Blood Institute suggests that gonadarche and adrenarche may be occurring earlier in females, particularly those of African-American descent [16]. However, we continue to recommend that caution be exercised in assigning a diagnosis of "normal variant" pubertal development to females younger than 7–8 years of age. This is because as many as 20% of girls with premature adrenarche will progress to central precocious puberty [47]. Benign premature adrenarche does not alter normal pubertal progression and is generally believed to be self-limited. However, recent studies suggest that premature pubarche may not be completely benign. These studies indicate an association with later hyperandrogenism, menstrual irregularities, and infertility in females, and that premature adrenarche may be the presenting feature of polycystic ovary syndrome (PCOS) [26]. Associations between premature adrenarche, elevated adrenal androgens (DHEAS), and insulin resistance have also been reported in individuals with a history of intrauterine growth retardation (IUGR) [48–50]. IUGR, in turn, has been associated with an increased risk of metabolic syndrome (insulin resistance, hypertension, central adiposity, and dyslipidemia) in adult life [51]. Since additional prognostic indicators for PCOS and metabolic syndrome do not exist, long-term follow-up of these individuals may be warranted [52].

Signs of excess virilization such as deepening of the voice, increase in muscle mass, clitoral enlargement in females, or testicular/phallic enlargement in males are not features of benign premature adrenarche. When present, they should raise concern of significant pathology. The differential diagnosis includes late-onset or nonclassical congenital adrenal hyperplasia (NCAH), rare virilizing adrenal or gonadal tumors, and central precocious puberty in boys. In addition, poorly controlled classical congenital adrenal hyperplasia may cause elevations in androgen levels and excess virilization.

Enzymatic defects in adrenal steroidogenesis may lead to hyperandrogenism. Congenital adrenal hyperplasia is the result of one of several autosomal recessive defects in one of a number of steroidogenic enzymes necessary for cortisol production (Fig. 26.6). Defects leading to virilization result from the inability to synthesize sufficient quantities of cortisol to adequately suppress hypothalamic corticotropin-releasing hormone (CRH) and pituitary adrenocorticotropic hormone (ACTH) secretion. This leads to elevated ACTH levels that stimulate steroidogenic acute regulatory protein (StAR) and p450SCC leading to an overabundance of intermediary precursors proximal to the enzymatic defect. The enzymatic block coupled with excessive production of these precursors leads to shunting of hormone production to androgens. Defects in P450c21, P450c11AS, and 3-beta-hydroxysteroid dehydrogenase (3βHSD) lead to increases in adrenal androgen synthesis. Classical defects cause severely compromised or absent enzymatic function causing marked and early hyperandrogenism during fetal development and ambiguous genitalia in newborn females. However, partial defects may lead to less severe or absent phenotypic findings in females and may escape detection in male infants. The less severe defects do not lead to substantial mineralocorticoid or glucocorticoid deficiency but instead may cause hyperandrogenism later in life (NCAH). The incidence of NCAH varies widely depending upon the ethnicity of the population being studied. Rates range from 0.3% in the general population to almost 4% in Ashkenazi Jews [53]. Studies of screening for NCAH in females with premature adrenarche report incidences ranging from 6 to 40% [52, 54, 55].

Site	Grade	Definition
1. Upper lip	1 2 3 4	A few scattered hairs of outer margin. A small moustache at outer margin. A moustache extending halfway form outer margin. A moustache extending to mid- line.
2. Chin	1 2 3 & 4	A few scattered hairs. Scattered hairs with small concentrations. Complete cover, light and heavy.
3. Chest	1 2 3 4	Circumareolar hairs. With mid-line hair in addition. Fusion of these areas, with three-quarter cover Complete cover.
4. Upper abdomen	1 2 3 & 4	A few mid-line hairs Rather more, still mid-line. Half and full-cover.
5. Lower abdomen	1 2 3 4	A few mid-line hairs. A mid-line streak of hairs. A mid-line band of hair. An inverted V-shaped growth.
6. Arm	1 2 3 & 4	Sparse growth acting not more than a quarter of the limb surface. More than this; cover still incomplete. Complete cover, light and heavy.
7. Foream	1, 2, 3, 4	Complete cover of dorsal surface; 2 grades of light and 2 of heavy growth.
8. Tnigh	1, 2, 3, 4	As for arm.
9. Leg	1, 2, 3, 4	As for arm.
10. Upper back	1 2 3 & 4	A few scattered hairs. Rather more, still scattered. Complete cover, light and heavy.
11. Lower back	1 2 3 4	A sacral tuft of hair. With some lateral extension. Three-quarter cover. Complete cover.

Fig. 26.5 The Ferriman and Gallwey system for scoring hirsutism. A score of 8 or more indicates hirsutism (Based on Ferriman and Gallwey [180])

Virilizing tumors of the adrenal gland and testes may rarely present as premature pubarche. Such tumors generally lead to marked virilization, growth acceleration, and skeletal advancement. This occurs in the absence of testicular enlargement in the male when the tumor is in the adrenal gland, and typically unilateral testicular enlargement when the tumor is in the testis.

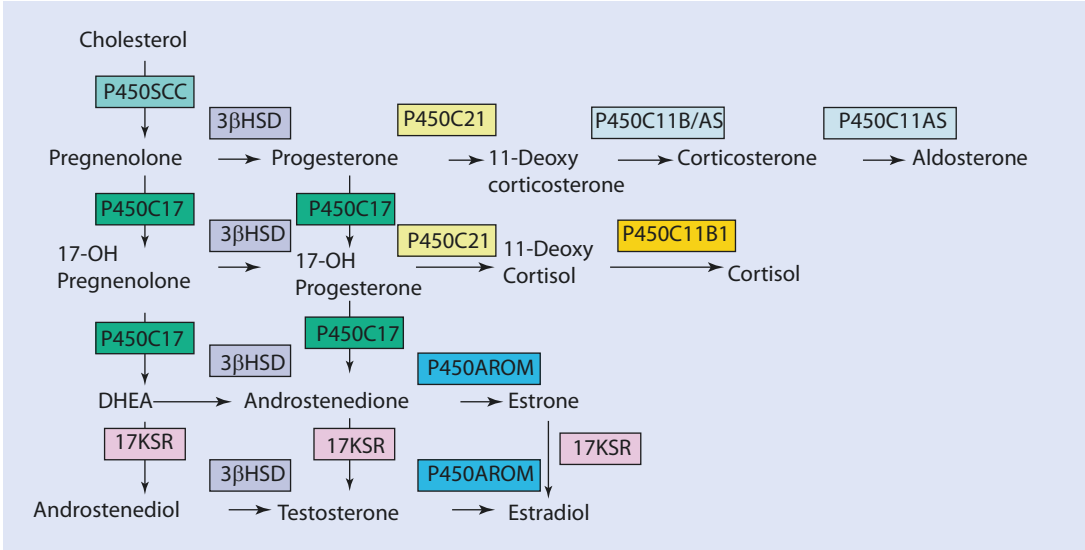

Fig. 26.6 Biosynthetic pathway of adrenal steroidogenesis

Evaluation of a child with premature pubarche may not require extensive evaluation. Typically, a bone age X-ray reveals that skeletal maturation is within 2 SD of chronologic age. Androgen levels in premature adrenarche are usually consistent with those seen in Tanner stage II–III pubic hair development [56]. We recommend obtaining a DHEAS level to help confirm this diagnosis. Clitoromegaly, rapidly progressive or excess virilization, and advanced skeletal maturation warrant determination of additional androgen levels including 17-OH progesterone, androstenedione, and testosterone. ACTH stimulation testing with 250 μg per square meter of body surface area of i.v./i.m synthetic ACTH (Cortrosyn) allows one to compare baseline and stimulated levels of adrenal steroid hormone precursors in order to pinpoint possible enzymatic defects. Adrenal magnetic resonance imaging (MRI), computed tomography (CT) scan or ultrasound may be used to identify mass lesions if significant virilization is present and hormonal evaluation does not reveal an adrenal enzymatic disorder. Tumors may be differentiated from CAH as they do not typically respond to ACTH stimulation or glucocorticoid suppression. Certain dedifferentiated tumors are unable to sulfate DHEA, and in these patients, DHEA rather than DHEAS levels are elevated and help make the diagnosis.

Therapy, if indicated, is dictated by the etiology of the excess androgens. In the case of NCAH, glucocorticoid replacement therapy is recommended to inhibit excess hypothalamic–pituitary stimulation of adrenal steroidogenesis and consequent hyperandrogenism. Virilizing tumors require surgery, preferably by a pediatric surgeon experienced in such procedures. Although surgical excision is often curative, chemotherapy may be indicated for cases with evidence of tumor extension or for recurrence. The recent association of premature adrenarche and PCOS may support a search for evidence of hyperinsulinism with a consideration of oral contraceptive and/or insulin sensitizers or metformin therapy in postmenarchal females [57–60].

26.4 Central Precocious Puberty

Central precocious puberty (CPP) is a gonadotropin-dependent process. It results from premature activation of the hypothalamic–pituitary–gonadal axis. Secondary sexual characteristic development follows the sequence of normal puberty, but begins at an earlier age. The American Academy of Pediatrics has advised that in girls, progressive breast development before 8 years of age with crossing of height percentiles upward on the growth chart, and in boys both testicular and penile enlargement before 9 years, are concerning for central precocious puberty [25]. The etiology is most commonly idiopathic in girls but is identified in the majority of boys [61, 62]. The reason for this sex difference is unclear. However, it is proposed that the female axis is more readily

activated by various factors. As listed in Differential Diagnosis above, a multitude of CNS abnormalities can lead to CPP, presumably by interrupting the normal prepubertal neuronal pathways that typically inhibit activation of the hypothalamic–pituitary–gonadal axis.

Recently, genetic causes have also been identified, including inactivating mutations in *MKRN3* and *DLK1* and activating mutations in *KISS-1* and *KISS1R* that cause familial CPP. The maternally imprinted makorin RING finger protein 3 (*MKRN3*) gene is located on chromosome 15q11–q13 (in the Prader–Willi critical region) and is necessary for hypothalamic inhibition of GnRH secretion. Mutations in *MKRN3* lead to withdrawal of this inhibition and result in pulsatile GnRH secretion, leading to CPP. The gene is expressed only from the paternal allele, and mutations inherited from the father affect boys and girls equally. A high frequency of these mutations has been reported in familial cases of male CPP previously classified as idiopathic [63]. Of note, though precocious, pubertal onset in boys with *MKRN3* mutations in this study was later than in other boys with CPP. Interestingly, there is at least one report of paternally inherited mutations not resulting in CPP in two boys [64]. A paternally inherited *DLK1* deletion has also been reported to cause familial CPP [65]. Activating mutations of the kisspeptin gene (*KISS-1*) and its receptor (*KISS1R*) are rare causes of CPP.

The most common identifiable cause of CPP is a hypothalamic hamartoma, which accounts for 10–44% of CPP cases [62]. These "tumors" are usually benign congenital malformations arising from disorganized central nervous tissue including GnRH neurosecretory neurons. They are best visualized by MRI and typically appear as a pedunculated mass attached to the hypothalamus between the tuber cinereum and the mammillary bodies, just posterior to the optic chiasm [62, 66]. Rarely, these tumors are associated with gelastic (laughing) seizures, secondary generalized epilepsy, behavioral difficulties, and variable cognitive deficiencies [66] (as in Pallister Hall syndrome). The Pallister–Hall syndrome is a rare genetic disorder caused by GLI3 mutations associated variably with polydactyly, cutaneous syndactyly, a bifid epiglottis, imperforate anus, and kidney abnormalities. Hamartomas rarely enlarge or cause mass effects or increased intracranial pressure; thus, invasive surgery is not indicated. Furthermore, some studies have shown that complete resection of these lesions can fail to halt pubertal progression [67]. Similar to other children with CPP, children with hypothalamic hamartomas respond well to GnRH analog therapy [66, 67].

A variety of other CNS lesions can result in central precocious puberty (see Differential Diagnosis above). These lesions likely disrupt tonic inhibitory signals to the hypothalamus, leading to pulsatile GnRH release and activation of the hypothalamic–pituitary–gonadal axis. Such activation has been seen with optic gliomas of the chiasm in neurofibromatosis type I [68], CNS developmental defects such as septo-optic dysplasia and myelomeningocele, and in isolated hydrocephalus. High-dose cranial irradiation used in the treatment of pediatric malignancies has also been shown to cause precocious puberty in up to 25% of cases [69]. CNS irradiation with doses as low as 18–24 Gy used in CNS prophylaxis therapy for childhood leukemia can predispose children to CPP. In these cases, the age of onset of precocious puberty is often correlated with age at the time of radiation therapy.

CPP may also arise following exposure of the hypothalamus to elevated sex-steroid levels associated with peripheral precocious puberty. We consider this secondary central precocious puberty because the activation of the hypothalamic–pituitary–gonadal axis occurs secondary to the primary peripheral cause of precocious puberty. Patients with uncontrolled congenital adrenal hyperplasia, virilizing adrenal tumors, McCune–Albright syndrome, familial male limited precocious puberty, ovarian tumors, and exogenous sex-steroid exposure have all been reported to develop CPP, usually after successful treatment of the primary disorder has lowered sex-steroid levels [70–73]. The mechanism responsible for activation of the GnRH neurosecretory neurons in these cases is unknown. It is hypothesized that "maturation" of the axis leads to disinhibition of the GnRH pulse generator. The degree of bone age advancement is correlated with the time of onset of CPP. The majority of reported cases have had bone ages in excess of 10 years at onset of central activation. An exception is the report of a 5-year-old girl who developed CPP at a bone age of only 5.5 years following removal of an ovarian tumor that had been diagnosed at 7 months of age.

The evaluation of a child with premature sexual development includes a detailed medical and

family history and a history of potential exogenous sex-steroid or endocrine disruptor exposure. Previous growth data are invaluable in determining if growth acceleration has occurred. A thorough physical examination is critical to accurately document growth parameters and to assess for the presence of acne, café au lait spots, adult body odor, breast development, axillary and pubic hair, estrogenization of the vaginal mucosa, and physiologic leukorrhea. Both breast and pubic hair development in girls, and bilateral symmetrical testicular enlargement with penile enlargement in boys, is necessary for the diagnosis of CPP; sexual development is congruent with the sex of the child. A bone age determination to evaluate the degree of skeletal maturation is essential for both diagnosis and long-term therapy.

The "gold standard" for detecting activation of the hypothalamic–pituitary–gonadal axis is a GnRH stimulation test. In the most widely utilized version of this test, synthetic GnRH (Factrel®) is administered intravenously, and gonadotropin levels are drawn at baseline and at subsequent intervals over 1 h. A quiescent axis under tonic inhibition does not respond to a single GnRH stimulus, whereas an activated axis generates a brisk response in gonadotropins and, consequently, sex steroids. Variations on this method have utilized subcutaneous GnRH followed by a single measurement for gonadotropins [74] and the use of GnRH agonists, such as leuprolide and nafarelin to induce gonadotropin release [75–77]. In the United States, GnRH is no longer available, and leuprolide is most commonly used in its place. Although an "LH predominance" is classical in CPP, intermediary responses have been described in early puberty and premature thelarche. Higher-sensitivity assays have more recently revealed that circulating LH and FSH levels are pulsatile in prepubertal children, albeit at much lower frequencies and pulse amplitudes [78–81]. Peripubertal increases in LH are first seen at night followed by greater increases in daytime levels [82]. The development of ultrasensitive LH assays may be sufficient to differentiate prepubertal from pubertal levels of gonadotropins, without the aid of GnRH stimulation of the pituitary gonadotropes, thus replacing the need for a GnRH stimulation test. Typically, a serum LH concentration > 5 U/L after GnRH or leuprolide administration, or a basal LH > 0.3 U/L using an ultrasensitive assay, is considered diagnostic of CPP [23]. In girls, a ratio of peak PH/peak FSH of >0.66 following GnRH stimulation is also considered diagnostic of CPP.

If CPP is suspected, a cranial MRI is indicated to determine the anatomy of the hypothalamic–pituitary area and to rule out potential pathology. A bone age radiograph helps determine the degree of sex-steroid exposure based on the extent of skeletal maturation with ascertainment of growth potential. Pelvic ultrasonography can reveal adrenal and ovarian lesions and document ovarian and uterine size. Multiple studies have shown that 53–80% of prepubertal females have small (<9 mm) ovarian cysts. Cyst size does not vary with age, and the finding of multiple cysts within a single ovary is not rare. Pubertal ultrasound findings include a uterine length greater than 3.5 cm and a fundus to cervix ratio of >1 on midline endometrial measurement [83]. Therapy for gonadotropin-dependent precocious puberty must first address the etiology of the disorder. Consultation with an experienced pediatric oncologist and/or pediatric neurosurgeon should be sought for potentially invasive CNS lesions. Early exposure of the epiphyses to elevated estrogen in the female as well as in the male (via aromatization of androgens) leads to premature epiphyseal fusion and a compromise in adult height. Thus, a decision to treat a child is primarily based on the risk for adult short stature. Secondarily, treatment is aimed at slowing the rate of progression of secondary sexual development.

Until the early 1980s, therapy for CPP was limited to progestational agents such as medroxyprogesterone acetate (MPA, Provera®). These act by interfering with steroidogenesis and directly inhibiting both GnRH and gonadotrope secretion. Although they were successful in preventing menses and providing at least partial regression of secondary sexual characteristics, they were unsuccessful in slowing skeletal maturation with the resultant effect being a compromise in adult height. "Super-agonist" therapy with long-acting GnRH analogs is currently the most widely used and effective therapy for CPP. Modification of the GnRH decapeptide in the sixth and tenth positions results in greater receptor affinity with resultant increases in GnRH potency and duration of action. The rapid and sustained binding of these analogs to GnRH receptors typically causes a brief (<4–6 weeks) stimulation of gonadotrope release and sex-steroid production, followed by a decrease in LH and FSH subsequent to receptor downregulation, which

results in a decrease in gonadal sex-steroid production and release. The frequent administration required of early generation subcutaneous and intranasal agonists led to difficulties in maintaining compliance, and on occasion, there was actually progression of pubertal development secondary to intermittent agonist administration.

Currently, one the most widely used agents in the USA is depot leuprolide acetate. Depot leuprolide may be given at a dose of 0.2–0.3 mg/kg i.m. every 28 days. Longer-acting preparations of depot leuprolide (11.25 and 30 mg doses) are also available for administration every 3 or 6 months. Recent data suggest insufficient hormone suppression with the 11.25 mg dose compared to higher doses for 3-month use; however, it remains unclear whether this also translates to suboptimal efficiency in suppressing pubertal progression [84–86]. Sterile abscesses occur in 1.5–3% of patients.

The GnRH agonist histrelin, administered as a subdermal implant, is gaining favor with a persistent decrease in bone age advancement and improvement in adult height prediction [87]. Although the implant was initially approved for yearly replacement, studies have demonstrated suppression of LH levels until 2 years [88]. Adverse events include pain and bruising at the site of insertion (in 61%) and brittleness of the implant after a year leading to breakage during removal (in 16 and 22% of cases at 1 or 2 years) [87]. Sterile abscesses are rare, but may occur.

Adequacy of therapy is assessed by clinical, radiographic, and biochemical means. Slowing the rate of breast and pubic hair development is common and gonadotropin and sex-steroid levels are suppressed [89]. The return of ovarian and uterine volumes to age-appropriate sizes usually occurs within 6 months of initiation of therapy. Similarly, testicular volume decreases in boys. Children treated with GnRH analogs achieve significant long-term improvement in adult height when compared with predicted adult height at the start of therapy and with untreated historic controls [90–92]. Studies to document final adult height data are vital because the predicted adult height at the completion of therapy frequently overestimates final height. This may be a consequence of rapid pubertal progression occurring after agonist therapy is discontinued [93]. Gains in adult height are greater in patients treated with GnRH analog therapy before 6 years of age. Gains are less when therapy is initiated after 6 years of age, particularly for girls.

Following initiation of GnRH agonist therapy, there is a deceleration in growth velocity. On occasion, especially when the bone age is greater than 11 years, the growth velocity is significantly decreased (<4 cm/year). This has led to investigation of the role of sex steroids in the GHRH–growth hormone axis [94, 95]. In a number of studies, addition of growth hormone therapy has been shown to improve growth velocity and predicted adult height [96]. The effect on final adult height has been favorable with an average gain of 7.9 cm [97]. GnRH agonist therapy may decrease bone mineral density (BMD) somewhat during the course of therapy. However, most reports indicate that BMD remains normal within the range for chronologic age and/or bone age during therapy [98] and at the attainment of final height [99]. For the individual clinician and patient, it is important to weigh the potential benefit in height against the financial cost of this therapy.

Although there is a single report that suggests an increased risk for the development of PCOS in girls with a history of CPP treated with GnRH agonists, this finding has not been confirmed by other investigators [100].

The decision as to the appropriate time to discontinue GnRHa therapy should be reached by individualized discussions between the physician and the family. The most important factors include the child's predicted adult height and maturity level and ability to adjust to progressive sexual development and, for girls, menstrual cycles. Most girls experience menarche and develop appropriate ovulatory cycles within 1–2 years of terminating therapy [101]. Following removal of the histrelin implant, unstimulated gonadotropin levels have been reported to increase by 3 weeks, and higher estradiol levels are noted in 6 weeks.

26.5 Peripheral Precocious Puberty

It is important to differentiate gonadotropin-dependent and gonadotropin-independent forms of puberty because the differential diagnosis and therapeutic approach differ. Peripheral precocious puberty or gonadotropin-independent precocious puberty can arise from a variety of disorders (see Differential Diagnosis above). These range from exposure to exogenous sex ste-

roids to carcinomas. The diagnostic and therapeutic approaches to these children are dependent on the sex of the child and whether there are signs of virilization, feminization, or both. Prolonged sex-steroid exposure may cause maturation of the hypothalamic–pituitary–gonadal (HPG) axis and lead to CPP secondarily.

26.5.1 Exogenous Sex-Steroid Exposure

The evaluation of every child with sexual precocity should include a thorough review of potential exposure to exogenous sex steroids. Ingestion of oral contraceptives, estrogen-contaminated foods, and topical exposure to transdermal preparations of estrogens and androgens have all been shown to cause gonadotropin-independent precocious puberty [102–104]. More recent investigations have also suggested that specific environmental pollutants may exhibit estrogenic effects and cause premature sexual development [39].

26.5.2 Ovarian Cysts

Before widespread use of ultrasound imaging, the prepubertal ovary was believed to be dormant, and the frequency of ovarian cysts among prepubertal girls was thought to be low. It is now appreciated that the ovary undergoes continuous change from fetal development to puberty and through adulthood. In utero, follicular cysts develop under maternal, placental, and fetal hormones. Cysts have been detected as early as 28 weeks of gestation. After birth and removal from hormonal stimulus, regression of both follicular and luteinized cysts often occurs. Small follicular cysts (9 mm) are present throughout childhood [105], and 50–80% of prepubertal girls have small cysts detected by ultrasound. Cyst size does not appear to vary with age and multiple cysts within a homogeneous ovary are not uncommon. Cysts are bilateral in up to 23% of cases and usually suggest gonadotropin stimulation rather than intraovarian stimuli. Typically, prepubertal cysts do not release appreciable quantities of estrogen; however, they may become transiently functional, thereby elevating estradiol levels and causing transient breast development or even a period of brief spotting (following cyst rupture and a decrease in estradiol levels).

26.5.3 McCune–Albright Syndrome

McCune–Albright syndrome (MAS) is characterized by the triad of gonadotropin-independent precocious puberty (more common in affected girls than boys), polyostotic fibrous dysplasia of bone, and irregular café au lait spots ("coast of Maine") [106]. However, the syndrome can be difficult to diagnose due to its variable phenotypic expression. Some girls have waxing and waning breast development, with or without episodes of vaginal bleeding, and minimal skeletal advancement, whereas others have progression through puberty and repeated episodes of vaginal bleeding [23]. The clinical findings result from the autonomous hyperactivity of tissues that produce products regulated by intracellular accumulation of cyclic adenosine monophosphate (cAMP). The constitutive overproduction of cAMP is caused by an autosomal dominant somatic mutation of the gene, *GNAS*, encoding the alpha subunit of the stimulatory guanine nucleotide-binding protein (G protein), *Gs*α [107, 108]. The guanosine triphosphate (GTP)-binding proteins consist of three subunits (α, β, and γ). The activated α-subunit stimulates adenylate cyclase, increasing the production of cAMP. It also acts as a guanosine triphosphatase, catalyzing the hydrolysis of bound guanosine triphosphate to guanosine diphosphate and inactivating the G protein. Normally, this leads to a decrease of intracellular cAMP and resetting of cellular quiescence. The mutation resulting in MAS leads to unrestrained production of gene products derived from the affected cells. The defect occurs early during embryogenesis and leads to mosaicism. The distribution of the progeny of the affected somatic cell determines the cell types affected and the phenotype of the individual patient.

Mutations of the *GNAS* gene are found in both endocrine and non-endocrine tissues of patients with MAS [109]. The clinical presentation is extremely variable ranging from patients with multiple endocrine and non-endocrine abnormalities to hyperfunction of the ovary alone [110]. Affected endocrine organs may include the gonads, thyroid, pituitary, and parathyroid glands [111–113]. Non-endocrine disorders may include hepatobiliary dysfunction; hyperplasia of the thymus, spleen, and pancreas; gastrointestinal polyps; and abnormal cardiac muscle cells [114]. MAS also can present with only fibrous dysplasia

lesions of bone and precocious puberty in the absence of cutaneous lesions [111].

The inheritance of MAS is sporadic and has been reported in all ethnic groups [115]. It is most frequently diagnosed in females, although it occurs in both sexes [116]. The most common presentation is a girl with precocious puberty secondary to estrogen production of autonomously functioning ovarian tissue [117]. Vaginal bleeding may appear as the result of spontaneous cyst regression or unopposed estrogen leading to breakthrough bleeding. Menses have rarely been reported to occur prior to significant breast development [115].

Laboratory evaluation of MAS children with precocious pubertal development reveals periodic elevations of sex steroids with prepubertal gonadotropin levels. GnRH stimulation test results indicate that gonadotropin levels are suppressed, unless sufficient sex-steroid exposure has occurred to cause secondary maturation of the HPG axis (secondary CPP).

The variable presentation of the precocious puberty in children with MAS and the waxing and waning nature of the autonomous gonadal function have made assessment of therapy difficult. In the absence of hypothalamic activation, the precocious puberty of MAS is unresponsive to GnRH analog therapy. However, in cases of "secondary CPP," GnRH agonist therapy has proven beneficial [72, 118]. Therapeutic interventions have focused on ameliorating the hyperestrogenic state by inhibiting estrogen production or blocking estrogen action. Cyproterone acetate, a steroidal antiandrogen possessing progestin and antiestrogenic effects, has been used in Europe with limited success [119]. It modestly controls breast development and menses; however, growth velocity and skeletal maturation are unaffected. Testolactone, a weak aromatase inhibitor, decreases estrogen levels, prevents menses, and also improves predicted height [120]. Unfortunately, compliance is hindered by side effects (headache, diarrhea, and typically transient abdominal cramping), the large number of pills required to achieve adequate dosing, and a rapid increase in serum estradiol levels with cessation of therapy [121]. The third-generation aromatase inhibitor, letrozole, has been shown to decrease vaginal bleeding, growth velocity, and bone age advancement, without changing ovarian volume [122]. Further, the nonsteroidal estrogen–antiestrogen tamoxifen may have a role in the suppression of estrogen action and precocious puberty in patients with MAS, with results superior to those achieved with aromatase inhibitors [123]. Recently, a study of fulvestrant, a pure estrogen receptor blocker, has shown promising results [124]. Monthly 4 mg/kg i.m. injections of fulvestrant led to a 50% reduction in the number of days of bleeding in 74% of patients, and the rate of skeletal maturation decreased from 1.99 to 1.1 over a year. However, there was no difference in adult height prediction, uterine or ovarian volume, or frequency of ovarian cysts. Despite these difficulties with management, female MAS patients can achieve normal menses and fertility and mildly affected patients have achieved normal adult height. The skeletal lesions generally increase in severity and number with increasing age in childhood and then stabilize after puberty. Adult patients may suffer conductive hearing loss secondary to temporal bone sclerosis. In severe cases, Cushing syndrome, growth hormone excess, and bone disease cause significant morbidity. Anecdotally, there appears to be an increased prevalence of ductal breast cancer in young women who had a prior history of precocious puberty [125].

26.5.4 Familial Male-Limited Precocious Puberty

Familial male-limited precocious puberty (FMPP), or testotoxicosis, is a male-limited form of gonadotropin-independent precocious puberty. It is caused by a heterozygous mutation of the LH receptor, leading to constitutive activation in Leydig cells. Mutations have been identified in the first, second, third, fifth, and sixth transmembrane domains and in the third intracellular loop of the receptor [126–130]. The Leydig cell produces testosterone constitutively despite suppressed gonadotropins. Mutations are typically autosomal dominant; however, sporadic mutations have also been identified [131]. The finding that females with these mutations do not manifest precocious puberty is not surprising given that both LH and FSH are required for ovarian sex-

steroid production [111]. It is interesting to note that no other signs of LH hypersecretion in females, such as polycystic ovary syndrome, have been reported.

Boys with FMPP typically present by 4 years of age with a family history of precocious puberty in males, progressive virilization (acne, pubic and axillary hair, minimal to modest testicular enlargement, penile growth, increased musculature, bone age advancement), and growth acceleration. The testes are small for the degree of virilization and demonstrate Leydig cell hyperplasia [132]. Serum testosterone levels are in the adult male range and baseline and GnRH-stimulated gonadotropin levels are suppressed [133]. These boys mature rapidly and premature epiphyseal fusion causes short stature. Fertility is generally normal, but oligospermia and testicular dysfunction have been reported in some adult patients [134–136].

Therapy targeting androgen synthesis and action has been fairly effective. Early therapy utilized medroxyprogesterone acetate (MPA) [137] to inhibit steroidogenesis. It was modestly effective in decreasing testosterone levels and growth velocity. However, its effects on glucocorticoid synthesis and testicular morphology limit its application. More effective therapies include ketoconazole [138] (an antifungal and inhibitor of the 17,20-lyase activity of P450c17—Fig. 26.6) and a combination of spironolactone (an androgen receptor blocker) and testolactone (an aromatase inhibitor), both of which need multiple-daily dosing [139]. Ketoconazole has been associated with toxicity (rash, nausea, headache, hepatotoxicity, pneumonitis, and renal failure) and requires careful monitoring [140]. The addition of an aromatase inhibitor to the antiandrogen treatment is necessary because the elevated androgens may undergo aromatization leading to feminization and an enhanced estrogen effect on skeletal maturation. The BATT (Bicalutamide Anastrozole Treatment for Testotoxicosis) study used the newer androgen receptor, bicalutamide, with the aromatase inhibitor anastrozole in 14 boys with FMPP and reported a reduction in growth rate and skeletal maturation, with once-daily dosing [141]. One study has reported long-term data on two boys treated with these agents for 4.5–5 years, with continued suppression of androgenization, reduced skeletal maturation, and improved adult height prediction [142]. As in MAS, GnRH agonist therapy is indicated if CPP occurs following long-term sex-steroid level elevations [143].

26.5.5 Congenital Adrenal Hyperplasia

Congenital adrenal hyperplasia (CAH) is the result of an autosomal recessive defect in one of a number of steroidogenic enzymes necessary for cortisol production (Fig. 26.6). As detailed above, nonoptimal glucocorticoid therapy of patients with classical CAH and partial defects in P450c21, P450cl1AS, and 3-beta-hydroxysteroid dehydrogenase (3βHSD) insufficient to cause phenotypic changes in the neonate may lead to increased adrenal androgen synthesis later in life and presentation of nonclassical CAH (NCAH).

26.5.6 Tumors

Although sex-steroid-producing tumors are rare in children, their diagnosis is critical. The presentation depends upon the tumor location and the class and quantity of the sex steroids produced.

26.5.6.1 Ovarian Tumors

Primary ovarian tumors are quite rare in children although they comprise approximately 6% of all tumors in adult women. The neoplasms may originate from sex cord/stromal tissue, epithelium, or the germ cell line [144]. Granulosa cell tumors account for 5% of all ovarian tumors, but the juvenile variety is the most common before 20 years of age. The most common presentation of juvenile granulosa cell tumors is precocious puberty, frequently in a precipitous manner of weeks to months. The mean age of presentation is 10 years, but ovarian tumors have been discovered in infants [145]. The majority of ovarian tumors produce estrogen, causing feminization, but they may also produce androgens, causing virilization [144, 146]. Tumors frequently cause local symptoms including pain, distension, ascites, and mass effects. The frequency of precocious

puberty varies but has been reported to be as high as 70% in 1 series of 17 granulosa/theca cell tumors [147]. The diagnosis is usually based on the identification of a solid ovarian mass and an elevated serum estradiol in conjunction with suppressed gonadotropins. The association between CPP and granulosa cell tumors prompted an examination of four females with juvenile granulosa cell tumors. No activating mutations were identified in exon 10, and it was suggested that activating mutations at other exons of the FSH receptor or associated G proteins might be responsible for granulosa cell tumors [148]. Surgical resection with a unilateral salpingo-oophorectomy is typically the only required therapy and carries a good prognosis, particularly in the case of juvenile granulosa cell tumors. Pubertal regression should ensue.

26.5.6.2 Testicular Tumors

Leydig cell tumors account for only 3% of all testicular neoplasms [149], but they are the most common gonadal stromal tumors associated with precocious puberty in boys. Although 10% of these tumors are malignant, they are typically benign in children. Boys usually present between 5 and 9 years of age with virilization, palpable unilateral testicular enlargement, and elevated testosterone levels. The rare Sertoli cell tumor, most commonly seen in Peutz–Jeghers syndrome, may present with gynecomastia in addition to virilization [150]. Surgical resection of these tumors will halt pubertal development [151]. It should be noted that testicular adrenal rest hyperplasia in the male with poorly controlled CAH may present with testicular enlargement (more commonly bilateral) secondary to the stimulatory effects of elevated ACTH levels. ACTH and GnRH stimulation testing and testicular ultrasound and biopsy may be necessary in order to distinguish adrenal rest tissue from other tumors.

26.5.6.3 Adrenal Tumors

Adrenal tumors typically present with virilization; however, feminization may occur [152]. As noted above in the segment on premature pubarche, adrenal tumors may be differentiated from CAH as they do not typically respond to ACTH stimulation or glucocorticoid suppression. Adrenal adenomas often produce DHEAS (or DHEA if dedifferentiated enough to lack DHEA sulfotransferase), whereas androstenedione and testosterone are the primary products of carcinomas. Surgical resection is often curative with resolution of the precocious puberty. However, chemotherapy may be required if there is evidence of tumor extension or ill the event of recurrence.

26.5.6.4 hCG-Producing Tumors

Human chorionic gonadotropin (hCG) and LH possess identical α-subunits and similar β-subunits. It is therefore not surprising that germ cell tumors secreting hCG may cause precocious puberty. They have been reported to arise in the liver [153], lungs [154], mediastinum [155], pineal gland [156], basal ganglia, thalamus, and hypothalamus [157]. In boys, hCG simulates Leydig cell production of testosterone and manifests with minimal to moderate testicular enlargement that is bilateral. Precocious puberty in the setting of hCG-producing tumors is quite rare in females because both LH and FSH are typically required for ovarian follicular development. One reported case of a female with a suprasellar germinoma was explained by the demonstration of aromatase activity in the tumor [158]. An hCG-secreting tumor should be suspected in a boy presenting with marked virilization but without significant testicular enlargement. Hepatoblastomas comprise the majority of these tumors, although hCG may arise from pinealomas, intracranial germinomas or choriocarcinomas, and thymic or testicular germ cell tumors [159]. Tumor markers that have been quite useful in the diagnosis and follow-up of these tumors include alpha-fetoprotein, hCG, and pregnancy-specific beta-1-glycoprotein [160]. The diagnosis of an extratesticular germ cell tumor should prompt an evaluation for Klinefelter syndrome because such tumors are 50 times more common in these individuals [161]. This increased tumor risk has been identified even in those males with low-level mosaicism for Klinefelter syndrome [159].

26.5.7 Severe Hypothyroidism

Severe hypothyroidism may rarely present with precocious puberty (Van Wyk–Grumbach syndrome). The cardinal sign of this disorder is the child presenting with sexual precocity, poor growth, and skeletal delay. Girls may present with breast development, galactorrhea, ovarian cysts, and vaginal bleeding [162, 163]. Boys may develop testicular enlargement with minimal virilization. Thyroid hormone replacement results in regression of the secondary sexual characteristics with the exception of the macroorchidism [164]. The mechanism for the sexual precocity is still somewhat unclear. There is evidence for mechanisms at both the gonadal and pituitary levels; cross-reaction of high levels of TSH and α-subunit can occur at the gonadal FSH receptor [165], and stimulation of gonadotrope FSH secretion may occur by elevated TRH levels [164]. We have seen several patients with primary hypothyroidism and precocious puberty that had a pubertal gonadotropin profile classical for central precocious puberty. Galactorrhea occurs from increased hypothalamic TRH secretion, which in turn increases secretion of prolactin.

26.6 Gynecomastia

The reported prevalence of pubertal gynecomastia in boys has ranged from 30 to >90% [166]. Prepubertal gynecomastia, on the contrary, is quite rare and almost always abnormal. The published data for prepubertal gynecomastia report an age of onset between 2 and 7 years with both unilateral and bilateral breast development. The etiologies include gonadal, adrenal, and hCG-secreting tumors [167], exogenous estrogen exposure [104, 168, 169], endocrine disruptors, and "idiopathic" [170]. It is likely that some familial cases of gynecomastia are secondary to an aromatase excess syndrome [171]. A thorough evaluation for sex-steroid origin is required in the male with prepubertal gynecomastia. Tumor excision permits pubertal regression. In the case of idiopathic gynecomastia, surgical excision of glandular tissue is curative. Antiestrogens or aromatase inhibitors have being investigated for this indication with limited success.

26.7 Conclusion

Physicians involved in the care of children commonly encounter premature sexual development. The first step in evaluating such a child is the ascertainment of secondary sexual characteristics through a thorough physical examination. Isolated breast development in a female between 12 and 30 months of age may be benign premature thelarche. On the other hand, accelerated linear growth and advanced skeletal maturation suggest a more serious or progressive disorder. Isolated virilization in a female indicates excess androgen production. Arriving at a correct diagnosis permits the selection of the appropriate therapy. The diagnostic approach to evaluating precocious puberty in boys and girls is illustrated in Figs. 26.7 and 26.8, respectively.

Although we have gained much insight into the diagnosis and management of precocious puberty, numerous areas of controversy remain. The age of onset of normal puberty is still being debated. GnRHa therapy has revolutionized treatment for children with central precocious puberty. For some girls with idiopathic central precocious puberty, progression can be quite slow, without an apparent compromise in final height [172–175]. Follow-up of these selected patients suggests that therapeutic intervention may not be warranted [47, 176, 177]. Advances have been made in the molecular diagnosis of several forms of gonadotropin-independent precocious puberty. However, therapy for these disorders remains suboptimal. While much has been learned, much remains to be discovered.

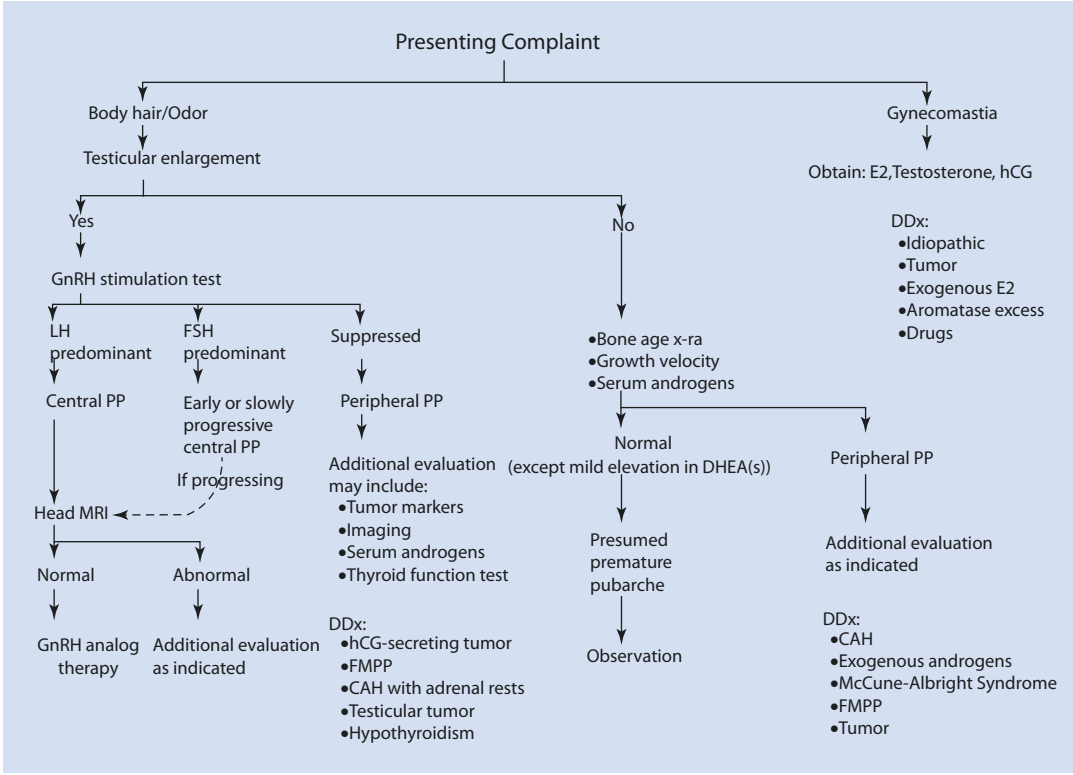

Fig. 26.7 Diagnostic approach to evaluation of precocious puberty in boys (Data from Eugster and Pescovitz [181])

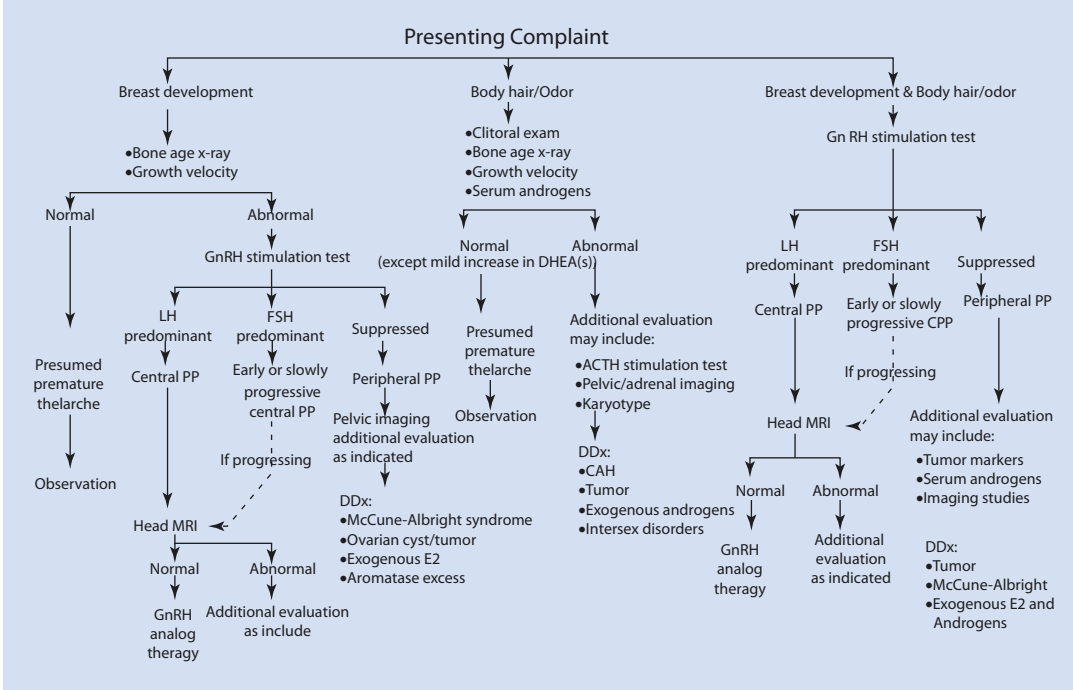

Fig. 26.8 Diagnostic approach to evaluation of precocious puberty in girls (Data from Eugster and Pescovitz [181])

Case Study

A 7-year-old Latina presents after her mother noticed breast development about 6 months ago. She had a normal prenatal course, was born AGA, and reached normal developmental milestones. Mom is concerned that she is "hanging around" girls in the fourth grade. Mom had menarche at 11 ½. Her height is at the 75th percentile for age, her weight is at the 90th percentile for age, and her midparental height is at the 50th percentile. Her examination was unremarkable except for Tanner stage II breast development without pubic hair. She had a normal neurologic exam. Laboratory studies show an AM LH 2.2 U/L, FSH < 0.2 U/L, and estradiol 40 pg/ml. Her bone age is 8 years.

Review Questions

1. The best next step in evaluating this child would be:
   A. Brain MRI
   B. Pelvic ultrasound
   C. Reassurance and follow-up in 3–6 months
   D. GnRH agonist stimulation test

Case Discussion

Recent studies suggest that the average age of pubertal onset is decreasing in American girls, sparking controversy in defining the age at which puberty is considered precocious. Studies have also shown ethnic differences in the trend toward early breast development, especially among African-American and Latina girls. Obesity has been suggested to play a role in the possible early onset of puberty in girls, as girls with early onset of breast budding have higher BMI scores than age-matched girls without budding. Significant implications arise regarding this diagnosis, including extensive and expensive testing for precocious puberty and consideration of therapy. The only permanent physical complication of true isosexual precocity is short adult height. Excessive sex hormone production causes early maturation of the epiphyses resulting in their premature closure. About half the girls with this disorder reach an adult height of 53 to 59 inches, and the remainder is over 60 inches tall. This young girl has no clinical evidence of progressive puberty; hence, close follow-up is indicated. However, 6- to 8-year-old girls with a suggestion of rapidly progressive or excessive androgenization or feminization, neurologic symptoms, linear growth acceleration, or significant bone age advancement should be more completely evaluated. Ultrasonography is indicated to screen for abdominal or pelvic masses when progressive feminizing or virilizing disorders are suspected. MRI of the hypothalamic–pituitary area is indicated in progressive true sexual precocity, especially those less than 6 years old or those at risk of organic causes by virtue of their underlying condition or neurologic symptoms and signs. If the precocious development continues over the next 3–4 months, a GnRH test may be considered, especially if GnRH agonist therapy is being considered. A post-GnRH peak LH >6.9 U/L has been reported to be 92% sensitive and 100% specific [178], whereas a post-GnRH agonist peak LH >4.0–5.0 U/L has been reported to be ≥90% accurate for the diagnosis of central precocious puberty. The GnRH agonist test also permits assessment of the ovarian gonadotropin-responsiveness: an estradiol peak ≥34 pg/ml is approximately 90% sensitive and ≥60 pg/ml 95% specific for puberty. FSH levels are not as helpful diagnostically since prepubertal values overlap considerably with pubertal ones and they may be elevated in premature thelarche. *Thus, the answer is C.*

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Management of Infants Born with Disorders/ Differences of Sex Development

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Key Points
- Disorders of sex development
- Medical management of DSD
- Gender change
- Gender dysphoria

27.1 Introduction and Background

In 2006, the European Society for Pediatric Endocrinology (ESPE) and the Pediatric Endocrine Society (PES) replaced the terms intersex, hermaphroditism, pseudohermaphroditism, sex errors of the body, and ambiguous genitalia with an umbrella term, disorders of sex development (DSD) defined as "congenital conditions in which development of chromosomal, gonadal, or anatomical sex is atypical" [1]. The reason for the change in nomenclature was that traditional terms were not only confusing to patients and professionals alike but also considered derogatory by some. DSD are rare conditions, and the lack of precise medical codes precludes collection of accurate population statistics for DSD. However, the estimated incidence is 0.1–0.5% of live births [2]. Approximately 1 in 4000 infants is born with atypical external genitalia and will receive a DSD diagnosis [1].

The current genetic-based classification system of DSD highlights the importance of chromosomal analysis in the diagnosis and management of individuals with DSD [1].

Since its inception, the term DSD has been accepted and used widely by the medical profession; however, critics have raised concerns because of the negative connotations associated with the words "disorder" and "sex" [3, 4]. Some professionals and their organizations are now defining "DSD" as differences of sexual development, hence the two terms used in the title of this chapter.

27.1.1 Purpose

The aims of this chapter are to (1) provide a concise update on the mechanisms controlling typical and atypical sexual differentiation, (2) share a protocol used at our institution for the practical management of infants born with DSD, (3) provide information to guide the physician when making a DSD diagnosis and designing its medical management, (4) provide new behavioral information on DSD, and (5) highlight current differences of opinions about the care of infants born with DSD.

27.2 Etiology

27.2.1 Mechanisms Involved in Sexual Differentiation: Tissues, Genes, and Hormones

27.2.1.1 The Bipotential Gonad

The bipotential gonad is destined to become either a testis or an ovary depending on the sex chromosome constitution of the germ cells (classified as gonadal or primary sex) as well as other critical sex-determining genes that control male vs. female pathways of sex development (Table 27.1). At conception, the genetic sex (XX or XY) of the fetus is determined when an X- or Y-bearing sperm fertilizes the ovum. During the next few weeks, the developing germ cells migrate from their point of origin in the endoderm of yolk sac wall to their final destination within the primitive gonad.

27.2.1.2 Testicular Differentiation

Testicular differentiation depends primarily on the sex-determining region Y (SRY) gene located on the short arm of the Y chromosome. The SRY gene product codes for a 204 amino acid transcription factor which binds to and bends the DNA strands, thus allowing access by other transcription factors. However, the mechanism by which SRY determines gonadal sex is not fully understood. Two mechanisms have been proposed: (1) SRY activates a cascade of genes needed for male development, or (2) SRY inhibits a repressor of male determining genes. In the activator model, the male pathway is dominant and the female pathway is the default pathway. In contrast, the repressor model suggests an active female development pathway, which must be suppressed by a male gene. In humans, SRY is expressed in the testes and a variety of brain structures; whether expression in the brain influences sexual behavior is unknown. SRY expression is tightly regulated. It is seen early in testicular development and is transient. SRY induces Sertoli cell development, followed by

Table 27.1 Genes controlling sexual differentiation.

Gene/chromosome	Family/function	Clinical phenotype
SRY, Yp11.3	HMG protein, transcription factor	XY gonadal dysgenesis
WT1, 11p13	Zinc finger protein transcription factor	Denys-Drash syndrome Frasier syndrome
SF1, 9q33	Orphan nuclear receptor transcription factor	XY gonadal dysgenesis Adrenal insufficiency
DAX1, Xp21.3	Orphan nuclear receptor transcription factor	Duplication causes XY sex reversal Mutation causes XY adrenal hypoplasia congenita (AHC) Gonadotropin deficiency
SOX9, 17q24.3	HMG protein transcription factor	Mutation causes XY sex reversal Campomelic dysplasia
Wnt-4, 1p32–36	Growth factor	Duplication causes 46,XY sex reversal Deletion causes Masculinization of 46,XX female

differentiation of seminiferous tubules and the formation of Leydig cells. The middle third of the SRY protein has a DNA-binding domain known as the HMG (high mobility group) box protein, which belongs to a family of transcription-regulating proteins known as the HMG box proteins. Mutations within the HMG region of the SRY gene result in failure of testicular development and sex reversal with a female phenotype or genital ambiguity [5–7].

SRY mutations are not responsible for all cases of sex reversal in 46,XY females, nor do they explain the presence of testes in 46,XX males with genital ambiguity. Only 25% of 46,XY females who present with gonadal dysgenesis (streak gonads) and female phenotype have an SRY mutation. Further, SRY is present in only 10% of individuals with 46,XX ovotesticular DSD and 10% of those with 46,XX DSD. In contrast, among 46,XX males with normal male genitalia and testes, the majority are SRY positive. It is possible that the role of SRY may be underestimated in individuals with sex reversal if it is present only in the gonads and not in peripheral blood lymphocytes [5].

The importance of certain autosomal genes in testicular differentiation has become more obvious (► Box 27.1). The most notable among these is SOX (SRY-homeobOX) 9 gene. The contribution of this gene to testicular differentiation was discovered when a 46,XY infant with a SOX9 mutation presented with skeletal dysplasia (campomelic dwarfism), female phenotype (sex reversal), and streak ovary-like gonads [6, 8]. This clinical presentation has been attributed to haploinsufficiency resulting from loss of one copy of SOX9. Since SOX9 is located on 17q24–25, the female phenotype in 46,XY males with this mutation has been classified as autosomal sex reversal [9, 10]. SOX9 mediates Sertoli cell development

Box 27.1 Causes of 46,XY Disorders of Sex Development (DSD)

1. XY gonadal dysgenesis
2. Denys-Drash syndrome – WT-1 mutation, also nephropathy, Wilms' tumor
3. Frasier syndrome – WT-1 mutation, also nephropathy, gonadoblastoma
4. XO/XY mosaicism-mixed gonadal dysgenesis
5. Campomelic dysplasia – SOX9 mutation
6. DAX1 duplication (DSS syndrome)
7. Wnt-4 duplication
8. SF1 (NR5A1 gene) mutation
9. Deletion of 9p-, 10 q-
10. Duplication: X p +

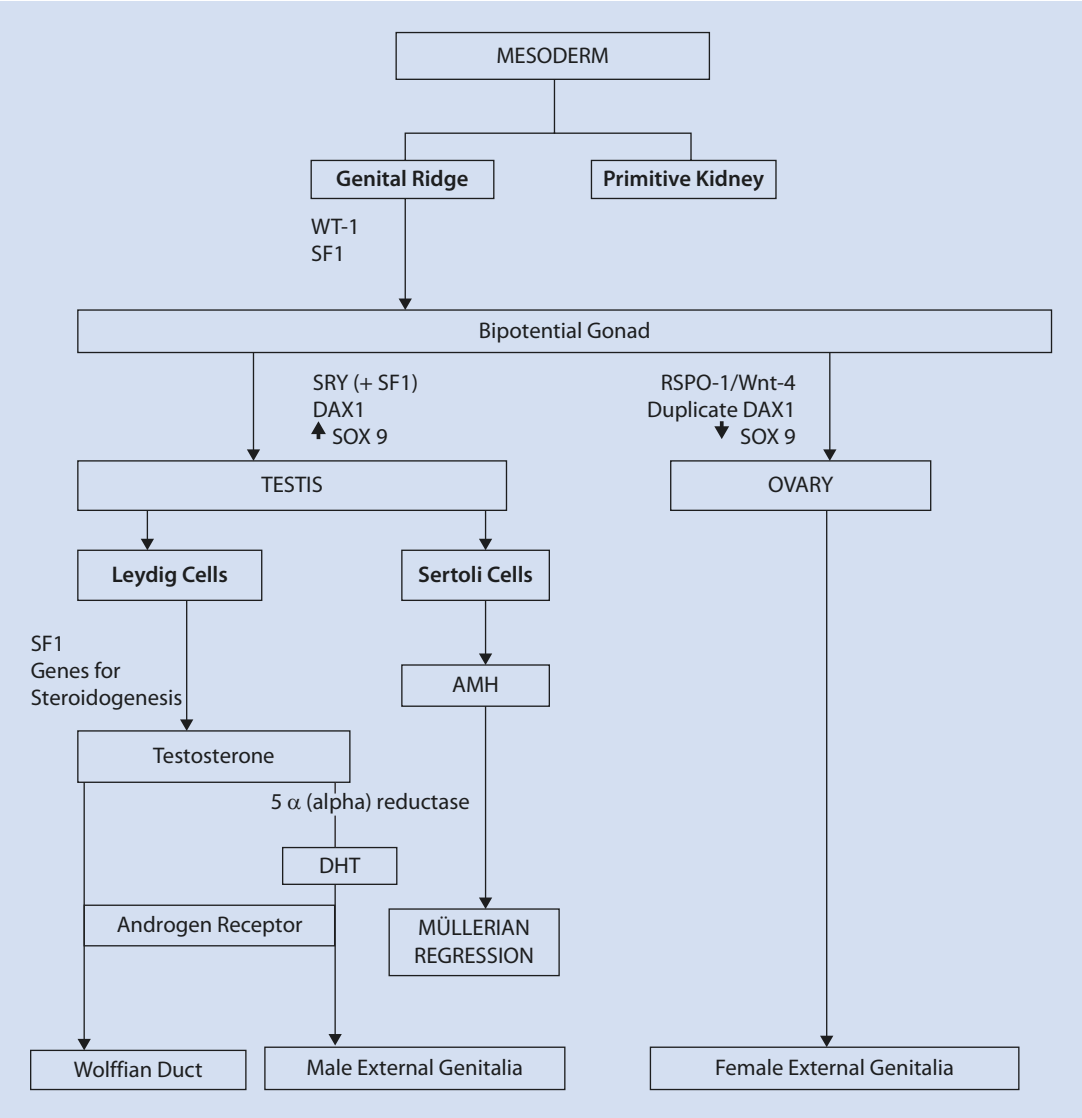

Fig. 27.1 Pathway of sexual differentiation

and testis differentiation [8] along with initiation of anti-Müllerian hormone (AMH) expression [11]. Consequently, SOX9 is an autosomal gene, which plays a pivotal role in male sexual development (Fig. 27.1). Two additional genes, steroidogenic factor 1 (SF1) and Wilms' tumor 1 (WT1), have a synergistic role in testicular development [12]. SRY is transactivated by the WT1 gene product [13]. In the testis, SRY and SF1 cooperatively upregulate SOX9 [14].

The role of DAX1 (dosage-sensitive sex reversal-adrenal hypoplasia congenita critical region on the X chromosome, gene 1) in testicular development needs special mention. Duplication of DAX1 inhibits SRY [15, 16] and inhibits the synergy between WT1 and SF1 [12] blocking subsequent testicular development. Mouse models have additionally demonstrated a "pro-testis" effect of DAX1 [17]. The animal models indicate that a precise dose of DAX1 is important for testicular development, "too much or too little can prevent cord formation" [18].

27.2.1.3 Ovarian Differentiation

Ovarian differentiation is poorly understood but recent evidence suggests that the process is actively regulated by a network of genes and transcription factors. It is no longer believed that

ovarian development is a passive or default process. Ovarian development appears to depend on suppression of testis-inducing genes, along with activation of ovary inducing genes. The probable candidate genes for suppression of testis differentiation include DAX1 and Wnt-4. The DAX1 gene is located on the X chromosome, which is inherited from the mother in males. DAX1 duplication inactivates SRY [15, 16] and SOX9 function, thereby blocking testicular development and anti-Müllerian hormone (AMH) expression [11]. DAX1 appears to be an important anti-testis gene in the ovary. 46,XY individuals with a duplication of DAX1 have a female phenotype (sex reversal) and gonadal dysgenesis [19, 20]. The DAX1 duplication may be located on the X chromosome, or, alternatively, one DAX1 gene can be normally located on the X chromosome along with a second translocated DAX1 gene on the Y chromosome.

In the differentiating ovary, autosomal genes also play a significant role on ovarian development. R-Spondin-1 (RSPO-1) affects Wnt-4 signaling [21, 22], which in turn appears to inhibit testicular vascular development, stabilize oocytes, and induce Müllerian development [23]. Deletion of Wnt-4 leads to masculinization in 46,XX individuals and degeneration of Müllerian duct derivatives [24]. Wnt-4 appears upstream of and in concert with DAX1. In 46,XY individuals, duplication of Wnt-4 causes upregulation of DAX1 in the testes, and suppression of SOX9, resulting in a female phenotype [25]. Hence, duplication of either DAX1 or Wnt-4 causes dosage-sensitive 46,XY sex reversal.

Genes that support ovarian development have not been completely identified. However, they are probably located on the X chromosome and may have dosage-sensitive functions as women with Turner syndrome, a condition associated with partial or total absence of one X chromosome, have a loss of ovarian germ cell development.

The sequence of genetic events in ovarian development is depicted in the following theoretical model:

1. Activation of DAX1 antagonizes SF1 and SOX9.
2. Granulosa cells develop from the supporting cells while Sertoli cell differentiation is blocked.
3. Wnt-4 induces differentiation of Müllerian structures into the uterus, fallopian tubes and upper third of vagina.
4. Wnt-4 produced by ovarian somatic cells prevents interstitial precursor cells from developing into Leydig cells (see Fig. 27.1).

Certain mechanisms have been shown to cause sex reversal. Duplication of SOX9 is an autosomal cause of sex reversal in 46,XX individuals [26], while mutations in the SF1 gene and other sex-determining genes are also known to cause 46,XY gonadal dysgenesis and a female phenotype.

27.2.2 The Paired Internal Ducts: Wolffian and Müllerian Ducts

27.2.2.1 Wolffian Ducts

The differentiation of the Wolffian duct into the epididymis, vas deferens, seminal vesicles, and ejaculatory ducts in 46,XY males is dependent on the local (paracrine) production of testosterone by the Leydig cells [27, 28], which is initially regulated by human chorionic gonadotropin (hCG) [29]. Genetic males who are agonadal exhibit involution of the Wolffian ducts and also have Müllerian ducts because AMH is not produced [27, 30]. Normally, AMH is produced by the Sertoli cells in the fetal period, and the production continues until the age of 8 to 10 years in boys, after which it declines. Hence, AMH is a marker of Sertoli cell function in patients with DSD [31].

27.2.2.2 Müllerian Ducts

Müllerian duct differentiation progresses in the absence of AMH and results in the development of the fallopian tubes, uterus, cervix, and upper third of the vagina. The transcription factor Wnt-4 plays an important role in Müllerian duct differentiation and in ovarian function during fetal life. Female Wnt-4 knock-out mice fail to develop Müllerian structures and lose oocytes [24]. Additionally, they manifest hypersecretion of ovarian androgens derived from Leydig cell precursors in the interstitium and exhibit masculinization of the Wolffian ducts [32]. Thus it appears that Wnt-4 acts to suppress androgen production by precursors of Leydig cells in the interstitium of the ovary in addition to being a regulator of Müllerian duct development.

27.2.3 The Bipotential External Genitalia

The primordial structures of the male and female external genitalia are homologous, and the external genitalia of genetic males cannot be distinguished from genetic females at 8 weeks of life. The genital tubercle becomes either the clitoris or the penis. The urethral folds become either the labia minora and clitoral hood or the skin covering the penis including the foreskin. The labioscrotal folds or genital swellings become either the labia majora or fuse together to become the scrotum. Future development and differentiation of the bipotential external genitalia from this homologous state into male structures is dependent on the presence of androgens. Female-appearing external genitalia will differentiate and develop if androgens are absent as seen in the fetus with no gonads, streak gonads, and non-functional testes or if androgen function is defective as in androgen insensitivity due to genetic defects.

27.2.3.1 Male External Genitalia

Development of male external genitalia depends on the adequacy of testosterone (T) and 5α (alpha)-dihydrotestosterone (5α-DHT) production as well as functional androgen receptors (AR). The testis is formed by 6 to 7 weeks of fetal life, and the Leydig cells are stimulated by placental hCG in the first trimester [29] and by fetal pituitary LH in the second and third trimesters [33]. Male external genital differentiation is complete by 12 weeks; additional penile enlargement in the second trimester is dependent on Leydig cell stimulation by LH from the fetal pituitary gland.

Complete masculinization of external male genitalia requires the conversion of T to 5α-DHT; the enzyme responsible for this conversion is 5α-reductase, which is present in genital tissue. 46,XY infants with 5α-reductase deficiency have normally functioning testes; the predominantly female phenotype in early childhood is due to defective conversion of T to 5α-DHT during early fetal life. In many societies where rates of 5α-reductase deficiency are high, spontaneous gender reassignment to male usually occurs in puberty. A number of reasons have been suggested for this transition. 5α-DHT is critical for normal external male genital differentiation in fetal life and virilization of males during puberty [34–36]. Cultural practices and preferences play another important role in gender reassignment. In many of these cultures, male identity is valued over female identity. Historically these cultures have come to expect a change from female to male and have even codified such expectation in their language [35].

27.2.3.2 Female External Genitalia

Female external genitalia will be normal except when exposed to androgens in early fetal life. Androgens may be derived from: (1) the mother (ingested or endogenously produced); (2) the fetus (congenital adrenal hyperplasia (CAH)); (3) due to homozygous or heterozygous mutations in the placental aromatase gene, which leads to a failure to convert maternal androgens to estrogens, with virilization of both the mother and the fetus during the pregnancy; (4) ovotesticular DSD; (5) mixed gonadal dysgenesis [37–39]. The degree of virilization is dependent on the quantity, timing, and actions of androgens to which the 46,XX fetus is exposed. Thus virilization ranges from clitoromegaly alone to severe posterior fusion of labia majora, absence of the labia minora and vaginal orifice, or a urogenital sinus with a large phallic-appearing clitoris that resembles a hypospadiac penis. Rarely, extreme virilization causes complete labioscrotal fusion and a "penile urethra" (Prader 5). In the last example, the external genitalia appear normal male, and the only clue to the possibility of a genetic female is the absence of palpable gonads, which is mistakenly thought to represent cryptorchidism.

27.3 Clinical Approach to Care of Infants with DSD

The care of a baby born with DSD starts with the recognition of the presence of a genital abnormality. The second step is to inform the parents of this finding and to reassure them that a team of experts will care for their baby. A patient-centered approach requires the physician to be mindful that parents usually are aware that something is wrong and are at a loss if the physician avoids talking with them openly. Avoidance increases the anxiety the parents are experiencing. Parents are always relieved to learn that their infant will

be cared for by a team of experts who have cared for many babies with similar problems.

The following is an outline of the protocol we follow at our institution when a baby is born "in-house" or "is transferred in" from a regional hospital (Fig. 27.2). Our team consists of pediatric endocrinologists, a pediatric psychologist with extensive endocrine expertise, pediatric urologists, geneticists, radiologists, the child's primary care physician, and other specialists as required.

27.3.1 DSD Protocol

1. The first physician to see the infant alerts team members that they need to see the infant as soon as possible; i.e., within a few hours. It is important to determine if the baby was announced as a boy or a girl in the delivery room. If a gender assignment has already been made or a name given to the baby, we do not change this information. However, we inform parents that diagnostic studies may indicate the need to consider reassignment of the baby's gender. If the infant arrives with no chosen name or gender, the entire staff and parents are asked to refer to the baby as "baby or infant" usually with the family name included, i.e., "baby Jones." Physical examination by professionals not directly linked to the case is avoided to decrease stress to the family.
2. Our multidisciplinary team has a team leader who not only organizes the exchange of information with team members but also acts as a liaison between the family and the panel of doctors. The team leader is often the pediatric endocrinologist who meets the family and examines the infant. However, in some instances, the psychologist serves in this role. The team leader provides the parents with the differential diagnosis, details about radiologic studies and biochemical tests that will be ordered, and a timeline for expected results. In addition, the team leader outlines any acute medical management that must be provided for their child. Parents are informed that all test results will be given after they are finalized and reviewed by the panel of doctors. It has been our experience that parents appreciate this approach as it minimizes conflicting opinions and decreases the likelihood that misinformation will be transmitted. Parents are assured that they will be part of the decision-making team when the time arrives to discuss what is best for the baby.
3. At our institution, the psychologist is in daily contact with the parents. At the initial visit, the family members and other close relatives are educated about the typical process of sexual differentiation in males and females and how DSD may occur during fetal development. Information regarding gender identity development is also discussed. Diagrams and current source materials [40, 41] are helpful and repetition is essential. Examining the infant with the parents helps them to understand the changes that have led to the appearance of the genitalia. The pediatric psychologist provides support and assurance that all team members are working together to provide the best possible care for the child on a daily basis. Parents may reveal their most hidden fears to this individual and will tell of other stresses in the family that may complicate the acceptance of their infant. Such frequent contact also allows the parents to grieve over the birth of their infant who has anatomical differences and may have serious medical problems. One common concern of parents is how to inform siblings, relatives, and friends of the baby's condition or reassignment of gender if such a decision is made. The psychologist guides the family in developing strategies to handle these concerns.
4. While the psychologist is educating the parents and close relatives, the endocrinologist and other team members promptly begin the medical management as outlined in the next section (see ▶ Sect. 27.4). Diagnostic studies include hormone assays, genetic studies, ultrasound examination of the pelvic and abdominal structures (uterus, gonads, and renal anatomy), a vaginogram, and a voiding cystourethrogram, if needed.
5. Once all test results are available, the DSD team meets to review the results of the diagnostic studies, arrive at a consensus on the diagnosis, recommend the most suitable gender assignment, and decide which members of the team will meet with the parents. Often, a specific etiology is not readily available. Regardless, the team presents a unified decision to the parents, fully aware that the parents may not accept the team's advice about the gender assignment recommendation.

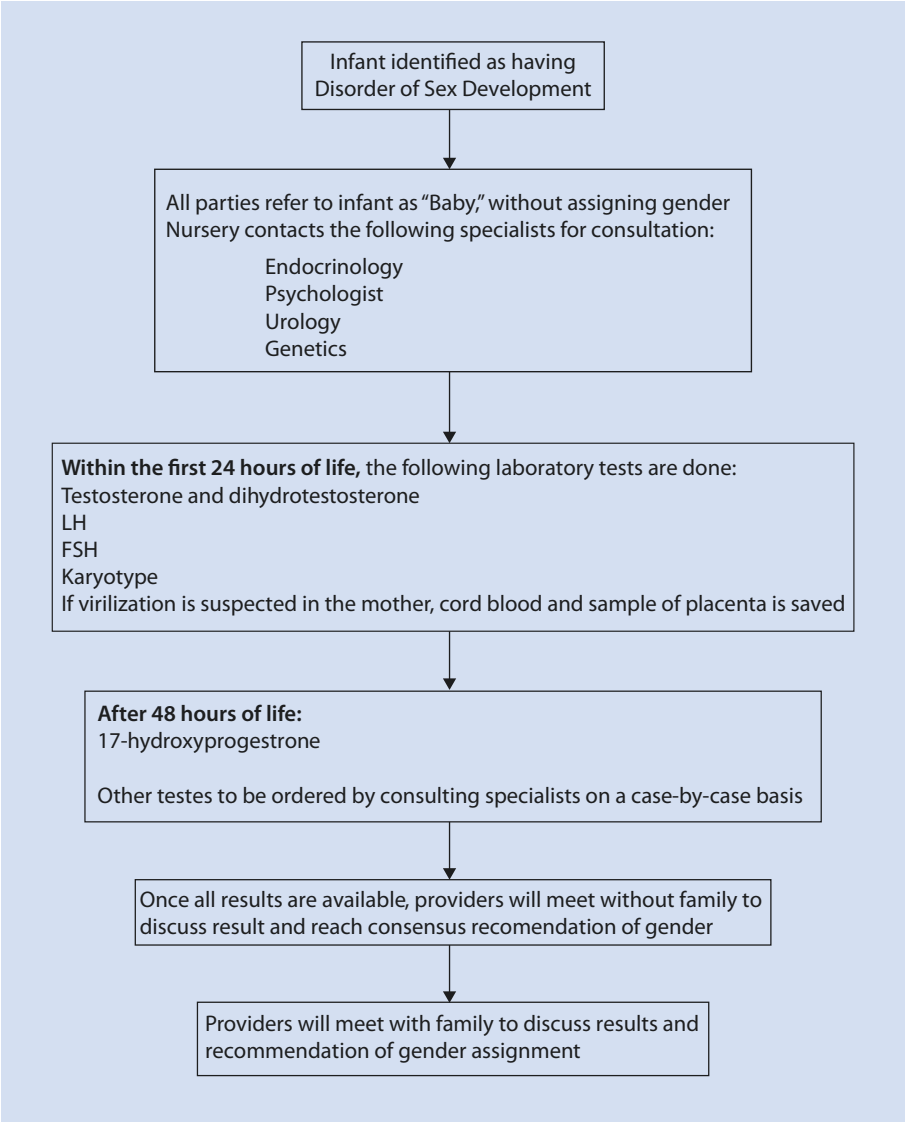

Fig. 27.2 DSD Protocol: An outline of protocol we follow at our institution when a baby with DSD is identified

6. The designated team members meet with the family for the following purpose:
   - To provide full disclosure of all the results and the most likely diagnosis. If the medical diagnosis is still in question, the family is informed of this as well. The medical/surgical interventions associated with the diagnosis are reviewed.
   - To present the pros and cons of gender assignment as male or female and the consequences of each choice.
   - To give outcome information regarding fertility, the need for hormonal therapy, and psychosexual data if known.
   - To answer parents' questions and get their feedback. We recognize that parents may need time to make a decision. Parents are reassured that the medical care of their infant will not be compromised should they decide on a gender different from the team's recommendation.
   - Gender assignment, following which the name of the baby is announced.
   - To inform the parents that a summary of their infant's condition along with important time periods to remember, for example, for initiation of hormone treatment, will be provided.

7. Follow-up care is scheduled with the medical and surgical team members. The psychologist sees the family at regular intervals to support and educate them regarding what and how to inform their child about his/her condition at developmentally appropriate times. Ideally, the child will meet the psychologist, who will, along with the parents, educate him/her over time about the DSD diagnosis [3].

27.4 Clinical Presentation and Medical Management of DSD

27.4.1 Medical History

From the outset, the following simple questions guide the physician with the differential diagnosis and what tests to order:

1. Is the infant a genetic female (46,XX) exposed to fetal androgens (i.e., 46,XX DSD)?
2. Is the infant an undervirilized genetic male (46,XY) due to underproduction or decreased action of androgens (i.e., 46,XY DSD)?
3. Does the infant have a complex sex chromosome disorder (such as ovotesticular DSD in which 80% of patients have a 46,XX karyotype or mixed gonadal dysgenesis, 45,X/46,XY)?
4. Is the genital defect the result of a birth defect in structures that distort the genitalia (i.e., epispadias, cloacal exstrophy, or aphallia)?

A thorough history often provides important information that helps in the diagnosis. Special attention is given to the history of maternal drug or medication use (anabolic steroids or androgens in oral contraceptives), general health and endocrine status (maternal hirsutism or virilization, including virilization during the pregnancy), family history (such as infertile non-menstruating maternal female relatives with scant or absent pubic and axillary hair, which suggests androgen insensitivity with an X-linked recessive inheritance pattern [42]), and consanguinity in the parents (for autosomal recessively inherited conditions such as 5α-reductase deficiency). Antenatal data such as ultrasound examinations and genetic studies obtained from chorionic villus sampling or amniocentesis are invaluable in the diagnostic process. Discordance between fetal karyotype (46,XY) and antenatal ultrasound genital findings (no phallus) may provide early evidence of androgen resistance or aphallia.

27.4.2 Physical Exam

A gonad located in the inguinal canal by either manual palpation or by ultrasound exam is highly informative. In nearly all cases, an external gonad is a testis. In the absence of an external gonad, the genetic sex of the infant cannot be identified by inspection if the infant has (1) 46,XY DSD with incomplete virilization, (2) 46,XX DSD with excess virilization, (3) ovotesticular DSD, or (4) mixed gonadal dysgenesis.

It is best to avoid using definitive terms such as penis or scrotum until the diagnostic studies are completed. Instead, the phallic structure (clitoris or penis) is measured and examined for presence of chordee, a downward curving phallus due to a shortened ventral surface. The phallic measurement may not be easy because an erectile mass is sometimes buried in pubic fat and is curved. A tape measure placed on the point of origin on the pubic ramus along the dorsum to the "erectile mass" gives a reasonable estimate of length [43, 44]. If the stretched length is less than 2.0 cm, the diagnosis of microphallus is made [45]. The width of the erectile tissue and its consistency should also be recorded. Hypospadias is defined by the location of the urethral opening and is classified as first degree (asymmetric on "glans"), second degree (mid phallic shaft), third degree (junction of phallus with the scrotum), or fourth degree (perineal, closer to the anus). Rarely, 46,XX infants with salt losing CAH (21-hydroxylase deficiency) have extreme virilization classified as Prader 5 because they have a penile urethra, totally fused empty scrotum, and normal looking genitalia. They resemble male infants with cryptorchidism.

The presence of a vaginal dimple or introitus should be recorded. Babies with complete androgen insensitivity syndrome (CAIS) have normal female-appearing external genitalia with a normally positioned urethra and a blind vaginal pouch.

In most infants with DSD, the labioscrotal folds look like two separate sacs separated by an indentation giving the appearance of a "bifid scrotum," either flat or rounded. The labioscrotal folds may be smooth or rugated with many linear

creases on the surface. Posterior perineal fusion of the labioscrotal folds may be partial or complete; the latter is indistinguishable from a scrotum. Often the physician is misled as to the actual location of the urethral opening, which may be more posteriorly located but is hidden because the phallus is "enwrapped" in the fused labioscrotal folds.

In addition to the genital appearance, a record should be made of the presence or absence of dysmorphic features, skeletal abnormalities, or other physical exam findings that might be useful in establishing a diagnosis. For example, campomelic dwarfism is an eventually fatal condition in 46,XY individuals who have striking skeletal dysplasia and female phenotype [8].

27.4.3 Diagnostic Evaluation

The following tests are informative:

1. Karyotype (may include genetic studies SRY (FISH for SRY gene may have faster turnaround time), SOX9, DAX1, WT1, etc.)
2. Serum hormone levels:
   - Within 24 h of life
     - Testosterone
     - DHT
     - LH
     - FSH
   - After 48 h of life
     - 17-OH progesterone
     - Androstenedione
     - 17-OH pregnenolone
     - DHEAS
     - Renin
3. Electrolytes
4. Imaging studies:
   - Ultrasound of the pelvis/inguinal canal to identify the presence of the uterus and gonads
     - Ultrasound of kidneys
   - Urethrogram/vaginogram/voiding cystogram – to determine the position of the urethra, presence of a vagina, and reflux

27.5 Challenges in Diagnosis and Treatment of DSD

An algorithm that links the diagnostic tests to clinical diagnosis is provided in Fig. 27.3.

The most common cause of DSD in a 46,XX genetic female with the diagnosis of CAH is 21-hydoxylase deficiency; 75% are salt losers who will develop low sodium, high potassium, and significant weight loss during the second week of life. Prior to clinical dehydration, the asymptomatic salt loser can be identified by high renin levels. 46,XY males with CAH are at high risk for shock because their genitalia are entirely normal. In the United States, screening of newborns for CAH by measuring 17-hydroxyprogesterone has protected infants from high morbidity and death. Female gender assignment is relatively straightforward in virilized CAH genetic females because the infants have a uterus and ovaries [37]. Many 46,XX CAH individuals with a penile urethra are being reared as girls following phallectomy and genital reconstructive surgery, while others have been successfully reared as males and have undergone gonadectomy and hysterectomy. There is ongoing debate as to the best choice for these females who have unambiguously male external genitalia with female internal sex structures. The International Consensus Conference on Intersex 2005 on CAH recommend that all 46,XX CAH patients be reared female. The CAH consensus statement indicated that "there is insufficient evidence to support rearing a 46,XX infant at Prader 5 as a male." Recently, evidence has been reported on twelve 46,XX individuals diagnosed with CAH and born with Prader 4 or 5 genitalia [46], all of whom were assigned the male gender at birth. Ten of the 12 individuals always lived as a male. Two were reassigned the female gender in childhood but eventually self-reassigned themselves as male. At the time of the study, the age range of the men was between 35 and 69 years. Based on these findings, Houk and Lee proposed "consideration of male assignment for 46,XX patients who have fully developed male genitalia" [46, 47]. In a third paper, Lee and Houk [48] reported on other cases, although rare, of individuals who were assigned the male gender at birth, which was firmly established as male in adulthood. Given this new evidence, we believe that the best standard of care requires that parents be fully informed of this new information when initial gender assignment is considered.

The most difficult decisions associated with gender assignment revolve around 46,XY infants with severe micropenis, either isolated or associated with hypospadias. The testes may be of

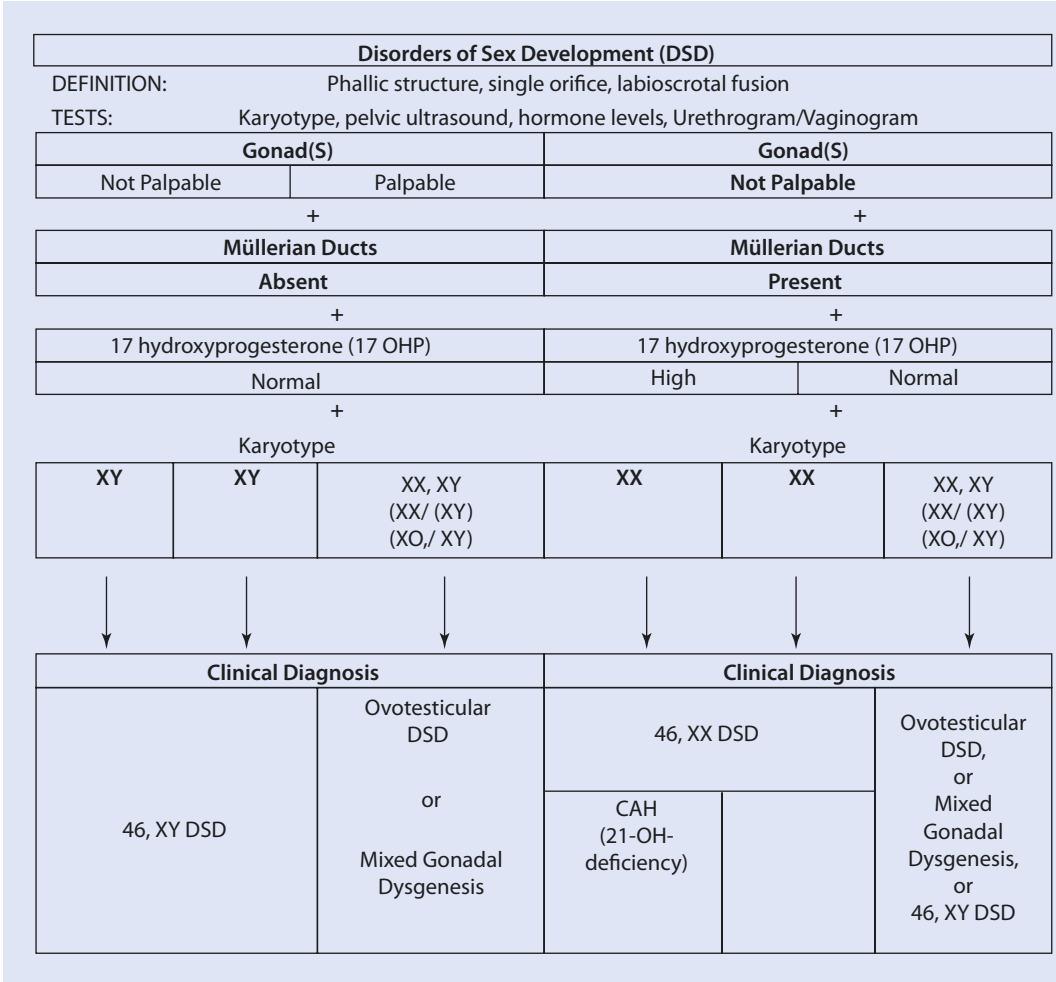

Fig. 27.3 Differential diagnosis of disorders of sex development (DSD): The main features included in this diagram are the presence or absence of external gonads, the structure of Müllerian duct derivatives, the serum concentrations of 17-hydroxyprogesterone, the karyotype, and possible clinical outcomes using this information. DSD resulting in normal-appearing genitalia are not included in this diagram (i.e., 46,XY complete androgen insensitivity syndrome, 46,XY 17-α (alpha)-hydroxylase deficiency, 46,XX males and 46,XY females)

normal size or hypoplastic. Partial androgen insensitivity is a concern because in this condition, male genital growth is permanently compromised. In the past, 46,XY infants with micropenis, with or without hypospadias, were assigned the female gender [49, 50]. This recommendation was based on the belief that it was easier to make a functional vagina than a functional penis. Thus, a female gender assignment was predicted to result in a more favorable psychosexual outcome. The proponents of this recommendation appreciated the influences of intrinsic genetic forces (nature) as well as the impact of environmental factors (nurture) but reasoned that environmental forces within the family were more dominant than the genetic forces if the gender reassignment was done within the first year of life [51]. With this strategy, genital reconstruction was done in infancy without a prior trial of testosterone to enlarge the micropenis because it was believed that the penile response to treatment would not accurately predict the size of the penis in adulthood. Also, it was held that a micropenis at birth was synonymous with micropenis in adulthood. This approach has been challenged by multiple observations. 46,XY infants with micropenis reared as males have reported satisfactory sexual function as an adult, even though they have expressed concerns about their penile size [52–54]. At present, many pediatric endocrinologists believe that 46,XY

infants with isolated micropenis and a fused scrotum should receive a short course of depot testosterone (25 mg IM once a month for three injections) in infancy and reared as males. They believe that rapid increases in length and width of the phallus are good prognosticators of future penile growth. In addition, for 46,XY infants with micropenis and hypospadias, increases in penile growth following testosterone treatment facilitate reconstructive surgery to correct hypospadias. The outcome of these infants in adult life appears to be reasonably good, even though penis size may not be in the normal range [52–54]. In the most severely affected 46,XY males with negligible phallic tissue, the urologist often advises the team and parents that penile reconstruction is not possible.

Gender assignment is an "imperfect art" [41] based solely in the past on anecdotal evidence and "the medical team's fear of the worst outcome." The notion that feminizing surgery has better long-term outcome is also being challenged. Early feminizing genitoplasty and female gender assignment has been criticized by some women who complained of genital discomfort and lack of arousability in adult life [55]. We now know that female sexual arousal and functioning is more complicated than was assumed previously. It requires preservation of erectile tissue and neurovascular anatomy. A constructed vagina that lacks intravaginal sensory responses or the ability to lubricate will likely not result in satisfactory adult female sexual function. The reconstructive vaginoplasty may be associated with scarring of the introitus, further affecting sexual satisfaction. Additionally, it may carry the risk of neoplasia [56].

The timing of various surgical procedures has become a point of great debate with the consensus favoring postponement of genital surgery until adolescence unless medically necessary for an individual's health [41]. For example, early surgery may be medically indicated for infants with a urogenital sinus connecting to the upper vagina. Although some centers advocate early correction for all genital differences, vaginal reconstructive or cosmetic constructive surgery is usually done in late adolescence by an experienced surgeon. In the past, early surgery (i.e., clitoral reduction) was promoted to maximize psychosexual adjustment [57, 58]; however, this approach is now debated. Because female patients must actively participate in post-operative care in order to increase the likelihood of a fully functional vagina, the level of maturity and commitment of the patient are crucial variables in selecting the timing of surgery in adolescent patients. In addition, "nerve sparing" clitoral surgery with focus on functional rather that cosmetic outcome is being promoted [41].

Some young women complain of discomfort resulting from prior genital surgery. Further data measuring outcome parameters such as vaginal width, depth, and comfort during intercourse may help resolve the debate on early vs. later vaginal surgery. The variability of postsurgical and psychosexual outcomes presently tips the balance in favor of waiting until the patient is able to actively participate in the decision-making process and give full consent.

Legally, the parents make the final decision about accepting a female gender of rearing for a 46,XY individual and the possible need for gonadectomy and later vaginal construction. However, religious and social beliefs may influence the decision of parents regarding rearing of 46,XY individuals as male regardless of penile size or future function. It is essential to give parents all the options regarding gender assignment including the pros and cons of each choice. If language barriers exist, an interpreter must assist communication. In some cultures, a male gender of rearing is considered an advantage regardless of the genital deformities. Parents have to be comfortable with the gender assignment decision since they are responsible for rearing their child. If they oppose the recommendation of the medical team and are forced to accept a decision, the emotional well-being of the child and family is placed in jeopardy. The level of parental understanding may require prolonged discussions and great patience by the medical team. The trained psychologist is particularly helpful in this situation.

DSD may be present in infants with normal or nearly normal external genitalia [43, 59] and may prove to be a diagnostic challenge in these infants. This includes 46,XY males who have complete androgen insensitivity syndrome (CAIS) or 17α-hydroxylase (P450C17 or CYP17) deficiency and are born with normal-appearing female genitalia. 46,XY females with CAIS present either in childhood with a hernia containing a testis or in late adolescence or adulthood with primary amenorrhea or absence of sexual hair. 46,XY females with 17α-hydroxylase deficiency may also present with primary amenorrhea but

are hypertensive due to ACTH-mediated excess of mineralocorticoids. The latter condition is autosomal recessive and consanguinity may be present in the parents. These patients fail to make all sex steroids because this enzyme plays a pivotal steroidogenic role in both the testes and adrenal glands. They lack sexual hair and are sometimes misdiagnosed as having CAIS. The Sertoli cells of the testes function normally; therefore, Müllerian structures are absent, similar to individuals with CAIS. A 46,XX female with the same genetic defect lacks sex hormones and is hypertensive but will menstruate when given estrogen replacement because of the presence of a uterus. Androgen replacement in a 46,XY individual with 17α-hydroxylase deficiency will result in sexual hair growth, distinguishing them clinically from individuals with CAIS.

27.5.1 Gender Change Following Initial Gender Assignment

Gender change from the initial gender assignment has been reported in patients with congenital adrenal hyperplasia [60], 5α-reductase deficiency, 17β (beta)-hydroxysteroid dehydrogenase deficiency [61], partial androgen insensitivity syndrome [62], and 46,XY individuals with penile agenesis, cloacal exstrophy of the bladder, or penile ablation who are raised female [63]. Recently there was the first reported case of an individual with a confirmed diagnosis of CAIS changing gender from female to male [64], and most recently there was the first case report of a gender change from male to female in a 46,XY individual diagnosed with CAH due to 21-hydroxylase deficiency [65]. Gender change from male to female or female to male has not been reported in patients with micropenis, isolated or when associated with other conditions (e.g., hypogonadotropic hypogonadism) [62]. All individuals with the diagnosis of Mayer-Rokitansky-Kuster-Hauser (MRKH) are assigned female, and to date there are no reports of gender change to male [66].

Several conclusions can be drawn about gender identity and gender change in individuals with 46,XY DSD conditions. First, the prevalence of individuals who undergo gender change varies markedly between the various conditions. Second, gender change from female to male occurs more often than from male to female; the only condition where gender change occurs in both directions is in partial androgen insensitivity syndrome. Third, gender change is not 100% in any given condition (even in persons reared female with a history of prenatal androgen exposure). These data do not support biological factors determining adult gender identity to the exclusion of others, but suggest an indirect influence of androgens on such development as gender change appears more common in conditions with relatively high androgen exposure [63].

Summarizing the data on gender change in 46,XY individuals, it is clear that gender change occurs in various frequencies in various conditions with the exception of micropenis where no gender change has yet been reported. These data also suggest that the best predictor, although not perfect, of what the established adult gender identity will be is the initial gender assignment. In cases of isolated micropenis, the extant literature showed no gender change in individuals from their initial gender assignment of male or female. However, the number of individuals with micropenis assigned and reared female is small, and many were young at the time of the review, ranging in age from 1 week to 29 years of age; consequently, we do not know what their established adult gender identity will be. Until further evidence proves otherwise, we recommend an initial male gender assignment in individuals with isolated micropenis.

27.5.2 Gender Dysphoria Diagnosis in Individuals with DSD

A person who desires to change his/her gender has a gender identity disorder (GID) (diagnosed by mental health professionals) using the criteria found in the fourth version of the *Diagnostic and Statistical Manual of Mental Disorders* (DSM-IV-TR). The recently published fifth version (DSM-5) replaced the term GID with gender dysphoria and allowed this diagnosis to include persons with a DSD diagnosis. DSD was an exclusionary criterion for GID in DSM-IV-TR. The main reason for this change was the evidence mentioned above that self-gender change occurs in many different DSD. Perhaps another finding that was also influential but not mentioned by the DSM-5 working group was survey [67] data

that indicated that 40% of professionals working with adult transgender persons were providing services for people with a DSD diagnosis. These professionals were members of the World Professional Association for Transgender Health (WPATH). At the time of the survey, WPATH was the Harry Benjamin International Gender Dysphoria Association (HBIGDA). This change is not accepted by everyone [68, 69] primarily because there are historical, medical, and management differences between those who have gender dysphoria and a DSD condition versus those who do not [70].

27.5.3 Genetics and DSD

Diagnosis of patients with a DSD has traditionally been through the assessment of the patient's phenotype, including physical examination of external genitalia, imaging of the internal reproductive structures, endocrine assessment in basal and stimulated states, and molecular analysis of a small number of genes known to cause DSD. Modern technology along with increasingly wide availability and decreasing prices of genetic testing has allowed genetic investigation to be utilized earlier in the diagnostic process. Potential benefits of new genetic diagnostic tools include a shorter diagnostic time, improved genetic counseling and an understanding of reproductive options for the family, and improved monitoring [71].

27.5.4 Disagreements Over Nomenclature and Who Is Considered to Have a DSD Diagnosis

It has been 11 years since the consensus meeting in Chicago. While scientific and medical communities rapidly adopted the new nomenclature, some vocal elements of the patient community strongly opposed the word "disorder" because they said it implied pathology of the genitals which appeared different from the norm. Consequently, they disliked the words, "disordered and pathology" because they believed these words embedded a bias which often led medical professionals toward performing potentially harmful surgical "normalization" procedures [4]. Some affected individuals prefer the term "differences" to "disorders," i.e., "differences of sex development," and others reject DSD entirely, still preferring the term "intersex" – referring to an identity, rather than a diagnosable medical condition. A global initiative was created to address a number of issues since the 2005 consensus meeting including nomenclature [72]. Perhaps not surprisingly, there was no resolution. Therefore, the abbreviation DSD may indicate a "disorder of sexual development" or "differences of sexual development." Further, there are those who prefer to be referred to as intersex. There also remains disagreement on whether conditions such as Turner syndrome, Klinefelter syndrome, and hypospadias constitute a DSD.

27.5.5 DSD National Registries

National registries provide a large database for research and clinical purposes. Categorization and standardization of information allow for improved clinical care, research, and general understanding. While there have been registries for a number of medical conditions for years (e.g., cancer), only recently has there been creation of national registries for DSD. There are currently two such registries; the International DSD (I-DSD) in Europe and the National Institutes of Health funded Disorders of Sex Development-Translational Research Network (DSD-TRN) in the United States [73]. In addition to creating a large database for research purposes, the DSD-TRN is designed to delineate the process of clinical care with the hope of providing a more standardized non-categorical [74] approach to patient and family care, thus improving outcomes.

In addition to these two registries, there are currently two ongoing research projects. The one in the United States (Short-Term Outcomes of Genitoplasty) is a prospective study of the short-term outcomes of feminizing or masculinizing surgeries for children under the age of 2. There are three aims of this investigation: (1) the cosmetic outcomes of current surgical techniques to feminize or masculinize external genitalia in young children with ambiguous genitalia, (2) the incidence of urinary tract infections and surgical complications associated with different types of feminizing and masculinizing genitoplasty currently in use for young children with ambiguous genitalia, and (3) psychological outcomes

following parents' decisions to proceed with genitoplasty for their child (or not), including parent depressive, anxious, and posttraumatic stress symptoms, parent quality of life, uncertainty about their child's illness, and decisional regret regarding their choices at the time of surgical decision-making. Achieving these three aims will provide much needed information regarding the risks and benefits of genitoplasty procedures currently performed in young children with DSD as well as the impact of these decisions on parents.

The study in Europe (Comprehensive European Clinical Outcome Study) is focusing on the long-term outcomes of adults with various DSD diagnoses.

27.5.6 Global Legislation

Legislatures in several countries have passed laws accommodating those born with a DSD and who identify as other than male or female [75]. In 2012, Argentina passed the Gender Identity Law, which allows for name and sex changes on official documents without proof of surgical reconstruction or other medical or psychiatric treatment. This law was the first to allow gender reassignment based solely on individual proclamation and is applicable to both DSD and transgender cases [75]. In 2013, the Australian Senate passed the Sex Discrimination Amendment Bill [76] that introduced legislation prohibiting discrimination on the basis of DSD status. The new guidelines on gender recognition state that individuals should be given the option of selecting male, female, or intersex on their personal documents. In the same year, Germany introduced an "indeterminate" sex option on birth certificates. Also in 2013, the Council of Europe [77] adopted a resolution on the protection of children's rights to physical integrity, demanding no one be "subjected to unnecessary medical or surgical treatment that is cosmetic rather than vital for health during infancy or childhood."

Greater emphasis on "patient-centeredness" in healthcare delivery and increased awareness of DSD have been accompanied by controversy, especially about the utility of elective genital surgery in infancy or early childhood. Legal questions have been raised, and there is currently an ongoing lawsuit regarding the legality of surgical intervention in the management of a child with a DSD [78].

27.5.7 Lawsuits and DSD

While there have been lawsuits brought by individuals with a DSD, or their parents, regarding unnecessary medical surgery, these have largely been settled out of the public's eye [79]. The first public lawsuit challenging early gender assignment surgery on a child with a DSD was filed in the United States. The child was diagnosed with ovotesticular DSD, assigned a female gender, and received feminizing genital surgery at 16 months of age, after obtaining parental consent. Shortly thereafter, the biological parents abandoned their child, who then came under the care of the South Carolina Department of Social Services and was later adopted. Despite the feminizing surgery, the child eventually identified as male, and the adoptive parents alleged that the medically unnecessary early genital surgery, which occurred without the child's consent, violated the child's constitutional rights. In Germany, a woman with 46,XX CAH successfully sued the surgeon who removed her fully intact female internal organs when she was 18 years old. Her diagnosis of CAH occurred following an appendectomy in which a uterus and ovaries were discovered; she had previously been reared as a male and undergone surgery to correct hypospadias in childhood. She sought 100,000 euros in damages for pain and suffering, claiming the surgeon failed to fully inform her about the consequences of the operation or about possible alternatives [72]. In both cases, the issue of informed consent with regard to elective surgery appears to lie at the heart of the matter.

27.5.8 Shift in Research Focus and Clinical Care

For years, the clinical research and management of persons with DSD focused on the relationship between gender identity, gender role, sexual orientation, sexual functioning, and pre- and postnatal hormones because there was evidence from animal research that hormones, primarily androgens, affected sexual behavior. The reviews above mirror that focus. They do not report on the quality of life for individuals regardless of gender assignment and rearing or even in those who changed gender because if such data are available, they are not sufficient to draw any meaningful conclusions. They also do not focus on the influence of genes on brain sexual differentiation and

development, an area that is only beginning to be explored [80].

This approach was categorical, meaning that the focus was on investigating and managing psychosexual development and hormones to the exclusion of other variables of human development (i.e., quality-of-life variables, in addition to psychosexual status and hormones). Recently, Sandberg and Mazur [74] outlined an approach that encompasses psychosexual development in persons with a DSD in a broader, non-categorical approach to clinical care. This non-categorical approach guides theory development and clinical care by taking into account a growing body of evidence that successful developmental trajectories of persons with chronic medical conditions are influenced as much by psychosocial environment, supports, and organization of healthcare delivery as by the specific nature of the person's medical condition [81, 82]. Such an approach holds the potential to systematically account for variability in DSD patient quality-of-life outcomes and lead to translating clinical interventions, proven effective in the management of other chronic conditions, to the circumstances faced by those affected by DSD.

While investigation on basic biological mechanisms will continue, so will quality-of-life studies in individuals with various DSD syndromes. Examples of this work include those by Wisniewski et al. [83] and Stout et al. [84] on CAH, Schonbucker et al. [85] on sexual quality of life in 46,XY individuals with a DSD diagnosis, Mazur on a small sample of five 46,XY individuals assigned and reared female [86], and Bean's review of MRKH [66]. Recent reports have also focused on the effects of having a child with a DSD on parenting characteristics [87–89] and on peer relations [90].

27.5.9 Advocacy

Since Hermaphrodites with Attitude, the first advocacy group in North America, numerous advocacy organizations have formed for the purpose of advocating for individuals with DSD and their families. Advocacy and peer support groups have grown internationally [91]. These different advocacy organizations hold meetings to provide support as well as to allow individuals to meet others, voice concerns, and educate themselves and others on their conditions. The link ▶ http://www.accordalliance.org/ is a portal through which one can access many of these advocacy/support groups.

27.5.10 Unnecessary Medical Surgery

Performing surgery on infants that is not medically necessary for survival, but rather is cosmetic, is questioned and remains a major controversy [1]. Research on benefits of such surgery is lacking, and there is no evidence that advanced surgical techniques have resulted in improvements for the individual. On the other hand, there is evidence in some individuals that operations to create the appearance of typical male or female genitals have impaired sexual function and sensation. Furthermore, such operations to create sex-typical genitals can be damaging should a child's eventual gender identity not match the birth assignment. This controversy has prompted surgeons to improve their surgical techniques, but whether or not these will result in benefits to the individual remains to be proven. A second controversial issue is the timing of cosmetic surgery. Some surgeons believe healing is superior if performed in early childhood, preferably the within the first 2 years of life, while others advise waiting until the child can participate in the decision-making process. It is important that parents are fully informed of the current controversy so they feel knowledgeable, know that nothing is kept from them, and trust their providers to work with them in giving their child the best possible care.

27.5.11 Patient-Centered Care Emphasized

The consensus statement not only created a new nomenclature (DSD) to replace old stigmatizing and confusing terminology but also emphasized the importance of a "multidisciplinary team" to provide the best possible care for individuals with a DSD diagnosis and their families. The focus of this team is patient-centered care. Such care requires that the team of professionals pay attention to the patient's and family's needs, preferences, and beliefs, which the traditional hierarchal medical model typically overlooks. Within this context, support groups and other "interested parties" in improving the care of patients with a diagnosis of

a DSD and their families can be helpful as long as their approach is constructive [92]. In essence, the patient (when old enough) and family become part of the team interacting in open communication with each other. A nonprofit organization (Accord Alliance) was created to assist institutions in establishing successful interdisciplinary teams [93]. This organization also maintains a web-based clearinghouse ▶ http://www.accordalliance.org/ for educational tools and information about living with and caring for those with a DSD, which includes *Handbook for Parents* [40] and *Clinical Guidelines for the Management of Disorder of Sex Development in Childhood* [41].

Case Study

27

An Example of a DSD Non-categorical Approach

Birth history: A full-term infant, born vaginally, with normal APGAR scores, was transferred to our hospital from an outlining hospital soon after delivery because of "ambiguous genitalia." At birth the parents were expecting a girl based on prenatal ultrasound findings and were told at delivery that they had a little girl. Shortly after the delivery, they were informed that the female gender assignment might not be correct based on further inspection of the external genitalia. The parents were reported to be upset. Once transferred to our hospital, a member of our DSD team (TM) was informed, and our protocol (see DSD Protocol) was immediately initiated. He contacted all other members of the team and immediately went to meet the parents to inform them that we had extensive experience in helping parents whose infant was born with features such as those present in their infant. He told the parents that they were in the right place and explained our protocol to them, which included referring to their baby as "baby" with no name until a diagnosis, if possible, could be established. He also explained that once all results are reported, they would be part of a complete discussion of all findings. They would be informed as to what to expect in terms of what will be currently needed medically and in the future as their child develops and the psychological findings on parents with a child with a diagnosis as well as what we know about the child's psychological and behavioral development and needs in the years ahead. They would also be informed of the team's recommendation for gender assignment and the reasons for this recommendation which they can either agree with or not. TM also identified for them each member of the team and their respective specialty. He also began to demystify why the gender of assignment was in question by educating them as to how the internal and external sexual/reproductive systems develop and differentiate.

Physical examination: Healthy infant with no dysmorphology other than the external genitals; enlarged penoclitoral structure (longitudinal length = 2 cm) with opening at the base, fused labioscrotal folds with hyperpigmentation, giving the appearance of a bifid scrotum, no gonads palpated.

Test results: Laboratory tests were done as per the hospital protocol outlined. Serum LH, FSH, testosterone (18 ng/dl (normal for female, 20–64)), and DHT (10 ng/dl (normal for female <2–15)) concentrations were drawn within 24 h of life and were normal. Child's karyotype was 46,XX. At 72 h of life, blood tests were done at a reference laboratory: serum 17-hydroxyprogesterone, 571 ng/dl (normal, day3 < 78); 17-hydroxypregnenolone, 887 ng/dl (normal, 10–829); androstenedione, 210 ng/dl (normal <10–279); and DHEAS, 166 µg/dl (normal, 88–356). Serum electrolyte concentrations and plasma renin activity were normal. Pelvic ultrasound showed a uterus but no gonads; VCUG showed a normal bladder and a vagina that opened into the urethra.

Diagnosis: Congenital adrenal hyperplasia, non-salt-losing likely due to 21-hydroxylase deficiency.

Gender assignment: Based on the evaluation, the DSD team and the parents agreed that the initial assignment of female was the correct one. A female name was given.

Treatment: Hydrocortisone, 15 mg/m2/day, and Florinef, 0.1 mg once daily, were initiated. The benefits and risks of surgery for the enlarged clitoris and the urogenital sinus were eventually discussed using the article by Minto and Creighton [94]. After discussion with TM and the urologist, the parents decided not to have clitoral surgery given that the clitoris recedes as the child grows. They did, however, decide to have posterior flap vaginoplasty. They understood that such surgery might require a revision during adolescence if there was scarring or if it was not adequate for intercourse.

Follow-up: Periodic follow-ups are continued by the DSD team to monitor medication, growth and development, and general quality of life for the child and the family. We referred the family to various support groups such as CARES as well as the website ▶ www.accordalliance.org. They also became part of our small group of families, three, who each have daughter with CAH. These four children were born within a year or two of each other. A brief synopsis of their daughter's medical history was given to the parents shortly after discharge. This synopsis was to act as an educational tool summarizing information in language understandable to them and others regarding their daughter's CAH and to also act as a guide and a roadmap, as to what needs attention in the years ahead.

The family continues to be seen in follow-ups by both medical and behavioral specialists in accord with the 2006 consensus statement.

27.6 Summary

What becomes immediately evident to one who reviews the three editions of this chapter is that there has been little change in the endocrine treatment of DSD over last few decades. The advances are mostly in the domain of genetics. On the other hand, one reading all three editions of this chapter sees an evolving dynamic on many fronts involving:

1. Debates about the efficiency and timing of medically and nonmedically necessary genital surgery (should this wait until the child can consent or not, its effects on sexual arousal, and even whether it should or should not be performed).
2. The creation of laws regarding unnecessary surgery and gender.
3. More transparency from physicians and other health professionals with family and patients about the condition and what options are available to them – in brief patient- and family-centered care.
4. The creation of decision-making tools [95] which become the vehicle for "shared decision-making" on issues such as genital surgery, meaning that the physician or health professional enters into a dialogue with the family and patient about the risks and benefits of various treatments such as genital surgery. Such an approach is client or patient centered and follows the recommendation of the consensus report.
5. This approach has been greatly influenced by the rise of advocacy in this area. In essence, there has been an evolving shift to a "patient, family first" approach and attention to quality of life.

As a result of this continuing interaction between support groups, patients and families, and patient advocates and the professional healthcare providers, gender assignment decisions are no longer hurried with parents given incomplete information for fear of increasing their level of anxiety. Current recommendations promote full disclosure with an active involvement of the families in the gender assignment process. While urgency is still present, informed parents are willing to accept longer waiting times in order to obtain all the data from molecular studies, other diagnostic tests, and equally important behavioral information about what is known and not known about both short- and long-term quality of life including functioning in the common domains of living: school, friends, romance, sexual functioning, fertility, and general overall mental health. Furthermore, recent evidence underscores the fact that gender assignment in the newborn period still is an imperfect art. Self-gender change may occur later in the child's life despite the best intentions of the parents and the DSD team. Full disclosure demands that parents be made aware of this possibility.

Knowledge is still incomplete, and gender identity development and differentiation remains a complex and incompletely understood phenomenon. The challenge for practitioners, and parents working with limited knowledge, is how to maintain open, honest communication in order to build a foundation of trust, which can only result in the best care possible for the child. All of these changes and the awareness that they bring will produce a better standard of care in the years ahead.

Review Questions

1. There is no evidence that genital surgery on girls with a DSD diagnosis (e.g., CAH) can have negative consequences on their sexual functioning: True/False
2. DSD is a gonadal-based classification system that highlights the importance of gonadal analysis in diagnosis and management of individuals with DSD: True/False
3. The terminology disorders of sex development (DSD) has been universally embraced by all professionals, advocates, and patients: True/False
4. A child with a DSD will never change from their initial gender assignment (e.g., "once assigned a girl always a girl"): True/False
5. There is a new approach to the psychosocial care of DSD. It is called the non-categorical approach: True/False

Answers

1. False
2. False
3. False
4. False
5. True

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Menstrual Disorders and Hyperandrogenism in Adolescence

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Key Points

- Anovulatory disorders that feature a low estradiol level are caused by hypogonadism (hypergonadotropic or hypogonadotropic).
- Anovulatory disorders that feature normal estradiol may be due to pregnancy, hyperandrogenism, hyperprolactinemia, or hypogonadism in its early phases.
- Tandem mass spectroscopy after preliminary chromatography is the most accurate and reliable method to accurately measure estradiol and testosterone.

28

28.1 Introduction and Background

The evaluation of menstrual disorders in adolescents requires special consideration. Adolescents are in the midst of developing physically and physiologically to achieve adult reproductive function. Thus, the normal variation in the age of onset of puberty and subsequent menarche should be taken into account when evaluating adolescent girls. Menarche will be delayed if puberty is delayed in onset. The average age of menarche is 12.6 years in the normal-weight general American population, with the normal range being 11.0–14.1 years [1]. It occurs approximately 0.5 years earlier in overweight girls and in non-Hispanic Black girls, with Mexican-American girls being intermediate. Although breast development may be beginning earlier in American girls in the last three decades [2], available data do not indicate as dramatic a change in age of menarche [1].

Because of the immaturity of the hypothalamic-pituitary-ovarian axis, about half of menstrual cycles are anovulatory or have attenuated ovulation during the first 2 years after menarche [3–7]. This "physiologic adolescent anovulation" accounts for the greater menstrual irregularity and longer average intermenstrual length in the early post-menarcheal years than in adults (Fig. 28.1) [8]. Because immature cyclic follicular function is occurring in most "anovulatory" cycles, most menstrual cycles are 21–45 days in length, which is only slightly different from adult standards, so menstrual regularity and frequency is greater than ovulatory frequency. In contrast, menstrual irregularity always indicates ovulatory irregularity.

The types of abnormal uterine bleeding (AUB) patterns that should concern endocrinologists are *primary amenorrhea* (failure of menses to begin at a normal age), *secondary amenorrhea* (cessation of menstrual periods for 90 days or more after initially menstruating), o*ligomenorrhea* (fewer than normal menstrual periods a year), and *excessive anovulatory bleeding* (formerly termed "dysfunctional uterine bleeding": bleeding that occurs more often than at 19–21-day intervals or is excessive) (Table 28.1) [4]. Normally, cyclic menstrual bleeding usually occurs at 21–45-day intervals even during the first post-menarcheal year, and cycles outside 19–90 days are always abnormal. Because these menstrual patterns are statistically abnormal, even within the first year after menarche, (occurring in less than 5%

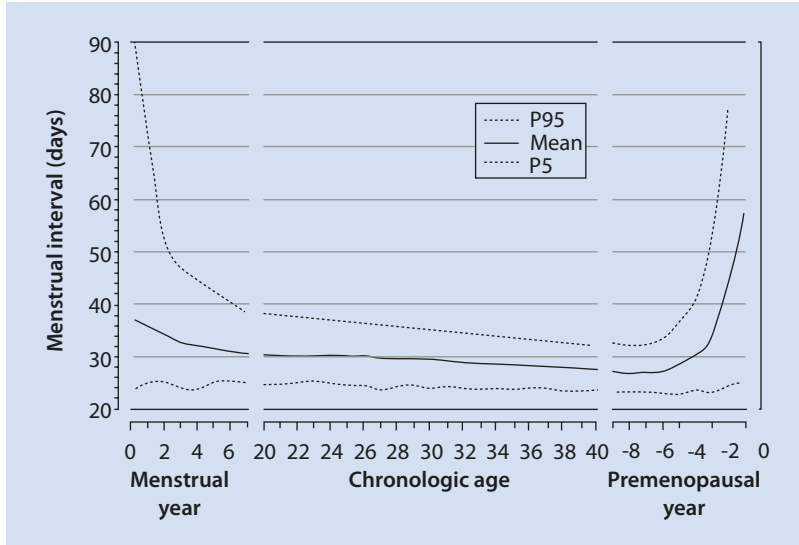

Fig. 28.1 Menstrual cycle lengths throughout reproductive life from menarche to menopause. Tenth, fiftieth, and nineti-eth percentiles are shown (Reproduced with permission from Treloar et al. [8])

Table 28.1 Definition of abnormal uterine bleeding (AUB) in adolescence

Descriptor	Definition
Primary amenorrhea	Lack of menarche by 15 years of age or by 3 years after the onset of breast development[a]
Secondary amenorrhea	Over 90 days without a menstrual period after initially menstruating
Oligomenorrhea	Post-menarcheal year 1: average cycle length > 90 day (<4 periods in the year) Post-menarcheal year 2: average cycle length > 60 days (<6 periods in the year) Post-menarcheal years 3–5: average cycle length > 45 days (<8 periods per year) Post-menarcheal years ≥6: cycle length > 38–40 days (<9 periods per year)
Excessive anovulatory AUB[b]	Menstrual bleeding that occurs more frequently than every 21 days (19 days in year 1) or is excessive (lasts >7 days or soaks >1 pad or tampon every 1–2 h)

Modified from Rosenfield [5]
[a]Bone age of 15 years may be substituted for chronologic age in girls with earlier than average age at puberty onset
[b]Encompasses frequent, intermenstrual, heavy, and/or prolonged AUB. Formerly termed "dysfunctional uterine bleeding"

of adolescents), these patterns represent "symptomatic" rather than the usual "physiologic" adolescent anovulation, and evaluation is suggested if they persist. The risk of persistent menstrual irregularity is slightly over 50% if symptoms persist for 1 year beyond menarche and nearly two-thirds if symptoms persist 2 years beyond menarche; thus, evaluation is indicated if symptoms persist for 1–2 years after menarche [9].

28.2 Etiology

Two general types of disorders cause menstrual abnormalities: those that are associated with genital tract disorders and, more often, those that result from anovulation. The causes of menstrual disorders are presented in ▶ Box 28.1 [9].

Box 28.1 Causes of Adolescent Menstrual Abnormalities

- *Abnormal genital structure*
  - Aplasia[a]
    - Hymenal
    - Vaginal
    - Müllerian
    - Disorders of sex development
  - Endometrial adhesions
  - Ambiguous genitalia
    - Disorders of sex development
- *Anovulatory disorders*
  - Hypoestrogenism: FSH elevated (hypergonadotropic hypogonadism)
    - Primary ovarian insufficiency
      - Congenital
        - Chromosomal disorders
        - Genetic disorders
        - Resistant ovaries
        - Bioinactive gonadotropin
        - Steroidogenic block
      - Acquired
        - Oophorectomy
        - Radiotherapy or chemotherapy
        - Oophoritis
        - Idiopathic
  - Hypoestrogenism: FSH not elevated (hypo- or eugonadotropic hypogonadism)
    - Gonadotropin deficiency
      - Congenital
      - Acquired
        - Organic
        - Functional (nonorganic)
    - Delayed puberty
      - Constitutional delay[a]
      - Growth-retarding disease
    - Primary ovarian insufficiency (before FSH elevation is evident)
      - Complete, if BA <11 year[a]
      - Incomplete, if BA >11 year
    - Virilization
  - Estrogenized: FSH not elevated
    - Pregnancy
    - Functional hypothalamic anovulation
      - Athletic amenorrhea
      - Psychogenic amenorrhea
      - Idiopathic functional hypothalamic amenorrhea
      - Post-pill amenorrhea
    - Secondary hypothalamic anovulation
      - Chronic disease
      - Undernutrition or obesity
      - Cushing's syndrome
      - Hypothyroidism
      - Hyperprolactinemia
    - Hyperandrogenism

Modified from Rosenfield et al. [9]
[a]Cause only primary amenorrhea

28.2.1 Genital Tract Disorders

Primary amenorrhea can result from structural abnormalities of the genital tract that occur independently or are secondary to a disorder of sexual differentiation (DSD). Varying degrees of vaginal and uterine aplasia are found in the Mayer-Rokitansky-Kuster-Hauser syndrome [10]. This syndrome occurs as a single gene defect or as an acquired teratogenic event which sometimes affects differentiation of the urinary tract. Uterine aplasia may occur alone or in combination with renal, cardiac, or limb abnormalities. A subtype due to *Wnt4* gene defects is associated with hyperandrogenism [11]. Obstruction of the genital outflow tract—most commonly due to imperforate hymen—causes hydrocolpos, which results in a fullness and bluish appearance at the vaginal introitus from the bulging imperforate hymen, or hydrometrocolpos when the uterus is involved, which may present as an abdominal mass. Uterine aplasia may also result from DSD syndromes in which anti-Müllerian hormone secretion by testicular tissue has occurred. For this reason, primary amenorrhea may be the presenting symptom of phenotypic girls with complete androgen resistance (testicular feminization syndrome), congenital deficiency in one of the enzymes necessary for testicular testosterone secretion, or in patients with ambiguous genitalia due to partial versions of these disorders or due to 5α-reductase deficiency.

Intrauterine adhesions (Asherman's syndrome) may result from trauma, such as postcurettage or as a complication of radiation therapy of pelvic disease or chronic inflammatory disease [12].

Abnormal bleeding, on the other hand, can result from genital tract trauma or infection such as from sexual abuse or a foreign body. Bleeding may also result from genital tract tumors.

28.2.2 Anovulatory Disorders

Anovulatory disorders, the most common cause of menstrual disorders, can be categorized into those disorders associated with hypoestrogenemia due to varying degrees of hypogonadism or those disorders associated with normal serum estrogen. If hypogonadism is complete and present prior to the onset of neuroendocrine puberty, breast development and feminization of puberty do not occur. If hypogonadism is slightly less severe or becomes manifest in the early teenage years, it may permit some feminization but too little to permit the onset of menses. In either case, primary amenorrhea is a result. Milder, partial, or incomplete forms of hypogonadism may cause either secondary amenorrhea or oligomenorrhea. At its mildest, hypogonadism may present with the anovulatory symptoms of excessive anovulatory bleeding or with frequent periods due to a short luteal phase.

Hypogonadism can be categorized according to whether or not gonadotropins, particularly serum follicle stimulating hormone (FSH) levels, are elevated (▶ Box 28.1).

Hypergonadotropic hypogonadism: Hypoestrogenism with elevated FSH (hypergonadotropic hypergonadism) indicates primary ovarian insufficiency (POI). The causes include both hereditary and acquired disorders. Acquired ovarian insufficiency may result from irradiation, chemotherapy, trauma to the ovary, galactosemia, or autoimmune disease. Gonadal dysgenesis due to deficiency of genes on the X chromosome causing Turner syndrome is the most common cause of POI, with an incidence of about 1 in 2500 liveborn girls. About one-third of girls with Turner syndrome enter puberty spontaneously, and about 5% go on to complete it, with most developing secondary amenorrhea [13]. In women and adolescents with spontaneous POI not due to gonadal agenesis, a cause is identified in only ~15% [14]. Fragile X chromosome permutation causes some cases of X-linked POI [14, 15]. POI may rarely result from hereditary gonadotropin resistance due to mutations in the LH or FSH receptor or along their signaling pathway, as in the context of Albright hereditary osteodystrophy, or to steroidogenic blocks in estradiol biosynthesis [16–20]. Mutations in bone morphogenetic protein 15 (BMP15), a critical regulator of folliculogenesis, FOXL2, a transcription factor, and NR5A1, a nuclear receptor, have been found in some families with POI [21]. Unexplained acquired POI has an autoimmune basis in about 4–5% of all cases [14].

Hypogonadotropic hypogonadism: Frank hypoestrogenism without elevated FSH levels usually indicates secondary ovarian insufficiency (gonadotropin deficiency, hypogonadotropic hypogonadism) because a normal gonadotropin

level is inappropriate in the setting of hypoestrogenism. Gonadotropin deficiency can be congenital or acquired.

Congenital gonadotropin deficiency can arise from diverse cerebral, hypothalamic, or pituitary disorders, single gene mutations in the pituitary development cascade [22], or a chromosomal disorder (such as Prader-Willi syndrome). Congenital isolated gonadotropin deficiency may result from autosomal dominant or recessive disorders [23], of which GnRH receptor deficiency is more common in women than the anosmia-associated Kallmann syndrome [24]. These and other specific gene defects that cause congenital isolated gonadotropin deficiency are discussed in ► Chap. 25. These disorders are typically associated with absent puberty but primary or secondary amenorrhea may be manifest.

Acquired gonadotropin deficiency may be organic or functional (nonorganic). Organic acquired gonadotropin deficiency can be a consequence of tumors, trauma, autoimmune hypophysitis [25], degenerative disorders involving the hypothalamus and pituitary [26], irradiation [27], or chronic illness of virtually any organ system [28]. Functional hypogonadotropinism (referred to as functional hypothalamic amenorrhea in sexually mature girls) is commonly caused by energy-deficit states such as eating disorders and excessive exercise (associated with low or normal weight) and emotional or psychological stress [29].

In some situations, it can be difficult to differentiate between gonadotropin deficiency and POI as a cause of low estrogen. The most common situation is in children who are too young to have undergone neuroendocrine puberty, as indicated by a bone age less than about 11 years; in these cases, the estrogen is low because puberty has not begun. The other situation occurs in incomplete or early POI because gonadotropin levels are often normal in the early stages as ovarian function is declining progressively yet intermittently, as during the menopausal transition [14, 30]. Suppression of gonadotropins and estrogens occurs in frankly virilizing disorders.

Functional hypothalamic amenorrhea occurs in sexually mature (e.g., feminized) adolescents. It occurs in patients who secrete sufficient gonadotropin tonically to feminize normally but have functional (reversible) disorders which interfere with normal GnRH pulsatility. In this group of disorders, there are disturbances of cyclic or pulsatile GnRH release that interfere with LH pulse frequency and the positive feedback mechanism necessary for ovulation. If the disturbance of GnRH release affects baseline LH pulse frequency, then a hypogonadotropic hypogonadism clinical picture results with hypoestrogenemia. If disturbance of GnRH pulsatile release primarily affects the positive feedback mechanism for ovulation, then amenorrhea with normal serum estradiol is the result. Functional hypothalamic amenorrhea is commonly caused by disorders of energy imbalance or psychological stress [31]. Anorexia nervosa is the prototypic eating disorder, but bulimia nervosa, the binge-eating/purging variant, is easily overlooked because the weight is often normal and vomiting surreptitious. Energy imbalance due to excessive exercise and disorganized eating may occur in adolescents with normal weight; insight can be provided by a careful exercise history. It is also seen in the setting of psychological stress. Implicit with its categorization as "functional," return of menses occurs with amelioration of energy imbalance or psychological stress. Even when these factors are not obvious, similar mechanisms seem to underlie idiopathic functional hypothalamic amenorrhea [32]. Post-pill amenorrhea may be suspected after the long-term use of hormonal contraceptives. However, this entity usually results from an undetected antecedent anovulatory disturbance or an intercurrent illness, so a diagnostic evaluation is required.

Hyperprolactinemia requires special consideration because it varies greatly in its presentation, depending to a great extent on the degree of gonadotropin deficiency. Prime among the multiple mechanisms of functional hypothalamic anovulation is disruption of GnRH pulsatility [33, 34]. Galactorrhea is present in about half of the patients, occurring among those with residual estrogen production, who may also have adrenal hyperandrogenism. The causes of hyperprolactinemia are diverse and include hypothalamic or pituitary disorders, medications, hypothyroidism, renal or liver failure, peripheral neuropathy, stress, or idiopathic [35]. It is not only in the differential diagnosis of hypogonadotropic hypogonadism and hypothalamic amenorrhea; it may cause short or inadequate luteal phase (characterized by menstrual cycles less than 22 days), excessive anovulatory bleeding, or hyperandrogenic symptoms.

Chronic disease of virtually any organ system can mimic gonadotropin deficiency or functional hypothalamic amenorrhea. Obesity may cause amenorrhea via suppression of LH levels, distinct from its roles in ovary syndrome, discussed below [36]. The main mechanism involves in part accelerated LH turnover [36, 37] and possibly in part gonadotropin suppression by inflammatory cytokines [38]. Glucocorticoid excess causes amenorrhea by multiple mechanisms, prime among which is interference with gonadotropin responsiveness to GnRH [39, 40]. Thyroid disorders are well-known causes of menstrual irregularity [41].

Menstrual disturbance in the presence of adequate estrogenization is probably the single most common problem that is encountered. Pregnancy and hyperandrogenism are the primary considerations. After pregnancy, hyperandrogenism is the most frequent cause of anovulation and is considered in more detail in the next section.

28.2.3 Hyperandrogenism

Hyperandrogenemia is of mainly ovarian origin in the majority of cases. It occasionally is of solely adrenal origin; in a few cases, it appears to be caused by obesity or abnormalities in the peripheral formation of androgen, and it is rarely caused by tumors or by self-administration of androgens. The differential diagnosis of hyperandrogenism is listed in ► Box 28.2 [36].

Box 28.2 Differential Diagnosis of Hyperandrogenism in Adolescents

1. Physiologic adolescent anovulation
2. Functional gonadal hyperandrogenism
   1. PCOS: Primary functional ovarian hyperandrogenism (common form of PCOS)
   2. Secondary functional ovarian hyperandrogenism
      1. Virilizing congenital adrenal hyperplasia
      2. Adrenal rests of ovary
      3. Ovarian steroidogenic blocks
      4. Insulin resistance syndrome
      5. Acromegaly
      6. Seizure disorder ± valproic acid therapy
   3. Disorders of sex development
   4. Pregnancy-related hyperandrogenism (placental aromatase deficiency and luteomas)
3. Functional adrenal hyperandrogenism
   1. PCOS primary functional adrenal hyperandrogenism (uncommon form of PCOS)
   2. Virilizing congenital adrenal hyperplasia
   3. Other glucocorticoid-suppressible functional adrenal hyperandrogenism
      1. Hyperprolactinemia
      2. Cortisone reductase deficiency (and apparent reductase deficiency)
      3. Dehydroepiandrosterone sulfotransferase deficiency, apparent
   4. Glucocorticoid-nonsuppressible functional adrenal hyperandrogenism
      1. Cushing's syndrome
      2. Glucocorticoid resistance
4. Peripheral androgen metabolic disorders
   1. Obesity
   2. Idiopathic hyperandrogenism
   3. Portohepatic shunting
5. Virilizing tumors
6. Androgenic drugs

From Rosenfield and Ehrmann [36]

28.2.3.1 Polycystic Ovary Syndrome

PCOS accounts for nearly 90% of cases of androgen excess presenting at or after the onset of puberty. The classic syndrome originally described by Stein and Leventhal includes various combinations of amenorrhea, hirsutism (defined as excessive male-pattern hair growth), obesity, insulin resistance, and polycystic ovaries. The etiology of PCOS is not fully understood, and the metabolic and endocrine manifestations differ widely among affected females. Consequently, there is no single test to diagnose PCOS; diagnosis is based upon meeting diagnostic criteria after excluding other causal etiologies. Diagnostic criteria for PCOS in adult women are encompassed in the "Rotterdam criteria" as various combinations of hyperandrogenism, oligo-anovulation, and polycystic ovary morphology (PCOM) (summarized in [36, 42]). Multiple societies have published guidelines to aid the clinician in diagnosing PCOS in adults [43–45]. These guidelines stress the relative importance of each diagnostic criterion differently, resulting in assignment of the PCOS diagnosis to certain mild phenotypes in some guidelines but not others. If adult criteria are applied to adolescents, the criteria will result in overdiagnosis of PCOS because normal physiologic events of adolescence may mimic some of the features of PCOS used to diagnose adults.

PCOS in adolescents should be diagnosed using the 2015 international consensus diagnostic criteria summarized in ▶ Box 28.3 [5, 46]. The criteria are essentially age- and pubertal stage-appropriate modifications of the "National Institutes of Health criteria" (otherwise unexplained hyperandrogenic anovulation); the paucity of normative data obviates the routine use of PCOM as a diagnostic criterion [5, 46]. Just as in adults, PCOS is a diagnosis of exclusion in adolescents. The definition of oligo-anovulation is dependent upon the years since menarche. Menstrual interval greater than 3 months is always abnormal, while menstrual intervals greater than 45 days or shorter than 20 days are abnormal 2 or more years after menarche. The "clinical evidence of hyperandrogenism" criterion may be applied if the manifestation is severe, such as moderate or greater hirsutism for the individual's ethnicity. There are no established normal ranges of serum testosterone or free testosterone in adolescent girls; and in the absence of these data, use of adult female norms is recommended. Polycystic ovaries are a physiologic variant in normal adolescent girls, and there is a paucity of normative data in adolescents; thus, use of ovarian morphology or size parameters in adolescents as diagnostic criteria is not recommended [46, 47]. To avoid mislabeling physiologic anovulation as PCOS, it is recommended that an otherwise healthy adolescent with menstrual abnormalities (▶ Box 28.3) and asymptomatic hyperandrogenemia (i.e., without cutaneous manifestations) of less than 2 years' duration be given only a provisional diagnosis of PCOS or of being "at risk of PCOS" [5, 46]. This recommendation is made in the attempt to accurately prognosticate PCOS persistence and should not prevent diagnostic evaluation of girls with hyperandrogenic amenorrhea.

Box 28.3 Diagnostic Criteria for Polycystic Ovary Syndrome in Adolescents

Otherwise unexplained combination of:

1. Abnormal uterine bleeding pattern
    1. Abnormal for age or gynecologic age
    2. Persistent symptoms for 1–2 years
2. Evidence of hyperandrogenism
    1. Persistent testosterone elevation above adult norms in a reliable reference laboratory is the best evidence
    2. Moderate-severe hirsutism is clinical evidence of hyperandrogenism
    3. Moderate-severe inflammatory acne vulgaris is an indication to test for hyperandrogenemia

From Rosenfield [5]

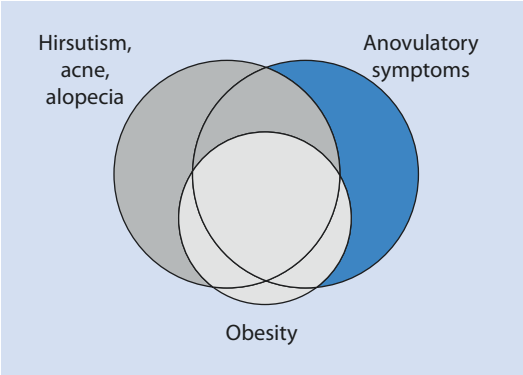

Fig. 28.2 Manifestations of polycystic ovary syndrome in approximate proportion to their relative incidence and coincidence. Cutaneous symptoms include hirsutism, acne, or acanthosis nigricans. Anovulatory symptoms include amenorrhea, oligomenorrhea, dysfunctional uterine bleeding, and infertility (Reproduced with permission from Rosenfield [48])

The manifestations of PCOS are quite variable (Fig. 28.2). Only about a third of cases have the classic Stein-Leventhal syndrome. Anovulatory symptoms vary from oligomenorrhea to excessive anovulatory bleeding to unexplained infertility and may not be evident at presentation with hirsutism, acne, obesity, or acanthosis nigricans. Over half of PCOS adolescents have an abnormal degree of hirsutism or acne, but hyperandrogenemia does not always have a cutaneous manifestation. Obesity and acanthosis nigricans, a sign of insulin resistance, are common presenting features [49]. Obesity is present in about half of cases. Females with PCOS have a high prevalence of the metabolic syndrome and are predisposed to type 2 diabetes mellitus [5]. In adolescents, there may be an antecedent history of premature adrenarche or early childhood obesity with a Cushingoid fat distribution or overgrowth (i.e., pseudo-Cushing's syndrome or pseudo-acromegaly) [50]. Congenital virilizing disorders, such as congenital adrenal hyperplasia, are a risk factor for PCOS at puberty.

Pathophysiology There has been considerable debate over whether PCOS is fundamentally a neuroendocrine disorder (in which hyperpulsatility

of GnRH is the origin of the problem), an ovarian disorder (in which intrinsic ovarian dysfunction is the origin), or a metabolic disorder (in which insulin resistance is a key element). Our research has led us to propose a minimal model that posits the essence of PCOS to be intrinsic functional ovarian hyperandrogenism (FOH) that is closely linked to the metabolic disorder [36]. FOH is characterized by an elevated free testosterone after suppression of adrenocortical function with dexamethasone. Two-thirds of these women also exhibit hypersensitivity to LH stimulation, as demonstrated by hyperresponsiveness to LH of 17-hydroxyprogesterone relative to other ovarian steroids.

The theory that PCOS is a fundamentally a neuroendocrine disorder was rooted in the early observation that baseline LH levels and responses to GnRH are elevated in PCOS. Compared to control women, women with PCOS have increased LH pulse frequency and amplitude. Since LH stimulates theca cell development, expression of steroidogenic enzymes, and steroidogenesis, the increased LH of PCOS was initially considered the cause of the androgen excess. However, this theory does not account for the subsequent finding that half of women with PCOS do not have elevated LH levels. LH levels are inversely correlated with the degree of obesity [37]. In addition, the normal response to excessive LH stimulation is homologous desensitization of theca cells. Desensitization involves downregulation of LH receptor expression and androgen secretion in response to further LH stimulation. Downregulation of androgen secretion is primarily exerted at the rate-limiting step in androgen formation, the 17,20-lyase activity of cytochrome P450c17, which has both 17-hydroxylase and 17,20-lyase activities: as a consequence, 17-hydroxyprogesterone levels rise in response to increased LH levels, but the downstream androgenic response to LH is limited [36]. Therefore, LH excess does not seem to be a fundamental cause of the hyperandrogenism although the disorder is gonadotropin-dependent, i.e., suppression of gonadotropin levels corrects hyperandrogenemia.

The excessive LH levels in women with PCOS can be explained by hyperandrogenism inhibiting estrogen-progestin negative feedback [36]. The LH pulse frequency of women with PCOS is less sensitive than that of controls to sex steroid suppression. Antiandrogen therapy can restore the inhibitory effect of estrogen-progestin on LH pulse frequency [51].

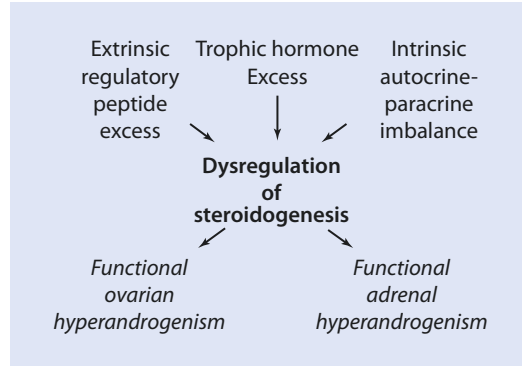

Fig. 28.3 Model of factors causing the common types of functional ovarian and adrenal hyperandrogenism. A mild degree of androgen excess can arise from excess trophic hormone (LH or ACTH) stimulation. Disturbances either extrinsic or intrinsic to these endocrine glands can amplify the effect of normal levels of trophic hormones. Extrinsic regulatory peptide excess is exemplified by hyperinsulinemia. Intrinsic peptides capable of inappropriately upregulating steroidogenesis include IGFs (Reproduced with permission from Rosenfield [52])

We favor the concept that the hyperandrogenism of PCOS is usually caused by intrinsic defects in the regulation of steroidogenesis. While this dysregulation may result from imbalance among various intrinsic and extrinsic factors involved in the modulation of trophic hormone action (Fig. 28.3), most cases appear to arise from intrinsic defects within the ovarian theca cells that make them hyperresponsive to normal or excessive LH stimulation, because these women have "escaped" from normal desensitization to LH. Overexpression of the steroidogenic enzyme cytochrome P450c17, which generates both 17-hydroxyprogesterone and the 17-ketosteroids androstenedione and dehydroepiandrosterone (DHEA), seems to be particularly important. In vitro studies indicate that the abnormal steroidogenesis is due to an intrinsic defect in the theca cells of PCOS patients [53, 54]. Recently, genome-wide association screening has led to the discovery of involvement of a previously unsuspected protein modulating steroidogenesis: DENND (differentially expressed in normal and neoplastic differentiation). The expression of the *DENND1A.V2* isoform is increased in PCOS theca cells, and its overexpression in normal theca cells reproduced the PCOS theca cell phenotype by upregulating steroidogenesis with prominent P450c17 activity [55].

A related steroidogenic defect sometimes seems to involve the adrenal gland. About a quarter of women with FOH have a related steroidogenic defect in adrenal formation of DHEA and its precursor, 17-hydroxypregnenolone, without evidence of a steroidogenic block in response to ACTH stimulation, as would occur in virilizing congenital adrenal hyperplasia. This dysfunction is termed primary functional adrenal hyperandrogenism (FAH). FAH had earlier been mistaken for nonclassical 3ß-hydroxysteroid dehydrogenase deficiency, which is now known to be a rare disorder and also considered to be "exaggerated adrenarche." Notably, *DENND1A* is also highly expressed in the adrenal androgen-forming zone [55].

Insulin-resistant hyperinsulinism is a common feature of PCOS [36, 56, 57]. The insulin resistance is confined to the glucose metabolic effects of insulin and is tissue-selective, primarily affecting skeletal muscle and hepatic cells but sparing the ovaries and adipose tissue. Insulin resistance is intrinsic: it is abnormal for the degree of obesity in about half of cases. Compensatory hyperinsulinemia appears to be an important factor in the pathophysiology of PCOS by sensitizing the ovary to LH [58]. Insulin counters homologous desensitization and steroidogenic downregulation in response to LH excess. Insulin also stimulates formation of testosterone by 17ß-hydroxysteroid dehydrogenase. It does so by stimulating expression of KLF15, a Kruppel-like transcription factor that is part of the gene's proximal promoter coactivator complex and that stimulates adipogenesis in adipocytes [59]. Thus, the hyperinsulinemia that is compensatory for the insulin resistance of PCOS seems to contribute to both androgen and fat excess in a state of resistance to the glucose metabolic effects of insulin. Obesity in turn aggravates insulin resistance. Insulin-lowering treatments, especially weight loss, lessen the hyperandrogenism of PCOS.

Follicular maturation and development of the dominant follicle is impaired in women with PCOS, leading to polycystic ovaries and anovulation. This dysregulation of folliculogenesis seems to be caused by premature follicular luteinization resulting from intraovarian androgen excess and in part by insulin excess, but an independent folliculogenesis defect cannot be ruled out [36, 60, 61].

Pathogenesis The cause of PCOS is unknown. Similar to type 2 diabetes mellitus, PCOS likely arises because of an interaction between congenital predisposing factors and environmental factors [36]. There is a strong heritable component to hyperandrogenemia and polycystic ovaries; each appears to be inherited as an independent autosomal dominant trait. Seventy-five percent of adolescents with PCOS have a parent with metabolic syndrome, indicating a close relationship to obesity, insulin resistance, and diabetes. Environmental influences that promote obesity and associated hyperinsulinemia are aggravating and possibly precipitating pathogenetic factors.

Prenatal androgen excess is a distinct predisposing factor [36]. All congenital virilizing syndromes are complicated by a high risk for PCOS. This is a common cause of persistent anovulation in well-controlled virilizing congenital adrenal hyperplasia. Experimental evidence suggests that the mechanism may involve reduction of hypothalamic progesterone receptor expression, with consequent hypersecretion of LH, by prenatal androgen excess [62]. Other proposed precipitating factors include intrauterine growth retardation, premature adrenarche, and heterozygosity for congenital adrenal hyperplasia [63].

28.2.3.2 Other Causes of Functional Ovarian Hyperandrogenism

Other functional causes of FOH can mimic PCOS (▶ Box 28.2). Extraovarian androgen excess (as in poorly controlled congenital adrenal hyperplasia) and ovarian steroidogenic blocks (such as 3ß-hydroxysteroid dehydrogenase, 17ß-hydroxysteroid dehydrogenase, or aromatase deficiency) are such causes and described in further detail below. Excessive stimulation via the LH receptor is a rare cause of hilus cell hyperplasia, and chorionic gonadotropin-related hyperandrogenism may occur during pregnancy [64, 65]. All known forms of extreme insulin resistance, including the hereditary cases which are due to insulin receptor mutations, as well as acromegaly [66], are accompanied by PCOS, apparently by excessively stimulating the IGF-I signal transduction pathway to cause escape from desensitization to LH. Functional ovarian hyperandrogenism may also result from adrenal rests of the ovaries in congenital adrenal hyperplasia or of gonadal origin in disorders of sex development. The

antiepileptic drug valproic acid also causes ovarian hyperandrogenism [67, 68].

Less than 10% of adrenal hyperandrogenism can be attributed to the well-understood genetic disorders listed in ▶ Box 28.2. Congenital adrenal hyperplasia arises from an autosomal recessive deficiency in the activity of any one of the adrenocortical enzyme steps necessary for the biosynthesis of corticosteroid hormones. Mild enzyme deficiency causes nonclassical ("late-onset") presentations, which lack the genital ambiguity of classic congenital adrenal hyperplasia and cause adolescent or adult-onset of anovulatory symptoms and/or hirsutism. Women with nonclassical congenital adrenal hyperplasia may have polycystic ovaries and high serum luteinizing hormone levels, but FOH seems to be unusual except in the presence of adrenal rests of the ovaries [69]. Nonclassical 21-hydroxylase deficiency is the most common form of congenital adrenal hyperplasia and accounts for about 5% of hyperandrogenic adolescents in the general population. Deficiencies of 3ß-hydroxysteroid dehydrogenase (3ß-HSD) or 11ß-hydroxylase are forms of congenital adrenal hyperplasia which have on rare occasions presented in adolescence. Cortisone reductase deficiency (and apparent reductase deficiency) is a rare autosomal recessive form of functional adrenal hyperandrogenism [70, 71]. Cortisone-reductase deficiency is associated with heterozygous mutations in 11beta-hydroxysteroid dehydrogenase type 1 [72]. Apparent DHEA sulfotransferase 2A1 deficiency is due to a genetic defect in DHEA metabolism that does not affect corticosteroid secretion [73]. Inactivating mutation of the sulfate donor to SULT2A1 (3′-phosphoadenosine-5′-phosphosulfate synthase 2, PAPSS2) prevents sulfation of DHEA and causes high DHEA yet undetectable DHEAS levels.

Dexamethasone-resistant forms of hyperandrogenism such as Cushing's syndrome and cortisol resistance are even more unusual than nonclassical congenital adrenal hyperplasia. Prolactin excess causes adrenal hyperandrogenism; Cushing's disease and hyperprolactinemia sometimes occur in association with polycystic ovaries [74, 75].

In approximately 10% of PCOS patients, a gonadal or adrenal source of androgen cannot be ascertained after thorough testing. Most of these cases have mild hyperandrogenemia, lack DHEAS and LH elevation, and seem to be due to obesity [36]. Adipose tissue is capable of forming testosterone from androstenedione [59] and suppressing gonadotropin levels in proportion to body mass index (BMI) [37, 38]. PCOS has also been reported as a rare complication of the impaired steroid metabolism which occurs as a complication of portosystemic shunting [76]. The hyperandrogenism of this condition has been attributed to a combination of impaired hepatic DHEA sulfation, which results in an increase in DHEA available for testosterone formation, and hyperinsulinemia, which results in postprandial hypoglycemia and ovarian hyperandrogenism [77]. Other idiopathic hyperandrogenemia cases may be due to hereditary quirks in peripheral metabolism of steroids. Tumor and exogenous ingestion of anabolic steroids are more rare causes of virilization.

28.3 Differential Diagnosis

Evaluation of primary amenorrhea should be undertaken if spontaneous menses have not occurred by 15.0 years of age or earlier if menses has not occurred 3 years after breast budding. A diagnostic approach to primary amenorrhea is shown in ◘ Fig. 28.4 [9]. The history should include a search for clues to chronic disease, eating disorders, excessive exercise, and emotional or psychological stress. The key features on physical examination are determinations of whether puberty is delayed (or indeed whether it has even begun), whether the child is underweight [79] or overweight, and whether the external genitalia are normal. All should have a panel of screening tests for chronic disease. Other key initial laboratory tests in the sexually *immature* patient are bone age radiograph and gonadotropin levels. Other key initial laboratory tests in the sexually *mature* patient are a pregnancy test, serum testosterone, and pelvic ultrasound examination.

In patients with secondary amenorrhea or oligomenorrhea, the occurrence of menarche indicates that a substantial degree of development of the reproductive system will have occurred. A pregnancy test and serum gonadotropin levels are the key tests with which to initiate the evaluation, as shown in ◘ Fig. 28.5 [9]. However, because breast development persists even if hypoestrogenism develops, the presence of a mature breast stage does not preclude the possibility of

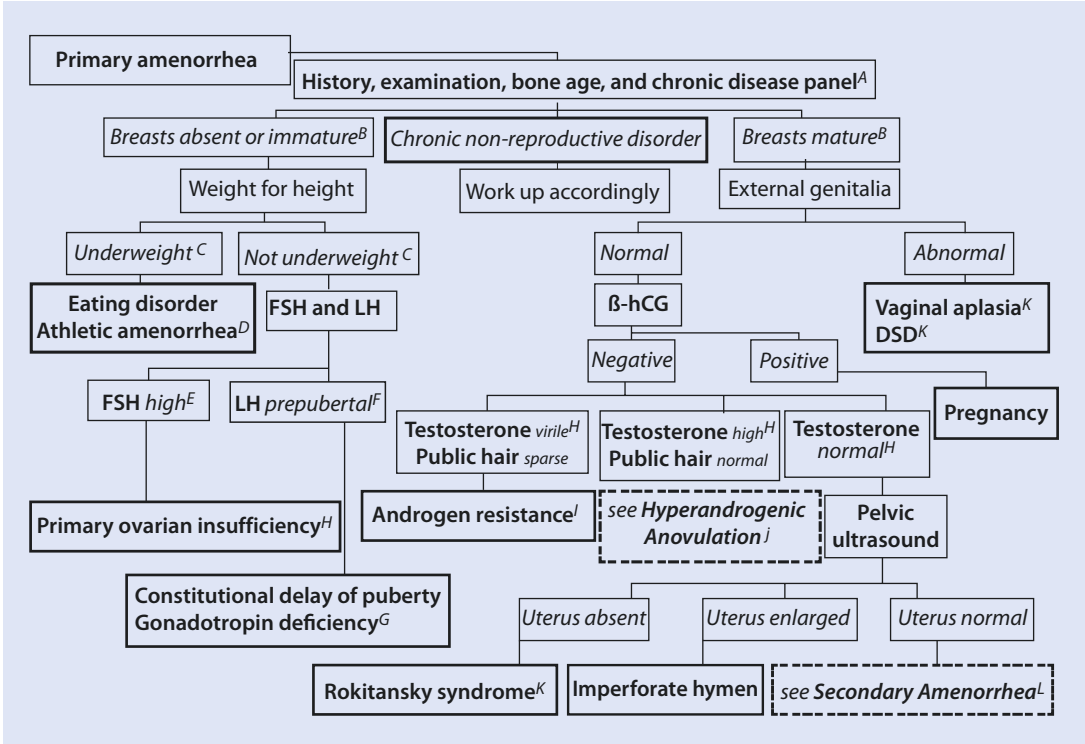

Fig. 28.4 Differential diagnosis of primary amenorrhea (Modified from Rosenfield [9]). Footnotes: A. Prime among the causes of primary amenorrhea are growth-retarding or growth-attenuating disorders. In the absence of specific symptoms or signs to direct the workup, laboratory assessment for chronic disease includes bone age radiograph if the adolescent is not sexually mature and a complete blood count and differential, sedimentation rate, comprehensive metabolic panel, celiac panel, thyroid panel, cortisol and insulin-like growth factor-I levels, and urinalysis. B. Breast development ordinarily signifies the onset of pubertal feminization. However, mature breast development does not ensure ongoing pubertal estrogen secretion (see Figs. 28.5 and 28.6). C. BMI <10th percentile generally corresponds to body composition <20% body fat but does not accurately reflect body fat in serious athletes or in bulimia nervosa. D. Low body fat is associated with amenorrhea in underweight girls and from athletic activity out of proportion to caloric intake in overexercisers. E. FSH is preferentially elevated over LH in primary ovarian insufficiency (POI). The most common cause of primary amenorrhea due to POI is gonadal dysgenesis due to Turner syndrome, but acquired causes must be considered (such as cytotoxic therapy). The workup of POI is considered in detail in the next algorithm (Fig. 28.5, secondary amenorrhea and oligomenorrhea). Lack of FSH elevation virtually rules out POI only when the bone age is appropriate for puberty (11 years or more). F. "Pediatric" gonadotropin assays sensitive to ≤0.15 U/L are critical to the accurate diagnosis of gonadotropin deficiency and delayed puberty. A low LH level is more characteristic of these disorders than a low FSH level. Congenital gonadotropin deficiency is closely mimicked by the more common extreme variation of normal, constitutional delay of puberty. G. History and examination may yield clues to the cause of hypogonadotropic hypogonadism, such as evidence of hypopituitarism (midline facial defect, extreme short stature, visual field defect) or anosmia (Kallmann syndrome) or functional hypothalamic disturbance (bulimia, excessive exercise). Random LH levels in hypogonadotropic patients are usually below 0.15 IU/L but often overlap those of normal pre- and mid-pubertal children. GnRH agonist testing (e.g., leuprolide acetate injection 10 μg/kg SC) may discriminate gonadotropin deficiency from functional causes. It may not be possible to definitively establish the diagnosis of gonadotropin deficiency until puberty fails to begin by 16 years of age or to progress at a normal tempo. H. Plasma total testosterone is normally 20–60 ng/dL (0.7–2.1 nM) but varies somewhat among laboratories. I. Androgen resistance is characterized by a frankly male plasma testosterone level when sexual maturation is complete, male karyotype (46, XY), and absent uterus. External genitalia may be ambiguous (partial form) or normal female (complete form). J. The differential diagnosis of hyperandrogenism is shown in Fig. 28.7. K. Vaginal aplasia in a girl with normal ovaries may be associated with uterine aplasia (Mayer-Rokitansky-Kuster-Hauser syndrome). When the vagina is blind and the uterus aplastic, this disorder must be distinguished from androgen resistance. If the external genitalia are ambiguous, it must be distinguished from other disorders of sex development. L. The differential diagnosis of secondary amenorrhea is presented in Fig. 28.5

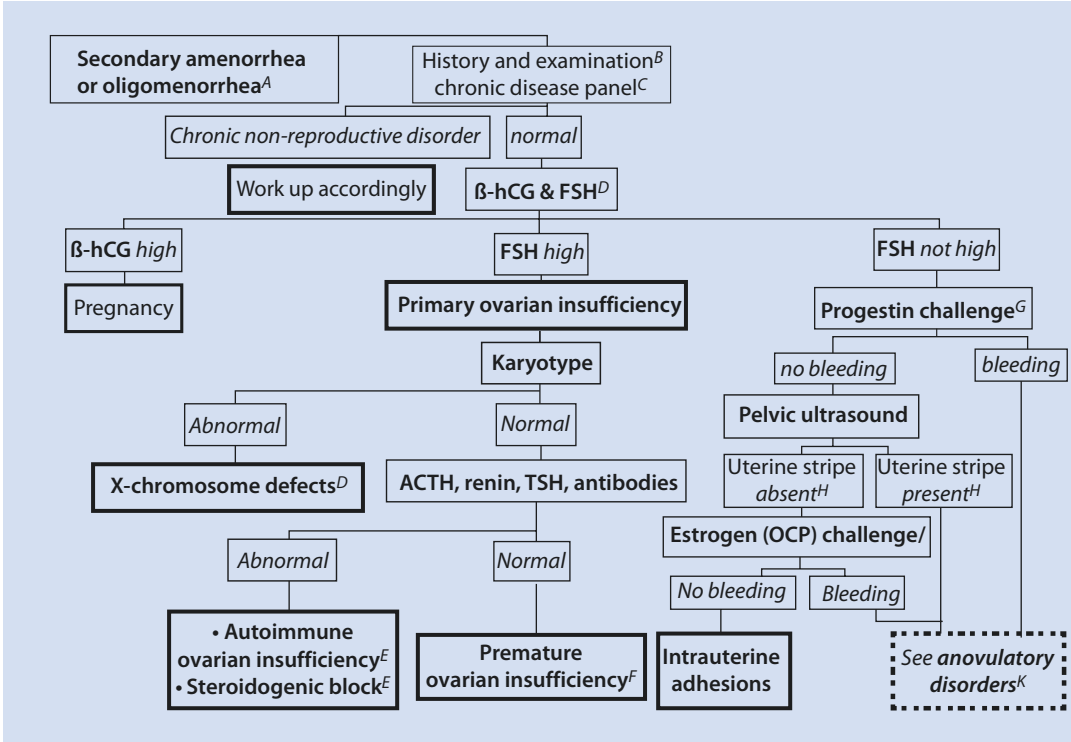

Fig. 28.5 Differential diagnosis of secondary amenorrhea or oligomenorrhea (Modified with permission from Rosenfield [9]). Footnotes: A. Mature secondary sex characteristics are characteristic because the occurrence of menarche indicates a substantial degree of development of the reproductive system. B. Diverse disorders of many systems cause anovulation. The history may reveal excessive exercise, symptoms of depression, gastrointestinal symptoms, radiotherapy to the brain or pelvis, or rapid virilization. Physical findings may include hypertension (forms of congenital adrenal hyperplasia, chronic renal failure), short stature (hypopituitarism, Turner syndrome, pseudohypoparathyroidism), abnormal weight for height (anorexia nervosa, obesity), decreased sense of smell (Kallmann syndrome), optic disc or visual field abnormality (pituitary tumor), cutaneous abnormalities (neurofibromatosis, lupus), goiter, galactorrhea, hirsutism, or abdominal mass. C. In the absence of specific symptoms or signs to direct the workup, evaluation for chronic disease in a sexually mature adolescent typically includes complete blood count and differential, sedimentation rate, comprehensive metabolic panel, celiac panel, thyroid panel, cortisol and insulin-like growth factor-I levels, and urinalysis. D. Patients missing only a small portion of an X chromosome may not have the Turner syndrome phenotype. Among 45,X patients, the classic Turner syndrome phenotype is found in less than one-third (with the exception of short stature in 99%). Ovarian function is sufficient for about 10% to undergo some spontaneous pubertal development and for 5% to experience menarche. If chromosomal studies are normal and there is no obvious explanation for the hypogonadism, studies for fragile X premutation and autoimmune oophoritis should be considered. E. Autoimmune ovarian insufficiency may be associated with tissue-specific antibodies and autoimmune endocrinopathies such as chronic autoimmune thyroiditis, diabetes, adrenal insufficiency, and hypoparathyroidism. Nonendocrine autoimmune disorders may occur, such as mucocutaneous candidiasis, celiac disease, and chronic hepatitis. Rare gene mutations causing ovarian insufficiency include steroidogenic defects that affect mineralocorticoid status (17-hydroxylase deficiency is associated with mineralocorticoid excess and lipoid adrenal hyperplasia with mineralocorticoid deficiency) and mutations of the gonadotropins or their receptors. Ovarian biopsy is of no prognostic or therapeutic significance. F. The history may provide a diagnosis (e.g., cancer chemotherapy or radiotherapy). Other acquired causes include surgery and autoimmunity. Chromosomal causes of premature ovarian insufficiency include X chromosome fragile site and point mutations. Other genetic causes include gonadotropin-resistant syndromes such as LH or FSH receptor mutation and pseudohypoparathyroidism. G. Withdrawal bleeding in response to a 5- to 10-day course of progestin (e.g., medroxyprogesterone acetate 10 mg HS) suggests an overall estradiol level greater than 40 pg/mL. However, this is not entirely reliable, and thus, in the interest of making a timely diagnosis, it is often worthwhile to proceed to further studies. H. A thin uterine stripe suggests hypoestrogenism. A thick one suggests endometrial hyperplasia, as may occur in polycystic ovary syndrome. I. A single cycle of an OCP containing 30–35 μg ethinyl estradiol generally suffices to induce withdrawal bleeding if the endometrial lining is intact. J. The differential diagnosis of other anovulatory disorders continues in Fig. 28.6

hypoestrogenism. A simple first step to assess the adequacy of estrogenization is to determine whether withdrawal bleeding occurs in response to a progestin challenge; a positive response indicates an estradiol level that averages ≥40 pg/ml [80]. The presence or absence of withdrawal bleeding is particularly helpful to identify a chronic estrogen-deficient state if available estradiol assays are not ultrasensitive and specific [81] and therefore unable to accurately detect a low serum estradiol.

If the above evaluation of a sexually mature girl does not yield a definitive diagnosis, further investigation for an anovulatory disorder should be undertaken (Fig. 28.6) [9]. Excessive vaginal bleeding is an alternate presentation of anovulatory cycles (Table 28.1). If present, other causes of excessive vaginal bleeding, such as sexual abuse, bleeding disorder, genital tract tumor, or feminizing cyst, should also be considered. The history of an adolescent with anovulatory symptoms should be carefully reviewed for evidence of the nutritional disorders, exercise activity, and physical or emotional stresses that are common causes of hypothalamic amenorrhea. The examination should particularly focus on the possibility of intracranial disorders, galactorrhea, and evidence of hirsutism or its cutaneous equivalents, treatment-resistant acne vulgaris, or male pattern balding. The workup is then directed differently according to the patient's estrogen and prolactin status (Fig. 28.6). GnRH agonist testing is helpful in confirmed hypoestrogenic cases. GnRH agonist testing yields and allows assessment of gonadotropin release and reserve in gonadotropin deficiency and also permits assessment of ovarian responsiveness to gonadotropins [82–85]. Imaging of either the ovaries or the brain is usually indicated.

Hyperandrogenism is the final consideration in the differential diagnosis of menstrual disorders (Fig. 28.7) [9]. The diagnosis may be difficult to establish [86]. Classically, when hirsutism or cutaneous hirsutism equivalents are present, this is considered as clinical evidence of hyperandrogenism. However, mild hirsutism and its cutaneous equivalents are common normal variants that are not associated with hyperandrogenemia. In addition, cutaneous manifestations are absent in nearly half of all patients with hyperandrogenism because there is considerable individual variability in pilosebaceous unit sensitivity to androgens. Therefore, hyperandrogenism is most firmly established if hyperandrogenemia can be reliably documented by biochemical testing. Measurement of total and/or free testosterone is recommended to document hyperandrogenemia [46].

Dependable testosterone assays may not be available to all practitioners, but are becoming more widely available. The most available, reliable methods to assay total testosterone in women and children involve preliminary chromatography followed by either specific radioimmunoassay or tandem mass spectrometry (abbreviated LC-MS/MS) [87, 88]. Most commercial specialty laboratories now use tandem mass spectrometry. Some direct total testosterone radioimmunoassays give comparable results to LC-MS/MS, but many do not [87, 89]. The most reliable method to detect free testosterone is to calculate it as the product of the total testosterone and the fraction that is free from sex hormone-binding globulin (SHBG) binding (free testosterone = total testosterone X percent free testosterone). Percent-free testosterone is calculated from the SHBG concentration or directly determined by dialysis methods. The automated assays of total testosterone, available in multichannel immunometric panels, and direct free testosterone assays are very inaccurate at the relatively low testosterone levels of women and children; thus they give misleading information about androgen status in women and girls.

It is reasonable to begin the evaluation with a total testosterone determination by a reliable method and, as suggested above, proceed according to the algorithms in Figs. 28.4 and 28.6. Most patients with PCOS have serum total testosterone concentrations between 40 and 150 ng/dL. A total testosterone over 200 ng/dL increases the likelihood of a virilizing neoplasm. DHEA-sulfate (DHEA-S) may be useful if cystic acne is a major symptom or there is a high suspicion for a virilizing tumor. DHEA-S levels are often markedly elevated (over 700 μg/dl) if a tumor of adrenal origin is present. Patients who have clinical features consistent with PCOS or otherwise unexplained anovulatory symptoms but an initial normal total testosterone should have an early morning total and free testosterone level (calculated from SHBG) performed in a reliable specialty laboratory.

If hyperandrogenemia is documented, we advise further diagnostic evaluation, as detailed in Fig. 28.7 [78]. An ultrasonographic examination

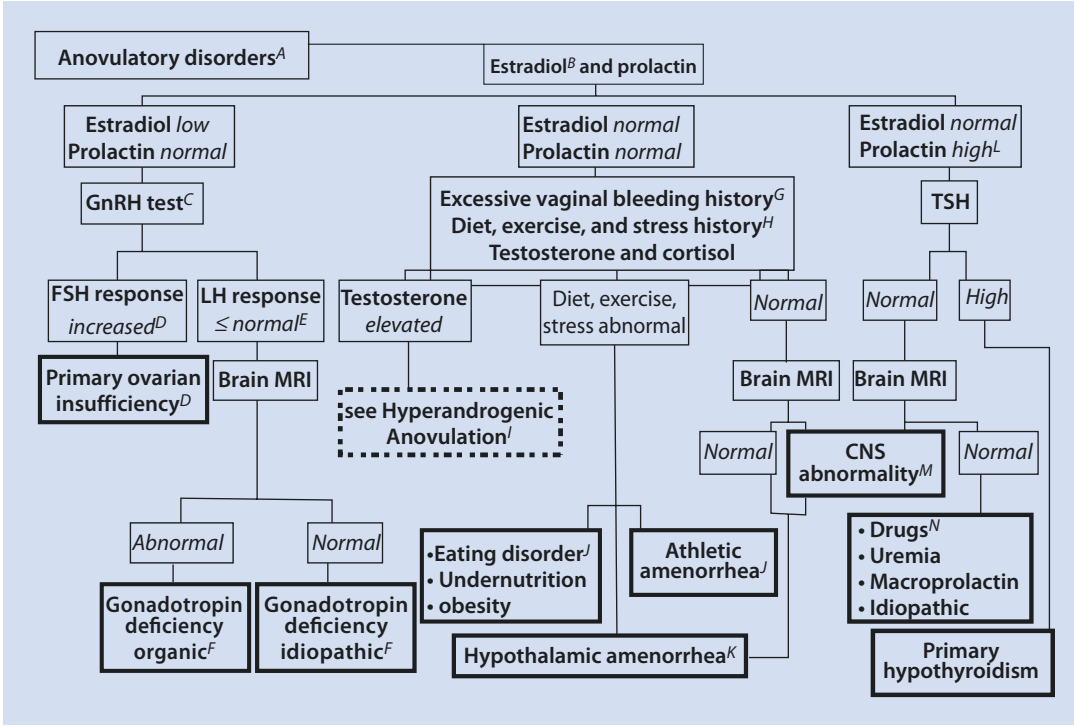

Fig. 28.6 Differential diagnosis of anovulatory disorders (Modified with permission from Rosenfield [9]) Footnotes: A. Anovulatory disorders should be considered in any girl with unexplained amenorrhea or oligomenorrhea, irregular menstrual bleeding, short cycles, or excessive menstrual bleeding. The workup in this algorithm progresses from negative studies in the Fig. 28.5 algorithm. B. Once breast development has matured, the breast contour does not substantially regress when hypoestrogenism develops. Hypoestrogenism is suggested if plasma estradiol is persistently <40 pg/mL in an assay sensitive to <10 pg/mL. However, a single estradiol level may be misleading because of cyclic or episodic variations. Functional hypothalamic amenorrhea may be associated with a low or a normal estrogen state. C. Baseline gonadotropin levels may not be low in gonadotropin deficient patients. GnRH agonist testing is performed by administering 10 mcg/kg leuprolide acetate subcutaneously and assaying LH and FSH at 3–4 h to assess gonadotropin reserve and at 18–24 h to assess the ovarian steroid response to endogenous gonadotropin release. D. Baseline gonadotropin levels may be normal as ovary function declines, but an exaggerated FSH response to GnRH agonist and subnormal E2 response to the gonadotropin elevation induced by acute GnRH agonist challenge are characteristic. The further workup is shown in Fig. 28.5. E. LH responses to GnRH agonist may vary from nil to normal in gonadotropin deficiency. Normal LH and FSH responses in the presence of hypoestrogenism indicate inadequate compensatory hypothalamic GnRH secretion. F. Gonadotropin deficiency may be congenital or acquired, organic or functional. Congenital causes include midline brain malformations or specific genetic disorders such as Prader-Willi syndrome, Laurence-Moon-Biedl syndrome, or Kallmann syndrome. Kallmann syndrome, the association of anosmia with gonadotropin deficiency, occurs in both the X-linked and autosomal-recessive forms. Special MRI views often demonstrate absence of the olfactory tracts. Acquired gonadotropin deficiency may be secondary to a variety of organic CNS disorders, varying from hypothalamic-pituitary tumor to radiation damage to empty sella syndrome. Autoimmune hypophysitis is a rare disorder, sometimes accompanying a polyendocrine deficiency syndrome. The prototypic form of functional gonadotropin deficiency is anorexia nervosa. Idiopathic hypogonadotropic deficiency may sometimes occur in families with anosmia, suggesting a relationship to Kallmann syndrome. G. Excessive vaginal bleeding or menorrhagia not controlled by progestin or OCP therapy additionally requires a pelvic ultrasound examination (for genital tract tumor or feminizing tumor), coagulation workup (which includes platelet count, prothrombin time, thromboplastin generation test, and bleeding time), and consideration of the possibility of sexual abuse. H. The equivalent of 4 miles per day or more is generally required before body fat stores fall to the point where amenorrhea occurs. Physical or psychosocial stress may cause amenorrhea. I. Hyperandrogenic anovulation evaluation is outlined in Fig. 28.7. J. Mild forms of stress disorders associated with low body fat (anorexia nervosa, bulimia nervosa, and athletic amenorrhea) may cause hypothalamic amenorrhea associated with normal or low plasma estradiol. The low body fat content of athletic amenorrhea may not be reflected by weight for height because of high muscularity. Dual-photon absorptiometry scan may be useful in documenting body fat below 20%. Patients with anorexia nervosa may become amenorrheic before or when

(continued)

weight loss begins, indicating an important psychological component to the etiology. If body composition does not improve, plasma estradiol falls and frank gonadotropin deficiency occurs. Obesity is also associated with anovulatory cycles. K. Hypothalamic amenorrhea is a diagnosis of exclusion. It is a form of partial gonadotropin deficiency in which baseline estrogen secretion is normal or low and a preovulatory LH surge cannot be generated. It may result from organic CNS disorders or be functional, due to stress, undernutrition or obesity, diverse types of endocrine dysfunction, chronic disease, or idiopathic. L. Hyperprolactinemia is heterogeneous in its presentation. Galactorrhea is present in half the patients. Some have normoestrogenic anovulation, which may be manifest as hypothalamic anovulation, hyperandrogenism, dysfunctional uterine bleeding, or short luteal phase. On the other hand, some are hypoestrogenic; these do not have galactorrhea. M. Hyperprolactinemia may be due to prolactinomas, which secrete excess prolactin, or may be secondary to interruption of the pituitary stalk by hypothalamic-pituitary tumors or other types of CNS injury. The latter cause variable pituitary dysfunction, which may include complete gonadotropin deficiency and various manifestations of hypopituitarism. N. Drugs, particularly neuroleptics of the phenothiazine or tricyclic type, may induce hyperprolactinemia

is useful to exclude tumor although the prevalence is only 0.2% and to reassure patients who have polycystic ovaries that the "cysts" are benign. The presence of polycystic ovaries is not specific for PCOS particularly in adolescents, and the presence of polycystic ovaries is not necessary for the diagnosis of PCOS [46, 69, 74]. Furthermore, the appropriate criteria for polycystic ovaries in adolescents are uncertain. Therefore, ovarian imaging may be deferred during the diagnostic evaluation, reserving it for girls with features of virilization, rapidly progressive hirsutism, or lack of response to therapy [5, 46, 78]. Transvaginal ultrasound is not recommended in virginal girls; transabdominal ultrasound is preferred although it has technical limitations. In girls with a significant amount of abdominal soft tissue and fat, the ovaries may be difficult to accurately visualize on transabdominal ultrasound. Anti-Müllerian hormone (AMH), produced by granulosa cells during early follicular development, has been found to be elevated in girls with PCOS. However, a threshold level that distinguishes girls with PCOS from girls with other causes of amenorrhea has not been clearly established. For this reason, AMH levels are not useful in the diagnostic evaluation [46]. Hyperandrogenic anovulation, in the absence of other causes of anovulation (Figs. 28.4, 28.5, and 28.6), including hyperprolactinemia, thyroid dysfunction, Cushing's syndrome, nonclassical congenital adrenal hyperplasia, and virilizing neoplasm, fulfills widely accepted criteria for the diagnosis of PCOS [5, 36, 43, 87].

PCOS may be mimicked by some rare disorders undetected by the above studies. Whether more extensive laboratory testing is performed varies upon the individual clinical features, circumstances, concerns, and preferences of each patient. If Cushing's syndrome is suspected based upon clinical history and multiple clinical features, evaluation should proceed according to published guidelines [90]. A history of rapid virilization, clitoromegaly, or rapid progression would be indications for a more extensive evaluation. It is our practice to initiate further workup for rare disorders in these select patients according to the algorithm presented in Fig. 28.8 [9, 78]. An early morning blood sample for free testosterone and steroid intermediates and 24-h urine for 17alpha-hydroxysteroids is obtained, followed by a dexamethasone androgen suppression test. We reserve ACTH testing, which is expensive if comprehensive, for the subset of patients with screening 17-hydroxyprogesterone levels that are suggestive of nonclassical congenital adrenal hyperplasia, or dexamethasone-suppressible androgen excess is present. There has been considerable confusion about the interpretation of moderately abnormal responses of steroid intermediates to this test. The experience to date indicates that mutations indicative of nonclassical congenital adrenal hyperplasia cannot be documented unless one of the steroid intermediates rises over 10 SD above average in response to ACTH [91–94]. If the source of androgen excess cannot be localized by these tests, one may be able to definitively demonstrate an ovarian source by a GnRH agonist challenge test [89]. If no source for the androgen excess can be found, one is dealing with idiopathic hyperandrogenism; such patients should be followed to ensure that their course is as benign as expected.

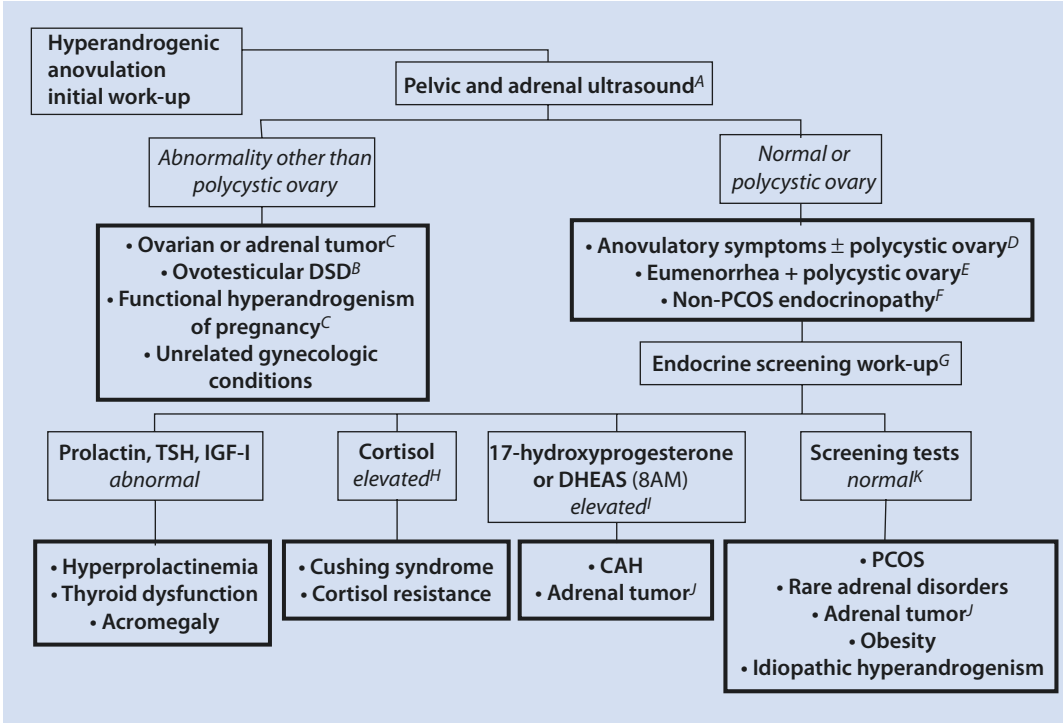

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Fig. 28.7 Initial workup of hyperandrogenism. This algorithm identifies the common causes of hyperandrogenism, which is most often due to PCOS (Modified from Rosenfield [78]). Footnotes: A. Ultrasonography is a study that detects polycystic ovaries and excludes ovarian pathology other than polycystic ovaries. It may be reserved for girls with rapid-onset symptoms, virilization, or lack of response to initial therapy. The abdominal ultrasound that is indicated for pelvic ultrasonographic imaging in virginal adolescents can also be used to screen for adrenal enlargement/mass. The current consensus criteria for polycystic ovary morphology in adults is an ovary with a volume > 10 cc and/or containing ≥12 follicles 2–9 mm diameter in the absence of a dominant follicle (≥10 mm diameter) or corpus luteum. One-third to half of normal adolescents meet these adult criteria. Until further research establishes definitive criteria, current evidence suggests that a mean ovarian volume > 12 cc (or single ovary >15 cc) be considered enlarged in adolescents; the normal range for follicle number in adolescents remains to be defined. Unless the ultrasound reveals an abnormality other than polycystic ovary morphology, further workup for hyperandrogenism is indicated. B. Ovotesticular DSD (disorder of sex development) was formerly termed true hermaphroditism. C. Virilization during pregnancy may be due to androgen hypersecretion by a luteoma or hyperreactio luteinalis. D. The presence of a polycystic ovary supports the diagnosis of PCOS in hyperandrogenic patients. The presence of a polycystic ovary is not necessary—nor does it suffice—for the diagnosis of PCOS in a patient with hyperandrogenic ovulation. E. In a eumenorrheic, symptomatically hyperandrogenic adolescent with normal menses, the presence of a polycystic ovary (which meets Rotterdam-AES diagnostic criteria in adults) is a risk factor for the diagnosis of PCOS. F. A polycystic ovary is not specific for PCOS; it has been reported in several specific endocrinopathies (e.g., hypothyroidism and Cushing's disease) and is also common in asymptomatic individuals. G. Further evaluation should include levels of serum prolactin, thyroid-stimulating hormone (TSH), insulin-like growth factor I (IGF-I), cortisol, 17-hydroxyprogesterone, and dehydroepiandrosterone sulfate (DHEAS). An abnormality of any of these endocrine tests is suggestive of one of the hyperandrogenic disorders that most commonly mimic PCOS. H. A midday or afternoon serum cortisol concentration of <10 mcg/dL is reassuring evidence against endogenous Cushing's syndrome in a hyperandrogenic obese girl. Patients with more elevated serum cortisol levels, or those with clinical features suggestive of Cushing's disease, warrant further evaluation (e.g., with 24-h urine collection for free cortisol and creatinine or midnight salivary cortisol). I. 8 AM 17-hydroxyprogesterone >170–200 ng/dl is approximately 95% sensitive and 90% specific for detecting common type (21-hydroxylase deficient) nonclassical congenital adrenal hyperplasia (CAH) in anovulatory or follicular phase women; it is often found in virilizing neoplasms. DHEAS >700 mcg/dl suggests adrenal virilizing tumor or a rare type of CAH (3ß-hydroxysteroid dehydrogenase deficiency). J. Computed tomographic scanning of the adrenal gland is a more definitive study for identifying adrenal tumor than is ultrasound. K. Exclusion of the preceding disorders in a hyperandrogenic patient with menstrual dysfunction meets standard diagnostic criteria for PCOS with approximately 95% reliability. However, this workup does not identify rare adrenal disorders (e.g., some types of CAH and related types of congenital adrenal steroid metabolic disorders), the rare testosterone-secreting adrenal tumor, or, most commonly, idiopathic hyperandrogenism (hyperandrogenism of unknown origin, which can arise from obesity or possibly metabolic abnormalities)

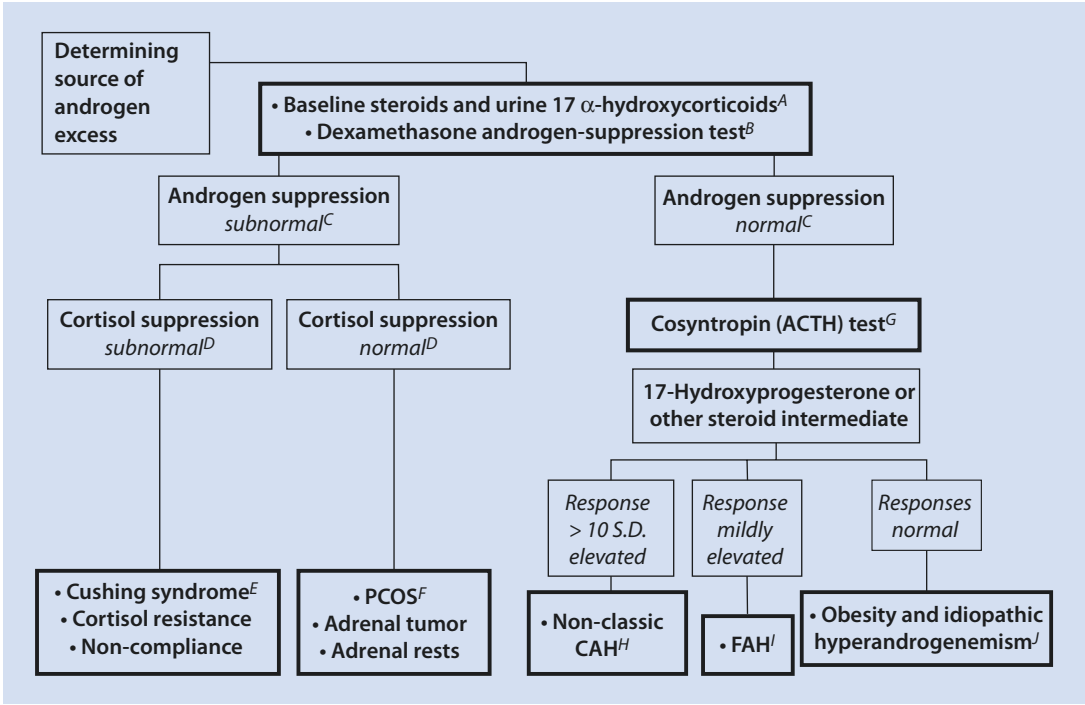

Fig. 28.8 Determining source of androgen excess (Modified from Rosenfield [78]). Footnotes: A. After obtaining an early morning blood sample for baseline steroid intermediates (e.g., 17-hydroxypregnenolone, 17-hydroxyprogesterone (17OHP), androstenedione, dehydroepiandrosterone) and a 24-h urine for 17 alpha-hydroxycorticoids, a dexamethasone androgen-suppression test (DAST) is performed. B. A long DAST is a definitive test. This consists of a 4-day course of dexamethasone 0.5 mg four times daily prior to an early morning posttest blood sample on day 5. A short DAST (sampling blood 4 h after a single noontime 0.5 mg dexamethasone) maximally suppresses total and free testosterone and 17-hydroxyprogesterone, but DHEAS and cortisol are not maximally suppressed in comparison with the 4-day DAST. C. Normal androgen suppression in response to the 4-day DAST is indicated in our laboratory (95% cutoff levels) by total testosterone of <28 ng/dL (1.0 nmol/L), free testosterone <8 pg/mL (28 pmol/L), DHEAS <40 mcg/dL (1.0 micromol/L) (>75% fall), and 17-hydroxyprogesterone <26 ng/dL (0.8 nmol/L). D. Normal corticoid suppression is indicated by serum cortisol <1.5 μg/dL (40 nmol/L) and urinary cortisol <10 mcg (27 nmol) per 24 h by the second day of dexamethasone administration. E. If androgen excess is suppressed by dexamethasone, further evaluation with a cosyntropin (ACTH) stimulation test is indicated. To perform the ACTH test, cosyntropin 250 μg is given, and blood is drawn for steroid measurements 60 min later. F. Subnormal suppression of testosterone (as well as androstenedione and 17-hydroxyprogesterone) and a normal suppression of cortisol and DHEAS is characteristic of PCOS, but the rare virilizing tumor or adrenal rests should be considered on the basis of clinical factors. G. If androgen and cortisol are both not normally suppressed, Cushing's syndrome and cortisol resistance should be considered. Poor suppression can also result from non-compliance with the dexamethasone regimen or the use of medications that accelerate the metabolism of dexamethasone. Thus, a dexamethasone level should be obtained. H. Steroid levels are assessed 60 min after ACTH administration. A post-ACTH 17-OHP value >1000 ng/dL (30 nmol/L) is highly suggestive, and >1500 ng/dL is definitive for nonclassical CAH due to 21-hydroxylase deficiency. A post-ACTH value of 17-hydroxypregnenolone >4500 ng/dl (150 nmol/L) suggests nonclassical 3ß-hydroxysteroid dehydrogenase deficiency, and a post-ACTH value of 11-deoxycortisol >4000 ng/dl (116 nmol/L) suggests nonclassical 11ß-hydroxylase deficiency. A serum cortisol level ≥ 18 mcg/dl (500 nmol/L) indicates that ACTH was administered properly. I. Primary functional adrenal hyperandrogenism (FAH) (suggested by a modest rise in 17-hydroxypregnenolone or 17-hydroxyprogesterone that does not meet the criteria for the diagnosis of CAH) is sometimes the only demonstrable source of androgen excess in PCOS. Cortisone reductase deficiency (or apparent CRD) is a rare mime of FAH and idiopathic hyperandrogenemia; baseline urinary corticoids consist primarily of cortisone metabolites rather than cortisol metabolites, so 17α-hydroxycorticoid excretion is elevated, but cortisol excretion is normal. J. When the source of hyperandrogenemia remains unexplained after intensive investigation (approximately 10% of cases), it usually appears to be due to obesity. Otherwise, the diagnosis is idiopathic hyperandrogenism (distinct from idiopathic hirsutism), if (apparent) cortisone reductase deficiency has been excluded

28.4 Management

Appropriate therapy of menstrual disorders depends upon the diagnosis. Amenorrhea due to genital tract outflow obstruction often requires surgical treatment, as in the case of vaginal aplasia or imperforate hymen. Some cases of intrauterine adhesions respond to hysteroscopic lysis. If a DSD is diagnosed, utmost sensitivity must be used when discussing the diagnosis with the patient and family. The gender identity and wishes of the patient must be considered when recommending hormonal therapy. Persons with ovotesticular gonadal dysgenesis are at higher risk for gonadal malignancy; close follow-up to monitor for gonadoblastoma is mandatory. In some cases, prophylactic surgical removal of dysgenetic gonadal tissue is advisable.

For some underlying causes, treatment of hypogonadism is achieved without hormone replacement. The treatment of choice for a prolactinoma is dopaminergic agents. Hyperprolactinemia will be maximally suppressed within 1 month, and the menstrual cycle will normalize within 3 months with an effective regimen. Cabergoline 0.25–1.0 mg once or twice weekly will usually control galactorrhea and shrink prolactinomas [35]. To minimize nausea, it is best to start with a low dose at bedtime. Two years of treatment will minimize recurrence. Transsphenoidal resection of prolactinomas is considered if the patient's condition or eyesight is critical and for the rare treatment failures. A link between cabergoline treatment and mild-moderate tricuspid valve regurgitation has been suggested in elderly patients with Parkinson's disease who often take large doses of the drug. Whether this link also exists in patients with hyperprolactinemia, who are generally prescribed five- to tenfold smaller doses, is yet to be established [95]. However, data to date are reassuring and suggest an increase in only trace to mild, but not moderate, valvular insufficiencies.

Hypogonadism due to chronic diseases such as cystic fibrosis, heart failure, cirrhosis, chronic renal failure, regional enteritis, or systemic lupus erythematosus is best treated by controlling the underlying illness. Anorexia nervosa is best managed by an experienced multidisciplinary team. Refeeding is the first priority, accompanied by long-term management of the psychodynamic issues [96]. Menses resume when psychotherapy is effective and body fat is restored to normal. Estrogen treatment of patients with an eating disorder may mask the psychopathology that underlies the menstrual disturbance by inducing regular menses, and oral estrogen-progesterone combination therapy does not yield the recovery of bone loss that occurs with weight gain [97], although more promising results have been reported with physiologic transdermal estradiol replacement [98].

Hormone replacement is the treatment of choice in hypergonadotropic hypogonadism and organic causes of hypogonadotropic hypogonadism, such as congenital or acquired hypopituitarism. Stature is an important consideration in sexually immature teenagers, especially those with Turner syndrome. The dose of estrogen in standard oral contraceptive pills (OCPs) will inhibit growth and lead to premature fusion of the epiphyses in sexually immature patients. Panhypopituitary patients require replacement of growth hormone, thyroid hormone, and cortisol deficits. Further discussion of full pituitary hormone replacement is beyond the scope of this chapter.

Optimal estrogen replacement therapy in the sexually immature girl requires the induction of puberty in a physiologic manner with extremely low doses of estrogen to maximize growth. Gradual, physiologic replacement of estrogen can be started at a peer-appropriate age without compromising height potential. This technique has been validated using intramuscular depot estradiol [99]. However, as intramuscular estradiol at the low doses required to mimic physiology at pubertal onset is most accurately provided with the support of a compounding pharmacy, transdermal E2 is a reasonable alternative [13]. There are various techniques to initiate therapy at an appropriate age at the very low doses required. The recommended starting dose averages 3–6 mcg daily. Two strategies have been employed to do this: daily administration of a fractionated patch or cyclic administration of a complete patch for a short period of time. For example, some administer one-quarter of a 25 mcg patch daily; others start with administration of a 25 mcg patch continuously for 7 days monthly. An alternate starting dose is 0.25 mg micronized E2 by mouth daily. The dose is increased every 6 months over a span of 2 years to adult replacement doses of transdermal estradiol 75–100 mcg daily or 2 mg micronized E2 by mouth daily. Conjugated equine

estrogens are not advised since doses as low as 0.325 mg daily inhibit growth [100]. Progestin should be added to estrogenic regimens after 2 years of estrogen replacement treatment or after bleeding occurs: we start physiologic replacement with micronized progesterone (Prometrium®) 100 mg daily for 7 days monthly and advance to a full maintenance dose of 200 mg daily for 10–12 days monthly as tolerated. In the setting of estrogen replacement at adult doses, the addition of progesterone is necessary to lower the risk of endometrial hyperplasia and endometrial carcinoma [101].

Hypoestrogenism in sexually mature girls may be managed by administration of an oral contraceptive (OCP) or transdermal patches with a progestin component. The currently available OCPs carry very little risk of venous thromboembolic disease (VTE) [102], and the progestational component protects against endometrial hyperplasia, but evidence in postmenopausal women suggests that transdermal estradiol is slightly safer [103, 104]. VTE risk is related to the dose of estrogen (combination OCPs have ethinyl estradiol doses that range from 10 to 35 mcg) and the type of progestin (with norethindrone and norgestimate having the lowest risk and antiandrogenic progestins, e.g., drospirenone, about 50% more risk). Obese patients may require higher doses of estrogen to achieve menstrual regularity. However, patients at risk of VTE or sensitive to estrogen because of conditions such as hypertension, migraine, or lymphedema are best advised to use a more physiologic form of therapy, such as 17β-estradiol delivered systemically, bypassing the liver. Systemic estradiol may be given intramuscularly as depot estradiol with medroxyprogesterone acetate or transdermal estradiol with norgestimate. When using estrogen alone, a progestin is administered for the last 7–12 days of each course of estrogen (e.g., micronized progesterone 100–200 mg orally daily); the more progestin administered, the less the risk of endometrial hyperplasia, but the greater the risk of premenstrual symptoms.

In sexually mature adolescents with menstrual irregularities who experience withdrawal bleeding in response to progesterone during the diagnostic workup (Fig. 28.4), oral progestin therapy may be repeated in 2–3-month cycles in order to detect the emergence of spontaneous menses that signals the resolution of the physiologic anovulation of adolescence. This treatment seldom causes side effects and has never been incriminated as a cause of post-pill amenorrhea; thus, it has the appeal of potentially disturbing the developing neuroendocrine system less than OCPs. However, patients must be made aware that this is not a contraceptive treatment; nor does it treat the underlying condition.

An acute episode of excessive vaginal bleeding requires the administration of estrogen, given together with a progestin as a low-dose OCP, one tablet four times daily for 7 days. Treatment is then stopped for 5 days, and the patient is warned that heavy withdrawal bleeding with cramps may occur. Therapy with a low-dose OCP, given as for contraception, is then begun to prevent recurrence of dysfunctional bleeding and is continued for about three cycles. Cyclic progesterone is an alternative treatment to oral contraceptive pills.

A patient who is hypovolemic because of rapid, heavy dysfunctional bleeding should be hospitalized and treated with intravenous fluids and blood products as necessary. Conjugated equine estrogens in a dose of 25 mg intravenously every 3–4 h for three to four doses is customary to reduce the bleeding. When medical management fails, a bleeding diathesis or uterine structural abnormality should be considered. If heavy bleeding persists, curettage should be performed by a gynecologist.

The management of hyperandrogenic states is individualized according to symptoms and patient goals—hirsutism, acne, and alopecia, menstrual irregularity, obesity, and insulin resistance—and the source of androgen excess. The hyperandrogenism associated with congenital adrenal hyperplasia, Cushing's syndrome, virilizing tumors, DSD, hyperprolactinemia, or acromegaly improves with appropriate treatment of the underlying cause. Undesirable side effects of glucocorticoid treatment of congenital adrenal hyperplasia can typically be minimized by using a modest bedtime dose (about 5–7.5 mg prednisone). Monitoring 17-hydroxyprogesterone and testosterone while on treatment is necessary for dose adjustment [105]. Control of androgens in congenital adrenal hyperplasia may not suffice unless nocturnal progesterone excess is also controlled [106]. This treatment will typically normalize the menstrual pattern in nonclassical congenital adrenal hyperplasia, but the effect in classic congenital adrenal hyperplasia is more problematic, since PCOS complicates many of

these cases as a result of congenital or perinatal masculinization [36, 69].

The management of PCOS is directed toward treating symptoms and monitoring for associated disorders. Treatment of menstrual irregularities in PCOS is recommended to prevent amenorrhea and the associated risk of endometrial hyperplasia and carcinoma. A combination OCP containing estrogen and progestin is the first-line treatment to induce regular menstrual cycles, especially in those with hirsutism or its cutaneous equivalents. OCPs containing non-androgenic progestins such as norgestimate generally have favorable risk-benefit ratios and optimize lipid profiles. Those on drospirenone may benefit from its weak antiandrogenic and moderate antimineralocorticoid effects, though it poses slightly more risk for thromboembolic events: drospirenone is available in the USA with 20 or 30 mcg ethinyl estradiol. Obese patients may require a higher dose of estradiol to provide menstrual regularity.

Progestin-only regimens can be used to induce menstrual regularity as detailed above, especially if hirsutism and its cutaneous equivalents are not a concern. Progestin-only regimens are also useful in patients in whom OCPs are contraindicated or in patients with objections to use of contraceptive therapy.

The hyperandrogenic manifestations of hirsutism and acne are treated by topical dermatologic and cosmetic measures and/or endocrinologic treatment. The choice between the treatment options depends upon symptoms and patient preference. Mild hirsutism can be treated by hair removal techniques such as shaving, bleaching, or waxing. Eflornithine hydrochloride cream (Vaniqa®) is a topical agent that is FDA approved for the removal of unwanted facial hair. Six to eight weeks of use is required before effects are seen, and it must be used indefinitely to prevent regrowth. It is often not covered by third-party payers. Laser therapy and electrolysis are techniques of permanent hair reduction. Because of expense, discomfort, and occasional scarring, these techniques are most appropriate for limited areas in patients who do not respond to other means of treating hirsutism.

OCPs are useful to treat hyperandrogenic symptoms as an adjunct to cosmetic treatments. The estrogen component decreases bioactive testosterone by suppressing LH secretion and ovarian androgen production while increasing serum SHBG. The decrease in bioactive testosterone, assessed 3–6 months after start of therapy, is associated with an improvement in hyperandrogenic cutaneous symptoms; acne can improve within 3 months and progression of hirsutism may arrest within 9–12 months in most PCOS patients. Endocrinologic treatment reduces the androgen-induced transformation of vellus to terminal hairs, and the effects of these agents are maximal at 9–12 months because of the long growth cycles of sexual hair follicles. Thus, a minimum of 6 months is required to determine improvement. OCPs are recommended as a first-line treatment of hirsutism [46, 68]. Many OCPs contain progestins that may have a mild androgenic component. It is logical, therefore, to treat hirsute women with OCPs that contain non-androgenic or antiandrogenic progestins, as detailed above. If after 6 months of treatment no substantial improvement in hirsutism occurs, antiandrogen therapy is suggested [68]. Spironolactone has been shown to be effective to the extent of lowering hirsutism score by about one-third [107], with considerable individual variation, and is probably the most potent and safe antiandrogen available in the USA. We recommend starting with 100 mg twice daily and then reducing the dosage to 50 mg twice daily for maintenance therapy after the maximal effect has been achieved. This dosage is usually well tolerated; however, fatigue and hyperkalemia at higher doses may limit its usefulness. It is potentially teratogenic to fetal male genital development and may cause menstrual disturbance in the patient; therefore it should be prescribed with an oral contraceptive. Flutamide is another antiandrogen of similar efficacy, but is not recommended because of potential hepatotoxicity and expense [68].

The risk of metabolic syndrome and type 2 diabetes mellitus is increased in PCOS; thus a fasting lipid panel and oral glucose tolerance test are recommended in patients with central obesity, hypertension, or family history of type 2 diabetes mellitus [108]. The 2-h blood sugar during an oral glucose tolerance test deteriorated at an average rate of 9 mg/dl/year over about a 3-year period in one study [56]. Primary relatives have been shown to have higher rates of diabetes mellitus and metabolic syndrome; thus, these tests are also recommended in obese or hypertensive primary relatives [109]. Women with obesity and PCOS are at risk for obstructive sleep apnea; thus,

questioning about sleep patterns with appropriate referral to a sleep clinic may be necessary [110]. Screening for fatty liver disease with serum AST and ALT may also be necessary with appropriate referral to a liver specialist as needed.

Weight loss improves ovulation, acanthosis nigricans, androgen excess, and cardiovascular risk in PCOS patients [91, 111, 112], while antiandrogens have only a modest effect on metabolic abnormalities [113]. This observation lends promise to the idea that insulin-lowering agents could be useful in the treatment of PCOS. The biguanide metformin is the most studied of the insulin-lowering agents in the treatment of PCOS. Metformin suppresses appetite and enhances weight loss. Randomized trials in adolescents demonstrate that metformin increases the frequency of menses and ovulation and modestly lowers testosterone levels [114–116]. The decrease in testosterone is not sufficient to improve hirsutism. In addition, it is unclear whether the effect of metformin on menstrual frequency is primary or secondary to the induced weight loss [117]. It is recommended that metformin be considered as an adjunct to weight-control measures in women with impaired glucose tolerance, especially if weight-loss measures fail [118]. Thiazolidinediones also increase insulin sensitivity [119], but their use in adolescents is not recommended because of the associated weight gain, risk of hepatotoxicity, and possible long-term cardiovascular side effects.

Psychological support is an important aspect of the management of teenagers with menstrual disorders. Girls with delayed puberty, whether it has an organic or functional basis, can almost always be assured that they will feminize and, if a uterus is present, that they will experience menses. Girls can be reassured that menstrual abnormalities will be regulated. In addition, patients with Turner syndrome or similar primary hypogonadism disorders can hold hope for the ability to carry a pregnancy, and patients with hypogonadotropic hypogonadism and chronic anovulatory disorders can hold hope for the ability to conceive, though in both situations this may require special care by a reproductive endocrinologist.

Case Study

H.C. is a 17-year-old girl who presents with lack of menses for the previous 7 months. She experienced menarche at age 11 and reports typical monthly menses until 2 years ago. At that time, her menses became more infrequent, but she gets at least one every 2–3 months per her memory. She reports significant weight gain between age 13 and 16, with her weight stable over the past year. She reports "bad" acne 2 years ago that improved with topical medications. She has noted hair development in her sideburn and umbilical areas. She shaves these areas about once every 2 weeks.

Her physical activity is confined to walking to and from school, about ¼ mile each way. She reports she only drinks water (not sugared beverages) and is trying to avoid snacking but professes a love for chips, indulging three to five times/week.

Her mother reports regular menses and no family history of irregular menses or infertility. HC has a sister who is 13 years old and started menstruating last year, with regular menses. Maternal grandmother has type 2 diabetes.

HC denies sexual activity, headaches, galactorrhea, voice changes, polyuria, or polydipsia.

On physical exam, HC is 93.2 kg, height 165 cm, with a BMI of 34.5. BP 118/64, pulse is 78 and regular. She does not have Cushingoid features, but does have generalized obesity. Head, neck, cardiac, lung, and abdominal exams are normal. She has mild hirsutism on her upper lip, sideburn area, mid and lower abdomen, thighs, and lower back (Ferriman-Gallwey score of 8). She does not have clitoromegaly. She has comedones on her face with scattered papules on her face, chest, and back. She has no striae.

Discussion: HC has secondary amenorrhea. Her clinical history is inconsistent with an eating disorder and athletic or stress induced amenorrhea. Her clinical history is most consistent with hyperandrogenism, but primary ovarian insufficiency, hyperprolactinemia, and thyroid disorders remain possibilities. Pregnancy is first on the differential of secondary amenorrhea and must be excluded in all cases. Evaluation should begin with a pregnancy test, FSH, estradiol, prolactin, TSH and testosterone levels, and if necessary an assessment of cortisol status.

Her urine pregnancy test is negative. Her FSH is 4.6, LH 5.5 U/L (not elevated), estradiol 36 pg/mL (normal), prolactin 6 ng/mL (normal), TSH 2.32 U/L (normal), late AM cortisol 9 mcg/dL (not elevated), and testosterone (by tandem mass spectroscopy) 86 ng/dL (elevated for women).

Discussion: HC has hyperandrogenemia. While it is customary to order LH simultaneously with FSH, serum LH is not as important diagnostically as in the differential diagnosis of hypogonadism; since

*LH in increased by hyperandrogenism and depressed by obesity, it is only supportive of the diagnosis of hyperandrogenism if elevated. Although the most common cause is polycystic ovary syndrome (PCOS), other causes must be excluded as well. Next steps in evaluation include an ovarian and adrenal ultrasound (using a transabdominal approach), although some providers defer this step if there is no virilization and testosterone level is < 100 ng/dL. A midday or afternoon serum cortisol concentration of < 10 mcg/dL is reassuring evidence against endogenous Cushing's syndrome in a hyperandrogenic obese girl. Patients with more elevated serum cortisol levels, or those with clinical features suggestive of Cushing's disease, warrant further evaluation (*e.g.*, with 24-h urine collection for free cortisol and creatinine or midnight salivary cortisol levels). To exclude late-onset congenital adrenal hyperplasia and other adrenal pathology such as a tumor, an 8 AM 17-hydroxyprogesterone and DHEA-S level is recommended.*

Her ovary and adrenal ultrasound indicates a right ovarian volume of 11 cc and a left ovarian volume of 12 cc. Approximately 15 small (4–8 mm) follicles are observed in each ovary. No ovarian or adrenal masses detected.

Her 8:15 AM 17-hydroxyprogesterone level is 55 ng/dL (not elevated) with a DHEA-S of 164 mcg/dL (not elevated).

Discussion: HC's normal morning 17-hydroxyprogesterone excludes late-onset congenital adrenal hyperplasia, and her normal DHEA-S and ultrasound exclude an ovarian or adrenal tumor. While her ovarian measurements would be abnormal for an adult, they are considered normal for an adolescent; furthermore, polycystic ovaries are not considered a diagnostic criterion in adolescents.

Given HC's non-severe clinical symptoms, physical exam findings, and evaluation thus far, a more extensive evaluation is not indicated unless she does not respond to initial therapy.

Treatment of her amenorrhea is indicated to prevent prolonged amenorrhea with risk of endometrial hyperplasia. A combination oral contraceptive pill is the treatment of choice as it will also improve her hyperandrogenic symptoms of acne and hirsutism. Progesterone withdrawal using 10 mg Provera for 10 days may be performed to promote shedding of the endometrial lining and diminish the chances of vaginal spotting upon initiation of oral contraceptive pills. After shedding of the endometrial lining, oral contraceptives can be started. An OCP combination of ethinyl estradiol at 30–35 mcg and drospirenone or a progesterone of low androgenic potency is preferred. HC should be counseled that the acne may improve in 2–3 months, but it will take 6–9 months of treatment to see improvement in hirsutism.

HC should also be counseled on the androgen-lowering effects of weight loss and encouraged to start a regular exercise regimen. A referral to a nutritionist would be helpful to give HC guidance on a balanced diet to prevent more weight gain. Screening for diabetes should take place once yearly. Metformin is not indicated unless lifestyle changes fail and/or diabetes develops.

After OCPs are started, HC should be seen again within 4 months to evaluate her tolerance of the OCP and her success in instituting lifestyle changes. Failure to normalize menstrual cyclicity and hyperandrogenemia would be unusual and cause for reevaluation.

28.5 Conclusion

Normally, cyclic menstrual bleeding occurs at 21–45-day intervals even during the first postmenarcheal year; cycles outside 19–90 days' duration are always abnormal. Menstrual disorders in adolescents are commonly encountered in general and subspecialty pediatric practices. They are due to a diverse range of causes, both hormonal and non-hormonal, but usually indicate an anovulatory disorder. The most common of these is polycystic ovary syndrome. Advances have been made in finding monogenetic causes of some disorders, but the underlying causes remain unknown for polycystic ovary syndrome and other polygenetic disorders. Only when the normal physiology and pathophysiology of reproduction and its disorders are fully teased out can optimally targeted treatments be developed.

Review Questions

1. What is the definition of secondary amenorrhea?
   A. Lack of menarche by age 15
   B. Six months without a menstrual period after initially menstruating
   C. Three months without a menstrual period after initially menstruating
   D. Less than 8 periods within a calendar year
2. Which of the following does *not* cause primary ovarian insufficiency?
   A. Turner syndrome
   B. Hyperprolactinemia

C. Autoimmune disease
D. Chemotherapy

3. Predisposing factors to developing PCOS include:
   A. Congenital adrenal hyperplasia
   B. Maternal PCOS
   C. Premature adrenarche
   D. All of the above

Answers

1. C
2. B
3. D

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Contraception

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Key Points

- It is imperative to address contraception with adolescents.
- Each contraceptive method has its own set of advantages and limitations.
- Hormonal contraception, including long-acting methods, may be particularly well-suited for adolescents.
- Hormonal contraceptive methods provide many noncontraceptive health benefits.

29

29.1 Introduction and Background

The vast majority, or 75%, of teen pregnancies in the United States are unintended [1]. Unplanned and teen pregnancies have important public health consequences, including prematurity and low birth weight [2] and may be particularly deleterious in women with endocrine disease. Pregnancy increases thyroxine requirements in many women with primary hypothyroidism [3], and overt hypothyroidism during pregnancy has been associated with pregnancy complications, such as prematurity, gestational hypertension, pregnancy loss [4], as well as a lower IQ in the children [5]. Because uncontrolled diabetes at the time of conception may increase the risk of fetal malformations, spontaneous abortions, and perinatal mortality [6], the American Diabetes Association recommends an A1C <7% prior to conception to minimize risk [7].

Given the risks of unplanned pregnancy, particularly in teens with endocrine disorders, it is imperative to address contraception with these patients. Contraception use has been shown to decrease rates of teen pregnancy. With increased use of contraception, teen pregnancy rates in the United States reached historic lows [8]. In 2011, there were approximately 553,000 teen pregnancies for a pregnancy rate of 52 pregnancies per 1000 women aged 15–19 years, which is dramatically lower than the peak rate of 117 per 1000 in 1990 [9].

Despite declining pregnancy rates, preventing unintended pregnancy among young women continues to be a vital health concern, particularly in the United States. Because US teens are less likely than European teens to use effective contraception, adolescents in the United States continue to have among the highest pregnancy rates of developed countries even though they do not have higher levels of sexual activity [10, 11]. In a US survey, between 2011 and 2013, 44% of female and 49% of male adolescents reported having sexual intercourse [12], but only 79% of female teenagers and 84% of male teenagers reported use of contraception at the time of their first intercourse [12].

Realistic choice of contraceptive method appears to be particularly important in the adolescent population. Although many forms of hormonal and nonhormonal contraception are available (Table 29.1), each contraceptive

Table 29.1 Available reversible contraceptive methods

Long-acting (3–10 years)	
Intrauterine devices (IUDs)	Copper IUD (ParaGuard®) Levonorgestrel IUS
Progestin-only contraception: Implantable contraception	Subdermal implant (Nexplanon®)
Intermediate-acting (months)	
Progestin-only contraception: Injectable contraception	Depo-Provera®
Shorter-acting (days to weeks)	
Combined hormonal Contraception	Combined oral contraceptives Transdermal patch Vaginal ring
Progestin-only contraception: Oral	Progestin-only pills
Coitally dependent	
Mechanical	Male condom Vaginal barriers Diaphragm Cervical cap Vaginal sponge Female condom
Behavioral	Withdrawal Periodic abstinence
Spermicidal	Foam Cream Suppository Gel Film

Table 29.2 Limitations of current reversible contraceptive methods

	Failure rate < 10% (typical use)	Prevents STD	Avoids daily patient responsibility	Preserves spontaneity	Avoids systemic side effects
Ideal method	Yes	Yes	Yes	Yes	Yes
Hormonal intrauterine device	Yes	No	Yes	Yes	Partially
Nonhormonal intrauterine device	Yes	No	Yes	Yes	Yes
Implantable contraceptives	Yes	No	Yes	Yes	No
Injectable contraceptives	Yes	No	Yes	Yes	No
Combined hormonal contraceptives	Yes	No	Variable (pills, daily; patch, weekly; ring, monthly)	Yes	No
Barrier	No	Some types	Yes	No	Yes
Behavioral	No	No	No	No	Yes
Spermicide	No	[a]See note	Yes	No	Yes

[a]Spermicides (even as an adjunctive measure) are not recommended for patients with HIV or AIDS or those at high risk for HIV due to an increased risk of genital lesions and disruption of cervical mucosa which may contribute to the transmission of HIV Curtis et al. [14]

method has its own set of advantages and limitations (Table 29.2). In choosing a contraceptive method, important considerations include effectiveness, side effects, contraindications, frequency of patient responsibility (e.g., daily, weekly, monthly, or longer acting), as well as the necessity of interrupting sexual spontaneity. All of these factors will affect efficacy but also continuation, which is demonstrably lower in adolescent women, with the exception of long-acting reversible contraceptives (Table 29.3). The selection of a contraceptive method for an adolescent patient with endocrine disorder may require additional considerations. Regardless of method, women under the age of 30 years will experience higher rates of contraceptive failure compared to older women [13].

The Centers for Disease Control and Prevention (CDC) has two sets of guidelines regarding contraception, and both were both updated in 2016. To assist clinicians in choosing safe contraception, particularly for women with preexisting medical conditions, the CDC provides evidence-based guidelines in the *US Medical Eligibility Criteria for Contraceptive Use (MEC)* [14]. Once a method is chosen, the CDC provided guidelines for effective and safe use in the *US Selected Practice Recommendations for Contraceptive Use* (SPR). These guidelines were adapted from the World Health Organization (WHO) and provide recommendations using a 4-point system (Table 29.4). Category 1 indicates there would be "no restriction for the use of the contraceptive method," while category 4 indicates that use of the contraceptive method "represents an unacceptable health risk." These guidelines are available on the CDC website and as a phone app.

In counseling patients, it should be emphasized that contraceptive use also confers health benefits unrelated to family planning. Hormonal contraceptive methods, in particular, have been used in management of dysfunctional uterine bleeding, dysmenorrhea, pubertal disorders, acne, endometriosis, hirsutism, menstrual migraine, and improvement of premenstrual dysphoric disorder symptoms. Hormonal contraceptives have

Table 29.3 Percentage of women experiencing an unintended pregnancy during the first year of typical use and the first year of perfect use of contraception and contraceptive continuation rates among all women and among young women at 1 year, United States

	% of women with an unintended pregnancy in the first year of use		Continuation rates among all women at 1 year (age 15–44)	Continuation rates among young women (14–25) at 1 year
Method	Typical use	Perfect use		
No method	85	85		
Withdrawal	22	4	46%	NA
Spermicide	28	18	42%	NA
Condoms	18	2	43%	NA
Diaphragm	12	6	57%	NA
Sponge: Parous	24	20	NA	NA
Nulliparous	12	9	NA	NA
Oral contraceptive	9	0.3	67%	32.7[a]
Patch	9	0.3	67%	10.9[a]
Ring	9	0.3	67%	29.4[a]
DMPA	6	0.2	56%	12.1[a]
All LARC methods	0.05–0.8	0.05–0.6	78–84%	84% (95% ci:79–89)[b]
IUD: Paragard	0.8	0.6	78%	74% (95% ci:61–87)[b]
LNG	0.2	0.2	80%	
Implant	0.05	0.05	84%	84% (95% ci:77–91)[b]

Source: (Unless otherwise noted) Trussell [19]
NA: Data not available
[a]Per 100 woman-years Raine et al. [42]
[b]% continuation at 1 year Diedrich et al. [184]

also been shown to reduce the risk of endometrial, ovarian, and colorectal cancers [15].

The potential prevention of sexually transmitted infections (STIs) may be an important consideration for young patients who are not in long-term stable relationships. Young adults, aged 15–24, accounted for nearly 9.7 million of the 19.7 million new cases of STI in the United States in 2008 [16]. Latex male condoms are recommended for preventing transmission of STIs [17], but are not a highly effective method of contraception, particularly among younger users [13]. Thus, dual method contraceptive use (with latex condom and more effective contraceptive method) should be encouraged for adolescents to prevent both pregnancy and STI transmission.

Because hormonal methods are metabolically active, it is necessary to consider the possible interactions with the patient's disease or medical treatment. Nonhormonal contraceptive methods can be classified as behavioral, spermicidal, barrier, and intrauterine. While there are no medical contraindications to behavioral and barrier contraceptive methods, these methods are associated with high typical-use failure rates [13]. The CDC MEC states that women with medical conditions associated with a high risk of adverse consequences from unintended pregnancy, including complicated insulin-dependent diabetes, should be advised that use of barrier or behavioral methods may not be an appropriate choice due to high typical-use failure rates [14].

Table 29.4 Categories of medical eligibility criteria for contraceptive use

Category	Condition
US MEC 1	A condition for which there is no restriction for the use of the contraceptive method
US MEC 2	A condition for which that advantages of using the method generally outweigh the theoretical or proven risks
US MEC 3	A condition for which the theoretical or proven risks usually outweigh the advantages of using the method
US MEC 4	A condition that represents an unacceptable health risk of the contraceptive method is used

Source: Curtis et al. [14]

29.2 Nonhormonal Methods of Contraception

29.2.1 Condoms

The male condom represents a mechanical barrier to fertilization and is the most frequently used method of contraception used at first intercourse. In 2006–2010, 68% of females and 80% of males reported using condoms at their first intercourse [18]. Numerous different types of male condoms are available over the counter and are similar in their efficacy in STI protection and pregnancy prevention. The Reality® female condom is a disposable method of contraception that was FDA approved for nonprescription sale in 1994. It consists of a polyurethane bag or sheath that fits into the vagina and is held in place by an internal vaginal ring.

With perfect use, condoms are an effective method of contraception with a failure rate for male condoms of 2% and for female condoms of 5% [19]. Perfect use requires placing the condom prior to any genital contact and withdrawal of the penis prior to the loss of the erection. Therefore, it is not surprising that typical-use failure rates are substantially higher than rates for perfect use with a failure rate for male condoms of 18% and for female condoms of 21% [19]. Users under 30 years old have a relative risk of 1.55 (95% confidence interval 1.11–2.16) for failure of male condoms compared to users aged 30 years and older [13].

In the adolescent population, the primary role for the male condom may be to minimize the spread of STIs. The male condom provides the best available protection from STIs, providing protection from both bacterial and viral infections, including HIV [20]. The CDC MEC recommends correct and consistent use of male latex condoms for reduction of STIs [14]. Although data regarding the female condom are limited, CDC MEC also recommended use of female condom for protection from STI transmission [14]. It should be noted that natural condoms made from sheep intestine may not be as effective as latex and polyurethane in the prevention of STIs [20].

Clinicians should counsel all adolescents who are at risk for STIs and at risk for unintended pregnancy to use both latex condoms and a more effective method of contraception. It should be emphasized to adolescents that prevention of STIs is a critical step for the preservation of their future fertility. In men, STIs may cause infertility through multiple mechanisms, including injury to the reproductive tract, and impairment of semen parameters [21]. In women, pelvic inflammatory disease (PID) usually results from ascending passage of bacteria from the lower reproductive tract into the tubal lumen [22]. It has been clearly demonstrated that the risk of infertility increases with the number of PID episodes. Infertility developed in 11.4% of women after one episode, in 23.1% of women after 2 episodes and in 54.3% of women after three episodes [23].

29.2.2 Behavioral Methods

Behavioral methods include coitus interruptus (withdrawal) and fertility awareness-based (FAB) methods with abstinence or barrier methods during the fertile period. Coitus interruptus refers to withdrawal of the penis prior to ejaculation so that sperm will not be deposited in the vagina. This method, however, is associated with an 18% failure rate in the first 12 months of use [13] and cannot be considered an adequate birth control method. FAB methods require careful monitoring of menstrual cycles, assessments of cervical mucous quality, and/or daily measurements of basal body temperature. Typical-use failure rates associated with FAB methods are as high as 25% in the first year of use [13]. Additionally, adolescents and patients with endocrine disturbances

are more likely to experience menstrual irregularities, which may complicate the use of FAB methods.

29.2.3 Spermicides

Vaginal spermicides are available, without a prescription, as foam, suppositories, gels, films, and cream, but do not represent an adequate birth control method for adolescents. In practice, the typical-use failure has been estimated at 28% [19], so that spermicidal agents can be recommended only as an adjunctive measure to increase the efficacy of barrier methods [24]. Spermicides (even as an adjunctive measure) are not recommended for patients with HIV or AIDS or those at high risk for HIV due to an increased risk of genital lesions and disruption of cervical mucosa which may contribute to the transmission of HIV [14].

29.2.4 Other Barriers Methods

The diaphragm, cervical cap, and contraceptive sponge prevent pregnancy by blocking the cervix, as well as holding spermicide around the cervix. Although studies have not determined the optimal timing, clinical practice has been to insert the barrier no more than 6 h prior to intercourse and to leave it in place for at least 6 h afterward [25], but no more than 24 h, due to concern for toxic shock syndrome (TSS) [26].

The diaphragm and cap are reusable latex barriers. They are available in several sizes, require fitting by trained personnel, and must be used in conjunction with spermicides for maximum effectiveness [25]. Additional spermicide is inserted prior to each act of coitus. With perfect use, the failure rate has been estimated at 6% [19], but with routine use, the failure rate has been reported to be twice as high (12%) during the first year of use [19]. Both the diaphragm and the cervical cap are associated with an increased risk of urinary tract infections [25], presumably as a result of alterations in vaginal flora [27, 28].

The vaginal contraceptive sponge is disposable and is available over the counter. It is impregnated with spermicide, must be inserted before intercourse, and is effective for multiple acts of coitus during a 24-h period. In typical use, the vaginal sponge appears to be twice as effective for nulliparous women with a similar failure rate (12%) in this group as the diaphragm and cervical cap, but failure rate was 24% in parous women [19].

The combination of inadequate protection against pregnancy without the proven STI protection of condoms makes these barrier methods poor candidates for use by adolescents. In addition, because these barrier methods require the use of spermicides, they are not recommended for women with HIV or AIDS, or those at high risk for acquiring HIV, due to concerns that spermicide use may increase the risk of HIV transmission, as discussed in ▶ Sect. 2.3.

29.3 Intrauterine Devices (IUDs)

The intrauterine device (IUD) is underutilized by women and adolescents in the United States. It has many of the characteristics that should make it highly appealing to adolescent women. It is easy to use, highly effective, allows privacy, does not require action at the time of intercourse, does not require partner cooperation, and does not require short-interval pharmacy or clinic visits (Table 29.2). Because of their ease of use, perfect-use and typical-use failure rates are nearly identical [19]. From 2002 to 2012, use of the IUD among young US women increased markedly, from 2.0% to 10.3% of current contraceptive users [29]. Among teens, 3% of contraceptive users utilized the IUD in 2012 [30].

There are two types of IUDs currently marketed in the United States (Table 29.1). The copper T 380A (TCu380A, Paragard®) is approved for use by the FDA for up to 10 years and contains no hormones. It has a typical-use failure rate of 0.8 per 100 women in the first year [19]. The other type of IUD available contains levonorgestrel and is referred to as a levonorgestrel-releasing intrauterine system (LNG-IUS). There are currently four products available, offering three different doses of LNG. The Mirena® IUS has been available the longest; it contains 52 mg of LNG and is approved for use by the FDA for up to 5 years, though its contraceptive efficacy has been shown to last for up to 7 years in clinical trials [31]. It has typical-use failure rate of 0.2 per 100 women in the first year [19]. These failure rates rival those of permanent surgical sterilization. The Liletta® IUS offers an identical dose of LNG and is currently

FDA approved for up to 3 years, though its contraceptive efficacy continues for at least 6 years. The Skyla® IUS is a slightly smaller device containing 13.5 mg of LNG. It is FDA approved for up to 3 years of use. Finally, the Kyleena® IUS was introduced in 2016. It contains 19.5 mg of LNG, is FDA approved for up to 5 years, and is the same size as the Skyla IUS.

The most common side effects of both types of IUDs are menstrual disturbances. For the TCu380A, menstrual cycles often become heavier and more painful. Irregular bleeding is less common but may occur during early use. However, for the LNG-IUS, users often experience irregular light bleeding for up to 6 months after insertion. After this resolves, most women report lighter cycles with an improvement in dysmenorrhea. The average decrease in menstrual blood loss with the 52 mg IUS is near 90%, with approximately 20% of users experiencing amenorrhea after 1 year of use [24]. The 52 mg LNG-IUS is FDA approved for treatment of heavy and painful menses. Amenorrhea is less common with the lower-dose IUS.

The preponderance of evidence supports the acceptability and safety of the IUD. Continued use of modern IUDs does not increase the risk of upper genital tract infection over a woman's baseline risk. However, there is an increased risk of upper genital tract infection in the first 20 days after insertion. This increased rate is likely secondary to insertion techniques or the presence of cervical infection at the time of insertion [32]. This finding warrants testing for cervical infection at the time of insertion, or soon beforehand, for all adolescent users due to their increased risk for STIs. In a prospective randomized trial, the 52 mg LNG-IUS protected against upper genital tract infection, compared to a copper IUD [33]. A case-control study of 1895 nulliparous women in Mexico provides the strongest evidence against a link between IUD use and subsequent infertility. The study compared cases with tubal infertility to controls with non-tubal infertility and to primigravid controls. Both comparisons showed no associations between tubal infertility and past use of a copper containing IUD [34].

In December 2007, the American College of Obstetricians and Gynecologists Committee on Adolescent Health Care issued a committee opinion encouraging healthcare providers to consider IUDs as a first-line contraceptive choice for both parous and nulliparous adolescent patients. The opinion states that "Intrauterine devices offer the long-term, cost-effective, highly reliable, and effective contraception needed by women, especially adolescents" [35]. A systematic review of IUDs for adolescents found reassuring results and also supported offering IUDs to adolescents as a first-line contraceptive option [36]. While the manufacturer's website recommends Mirena® "for women who have had a child," there is no evidence-based reason that nulliparous women and adolescents cannot use the Mirena®. There is no similar recommendation for the Liletta® IUS, which is identical in size and LNG dose.

29.4 Hormonal Methods of Contraception

29.4.1 Overview

Hormonal contraceptive methods are highly effective. In the past, there had been concern that use of hormonal contraception in adolescents might adversely impact the development of normal reproductive function and growth, but these fears have not been substantiated. After reviewing the literature in 1996, a consensus of 72 international experts concluded that healthy menstruating adolescents can take combined oral contraceptive therapy without any special assessment [37]. In fact, hormonal contraception may be particularly safe in adolescents because contraindications to oral contraceptive therapy, such as risk factors for cardiovascular disease, are rarely seen in the adolescent population.

Hormonal contraceptive agents can be delivered as subdermal implants, intramuscular injections, transdermal patches, contraceptive vaginal rings, and oral contraceptive pills (Table 29.1). Additionally, the levonorgestrel-releasing intrauterine system (LNG-IUS) is a hormonal method of contraception that acts via local release of a progestin (discussed earlier in ▶ Sect. 3). Oral contraceptive pills (OCPs) are taken daily and are available as progestin-only pills (POPs), also known as "mini-pills," or as combination oral contraceptives (COCs), containing both estrogen and a progestin. Other delivery methods for combined hormonal contraception (CHC) include the transdermal patch (Ortho Evra®, Xulane®) and the contraceptive vaginal ring (NuvaRing®),

which require weekly and monthly administration, respectively. Longer-acting progestin-only hormonal contraception is available as a 3-month depot intramuscular injection of medroxyprogesterone acetate (DMPA), sold under the brand name Depo-Provera® in the United States, and a subdermal implant that releases etonogestrel for a 3-year period (Nexplanon®).

Hormonal contraception prevents pregnancy via multiple mechanisms. At high circulating levels, progestins inhibit the mid-cycle surge of luteinizing hormone and block ovulation. Progestins inhibit sperm transport into the uterine cavity by producing a thick cervical mucus that is not receptive to sperm penetration [38]. Progestins also produce an atrophic endometrium in which embryo implantation would be unlikely [39]. In CHC methods, estrogens augment the progestational effects so that lower progestin doses are necessary. Estrogens prevent follicular development by inhibiting pituitary gonadotropin production and, in combination with progestins, reliably inhibit ovulation [40].

29.4.2 Hormonal Methods of Contraception: Efficacy

With perfect use, all methods of hormonal contraception are extremely effective with a failure rate of 0.05–0.3% in the first year of use [19]. In actual use, however, OCPs, transdermal patch, and vaginal ring are associated with a higher failure rates of 9% [19]. Adolescents are at high risk for inconsistent use and for discontinuation of OCPs. On average, adolescents miss up to 3 pills per cycle, and at least 20–30% of adolescents miss at least one pill each month. Unmarried African American adolescents have a failure rate as high as 18% [41]. In addition to inconsistent use, discontinuation of hormonal contraception among adolescent girls is common (Table 29.3). Within 6 months, up to 75% of DMPA and patch users and >50% of pill and ring users will discontinue method use [42]. The most common reason given for OCP discontinuation is the presence of side effects, including nausea, irregular bleeding, breast tenderness, and mood changes [41].

Proper counseling may be critical for compliance since discontinuation was found to be more likely in the first 2 months of CHC use, particularly if side effects were unexpected [43]. The more common side effects (bleeding irregularities, nausea, mood changes, breast engorgement, and headaches) should be reviewed with the patient, but it should be emphasized that breakthrough bleeding and nausea will usually resolve spontaneously with continued CHC use [44]. Since side effects decrease with continued use, patients with irregular bleeding and nausea should be encouraged to try CHC for three cycles prior to discontinuing.

29.4.3 Combined Hormonal Contraception (CHC)

Until 2002, the only method of CHC available to US women was the oral contraceptive pill (OCP). However, in 2001, the Food and Drug Administration approved two new delivery methods for estrogen/progestin combination birth control: a transdermal patch and a contraceptive vaginal ring. Both were first marketed in the United States in 2002. Both the monthly ring and the weekly patch provide longer-acting delivery systems and, therefore, do not require daily action. This may be especially important for adolescents, with their higher rate of inconsistent pill use. Despite these advances in delivery systems, adolescent women who use contraception continue to use the OCP more commonly than any other method. In 2012, 35% of teens used the OCP as their most effective method, whereas only 1% used the birth control ring or patch [8]. This is a decrease of 4% since 2007. In addition, these new delivery systems have not yet shown a benefit in terms of efficacy or continuation. A recent prospective study of high-risk adolescents showed higher pregnancy rates among those using the patch and ring than those using OCPs or DMPA [42].

29.4.3.1 CHC: Hormone Formulations and Dosing Regimens

CHC methods contain both estrogenic as well as progestational agents. The pills differ in their total estrogen content, in the progestational agent used, and in the ratio of estrogen to progestin. In monophasic preparations, the dose of estrogen and progestin is constant throughout the pill pack. In biphasic and triphasic preparations, the dose of the progestin or estrogen component varies during the cycle to minimize the total amount

of hormone required and to duplicate a normal menstrual cycle.

Ethinyl estradiol (EE), at doses of 10–35 mcg, is the estrogenic component of all low-dose combination OCPs available in the United States. The addition of an ethinyl group to estradiol makes the steroid orally active [45]. The metabolism of ethinyl estradiol varies between individuals so that the same dose can cause side effects in one woman and none in another [46, 47]. The contraceptive vaginal ring and transdermal patch also utilize ethinyl estradiol as their estrogenic components, released at 15 and 20 mcg per day, respectively.

There are several different progestins used in CHC methods [45]. The first-generation progestins were derived from testosterone and include norethindrone acetate, ethynodiol diacetate, and norethindrone. The addition of a methyl group to norethindrone increased its potency and created the second-generation progestins, norgestrel, and levonorgestrel (the biologically active optical isomer of norgestrel) [48]. The third-generation progestins, gestodene, desogestrel, and norgestimate are derived from levonorgestrel but have fewer androgenic effects. Gestodene containing products are not available in the United States. The active metabolite of desogestrel is 3-ketodesogestrel, also known as etonogestrel, which is the progestin used in both the contraceptive vaginal ring (NuvaRing®) and contraceptive implant (Nexplanon®) which are available in the United States. Additionally, the active metabolite of norgestimate is deacetylnorgestimate, also known as norelgestromin, which is the progestin used in the contraceptive patch.

Drospirenone is a newer progestin, which may have a pharmacologic profile more closely related to endogenous progesterone [49]. Drospirenone is an analog of spironolactone, the aldosterone antagonist and, like progesterone, has mild antimineralocorticoid activity [50]. It may result in fewer side effects resulting from fluid retention (e.g., breast tenderness and weight gain) by counteracting the estrogen-induced stimulation of the renin-angiotensin-aldosterone system [49]. In contrast to the other progestins, drospirenone has no androgenic activity and, in fact, appears to have mild antiandrogenic activity like spironolactone [49].

Combined hormonal contraception (pills, patch, or vaginal ring) was traditionally administered for 21 days, followed by a 7-day hormone-free interval to allow a withdrawal bleed to occur. Over time, however, the 21-day schedule and the necessity of a monthly withdrawal bleed have been questioned [51]. During the placebo week, some CHC users can experience hormonal withdrawal symptoms, such as pelvic pain, bleeding, and headache [52]. One strategy to reduce hormonal withdrawal symptoms is to decrease the hormone-free interval, by replacing the placebo week with ethinyl estradiol only or by shortening the placebo period to 4 days. Reducing the hormone-free interval to 4 days has also allowed reduction in total estrogen dose without sacrificing efficacy; there are a number of 24/4 regimens, utilizing 10–20 mcg of EE. Reducing the hormone-free interval to 4 days results in greater suppression of follicular development, with greater pituitary and ovarian suppression. Coupled with a shorter hormone-free interval, this allows pill users to take an overall lower daily dose of estrogen and experience fewer withdrawal side effects during the four hormone-free days.

Some COC preparations have placebo or ethinyl estradiol-only tablets 4 times per year after 84 hormone containing tablets, and some have eliminated the placebo week completely. In a study comparing an extended (84/7) regimen with a traditional (21/7) regimen, the number of unscheduled bleeding days was initially higher in the extended regimen group, but decreased over time with similar results at the end of a year [51]. Because irregular bleeding was more common in women who were initiating COC therapy, some recommend delaying extended regimens until a woman has used COCs for several cycles [53]. Although some women can take COCs continuously without placebo tablets, many experience breakthrough bleeding [54]. It has been suggested that women may individualize their COC regimen and begin a 4–7-day hormone-free interval when they begin to have breakthrough bleeding or at whatever interval is convenient.

29.4.3.2 CHC: Side Effects

Although the progestins are used at such low doses that the differences in their biologic effects should be negligible, different COCs may have slightly different side-effect profiles in a particular patient. The side-effect profile of a particular COC is due to differences in estrogen content, type of progestin, and ratio of progestin to estrogen. Side effects of COCs can be estrogenic, progestational,

Table 29.5 Relation of side effects to hormonal biological activity

Estrogen excess	Progestin excess	Androgen excess
Breast cystic changes Hypermenorrhea/menorrhagia Breast enlargement Mucorrhea Bloating Dizziness, syncope Edema Headache (cyclic) Irritability Leg cramps Nausea, vomiting Visual changes (cyclic) Weight gain (cyclic) Chloasma Chronic nasal pharyngitis Hay fever and allergic rhinitis Urinary tract infection Capillary fragility Cerebrovascular accident Telangiectasias Thromboembolic disease	Cervicitis Decreased withdrawal bleed Moniliasis Appetite increase Depression Fatigue Hypoglycemic symptoms Libido decrease Hypertension Leg vein dilation	Acne Cholestatic jaundice Hirsutism Libido increase Oily skin Rash and pruritis Edema
Estrogen deficiency	**Progestin deficiency**	**Androgen deficiency**
Absence of withdrawal bleed Irregular bleeding (days 1–9) Irregular bleeding (continuous) Pelvic relaxation symptoms Atrophic vaginitis Nervousness Vasomotor symptoms	Irregular bleeding (days 10–21) Delayed withdrawal bleed Hypermenorrhea/menorrhagia Bloating Dizziness, syncope Edema Headache (cyclic) Irritability Leg cramps Nausea, vomiting Visual changes (cyclic) Weight gain (cyclic)	Libido decrease

Adapted from Dickey [44]

and androgenic (Table 29.5). The hormone content of the COCs can differentially affect the endometrium as well [44].

The estrogenic effects of a particular CHC formulation is due to the dose of estrogen, the type of progestin used, the ratio of estrogen to progestin, and the delivery method. A small percentage of the first-generation progestins is metabolized to ethinyl estradiol and therefore may contribute to the estrogenic effect of the CHC [44]. Progestins, however, also reduce the biological activity of estrogen by acting as estrogen antagonists [44]. Consequently, for the same dose of ethinyl estradiol, a patient may experience symptoms of estrogen excess (breast engorgement, fluid retention, nausea, hypermenorrhea, chloasma) or estrogen deficiency (vaginal dryness, loss of hormonal withdrawal bleeding) depending on the dose of type of progestin used. If a patient experiences symptoms of estrogen excess or deficiency, a CHC preparation with different estrogenic activity can be tried. The contraceptive vaginal ring has been shown to have the lowest estrogen exposure, while the transdermal patch has the highest [55].

In tests of biological activity, the various progestins used in combination CHC can exert androgenic, anti-mineralocorticoid, as well as progestational effects. In combination with estrogen,

however, the specific differences between these progestins are less significant. For example, the number of progesterone receptors depends on the estrogen content [44]. CHC users, therefore, can experience symptoms of progesterone deficiency (breakthrough bleeding) or progesterone excess (decreased menstrual bleeding, increased appetite, depression) depending on the estrogen content and the estrogen to progesterone ratio.

With the exception of drospirenone, the synthetic progestins all have androgenic biological activity. These synthetic progestins can increase free androgen by depressing sex hormone binding globulin(SHBG) and also by displacing bound androgen from SHBG [44]. Nevertheless, all of the CHC methods exert an antiandrogenic effect. The estrogenic component increases SHBG to decrease free testosterone, and the progestational component suppresses the hypothalamic pituitary ovarian axis to decrease ovarian androgen production [56]. Some women may experience symptoms of androgen deficiency, such as decreased libido, due to these effects. In these women, a formulation with lower estrogenic and progestational activity may have a less suppressive effect on ovarian androgen production [44]. On the other hand, if a patient has symptoms of androgen excess, such as hirsutism or acne, a progestin with less androgenic activity may be worth trying. Norethindrone and desogestrel have a less depressive effect on SBG than norgestrel [44], and drospirenone appears to compete with androgen to exert an antiandrogenic effect [49].

Endometrial activity is measured by the ability of the method to prevent irregular bleeding while the active hormone pills are being taken [44]. Breakthrough bleeding results when the CHC cannot stimulate endometrial growth, which can result from relative estrogen deficiency, or when it cannot adequately support the endometrium due to progesterone deficiency. If breakthrough bleeding or amenorrhea persists after the third cycle, a CHC method with greater endometrial activity may be helpful. Irregular bleeding during the first nine pills is usually associated with estrogen deficiency, while bleeding after the 10th day is usually a result of a deficiency in the progestational activity [44]. When a patient presents with new irregular bleeding after many cycles of CHC use, other causes of bleeding per vagina, such as cervicitis, anatomic defects, or pregnancy, should be investigated.

It should also be emphasized to patients that there are many different formulations of CHC available today. If a particular side effect is still troubling after three cycles, a different preparation can be tried. The choice of a different preparation can be tailored to alleviate her particular symptoms.

29.4.4 Progestin-Only Contraception

Progestin-only contraception is useful for women who either cannot tolerate estrogenic side effects or have contraindications to estrogen use. These methods are available as progestin-only pills (POPs), as a 3-month depot intramuscular injection of medroxyprogesterone acetate (Depo-Provera®), or as a subdermal implant that releases etonogestrel for a 3-year period (Nexplanon®).

29.4.4.1 Progestin-Only Pills (POPs)

Only one type of progestin-only OCP formulation is available in the United States, norethindrone 0.35 mg, marketed under the trade names "Micronor" and "Nor-QD." Unlike COCs, they are packaged with 28 active tablets and are taken continuously. The progestin doses used in the POPs are lower than the doses used in COCs. At these low doses, ovulation is not reliably suppressed [57] and occurs in approximately half of oral progestin users [40]. Thus, the mechanism of action is the POP's effect on endometrium and cervical mucus [58]. With perfect use, the failure rate for POPs is 0.3% [19], but perfect use is difficult. Meticulous pill taking every 24 h appears critical for contraceptive efficacy, and a back-up contraceptive method is recommended if a pill is taken more than 3 h late [58]. For this reason, POPs are not usually used in the adolescent population.

29.4.4.2 Progestin-Only Injectable Contraception (Depo-Provera®)

Depot intramuscular injection of medroxyprogesterone acetate (DMPA) received FDA approval in 1992. DMPA use is more popular among adolescents than in any other age group. In 2012, 7% of 15–19-year-olds who currently used contraception reported using DMPA as their most effective method, in contrast to only 5.1% of 20–24-year-olds and 1% for women 35 and older

[59]; this is a slight decrease from 2007. It is given as an intramuscular injection of 150 mg DMPA in a crystalline suspension, every 12 weeks. After injection, crystalline deposits form in the tissue and are resorbed slowly [44]. As with other forms of hormonal contraception, the ideal time to initiate therapy is within the first 5 days of the menstrual cycle so that the contraceptive effect is immediate [44]. Administration at other times in the cycle is acceptable if pregnancy can be ruled out and the patient can use a back-up method of contraception for 7 days [60]. The dose of progestin is high enough to block the LH surge and ovulation [61]. Although ovulation does not occur for 14 weeks after injection, repeat injections are recommended every 12 weeks, and the recommendation is to rule out pregnancy in women who come after 13 weeks [61].

DMPA is one of the most effective contraceptive methods for adolescents, with a failure rate of 0.2% with perfect use [19]. With typical use, the failure rate after 12 months is 6% [19]. Increases in body weight and use of concomitant medications do not appear to reduce its efficacy. The return to fertility can be unpredictable after the last injection, which can be advantageous for the adolescent who is not meticulous about returning every 12 weeks. On the other hand, because the contraceptive effect is not immediately reversible, DMPA is not recommended for women who are planning pregnancies in the next 1–2 years [61].

The side effects of DMPA are related to estrogen deficiency or progestational excess [44]. DMPA reliably and consistently suppresses estrogen levels [62]. Because estrogen levels may reach the postmenopausal range [63], bone mineral density (BMD) may be affected in DMPA users. As for all progestin-only methods, the most prominent side effect of DMPA is irregular, unpredictable bleeding [64]. In a multicenter US study, 46.0% of women reported amenorrhea, and 46.2% of women reported irregular bleeding after 3 month of use [65]. With increased duration of use, menstrual bleeding decreased in frequency and duration, and in this multicenter study, 58.5% of users were amenorrheic after 9 months [65]. In another study, 73% of users were found to be amenorrheic after 12 months [66]. Many women, including adolescents, will view the amenorrhea as a benefit of DMPA therapy. It appears that pretreatment counseling may be the critical factor—if women are counseled about the menstrual changes prior to initiation of treatment, they are more likely to continue with subsequent injections [67].

An estimated 55% of adolescents will discontinue DMPA within 1 year of starting [68]. Irregular bleeding was the most common reason, cited by 60% of adolescents, for discontinuing DMPA. In addition, the following side effects were also cited by adolescents as a reason for discontinuing Depo-Provera®: weight gain (40%), increased headaches (26%), mood changes (20%), fatigue (20%), alopecia (20%), breast tenderness (14%), amenorrhea (14%), and acne (9%) [64]. The FDA package insert mentions depression as a side effect of DMPA [44], but published studies indicate that DMPA does not cause depression [61]. A large prospective study evaluated 393 women before and after 12 months of DMPA and found no increase in depressive symptoms, suggesting that use of DMPA would not exacerbate symptoms in women with preexisting depression [69]. The CDC MEC indicates that DMPA can be used without restriction in women with depressive disorders [14].

The most controversial topic regarding the use of DMPA by adolescents is its effect on bone mineral density (BMD). There have been many studies in adults that confirm that DMPA leads to at least a temporary loss of BMD and that it is more pronounced in the first 2 years of use [70–72]. In November of 2004, the US Food and Drug Administration (FDA) issued a black box warning, stating that this contraceptive is associated with significant loss of BMD, which may not be reversible and that use for more than 2 years should be limited. The FDA specifically targeted use by adolescents and young adults by warning that it is unknown whether use during this timeframe would adversely affect attainment of peak bone mass and increase the risk of osteoporotic fracture later in life [73].

The issue of decreased BMD is especially important in the teen population. Women gain 40–50% of their skeletal mass in adolescence, with the highest rate of accrual between 11 and 15 years. After the age of 18 years, total body skeletal mass increases only 10% and occurs in the following decade [74]. Peak bone mass is reached by the age of 16–22 years, and this measure is related to the risk of osteoporosis [75]. Studies of DMPA use in adolescents have shown overall similar results to those in adults: there is a statistically significant

loss in BMD in *current* users, and there is a trend toward regaining BMD after cessation of use [76–78]. Of note, no osteoporotic fractures have been seen in adolescents using DMPA. While it is true that the long-term effects of DMPA use on peak bone mass and on osteoporotic fractures later in life are unknown, the current data do not support denying this highly effective method of contraception to the adolescent population. The Society for Adolescent Medicine issued a position paper in 2006 as a response to the FDA warning, which stated that, in most adolescents, "the benefits of DMPA outweigh the potential risks." In addition, both the World Health Organization [79] and the American College of Obstetricians and Gynecologists [80] have supported the cautious use of DMPA in the adolescent population. The CDC MEC also indicate that the advantages of using DMPA for women under the age of 18 years exceed the risks [14]. None of the above-named organizations endorses routine bone mineral density testing in adolescents using DMPA, nor do they recommend restricted initiation or continuation of DMPA. Certainly, users of DMPA should be counseled about appropriate intake of calcium (1200 mg/day), vitamin D, and weight-bearing exercise, which have all been shown to have positive effect on bone density [81].

29.4.5 Progestin-Only Etonogestrel Implantable Contraception (Nexplanon®)

The etonogestrel implant is a single rod inserted into a woman's upper arm. It is the only implant currently being marketed in the United States. It was approved for use by the FDA in July of 2006 and initially marketed under the trade name Implanon®. A pharmacologically identical second-generation implant, radiopaque with a new inserter, was introduced in 2011 under the trade name Nexplanon®. This implant is highly effective at preventing pregnancy for up to 3 years; recent studies indicate it is equally effective for up to 5 years [31], with an overall failure rate of less than 0.05%. Because there is almost no opportunity for user error, the perfect-use and typical-use failure rates are nearly identical. Like DMPA, Nexplanon® is easy to use, is highly effective, allows privacy (although it can be palpated in the upper arm), does not require action at the time of intercourse, and does not require partner cooperation. In addition, it does not require regular visits to the clinic or pharmacy.

The number of teens using a long-acting reversible contraceptive method (implant or IUD) has increased from less than 1% in 2007 to greater than 4% in 2012; implant use accounts for less than one quarter of this total [8]. The most common reason for discontinuation of the etonogestrel implant is irregular vaginal bleeding. Although there is a tendency toward amenorrhea (14– 20% incidence) and infrequent bleeding, with an overall decrease in annual blood loss, the vaginal bleeding pattern with Nexplanon® is irregular and unpredictable. However, menstrual blood loss is not greater than that experienced with regular menses [82]. Additional side effects of the method are similar to those of other progestin-only methods and include acne (although most users experience decreased or no change in acne), headache, breast tenderness, and mood changes. As with DMPA, pre-use counseling about all side effects is essential. Insertion and removal are quick and well-tolerated [83, 84].

One potential advantage to use of Nexplanon® in the adolescent population is that it appears to have no significant effect on BMD. Although it is a progestin-only method as is DMPA, it does not result in suppressed estrogen levels. In a study of BMD among adult implant users compared to users of a nonhormonal IUD, there was no reduction in BMD in implant users [85].

29.4.6 Emergency Contraception

Emergency contraception (EC) represents the only form of postcoital contraception available to women and girls experiencing contraceptive failure (i.e., condom breakage) or nonuse. Although postcoital copper IUD insertion is the most efficacious method, with a pregnancy rate of <0.1% when placed within 5 days of unprotected sex [86], it requires a visit with a healthcare provider qualified to insert the IUD and access to the device. Oral methods, one of which has been available over the counter without a prescription to people of all ages since 2013, are used significantly more often than placement of a Copper IUD. Overall, emergency contraceptive methods have been used by 14% of teens between 2006 and 2010; this is a significant increase since 1995

[87]. Since EC became available over the counter in 2013, rates of use among teens have continued to climb. EC is both readily obtainable and safe in all adolescent populations and should be discussed and made available to teens, with some important caveats regarding timing, efficacy, and weight.

There are two dedicated emergency contraceptive products available in the United States: oral LNG and oral ulipristal acetate (Ella®, HRA Pharma, Paris, France), which is a selective progesterone receptor modulator. LNG products, including Plan B® OneStep® and its generic equivalents, contain 1.5 mg of LNG in a single dose and can be purchased off the pharmacy shelf or online without ID to any consumer regardless of age. Ella® requires a prescription. Both should be taken as soon as possible for maximum efficacy, within 72 h of unprotected intercourse. Both products work by suppressing the LH surge and thereby preventing ovulation; consequently they do not work if taken after the LH surge. Initial studies suggested that LNG emergency contraception had an efficacy rate of 95%, 85%, and 58% at 24, 48, and 72 h, respectively [88], but more recent data suggests that these numbers are optimistic, and actual efficacy rates are significantly lower. When compared with LNG EC, ulipristal acetate demonstrates 50% greater efficacy in preventing pregnancy in head-to-head trials [89].

Most importantly, it appears that the efficacy of LNG EC in women weighing >154 lbs. or a BMI > 36 is equivalent to placebo. UPA is efficacious up to 194 pounds or a BMI of 35, after which it also becomes ineffective at preventing pregnancy. Consequently, the greatest risk factor for failure of oral emergency contraception is weight, followed by intercourse at mid-cycle (at the time of or immediately following the LH surge) and then multiple acts of unprotected intercourse within the same cycle [89]. Adolescents should be counseled that while EC is more efficacious than using no contraception, it should not be relied upon regularly, especially among overweight and obese teens, for whom EC is no better than doing nothing at all. While EC use should not be discouraged, especially as a back-up for contraceptive failure, obtaining a history of EC use provides a good opportunity to counsel teens regarding more effective longer-acting methods of contraception.

29.5 Systemic Effects of Hormonal Contraception

Hormonal contraceptive methods have systemic metabolic effects. Studies performed in the 1960s and 1970s suggested that women using COCs were at increased risk for cardiovascular disease, but these data were derived from older pills, which contained higher steroid doses and different formulations compared with what is available today. Enovid, the first OCP released in the United States, contained 150 mcg of mestranol (equivalent to approximately 105 mcg of ethinyl estradiol [47], whereas today's low-dose combination oral contraceptive pill contains 10 mcg to 35 mcg of ethinyl estradiol. Reduction in the steroid content of COCs has been accompanied by a decrease in adverse metabolic effects, such as stroke, myocardial infarction, and deep vein thrombosis, as well as reductions in side effects, such as nausea and breast tenderness [45].

29.5.1 Cardiovascular Effects

With the decreasing estrogen content of COCs over the past years, their cardiovascular safety has improved dramatically [62, 90]. A review of the epidemiologic data found that in the absence of smoking, use of modern COCs, containing less than 50 mcg of ethinyl estradiol, is not associated with any meaningful increase in the risk of myocardial infarction or stroke—regardless of age [91]. The CDC MEC notes that the proven or theoretical risks of combination hormonal contraception usually outweigh the advantages in women 35 years of age and older who smoke [14], whereas healthy nonsmokers can continue to use CHC as long as they remain at risk for pregnancy [37]. In the adolescent population, cigarette smoking is not a contraindication for CHC use because they are at particularly low risk of cardiovascular disease. For women with hypertension, even young women with well-controlled disease, the CDC MEC warn that the risks outweigh the advantages of CHC use and that in severe hypertension (>160/>100) or if vascular disease is present, CHC represents an unacceptable health risk [14].

Atherosclerotic heart disease and coronary artery disease are associated with increased triglycerides, increased LDL cholesterol, and decreased HDL cholesterol [44, 92]. Hormonal

contraceptive agents have been shown to induce both favorable and unfavorable changes in the serum lipid profile [44, 92]. It is not clear, however, whether any of these changes in serum lipids increases the risk for cardiovascular disease, particularly in the adolescent. Women with multiple risk factors for atherosclerotic cardiovascular disease, i.e., hypertension, diabetes, and a poor lipid profile, should not use CHCs [14].

Many of the progestin-only methods have the same package labeling as for COCs despite the fact that the absence of the estrogen component in progestin-only contraceptive methods appears to eliminate or substantially reduce the cardiovascular risk. There are no restrictions for use of any of the progestin-only methods in smokers at any age [14]. Studies have not demonstrated significant changes in blood pressure with contraceptive methods containing only progestational agents [14] so that POPs, implants, and progestin-releasing IUD may be used without restriction in mild hypertension (up to 159/99), and advantages outweigh risks in more severe hypertension or when vascular disease is present. Although the advantages of DMPA use with mild hypertension outweigh the risks, DMPA's risks outweigh its advantages if there is more severe disease or vascular involvement [14].

29.5.2 Effects on Carbohydrate Metabolism

Insulin resistance increases the risk of diabetes, as well as atherosclerotic heart disease and coronary artery disease. Hormonal contraception has been associated with adverse changes in carbohydrate metabolism. COCs appear to impair glucose tolerance and elevate insulin levels [44, 92, 93]. Both estrogen and progestins influence carbohydrate metabolism, but since all types of COCs available in the United States contain the same estrogen component (ethinyl estradiol) at similar doses (10–35mcg), the differences in the carbohydrate metabolism between the various COC formulations have been attributed to the progestin component. In combination with estrogen, levonorgestrel appeared to have greater adverse effects on glucose tolerance and insulin resistance than those containing norethindrone or desogestrel. In contrast, progestin-only OCPs have minimal effects on carbohydrate metabolism [92].

Deterioration of glucose tolerance has also been demonstrated in healthy users of DMPA [94, 95]. In a large controlled study, users of nonhormonal contraception experienced little change in glucose or insulin levels, while DMPA users experienced a steady increase in glucose levels over 30 months along with rising serum insulin levels in the first 18 months of use [96]. Etonogestrel implants appear to induce mild insulin resistance without a significant change in serum glucose levels [97].

For women without diabetes, these alterations in carbohydrate metabolism do not appear to be clinically significant. Despite statistically significant increases in plasma glucose and insulin levels, glucose tolerance was not impaired in a study of 130 healthy women using triphasic COCs [98]. Similarly, glucose tolerance remained within normal limits for DMPA users after 12 months [94]. The most recent Cochrane review suggests that for women without diabetes, hormonal contraceptives have minimal effect on carbohydrate metabolism [99]. Even in previous gestational diabetics, the use of a low-dose COC is accompanied by a minimal risk of impaired glucose tolerance [100] and does not appear to influence their risk of developing diabetes [101]. CDC MEC do not consider a history of gestational diabetes to be a restriction for the use of any form of hormonal contraception [14].

Although there are theoretical concerns about prescribing hormonal contraception for women with insulin-requiring diabetes, the studies examining the use of hormonal contraception by women with well-controlled, uncomplicated diabetes have been reassuring [80, 102, 103]. Current data suggests that modern COCs have little effect on glycemic control or the insulin requirements of diabetic women [80, 102]. Furthermore, neither current, past, or duration of COC use was associated with current glycosylated hemoglobin or the development of diabetic sequelae, such as retinopathy, nephropathy, and hypertension [104, 105]. Similarly, levonorgestrel IUDs appear to have little effect on carbohydrate metabolism, even in women with diabetes. A randomized clinical trial comparing glucose metabolism in diabetic users of the copper T IUD with the levonorgestrel IUD found no differences in glycosylated hemoglobin, fasting serum glucose, or daily insulin requirements over the course of 12 months [106].

In contrast to COCs and the levonorgestrel IUD, another study of well-controlled diabetic

women found that 9 months after initiating treatment, users of DMPA had a statistically significant increase in their fasting blood glucose (102.7 to 112.9 mg/dl), while no change was found in the users of the copper T IUD [107]. The change in fasting blood glucose was thought to be clinical insignificant, however, since there was no associated change in their medication needs during this time.

Given that an unplanned pregnancy in a diabetic woman may be associated with poor glycemic control and poor pregnancy outcomes, CDC MEC indicate that the advantages of hormonal contraception generally outweigh the risks for both non-insulin and insulin-dependent diabetic women. However, for women with nephropathy, retinopathy, neuropathy, or other vascular disease, risks outweigh the advantages of use for CHC and DMPA, whereas advantages outweigh risks for POPs, the contraceptive implant, and the progestin-releasing IUD [14].

29.5.3 Risk of Venous Thromboembolism

An increased risk of venous thromboembolism (VTE), including pulmonary embolism, has been demonstrated in users of combined hormonal contraception (CHC). The increased risk appears to be related to estrogen, which increases hepatic production of coagulation factors in a dose-dependent manner [108, 109]. Accordingly, the risk of VTE has decreased along with decreases in the estrogen content of the COCs to less than 50 mcg ethinyl estradiol [110]. Nevertheless, even in users of modern CHC, the risk of VTE is increased approximately fourfold compared with nonusers [111]. It is important to remember, however, that the risk of VTE with CHC needs to be considered in the context of pregnancy. The absolute risk of VTE in users of CHC is very low (approximately 7 per 10,000 women-years) and is much lower than the risk of VTE associated with pregnancy (20 per 10,000 women-years) [111] or the postpartum period (40–65 per 10,000 woman-years [110].

Whether the progestin component or route of CHC administration also contributes to risk of VTE is controversial. There have not been large randomized controlled trials comparing the risk of VTE in various CHC preparations [110], and available studies have led to different results [111]. In the mid-1990s, several studies noted small increase in VTE among user of COCs containing the newer progestins (gestodene, desogestrel), and later, a similar phenomenon occurred with the even newer progestin (drospirenone) [110]. Others have questioned the results [112], citing biases, such as preferential prescribing, healthy user effect, and duration of use, in the original reports. While transdermal estradiol may confer a lower risk of VTE for postmenopausal women receiving hormonal therapy, this has not been definitively demonstrated for non-oral preparations of CHC [110].

It should be noted that risk of VTE with the use of CHCs periodically figures prominently in the media, especially when new data are released. Inaccurate and sensational stories propagated by traditional media, social media, and personal injury direct to consumer advertising can have a negative effect on CHC use among women and teens, who may stop using their contraceptive abruptly and without consulting with their physician. In 1995, three case-control studies appearing to demonstrate an increased risk of VTE among users of OCPs containing third-generation progestins were released in Europe. In advance of publication, the British Committee on Safety of Medicines released a warning regarding the increased risk of VTE, which was later demonstrated to be due to study bias. Hundreds of British women stopped taking their OCPs overnight, resulting in an increase in the unplanned pregnancy rate and an 8% increase in the abortion rate in less than 1 year [113]. The FDA chose to withhold judgment until the data could be better analyzed; consequently a similar rise was not seen in the United States. Physicians should be familiar with media reports of VTE risk and be prepared to answer questions and dispel fears about these methods or guide patients to choose another contraceptive method if compliance seems unlikely.

Some women may be at particularly increased risk for VTE with CHC. Smoking, age greater than 35 years, obesity, and family history of thrombosis are independent risk factors for VTE [110, 111]. Women with familial thrombophilia syndromes, such as factor V Leiden mutation, prothrombin G20210 A mutation, Protein C, Protein S or antithrombin deficiency, are at increased risk of VTE, and COCs have been shown to increase risk in these patients [110]. In particular, women heterozygous for the factor V Leiden mutation who

use COCs have a 30-fold higher risk of VTE compared with a six- to eightfold risk for carriers who do not use COCs [48].

The carrier frequency of the factor V Leiden mutation in Europe is higher than in the United States due to ethnic differences in the carrier rate, with Caucasian Americans having the highest carrier frequency of 5.27% [114]. Laboratory screening may be indicated to identify thrombophilic disorders in women with a family history of VTE or thrombophilic disorder, but routine screening is not currently recommended by the CDC since the absolute incidence of VTE is low [110].

Due to the increased risk of thromboembolism, however, CDC MEC recommend that individuals who are less than 3 weeks postpartum, have a personal history of VTE, or have a known thrombogenic mutation do not use CHC [14]. Because prolonged immobilization is also a risk factor for VTE, CDC MEC recommend that COCs should be discontinued when major surgery with prolonged immobilization is anticipated [14]. For all progestin-only methods of contraception, the CDC MEC gives no restrictions or states that the advantages generally outweigh the risks of method use for all conditions related to VTE, including acute DVT or PE [14].

29.5.4 Drug Interactions

Prior to initiating hormonal contraceptive therapy, a careful medication history should be obtained from patients, and patients should be counseled that the efficacy of hormonal contraception may be reduced by the concomitant use of certain medications [44]. Certain medications, through induction of liver enzymes, enhance the metabolism of estrogens and progestins. As a result, these medications may reduce the efficacy of hormonal contraception [80]. In particular, the CDC MEC indicate that the risks outweigh the benefits for combined hormonal methods (or oral progestin-only methods) with the use of certain anticonvulsants (phenytoin, carbamazepine, barbiturates, primidone, topiramate, oxcarbazepine, and lamotrigine) and antibiotics (rifampicin or rifabutin) [14]. The CDC MEC suggest that DMPA and the etonogestrel implant may be used with these medications.

The use of hormonal contraception may also modulate the effects of other medications. Because CHC may decrease drug level of fosamprenavir, the risk of using CHC may outweigh the benefits in users of this antiretroviral drug [14]. Steroids weakly inhibit hepatic drug oxidation but enhance glucuronosyltransferase activity [44]. For this reason, hormonal contraceptives may reduce the clearance (increase plasma levels) of certain medications and increase the clearance (lower plasma concentrations) of others. For example, increased serum levels of corticosteroids have been reported after high doses of estrogens [44] so that corticosteroid doses may need to be adjusted with COC use. Postmenopausal women with hypothyroidism have an increased need for thyroxine during estrogen therapy, presumably from increases in thyroid binding globulin [115]. It seems reasonable to believe that COC users may similarly experience a need for increased thyroxine doses. However, there are no restrictions to use of any method of contraception with goiter or with hyper- or hypothyroid disorder [116].

29.5.5 Reproductive Cancer

Fear of cancer is a major reason that women are reluctant to use hormonal contraception despite several reassuring cohort studies, which do not find an overall increased cancer risk in users of hormonal contraception [117]. To the contrary, several studies have even demonstrated a protective effect of hormonal contraception against some types of reproductive cancer [117]. The study of cancer risk and hormonal contraception is complicated by the fact that most patients develop cancer at an older age, many years after discontinuing contraception. Furthermore, most studies include only older COC preparations, which contained higher doses of hormones, so their relevance for current clinical practice, particularly with adolescents, may be limited.

The association between CHC and breast cancer has been studied extensively. A collaborative analysis, published in 1996, examined data from 53,297 women with breast cancer and 100,239 controls obtained in 54 studies conducted in 26 different countries [118, 119]. Regardless of duration of use, no increase in breast cancer risk was found in women who had not used COCs in the past 10 years. COC users were at a small increased risk of being diagnosed with breast cancer while they were using COCs and for 10 years

after discontinuation [118]. Similarly, results for DMPA have been reassuring. A large case-control study by the World Health Organization, published in 1991, provided reassurance that long-term users of DMPA were not at increased risk of breast cancer but did detect a slightly increased risk of breast cancer in the first 4 years of DMPA use, mainly in women less than 35 years old [120]. These results were interpreted to suggest that rather than causing new tumors, DMPA enhances growth of already existing tumors [61]. Compared to nonusers, cancers were diagnosed at an earlier stage in users—suggesting that the increased diagnosis of breast cancer may reflect a bias in cancer surveillance.

Subsequent studies of COC had similar findings, either finding no increase in breast cancer risk or if a risk was found, the effect disappeared after discontinuing [117]. More recent data from the Nurses Health Study, which followed 116,608 female nurses, aged 25–42, at time of enrollment, found marginally significant higher risk in current users of oral contraceptive pills with nearly all of the risk in users of triphasic preparations containing levonorgestrel [121].

Some have suggested, however, that the timing of exposure to hormonal contraception is an important consideration [122]. A large study suggested that women aged 20–34 years who had ever used OCPs had a slightly increased risk of being diagnosed with breast cancer compared to those who had never used OCPs [123]. Similarly, a 2006 meta-analysis of premenopausal breast cancer risk found a slightly increased risk of breast cancer in women who used COCs prior to their first full-term pregnancy [124]. There is concern that use of hormonal contraception prior to pregnancy or during adolescence when the breast is developing would make the individual particularly at risk for breast cancer.

An increased risk of cervical cancer has been demonstrated in long-term users of COCs, with decrease risk after discontinuation [122]. The major cause of cervical neoplasia is infection with particular types of human papilloma virus (HPV). A 2002 report by the International Agency for Research on Cancer pooled data from eight case-control studies and found among women infected with HPV risk of cervical cancer increased with duration of use. In women who had used COCs for more than 10 years, the risk of cervical cancer was four times higher [125]. Similarly, a pooled re-analysis of 24 studies found an increased risk of cervical cancer in current and long-term uses of COCs and a reduced risk after discontinuation [126]. A more recent prospective study also found positive associations between COC use and risk of cervical cancer and precancer, and similar to previous studies, the risk increased with duration of use and decreased with cessation of use [127]. A possible mechanism to explain the associations between OC use and cervical cancer risk is that estrogens and progestogens may interact with hormone receptors, mainly progesterone, present in cervical tissue and enhance the ability of HPV to induce carcinogenesis [127]. Because the overall risk of cervical cancer is very small, fear of cervical neoplasia should not be a deterrent to COC use, particularly with the availability of the HPV vaccine.

Strong epidemiologic data consistently demonstrate a protective effect of COCs against ovarian and endometrial cancers [117, 128, 129]. Furthermore, the benefits of COCs are enhanced with duration of use and persist years after OCPs are discontinued [37]. The Cancer and Steroid Hormone (CASH) Study demonstrated that the use of combination OCPs for as little as 3–6 months reduces the risk of ovarian cancer, and the protective effect persists 15 years after use ended [130]. Formulations with high progestin levels were associated with lower risk of ovarian cancer [131]. Even modern formulations, with ≤35ug EE, appear to be protective against ovarian cancer [122].

The CASH study also found combination OCPs to be similarly protective against endometrial cancer [132, 133]. Most studies found the protective effect against endometrial cancer persisted for up to 20 years after discontinuing COCs [117]. Daily exposure to progestins is thought to be the mechanism by which COCs protect the endometrium from endometrial cancer. Use of DMPA appears to reduce the risk of endometrial cancer at least as well as COCs [134] and may even reduce the risk further [135]. Similarly, levonorgestrel IUDs have a protective effect against endometrial cancer and have been used for the treatment of endometrial cancer [136], even in adolescents [137].

29.5.6 Weight Gain

It is widely believed that use of hormonal contraception may be associated with weight gain and may be a reason that adolescents are reluctant

to initiate hormonal contraception. Adolescents appear to be particularly concerned about maintenance of their body weight. In a survey of women ages 13–21, 50% reported that they would not accept a contraceptive method if it were associated with a 5 pound weight gain [138]. Adolescents cite fear of weight gain as a reason for discontinuing contraceptive methods [139]. In fact, 41% of adolescents listed weight gain as the primary reason for discontinuing contraception with long-acting progestin [64].

Theoretically, both the estrogenic and progestational components of CHC can contribute to weight gain. Progestins are thought to directly stimulate hypothalamic center to increase appetite [140] . Progestins have also been shown to increase insulin levels, which can be associated with symptoms of hypoglycemia and increased appetite [44]. Estrogen can result in increased subcutaneous fat deposition [44]. Additionally, fluid retention has been associated with both the estrogen and progestin component of COCs [44].

Despite the theoretical concerns about weight gain related to CHC use, the data does not support this concern. Several prospective studies have demonstrated that COC use was not associated with significant weight gain [141–144]. In another study, only 5% of women cited weight gain as a reason for discontinuing OCPs [145]. It appears that as many women lose weight as gain weight while taking COCs [44]. A 2014 review of randomized clinical trials found no association between CHC and weight gain [146].

Studies of progestin-only contraceptive users also do not show significant weight gain during use. A Cochrane review of the effects of progestin-only contraceptives on weight found little evidence for weight gain due to progestin-only contraceptives. In most studies, comparison groups using other birth control methods had similar weight gain (less than 2 kg) [147]. Similarly, in a large comparative study of progestin-only contraceptive users and Copper IUD users, participants using the etonogestrel implant had an average weight gain of 2.1 kg over 12 months, compared with DMPA users (2.2 kg), LNG-IUD users (1.0 kg) and Copper IUD users (0.2 kg). After adjusting for age and race, there were no statistically significant differences in weight change between users of progestin-only methods when compared to copper IUD users [148]. Interestingly, in this study, race was found to be an independent predictor of weight gain. Black race was associated with significant weight gain when compared to other racial groups [148].

In contrast to older studies, these more recent reports [147, 148] did not find significant weight increases in users of DMPA when compared to users of other contraceptive methods. Notably, FDA package labeling for DMPA states the method is associated with progressive weight gain, with an average weight gain of 5.4 pounds after the first year, 8.1 pounds after the second year, and 13.8 pounds after the fourth year. These weight changes were identified in a large US study involving 3857 women [149]. Later studies found that despite increases in weight by some women, many do lose weight while using DMPA. As many as 44–56% of DMPA users, including adolescents, were found to have lost or maintained their body weight in retrospective studies [150, 151]. Obese adolescents, however, may be at the highest risk for weight gain using DMPA. A prospective comparative study of 450 teens (aged 12–18 years) showed a 9.4 kg weight gain over 18 months for obese adolescents (BMI > 30) using DMPA, compared to a 0.2 kg weight gain for obese COC users, a 3.1 kg weight gain for obese controls not using any hormones, and a 4.0 kg weight gain for non-obese adolescents using DMPA [152].

29.5.7 Special Considerations for the Obese Adolescent

Obesity is an increasing problem among US adolescents. In 2011–2014, the prevalence of obesity among US girls aged 12–19 was 21%; this rate has increased steadily since the 1980s though it has plateaued over the last 3 years [153]. While the data regarding the effect of weight on adolescent sexual activity is mixed, overweight and obese adolescents are less likely to use contraception and less likely to use it consistently than their normal weight peers [154]. Adolescents also cite fear of weight gain as a reason for discontinuing contraceptive methods [139]; this may be of special concern to overweight and obese teens. There is also concern that hormonal contraception may be less effective in obese individuals. Thankfully, while there are few studies that explore contraceptive safety and efficacy in overweight/obese adults and virtually no studies of teens, there are few if

any restrictions against hormonal contraceptive use for obese teens who are otherwise healthy.

IUDs (both hormonal and nonhormonal) and etonogestrel implant provide excellent contraceptive efficacy regardless of BMI, demonstrating a pregnancy rate of less than one pregnancy per 1000 woman-years among all members of a cohort of nearly 6000 women, where >50% of participants were overweight or obese [155]. Further, IUDs are not associated with weight gain regardless of the weight of the user [148]. Levonorgestrel IUDs may be particularly useful in obese adolescents with abnormal uterine bleeding, significantly reducing menstrual bleeding overall and protecting against endometrial hyperplasia, which can occur with chronic anovulation associated with obesity [156].

For DMPA, there is no demonstrated difference in contraceptive efficacy between normal weight and obese users [157]. Adolescent users of DMPA, however, appear to gain more weight than adult users, and this may be particularly true for obese teens [152]. One study of adolescent DMPA users, aged 12–19, found that overweight teens had a mean weight gain of 13.6 lb, while the mean weight gain for the non-overweight users was 6.9 lb. [158]. Further, adolescents who gain >5% of their body weight in the first 6 months of use continue to demonstrate accelerated weight gain; this is particularly true among African American adolescents [159]. For an already obese adolescent, this may result in a further unhealthy increase in BMI with its attendant health risks, as well as compromised self-esteem and body image. DMPA may not be the first choice contraceptive method for an already overweight teen. Teens looking for a progestin-only method of contraception that decreases or eliminates menses may be better served by using an etonogestrel implant or a levonorgestrel IUD.

There is evidence that some methods of combined hormonal contraception may be less efficacious in obese women. One prospective cohort study of over 52,000 women assessing COC failure among normal weight and obese women demonstrated a slight but significant increase in failure rates as BMI increased: BMI greater than 35 was associated with a hazard ratio of 1.5 (1.3–1.8 95% confidence interval) for contraceptive failure [160]. The contraceptive patch was not initially studied in women weighing more than 90 kg. Subsequent analyses, though not powered to determine a difference in pregnancy rates by body weight, demonstrated that baseline body weight over 90 kg (198 lb) may be related to risk of failure: while the overall failure rate among all subjects was 0.8%, 33% of the failures were among women weighing >90 kg [161]. A 2017 systematic review also suggested, based on limited evidence, that increased weight and BMI may decrease effectiveness of the contraceptive patch, but found no direct evidence regarding the contraceptive ring [162].

Two systematic reviews, however, did not find an association between higher BMI or weight and effectiveness of hormonal contraceptives, but evidence was generally limited to fair and poor quality studies [162, 163]. There remains conflicting data regarding whether failure rates vary by specific CHC formulations or at highest BMI levels [162]. Many studies suggesting a decrease in CHC efficacy with increasing BMI were not designed to differentiate between failure based on pharmacokinetic effects vs compliance. Consequently, it is problematic to assume that obesity alone is responsible for the small decrease in efficacy. The CDC MEC consider the advantages of CHCs to outweigh the risks of obesity [14].

A particular concern regarding estrogen and obesity is an increase in the risk of VTE, which, while lower in adolescents than adults, is still double compared to normal weight peers. However, the overall risk of VTE is quite low and significantly less than the risk of VTE in pregnancy (60–100 per 100,000 vs 120–200 per 100,000) [164], and in an otherwise healthy obese adolescent, it is not a reason to withhold CHCs.

In prescribing CHC to overweight and obese adolescents, contraceptive patch should be avoided due to concerns of decreased efficacy. A pill with a shorter hormone-free interval (i.e., a 24/4 regimen as opposed to a 21/7) would be recommended, as pharmacokinetic studies have suggested an earlier return to follicular activity during 7 days as opposed to a 4-day or even shorter hormone-free interval among obese women. This carries the theoretical risk of increased contraceptive failure [165]. While head-to-head efficacy studies of 35 mcg vs 20 mcg EE pills among obese women have not been conducted, it is reasonable to assume that lower-dose pills may theoretically have decreased efficacy in obese women, as well as an increase in intermenstrual spotting, which women find bothersome and cite as a reason for

discontinuing OCPs. Consequently, monophasic oral contraceptives with at least 30 mcg of EE or a 24/4 regimen of lower-dose pills should be considered when caring for obese teens and adult women. Alternatively, the contraceptive ring provides a constant low dose of hormones with no demonstrated decrease in efficacy with increases in weight [166].

29.5.8 Screening and Follow-Up

Fear of complications should not be a deterrent to the use of hormonal contraception by patients and clinicians. Prior to prescribing hormonal contraception, candidates need to be screened to identify any contraindications. The screening, however, does not need to be overly burdensome. In 1996, an international consensus of 72 international experts concluded that the only two clinical assessments relevant to oral contraceptive use were blood pressure measurement and family and personal history, with particular attention to risk factors for thromboembolism and cardiovascular disease [37]. Because the adolescent population is at extremely low risk for cardiovascular disease, however, screening may be of limited utility.

The American College of Obstetricians and Gynecologists believe that oral contraceptives should be available over the counter since women should be able to self-screen for most contraindications using checklists [167]. Systematic review did not support benefit for clinical breast exam or pelvic exam prior to initiating CHC [168]. Similarly, systematic review found little support for laboratory screening of asymptomatic women prior to initiating contraception due to low prevalence of contraindications in reproductive age [169]. Permitting over-the-counter access to oral contraceptive methods without physical exam or laboratory screening would improve access to contraception and reduce rate of unintended pregnancy [169].

A follow-up visit may be beneficial, especially for adolescents who have high rates of incorrect use and discontinuation of contraceptive methods. An inability to attend a follow-up visit, however, should not preclude initiation of contraception. The follow-up should include an assessment of correct and consistent use of the method, with instruction and counseling about correct use. A patient may consider switching methods if the current method is not practical. This visit can also address minor side effects of hormonal contraception, with reassurance that most of these will subside with time. Because weight gain is common in reproductive age women, weight changes should not be immediately attributed to contraceptive therapy. Nevertheless, the potential for weight gain in the already obese adolescent patient starting DMPA is concerning, and new users may benefit from close attention to weight gain patterns.

29.6 Noncontraceptive Benefits of Hormonal Contraception

Aside from pregnancy prevention, there are numerous health benefits associated with the use of hormonal contraception. The protection from ovarian and endometrial cancer is well established and has already been discussed. In fact, compared with "never users," "ever users" of COCs were found to have a significantly lower rate of death due to all cancers, circulatory disease, or heart disease, resulting in a 12% lower mortality rate [170]. Hormonal contraceptive methods also offer protection from several benign conditions and are also used therapeutically [129].

Hormonal contraception has been shown to be beneficial for treatment of menstrual disorders, such as menorrhagia, dysmenorrhea, and both premenstrual syndrome (PMS) and premenstrual dysphoric disorder (PMDD) [15]. The symptoms of both PMDD and PMS were improved by COCs, containing 30 μg of ethinyl estradiol with drospirenone [171], and the NuvaRing was found to be effective for treating PMS [172]. All hormonal contraceptive methods, including the levonorgestrel IUD, will reduce the amount of menstrual blood flow and can be used to treat iron-deficiency anemia associated with menorrhagia [15, 44]. For heavy menstrual bleeding, a Cochrane review found the levonorgestrel IUD may be more effective than COCs [173].

In addition to increased iron stores [174], studies of users of COC [175], etonogestrel implant [82], and NuvaRing [172] have demonstrated improvement in dysmenorrhea. Hormonal contraception, including COCs, DMPA, etonogestrel implant, and the levonorgestrel IUD, has been shown to decrease the severity of dysmenorrhea and reduce the pelvic pain associated with

endometriosis [15, 129]. Hormonal contraceptive methods are used to treat endometriosis by producing an environment that promotes endometrial atrophy, and COCs have been a staple of medical management since before gonadotropin-releasing hormone agonists were available [44, 176].

In polycystic ovary syndrome, hyperandrogenism can be associated with anovulation, hirsutism, and acne. After serious illnesses, such as androgen-secreting tumor, Cushing's syndrome, and congenital adrenal hyperplasia are excluded, medical treatment can be initiated. COCs are the mainstay of medical therapy for hyperandrogenism [177, 178]. In combination, estrogens and progestins are antiandrogenic [15]. COCs decrease gonadotropin production and, as a result, suppress androgen production by the ovary. Estrogens increase the hepatic production of SHBG, decreasing the bioavailability of the androgens. COCs have been also been shown to decrease adrenal androgen secretion [179]. Additionally, COCs restore cyclic endometrial shedding and protect against the endometrial hyperplasia that is associated with chronic anovulation due to hyperandrogenism [56].

Patients with premature ovarian failure or insufficiency are at increased risk of developing cardiovascular disease and osteoporosis, and consequently, hormone replacement is recommended until the age of menopause [180]. Hormonal preparations for contraception, as well as hormone preparations designed for postmenopausal women, have been used for hormone replacement in this setting [181]. Younger patients may prefer contraceptive preparations due to familiarity and to be similar to peers [182]. It should also be noted that for unclear reasons, hormonal contraception may not be effective in suppressing ovulation in women with premature ovarian insufficiency [181, 182], and if pregnancy is not desired, another form of contraception will be necessary.

29.7 Conclusions

Women under 30 years of age experience higher rates of contraceptive failure and nonuse than their older counterparts [13] and consequently experience unplanned pregnancy at a much higher rate. While adolescent pregnancy rates are at historical lows in the United States, they are still higher than any other industrialized nation. This has a significant impact on the socioeconomic status and educational attainment of adolescent parents, particularly those who give birth to more than one child [183]. Consequently, providing effective contraception to adolescents is of particular importance.

Hormonal contraceptive methods are more effective than nonhormonal ones, with the exception of the copper IUD. Long-acting methods, in particular, have extremely low failure rates, presumably because these methods do not interrupt spontaneity and do not require daily patient responsibility. While overall efficacious and safe, each contraceptive method has its own set of limitations, and the limitations of hormonal contraception need to be considered. Hormonal contraceptive methods may have drug interactions that reduce contraceptive efficacy or alter require changes in medication doses. Disease processes, such as diabetes, may be altered by the use of hormonal contraception. Because hormonal contraceptive methods have systemic effects, untoward effects may occur in some individuals. The common side effects should be discussed prior to treatment since compliance has been demonstrated to improve with pretreatment counseling. Additionally, hormonal contraception does not offer any protection against STIs; a barrier method must also be recommended to individuals who are not in monogamous relationships.

Generally, the adolescent population is low risk, and these limitations should not be a deterrent to the use of hormonal contraception. In addition to providing reliable contraception, these hormonal methods also provide many noncontraceptive health benefits, including protection from certain cancer and benign medical conditions. Because these health benefits have not received as much publicity as the health risks, adolescents may not be aware that there are secondary benefits to hormonal contraception. Hormonal contraception is associated with decreased menstrual flow and decreased menstrual cramps. Although menstrual regulation is a lifestyle benefit, it has also been shown to improve iron-deficiency anemia.

In summary, hormonal contraceptive methods are particularly well-suited for adolescents since contraindications, such as risk factors for cardiovascular disease, are rarely seen in the

adolescent population. Hormonal contraceptive methods appear to be efficacious in this age group, providing reliable contraception with numerous noncontraceptive health benefits. Long-acting reversible contraceptives in particular may be well-suited to the adolescent population, as they are efficacious and well-tolerated and have the greatest continuation rates.

Review Questions

1. Combined oral contraceptives may be beneficial for which of the following disorders?
   A. Acne
   B. Menorrhagia
   C. Dysmenorrhea
   D. Endometriosis
   E. All of the above
2. Which contraceptive method would be LEAST likely to result in unintended pregnancy?
   A. Condoms
   B. IUD
   C. Combined oral contraceptive
   D. DMPA
   E. Contraceptive vaginal ring
3. Which would be the BEST contraceptive choice for an obese adolescent who is otherwise healthy?
   A. Contraceptive patch
   B. DMPA
   C. Combined hormonal oral contraceptive with 20 mcg of ethinyl estradiol and 7-day hormone-free interval
   D. LARC (IUD or etonogestrel implant)
   E. Barrier method (condom or diaphragm)
4. In adolescents, which of the following conditions would risks of combined oral contraceptive use outweigh the benefits?
   A. Cigarette smoking
   B. Uncomplicated, insulin-requiring diabetes
   C. Hypothyroidism
   D. Use of phenytoin
   E. 3 months postpartum
5. Which of the following is true about the risks of hormonal contraception?
   A. Progestin-only methods are contraindicated in smokers.
   B. The risk of venous thromboembolism with combined hormonal contraception is lower than the risk of venous thromboembolism associated with pregnancy.
   C. An increased risk of endometrial cancer has been demonstrated in users of combined oral contraception.
   D. Due to loss of bone mineral density seen with DMPA, DMPA users should have yearly measurements of bone mineral density.
   E. Significant weight gain has been demonstrated with hormonal contraception compared to users of IUDs.

Answers

1. (E) Hormonal contraception has been shown to be beneficial for treatment of menstrual disorders, such as menorrhagia, dysmenorrhea, and both premenstrual syndrome (PMS) and premenstrual dysphoric disorder (PMDD). Hormonal contraceptive methods have been used in management of dysfunctional uterine bleeding, dysmenorrhea, pubertal disorders, acne, endometriosis, hirsutism, and menstrual migraine.
2. (B) See Table 29.3.
3. (D) IUDs (both hormonal and nonhormonal) and etonogestrel implant provide excellent contraceptive efficacy regardless of BMI. Adolescent users of DMPA appear to gain more weight than adult users. For an already obese adolescent, this may result in a further unhealthy increase in BMI with its attendant health risks. DMPA may not be the first choice contraceptive method for an already overweight teen. Teens looking for a progestin-only method of contraception that decreases or eliminates menses may be better served by using an etonogestrel implant or a levonorgestrel IUD.

   In prescribing CHC to overweight and obese adolescents, contraceptive patch should be avoided due to concerns of decreased efficacy. A pill with a shorter hormone-free interval (i.e., a 24/4 regimen as opposed to a 21/7) would be recommended, as pharmacokinetic studies have suggested an earlier return to follicular activity during 7 days as opposed

to 4 days or even shorter hormone-free interval among obese women, which would theoretically increase risk of contraceptive failure. Barrier contraceptive methods are associated with high typical-use failure rates and would not be recommended.

4. (D) In the adolescent population, cigarette smoking is not a contraindication for CHC use because they are at particularly low risk of cardiovascular disease. Studies examining the use of hormonal contraception by women with well-controlled, uncomplicated diabetes have been reassuring. There are no restrictions to use of any method of contraception with goiter or with hyper- or hypothyroid disorder. The CDC MEC indicate that the risks outweigh the benefits for combined hormonal methods with use of certain anticonvulsants (phenytoin). Due to the increased risk of thromboembolism, the CDC MEC recommend that individuals who are less than 3 weeks postpartum do not use combined oral contraceptives.
5. (B) There are no restrictions for use of any of the progestin-only methods in smokers at any age. The absolute risk of VTE in users of CHC is very low and is much lower than the risk of VTE associated with pregnancy. Strong epidemiologic data consistently demonstrate a protective effect of COCs against ovarian and endometrial cancers. Routine bone mineral density testing has not been endorsed in adolescents using DMPA. Despite the theoretical concerns about weight gain related to CHC use, the data does not support this concern.

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Metabolic Disorders

Hypoglycemia

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Key Points

- Hypoglycemia disorders in children are rare but may have severe consequences if they are unrecognized or inappropriately managed.
- Evaluation for hypoglycemia disorders is recommended for:
  - Neonates with plasma glucose <60 mg/dL after the first 48 h of life
  - Infants and young children if plasma glucose concentrations are documented to be <60 mg/dL on laboratory quality assays
  - Older children and adolescent with documented Whipple's triad (symptoms/signs of hypoglycemia, a documented low plasma glucose, and relief of signs/symptoms when plasma glucose is restored to normal)
- The therapeutic plasma glucose goal for neonates, infants, and children with a hypoglycemia disorder is >70 mg/dL

30.1 Introduction and Background

Hypoglycemia is a medical emergency that may result in seizures, permanent brain damage, or even sudden death. Hypoglycemia can be the presenting sign of a large list of pathologies, and therefore it is necessary to have a comprehensive and systematic strategy for diagnosis and therapy. This chapter presents an approach to disorders of hypoglycemia based on the metabolic and endocrine systems involved in successful adaptation to fasting. This "fasting systems" approach takes advantage of the fact that almost all of the hypoglycemia problems in infants and children involve fasting. Since the integrity of these fasting systems is reflected in plasma levels of major fuels and counter-regulatory hormones at the time of hypoglycemia, the most important specimens for diagnosis are the ones obtained at the time of hypoglycemia, which is known as the "critical sample."

Within 1–2 h after birth, plasma glucose concentrations in newborns decrease to a nadir of 55–60 mg/dL and then steadily increase to reach a mean concentration of 80 mg/dL by 72 h of life [1]. Thus, the physiological plasma glucose range in neonates after the first 3 days of life is not different than for older children and adults (70–100 mg/dL). When plasma glucose decreases below this threshold, the glucose counter-regulatory systems are activated. A critical sample obtained when plasma glucose is <50 mg/dL reflects the metabolic response to hypoglycemia and allows the determination of the underlying cause of hypoglycemia.

30.2 Etiology

In infants and children, hypoglycemia, with few exceptions, is almost always fasting hypoglycemia. The physiology of normal successful fasting adaptation provides a useful framework that encompasses the diagnosis and treatment of potential hypoglycemia disorders [2].

Three metabolic systems regulate the physiologic response to fasting: (1) hepatic glycogenolysis, (2) hepatic gluconeogenesis, and (3) hepatic ketogenesis. These three metabolic systems are coordinated by the (4) endocrine system, which consists of suppression of insulin and production of counter-regulatory hormones that activate one or more of the three metabolic systems: glucagon (glycogenolysis), epinephrine (glycogenolysis, gluconeogenesis, ketogenesis, and suppression of insulin), cortisol (gluconeogenesis), and growth hormone (ketogenesis, via increased lipolysis) (Figs. 30.1 and 30.2).

The essential function of fasting adaptation is to maintain fuel supply to the brain, since the brain has no fuel stores of its own. As shown in

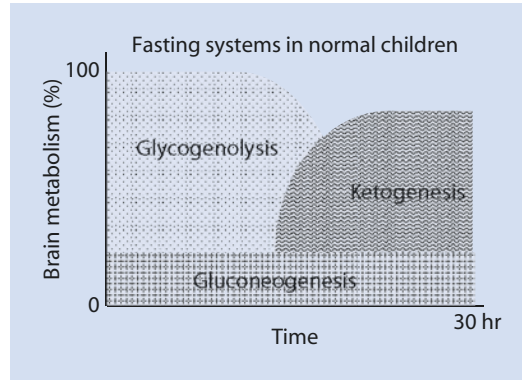

Fig. 30.1 Contribution of major fasting systems to brain metabolism over time in a typical normal infant. Note that glycogen stores are depleted by 8–12 h and that ketogenesis becomes the major fuel source for brain substrate by 24–36 h

Fig. 30.2 Hormonal regulation of fasting metabolic systems

Hormonal control of fasting systems

	Glycogenolysis	Gluconeo-genesis	Lipolysis	Ketogenesis
Insulin	–	–	–	–
Glucagon	+	+		
Epinephrine	+		+	+
Cortisol		+		
Growth hormone			+	

Fig. 30.3 Changes in plasma concentrations of major substrates during the course of fasting in a normal infant

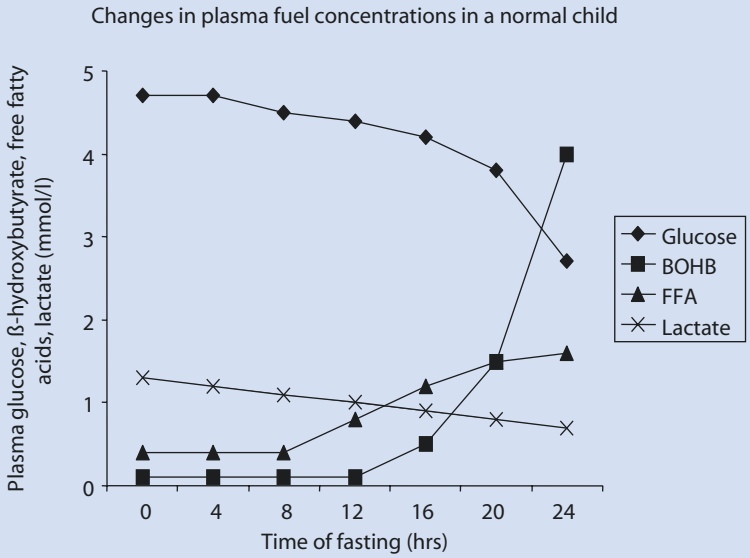

Fig. 30.1, early in fasting, glucose is the primary brain fuel and accounts for over 90% of total body oxygen consumption. Glucose is provided chiefly from hepatic glycogenolysis, supplemented by hepatic gluconeogenesis utilizing amino acids released by muscle protein turnover. After 12–16 h in normal infants (24–36 h in adults), glucose production declines, since the supply of liver glycogen is limited and the rate of gluconeogenesis from amino acids remains constant. At this time, a transition to fat as the major fuel for the body begins, with accelerated adipose tissue lipolysis and increased fatty acid oxidation in muscle and ketogenesis in liver. The brain cannot utilize fatty acids directly; therefore, ketones (β-hydroxybutyrate and acetoacetate) provide an alternative fat-derived fuel for the brain and permit a reduction of brain glucose consumption and the drain on essential muscle proteins. In late stages of fasting adaptation, fatty acid oxidation and ketone utilization account for 90% of total oxygen consumption.

The circulating levels of certain key fuels and hormones at the time of hypoglycemia reflect the integrity of these metabolic and hormonal systems of fasting. As shown in Fig. 30.3, in a normal infant fasted until hypoglycemia approaches at 24–30 h, i.e., at a plasma glucose of 50 mg/dL, (1) glycogen stores are exhausted (no glycemic response to glucagon) [3]; (2) gluconeogenic

substrate levels have declined modestly compared to the fed state (lactate <1.5 mM); (3) free fatty acids (FFA) have tripled (to 1.5–2.0 mM) and beta-hydroxybutyrate (BOHB), the major ketone, has risen 50–100-fold (to between 2 and 5 mM); and (4) insulin has declined to undetectable levels (<2 uU/mL). A comparison of these normal expected values to the values from a patient obtained at the "critical" time when fasting adaptation fails and the plasma glucose falls below 50 mg/dL provides the information "critical" to diagnosing the underlying cause.

30.3 Specific Disorders

30.3.1 Insulin-Mediated Hypoglycemia

30

Insulin is the most important regulatory hormone, and dysregulated insulin secretion with failure to suppress insulin during fasting is the most common cause of persistent hypoglycemia in infants and children. This can be due to a genetic defect affecting the various steps involved in fuel-stimulated insulin secretion, or in the high-risk neonate precipitated by perinatal stress (IUGR, infants of mothers with preeclampsia, etc.), or an insulin-producing tumor in older children and adolescents; but the possibility of exogenous insulin administration or hypoglycemic agents should also be considered.

30.3.1.1 Congenital Monogenic Hyperinsulinism (HI) [4, 5]

Caused by dysregulated insulin secretion of the pancreatic beta cells, HI presents with severe hypoglycemia, very short fasting tolerance, and a high GIR requirement (>10 mg/kg/min), although more mild forms exist. Diagnosis is based on evidence of excessive insulin effects on the metabolic systems, as insulin levels may not be elevated even at the time of hypoglycemia. Laboratory results consistent with the diagnosis are suppressed BOHB and FFA and a positive glycemic response to glucagon (>30 mg/dL rise in glucose) [3]. Mutations in ten genes are known to cause HI (◘ Fig. 30.4). The most common genetic forms are discussed in more detail below.

Recessive loss-of-function mutations of K_{ATP}-channel genes (*ABCC8*, encoding SUR1, sulfonylurea receptor; *KCNJ11*, encoding Kir6.2, potassium ion pore) [6, 7]. K_{ATP}-HI features include severe neonatal onset, LGA birthweight, protein-induced hypoglycemia, diazoxide unresponsiveness, and very high glucose requirement (up to 20–30 mg/kg/min).

Dominant loss-of-function mutations of ABCC8 and KCNJ11 [8]. Unlike the severe disease often associated with recessive mutations of SUR1, hypoglycemia in these cases is milder and typically responsive to treatment with diazoxide, although some mutations do not respond. Dominant *ABCC8* and *KCNJ11* mutations are also associated with protein-induced hypoglycemia [9].

Focal hyperinsulinism [10, 11]. This is a consequence of focal loss of heterozygosity for maternal 11p and expression of a paternally transmitted recessive K_{ATP}-channel mutation (either *ABCC8* or *KCNJ11*). The phenotype is very similar to recessive diffuse K_{ATP}-channel HI, and focal HI accounts for 40–60% of cases of severe diazoxide-unresponsive HI [12]. Preoperative localization of the lesion by 18F-fluoro-L-DOPA PET scan and resection of the lesion can result in cure of the hyperinsulinism [13, 14].

Dominant gain-of-function mutations of glutamate dehydrogenase (*GLUD1*): hyperinsulinism/hyperammonemia (HI/HA) syndrome [15–17]. Children with HI/HA typically present within the first year of life but may not be diagnosed in the neonatal period as they have normal weight at birth and can fast longer than neonates with K_{ATP}-HI. HI/HA is characterized by fasting and protein-induced hypoglycemia which is diazoxide-responsive and a persistent mild hyperammonemia (plasma ammonium, 50–200 micromols/L). They also have increased rates of seizures (typically absence) and learning disabilities, which are unrelated to the hypoglycemia [18].

Dominant gain-of-function mutations of glucokinase (*GCK*) [19]. Gain-of-function mutations in *GCK* result in a lower glucose threshold for insulin release. Children with GCK-HI have hypoglycemia of variable degrees of severity and are not usually diazoxide-responsive [20].

Recessive loss-of-function mutations of HADH (encoding SCHAD, the mitochondrial enzyme short-chain 3-hydroxyaxyl-CoA dehydrogenase) [20, 21]. In addition to its function in fatty acid oxidation, in the pancreatic beta cell, SCHAD functions as a negative regulator of glutamate dehydrogenase.

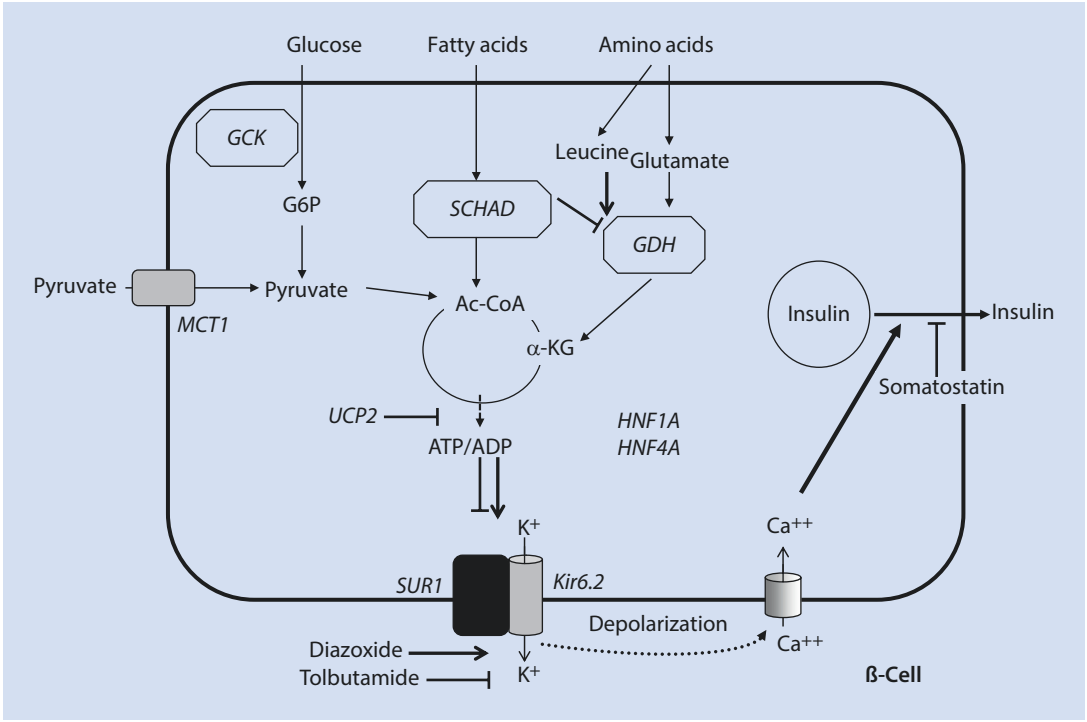

Fig. 30.4 Pathways of pancreatic beta-cell insulin secretion. Glucose and amino acids stimulate insulin secretion via an increase in ATP/ADP ratio which leads to inhibition of plasma membrane ATP-dependent potassium channels, membrane depolarization, and activation of voltage-gated calcium channels with the subsequent influx of calcium triggering insulin exocytosis. Note that leucine stimulates insulin secretion by allosteric activation of glutamate oxidation via glutamate dehydrogenase. Drugs may stimulate or inhibit insulin secretion by activation or inhibition of the plasma membrane ATP-sensitive potassium channel (e.g., diazoxide or tolbutamide) or by downstream inhibition of insulin release (e.g., octreotide). The known gene defects associated with hyperinsulinism are shown in italics. Abbreviations: GCK glucokinase, G6P glucose-6-phosphate, MCT1 monocarboxylate transporter 1, UCP2 uncoupling protein 2, SCHAD short-chain 3-OH acyl-coA dehydrogenase, Ac-CoA acetyl CoA, α-KG alpha-ketoglutarate, SUR1 sulfonylurea receptor 1, Kir6.2 potassium channel, GDH glutamate dehydrogenase, HNF1A hepatocyte nuclear factor 1A, HNF4A hepatocyte nuclear factor 4A

Thus, similar to HI/HA, SCHAD-HI presents with fasting and protein-induced hypoglycemia, which is responsive to diazoxide therapy, but without the hyperammonemia. The biochemical hallmark, in addition to markers of increased insulin action, is increased levels of 3-hydroxybutyryl-carnitine in plasma and increased levels of 3-hydroxyglutarate in urine.

Dominant loss-of-function mutations of hepatocyte nuclear factor 4 (HNF4A) and hepatocyte nuclear factor 1 (*HNF1A*) [22–24]. These transcription factors are well known causes of familial monogenic diabetes (MODY3 and MODY1, respectively). More recently, it has been recognized that the phenotype in individuals with these mutations is biphasic with neonatal hypoglycemia due to hyperinsulinism and diabetes later in life. The hyperinsulinism phase can last from a few weeks to up to 8 years. Children with these forms of HI respond well to diazoxide.

Dominant mutations of monocarboxylate transporter 1 (MCT1, encoded by *SLC16A1*) [25, 26]. Mutations in the promoter region of *SLC16A*1 result in aberrant expression of MCT1 in the beta cells and cause a rare form of HI, which is triggered by exercise. Characterized by episodes of hypoglycemia at the time of anaerobic exercise, MCT1-HI has been primarily identified in the Finnish population. The degree of hypoglycemia in response to exercise is variable.

Dominant mutations of uncoupling protein 2 (*UCP2*) [27]. Loss-of-function mutations in *UCP2*, which encodes an inner mitochondrial membrane carrier, result in a diazoxide-responsive form of HI.

30.3.1.2 Transient Neonatal Hyperinsulinism

Infant of diabetic mother. Maternal hyperglycemia triggers insulin hypersecretion by the fetal pancreatic beta cells, resulting in larger birth weight and hypoglycemia due to hyperinsulinism that typically resolves in 2–3 days.

Perinatal stress-induced hyperinsulinism [28, 29]. It is associated with SGA birth weight, birth asphyxia, and maternal toxemia. The mechanism is unknown. It can be clinically indistinguishable from the monogenic forms with high glucose requirements to maintain euglycemia (up to 20–30 mg/kg/min) and is diazoxide-responsive. The age of resolution is typically a few weeks to up to 2–3 months.

30.3.1.3 Syndromic Hyperinsulinism

Hypoglycemia due to HI can be the presenting symptom in children with Beckwith-Wiedemann syndrome (BWS), an overgrowth syndrome due to genetic or epigenetic abnormalities in the imprinted region of chromosome 11p. The severity of the HI in BWS is variable; infants with paternal UPD11p-associated HI have a persistent and severe HI phenotype compared to transient hypoglycemia of BWS patients caused by other etiologies [30]. Other syndromes associated with HI include Turner syndrome, Kabuki syndrome and congenital disorders of glycosylation (CDG).

30.3.1.4 Insulinoma

These are typically benign islet cell tumors, which are rare in the pediatric population. After diagnostic testing confirms evidence of insulin excess, imaging should be performed to localize the lesion. Multiple modalities (MRI, endoscopic ultrasound) may be necessary given the difficulty in localization. Resection is curative. Genetic testing for multiple endocrine neoplasia type 1 (MEN1) should be done in any child with an insulinoma.

30.3.1.5 Munchausen by Proxy

Induced hypoglycemia by administration of either insulin or hypoglycemic agents should be considered in the differential of insulin-mediated hypoglycemia. The clinical presentation can be undistinguishable from endogenous forms of hyperinsulinism; however, the abrupt onset of hypoglycemia of unpredictable pattern in an otherwise healthy child may trigger further investigation.

30.3.2 Hormone Deficiencies (Isolated Cortisol and Growth Hormone Deficiencies) and Panhypopituitarism

Hypoglycemia from growth hormone deficiency occurs primarily during infancy and presents as ketotic hypoglycemia. However, in the neonate, features may mimic hyperinsulinism, including high glucose requirement, low FFA and BOHB and a glycemic response to glucagon. This diagnosis should be suspected in neonates with the phenotype of HI but who also have midline malformations, microphthalmia, micropenis or cholestatic liver disease. Low growth hormone and cortisol levels obtained at the time of hypoglycemia are not diagnostic and prior to starting hormone replacement, appropriate stimulation tests should be performed to confirm the diagnosis [31], in addition to a brain MRI. The hypoglycemia resolves with replacement of the missing hormones.

30.3.3 Disorders of Gluconeogenesis

The most common disorder of gluconeogenesis is glucose-6-phosphatase deficiency (type Ia and type Ib glycogen storage disease) [32] (◘ Fig. 30.5). Infants present after feedings are spaced out longer than 3–4 h with failure to thrive, protuberant abdomen, and hepatomegaly. Shunting of glucose-6-phosphate to alternative pathways leads to markedly elevated blood lactate, triglycerides, and uric acid. Administering glucagon after a feed results in increased lactate levels with no change in plasma glucose. Diagnosis is straightforward based on these characteristic biochemical abnormalities and can be confirmed by genetic testing. GSDIb patients also have cyclic neutropenia. Fasting beyond 3–4 h in infants or 4–6 h in older children or adults results in a life-threatening metabolic acidosis.

30.3.4 Glycogen Storage Disorders (GSD)

Glycogen storage disorders include glycogen synthase (0), debrancher (III), liver phosphorylase (VI), or phosphorylase kinase deficiencies (IX)

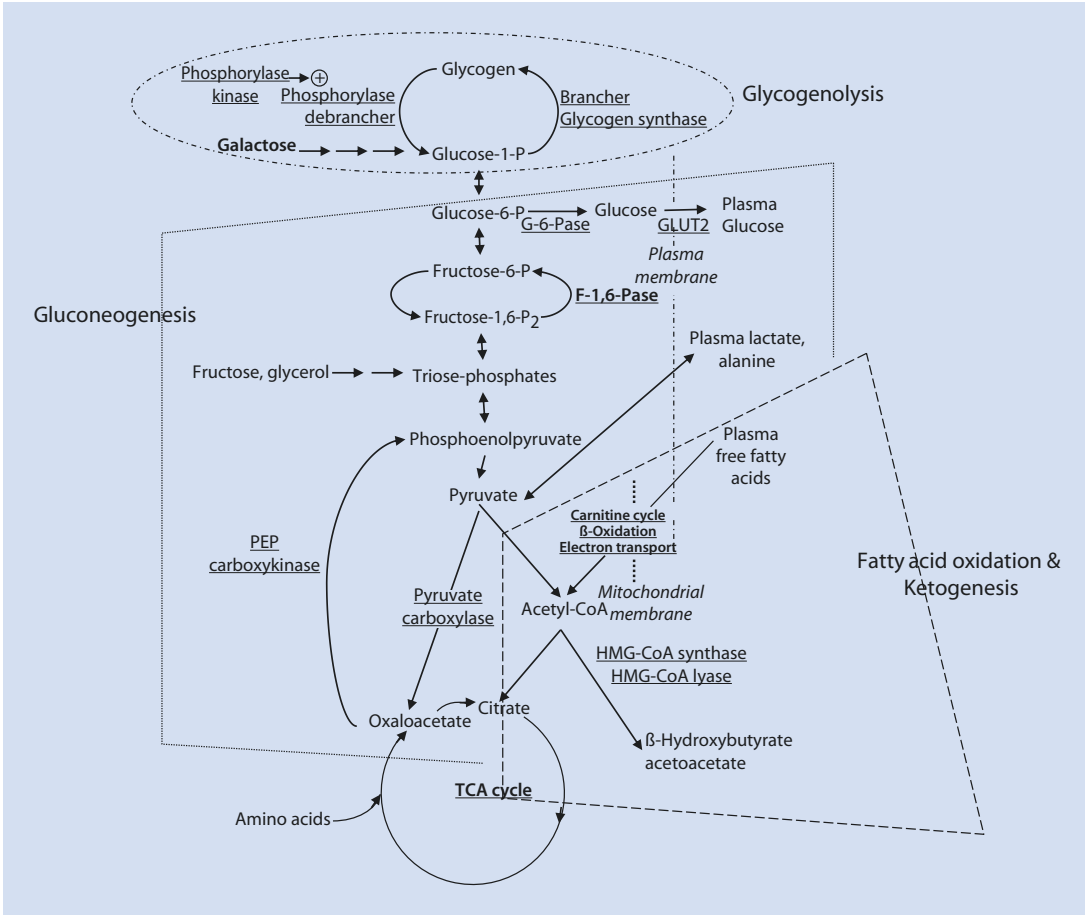

Fig. 30.5 Metabolic pathways of fasting adaptation. Sites of genetic defects are underlined

(Fig. 30.5). The hepatic GSDs are a heterogeneous group of disorders, which have varying severity of fasting hypoglycemia and hepatomegaly. GSD type III has the most severe presentation with failure to thrive, impressive hepatomegaly, hypertriglyceridemia, elevated liver transaminases, and cardiomyopathy. In contrast, GSD types VI and IX present with hypoglycemia following overnight fasting, milder hepatomegaly and growth retardation, and minimal metabolic and hepatic abnormalities. Liver biopsy as the gold standard for diagnosis has been replaced by genetic mutation testing.

30.3.5 Fatty Acid Oxidation Defects

The most common defect is due to medium-chain acyl-CoA dehydrogenase deficiency (MCAD) (Fig. 30.6) [33, 34]. These infants present with acute life-threatening episodes of illness, which are provoked by fasting stress beyond 8–14 h. Hypoketotic hypoglycemia, often with elevated liver transaminases, uric acid, or ammonia, but nearly normal levels of bicarbonate, is typical. The presentation mimics Reye syndrome [35]. Cardiac and skeletal muscle involvement occurs in the more complete defects. Most (but not all) can be diagnosed from plasma acyl-carnitine profiles by tandem mass spectrometry [36].

30.3.6 Ketotic Hypoglycemia

These are children, usually 1–4 years of age, with episodes of symptomatic fasting hypoglycemia, but do not have any identifiable metabolic or endocrine defect. In most instances, this can be thought of as merely a quantitative rather than a specific, qualitative abnormality of fasting

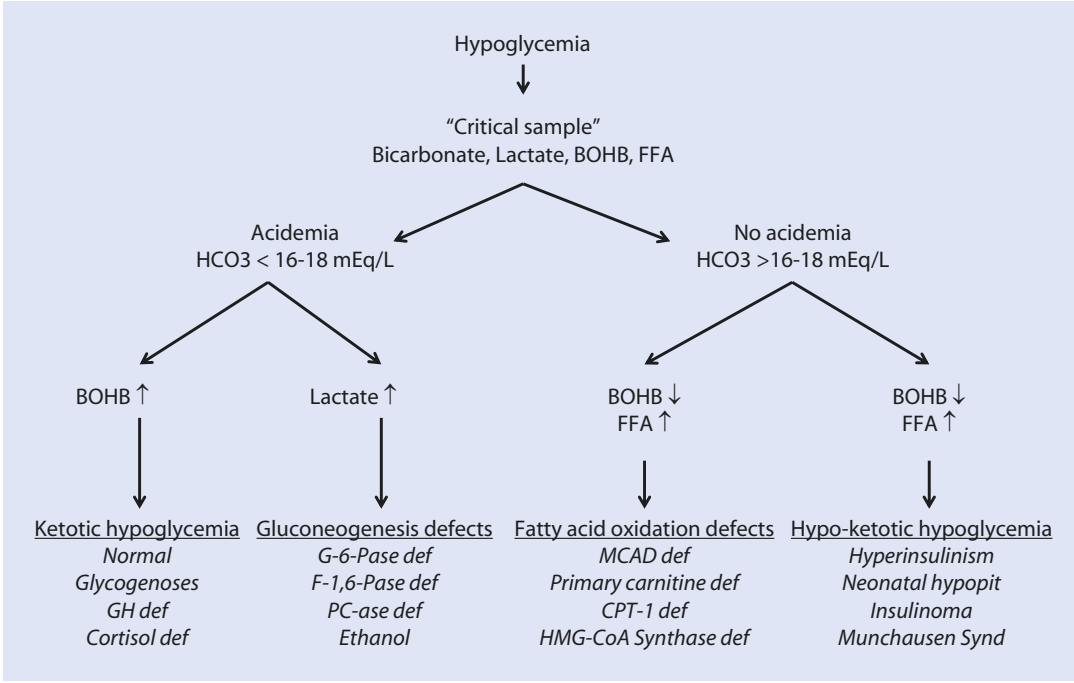

Fig. 30.6 Algorithm for diagnosis of hypoglycemia based on specimens obtained at time of fasting hypoglycemia

adaptation. These children may simply represent the lower end of the normal distribution of fasting tolerance. Note, however, that the features of abbreviated but otherwise normal fasting response are shared by the milder glycogenoses. Some cases of "ketotic hypoglycemia" have been found to have a mild GSD or inactivating mutations of monocarboxylate transporter 1 (MCT1) [37, 38].

30.3.7 Post-Fundoplication Hyperinsulinemic Hypoglycemia

This may occur in infants following fundoplication surgery for gastroesophageal reflux and may be severe enough to cause seizures and brain damage [39]. The mechanism involves rapid emptying of a meal into the small intestine, with early hyperglycemia followed by an exaggerated insulin surge and subsequent hypoglycemia, usually 1–2 h after the feed or meal. Increased secretion of the potent insulinotropic hormone, glucagon-like peptide-1 (GLP-1), by the small bowel after a meal may, at least in part, be responsible for the postprandial hyperinsulinemia [40]. The diagnosis is made by demonstration of the typical plasma glucose and insulin patterns in response to a mixed meal or formula tolerance test. Treatment includes feeds of longer duration, reduced high glycemic index foods, and inhibitors of gastric motility; the alpha glucosidase inhibitor, acarbose, may be useful as a means to delay digestion and absorption of complex carbohydrates [41].

30.4 Clinical Presentation

Older children and adults manifest Whipple's classic triad: neurogenic and neuroglycopenic symptoms/signs of hypoglycemia, a confirmed low plasma glucose value, and resolution of the symptoms/signs when the glucose level is restored to normal. However, neonates, infants, and younger children may not reliably demonstrate and/or lack the ability to communicate such symptoms. Hypoglycemic symptoms may be subtle and difficult to recognize in these age groups and include poor feeding, sweating, somnolence, apnea, and irritability. Myoclonic jerks and seizures may occur with severe hypoglycemia.

Hypoglycemia: the critical sample

	Hours	Lactate	BOHB	FFA	Response to glucagon
GSD I	2–4	↑	±↑	↑	–
GSD III	4–8	↓	↑	↑	–
F-1,6-pase	8–12	↑	↓	↑	–
MCAD	12–16	N	↓	↑	–
Hyperinsulinism	0–?	N	↓	↓	↑
Hypopituitarism	12–16	N	±↑	±↑	–

Fig. 30.7 Differential diagnosis of hypoglycemia disorders based on the "critical sample" obtained at a time of fasting hypoglycemia

30.5 Diagnostic Evaluation

Guidelines published by the Pediatric Endocrine Society detail which infants and children require evaluation for hypoglycemia disorders [42]. High-risk neonates (LGA, SGA, perinatal stress, maternal diabetes, those with congenital syndromes and family history of genetic hypoglycemia disorders) with plasma glucoses <60 mg/dL after the first 48 h of life should undergo additional evaluation prior to discharge. Evaluation should also occur in infants and young children if plasma glucose concentrations are documented to be <60 mg/dL on laboratory quality assays and in older children only if Whipple's triad is documented.

As outlined above, the integrity of the fasting systems can be evaluated by a critical sample obtained during a formal fasting test or during a spontaneous episode of hypoglycemia to establish the underlying cause of hypoglycemia (Fig. 30.7).

30.5.1 Formal Fasting Test Protocol

The success of the fasting test requires an experienced team of nurses and physicians, a blood-drawing IV, and rapid and accurate plasma glucose monitoring (N.B. Standard bedside glucose meters are not accurate enough). The fast usually begins with the 8 p.m. bedtime snack but may be adjusted later if very short fasting tolerance is suspected (consider monitoring for 24 h on usual diet before starting the fasting test to assess glucose stability). From the beginning of the fast, monitor plasma glucose and BOHB closely (e.g., every 3 h until <70 mg/dL, every 1 h until <60 mg/dL, then every 30 min to end). End the test at plasma glucose <50 mg/dL or when bedside measurement of BOHB indicates values >2.5 mM (or large urinary ketones in 2 subsequent occasions) or a specific duration has been reached (18 h if <1 month; 24 h if 1–12 months; 36 h if >1 year; 48–72 h for adolescents) or in case of any worrisome symptoms. At the end of the fast, obtain "critical sample" labs, which should include the basic metabolic panel, lactate, ammonia, insulin, c-peptide, BOHB, FFA, GH, cortisol, plasma acyl-carnitine profile, plasma total and free carnitine, and urinary organic acid profile. If considering hyperinsulinism (low BOHB during the fast), perform the glucagon stimulation test, 1 mg IV, to test liver glycogen reserve (at plasma glucose <50 mg/dL, appropriate glycemic response is <30 mg/dL within 15–30 min after glucagon administration; glycemic response above 30 mg/dL is consistent with hyperinsulinism) [3]. *Caution*: fasting tests are potentially hazardous provocative tests that, like the water deprivation test, must be closely monitored for patient safety. Sudden deaths during fasting tests have been reported in patients with fatty acid oxidation defects. Patients with fatty acid oxidation defects may develop life-threatening symptoms before plasma glucose levels fall below 60–65 mg/dL, including progressive lethargy, nausea, vomiting, or unexplained tachycardia; fasts should be terminated in these cases without waiting for plasma glucose to reach 50 mg/dL.

The serum bicarbonate from the critical sample allows segregation of the hypoglycemia disorders into two groups (Fig. 30.6), with and without acidemia (HCO3 < 16–18 vs. >16–18 mEq/L). The groups are then further divided based on the presence or absence of ketones and lactate:

1. Acidemia due to lactate accumulation typifies defects in hepatic gluconeogenesis: glucose-6-phosphatase deficiency (GSD type I), GLUT2 deficiency, fructose-1,6-diphosphatase deficiency, hereditary fructose intolerance, and ethanol ingestion.
2. Acidemia due to ketone accumulation typifies normal children, ketotic hypoglycemia (likely normal, but with shortened fasting tolerance), defects in glycogenolysis (GSD types 0, III, VI, IX), growth hormone and/or cortisol deficiencies, and ketone utilization defects.
3. No acidemia with ketones and free fatty acids both suppressed: congenital hyperinsulinism, prolonged neonatal hypoglycemia or "perinatal stress-induced" hyperinsulinism," insulinoma, exogenous administration of insulin or oral hypoglycemics, and neonatal hypopituitarism.
4. No acidemia with suppressed ketones but elevated free fatty acids: genetic defects in fatty acid oxidation and ketogenesis.

30.5.2 Other Tests

30.5.2.1 Plasma Acyl-Carnitine Profile

This test using tandem mass spectrometry measures the different fatty acids bound to carnitine to detect many (but not all) of the genetic defects in fatty acid oxidation. The method is now employed in most newborn screening programs using filter paper blood spots to screen for 20 or more inborn errors of metabolism. Medium-chain acyl-CoA dehydrogenase (MCAD) deficiency is particularly common (1/5000) and easily detected by this method [36].

30.5.2.2 Genetic Testing

Genetic testing for the most common forms of hyperinsulinism is available in commercial laboratories and should be obtained as soon as the diagnosis is confirmed. This is particularly important for infants with diazoxide-unresponsive hyperinsulinism as >90% of these cases will have a K_{ATP} channel defect and, therefore, an approximately 50% likelihood of having focal hyperinsulinism. Mutation screening is useful for glucose-6-phosphatase deficiency, since 80% of patients have one of five common mutations. Ninety percent of MCAD patients have the common A985G mutation. In addition, for children with repeated episodes of "ketotic hypoglycemia," genetic testing may identify those with an underlying defect in glycogenolysis or ketone utilization.

30.5.2.3 Cultured Cells

Lymphoblasts or fibroblasts are useful for diagnosis of some inborn errors of metabolism (such as fatty acid oxidation disorders) and as sources of DNA for mutation analysis for other genetic defects.

30.6 Outcomes

Severe or recurrent hypoglycemia results in neurologic dysfunction and, in rare cases, death. Therefore early recognition and treatment are essential to avoid neurologic damage and later risk of learning disabilities and epilepsy. Long-term studies of HI patients have found rates of neurodevelopmental abnormalities between 26% and 48% and epilepsy between 13% and 43% [43–45]. Despite a better understanding and recognition of the disease, individuals diagnosed with HI in the last 20 years have not demonstrated substantially improved outcomes compared to older individuals with HI [46]. This suggests that the neurologic insult from hypoglycemia occurs in the first several days of life and speaks to the importance of screening high-risk infants for hypoglycemia.

30.7 Treatment

The management of children with hypoglycemia should be guided by the following goals: (1) prevention of brain damage from recurrent hypoglycemia, (2) establishment of a specific diagnosis and therapy, and (3) encouragement of normal feeding behavior while assuring safe fasting tolerance.

To minimize the risk of brain damage, aim to maintain plasma glucose >70 mg/dL [42]. Ideally, treatment should maintain normoglycemia on a normal feeding schedule for age. It is advisable to periodically reassess efficacy of treatment in any form of hypoglycemia by a formal fasting study on treatment. Figure 30.8 depicts the management approach for children with hyperinsulinism and other causes of hypoglycemia.

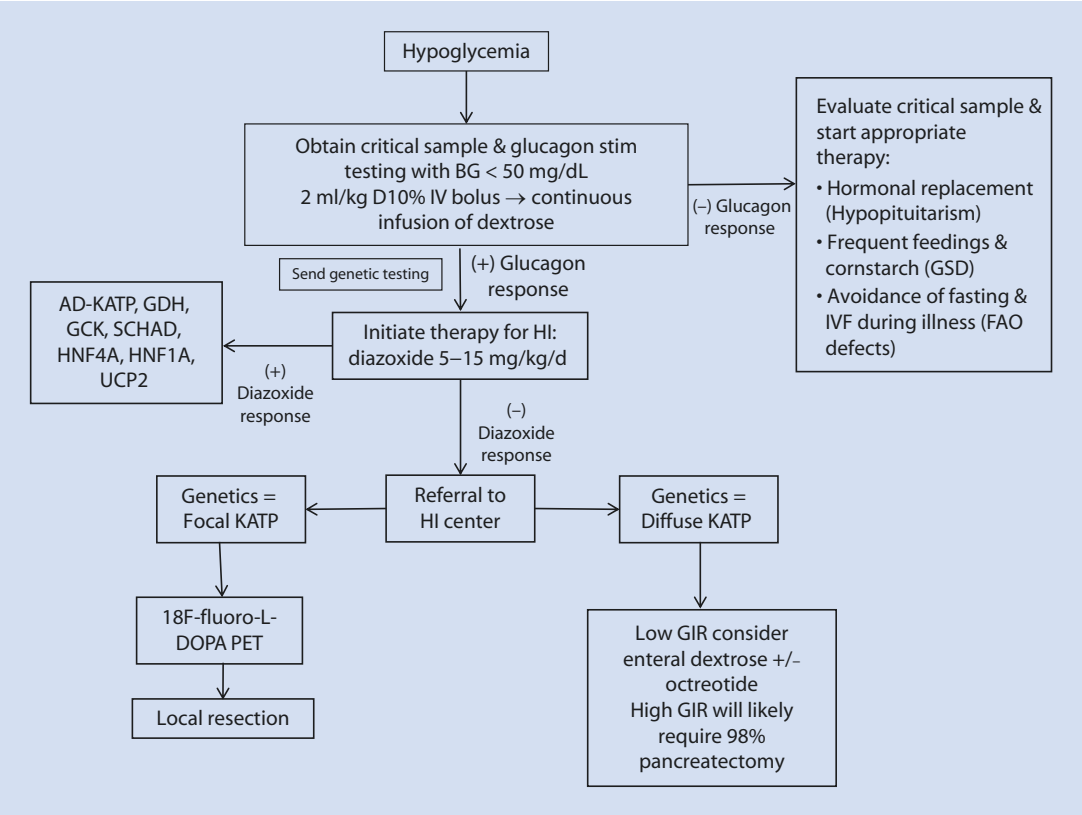

Fig. 30.8 Management approach to the child with hypoglycemia

30.7.1 Selected Drugs for Hypoglycemic Disorders

1. Dextrose (emergency Rx): IV 0.2 gm/kg bolus (2 mL/kg of D10), followed by D10 administered continuously. The glucose infusion rate is adjusted to maintain plasma glucoses greater than 70 mg/dL. Children with HI may need infusion rates of glucose as high as 20–30 mg/kg/min. Continuous dextrose (20%) can be given enterally as home management for GSD type I and certain forms of diazoxide-unresponsive HI.
2. Glucagon (emergency Rx only in case of insulin-induced hypoglycemia): 1 mg IM or IV. This can also be used as a continuous intravenous infusion in HI as a temporary measure to reduce glucose requirements, while the infant is in the hospital.
3. Diazoxide. 5–15 mg/kg/day divided into two oral doses for HI. The starting dose should be 5–10 mg/kg/day based on the severity, increasing to a maximum of 15 mg/kg/day if necessary. Fasting tolerance should be assessed after five full days of therapy (since the half-life is 24–36 h) [47]. The major side effects are fluid retention and hypertrichosis. Concomitant or preemptive use of a diuretic should be considered, especially in infants receiving intravenous fluids, to prevent congestive failure or pulmonary hypertension.
4. Octreotide: 5–15 mcg/kg/day SQ divided q 6–8 h. Tachyphylaxis is commonly encountered. Because of recent concerns about necrotizing enterocolitis in neonates treated with octreotide [48] and given the lack of a lasting response in most cases, we advise against its use in infants less than 2 months old. For older children who respond to octreotide, long-acting somatostatin analogs that can be dosed once a month are now available.
5. Cornstarch: 1.6 g/kg every 3–4 h for GSD type I [32]. This may also be used at bedtime for other forms of GSD or for young children with abbreviated fasting tolerance.

Case Study

A full-term baby boy with a birth weight of 4500 gm (LGA) was found to have a plasma glucose of 25 mg/dL shortly after birth. Due to persistent pre-feed plasma glucose values in the 30s, on DOL #2 he was started on dextrose containing IVF, which were subsequently weaned off over the next several days. His hypoglycemia was declared "resolved," and he was discharged home with no further evaluation. At 2 months of age, he had a generalized tonic clonic seizure and EMS was called. His plasma glucose was 30 mg/dL. He was admitted to the hospital where he was found to have persistent hypoglycemia. He underwent a fasting test, during which he fasted only 3 h with plasma glucoses above 50 mg/dL. His critical sample showed the following: glucose 45 mg/dL, HCO3 24 mmol/L, BOHB 0.1 mM, lactate 0.5 mmol/L, insulin 2 uU/mL, FFA 0.15 mmol/L, cortisol 18 mcg/dL, and GH 12 ng/mL. Glucagon 1 mg was given when his plasma glucose was 45 mg/dL and increased to 90 mg/dL by 20 min. He was diagnosed with hyperinsulinism and was started on diazoxide at a dose of 15 mg/kg/day. After 5 days, he continued to require a GIR of 16 mg/kg/min and could not be weaned off his IVF. Diazoxide was discontinued. Genetic testing of the HI genes had been sent on him and his parents and showed a paternally inherited autosomal recessive mutation of *ABCC8*. He underwent an 18F–fluoro-L-DOPA PET scan, which demonstrated focal uptake of the tracer in the tail of the pancreas. He was taken to surgery and underwent a 15% partial pancreatectomy after analysis of frozen samples confirmed focal HI histology. After recovery from surgery, he underwent another fasting test during which he was able to fast for 24 h with plasma glucose levels >70 mg/dL.

30.8 Summary

Hypoglycemia disorders are rare but have potentially severe consequences for children if unrecognized. An understanding of normal fasting adaption and a systematic approach to the interpretation of the critical sample allows for narrowing of the differential diagnosis and timely diagnosis and appropriate treatment.

Review Questions

1. A 9-month-old male presents to the emergency department with new onset seizures and a plasma glucose of 38 mg/dL. A critical sample obtained at presentation showed bicarbonate = 20 mmol/L, FFA = 0.82 mmol/L, BOHB = 0.95 mmol/L, plasma insulin = 3 uU/mL, lactate = 1 mmol/L, GH = 0.48 ng/mL, cortisol = 4.14 ug/dL, and ammonia = 120umol/L.

   What additional test would help establish the underlying cause of hypoglycemia?

   A. Arginine/clonidine GH stimulation test
   B. Fed glucagon stimulation test
   C. CRH stimulation test
   D. Glucagon stimulation test
   E. Both A and C

2. A 6-month-old female is referred for evaluation of poor growth and hypoglycemia. Laboratory testing ordered by the pediatrician showed a plasma glucose of 49 mg/dL, bicarbonate = 14 mmol/L, lactate = 6.8 mmol/L, AST = 120 U/L, ALT = 100 U/L, triglycerides = 800 mg/dL, and hemoglobin = 9 g/dL.

   The diagnosis is best confirmed by which of the following results?

   A. Following injection of glucagon 1 mg IV, the plasma glucose rises from 49 mg/dL to 80 mg/dL after 30 min.
   B. Following injection of glucagon 1 mg IV, the blood lactate rises from 6.8 mmol/L to 10 mmol/L after 20 min.
   C. Following ingestion of an oral protein supplement, 1.5 g/kg, the plasma glucose falls from 70 mg/dL to 40 mg/dL after 30 min.
   D. Following ingestion of oral glucose, 1.75 g/kg, the plasma glucose rises to 250 mg/dL at 1 h. and then falls to 35 mg/dL at 2 h.
   E. Following injection of galactose, 1 g/kg IV, the plasma glucose rises from 80 to 150 mg/dL after 30 min.

3. You are consulted to evaluate a 2-week-old male with persistent

hypoglycemia. He was born by emergency cesarean section due to non-reassuring fetal heart rate at 35 weeks of gestation and was SGA at birth. His initial plasma glucose was 10 mg/dL. He was on intravenous fluids with dextrose for 1 week and fortified formula every 3 h. Pre-feeding glucoses are now 55–60 mg/dL.

The most appropriate next step in the management of this infant is:

A. To initiate therapy with hydrocortisone 10 mg/m2/day.
B. No further evaluations are required since plasma glucose is now above 50 mg/dL.
C. To perform a diagnostic fasting test.
D. To recommend feeding the infant every 2 h.
E. To add cornstarch 1 g/kg to the formula.

Answers

1. (D) The findings of suppressed β-hydroxybutyrate and free fatty acids and a positive response to glucagon (> 30 mg/dL) at the time of hypoglycemia are diagnostic of hyperinsulinism. It is not unusual to find that insulin is not elevated in cases of hyperinsulinism; therefore, low plasma insulin at the time of hypoglycemia does not rule out the diagnosis. Accordingly, the diagnosis must often be based on the examination of other physiologic manifestations of excessive insulin secretion, such as suppression of glycogenolysis, lipolysis, and ketogenesis, which can be inferred by the finding of a glycemic response to glucagon and the suppression of plasma free fatty acids and β-hydroxybutyrate concentrations during hypoglycemia. The late presentation and the elevated ammonia are characteristic of GDH hyperinsulinism, or the hyperinsulinism hyperammonemia syndrome, caused by an activating mutation in *GLUD1*. Note that frequently GH and cortisol levels are not elevated in the critical sample in HI patients; also, except for the immediate postnatal period, deficiencies of these two hormones do not cause hypo-ketonemic, hypo-fatty acidemic hypoglycemia.
2. (B) The clinical picture of growth failure, hypoglycemia, hypertriglyceridemia, elevated liver enzymes, and lactic acidemia suggests glycogen storage disease type I (deficiency of glucose 6-phosphatase). The diagnosis can be confirmed by a fed glucagon stimulation test, which will show failure of glucose to rise and elevation of lactate as result of increased glycogenolysis.
3. (C) The finding of persistent hypoglycemia (beyond 2–3 days after birth) in this high-risk neonate suggests an ongoing problem with glucose regulation. The history suggests perinatal stress-induced hyperinsulinism that has not yet resolved. Prompt evaluation with a diagnostic fasting test will help determine the underlying cause and initiate appropriate therapy.

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Type 1 Diabetes in Children and Adolescents

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Key Points

- Successful pediatric diabetes management is best accomplished with a multidisciplinary team.
- Intensive diabetes management can be achieved in youth with T1D by using multiple daily injections or insulin pump therapy.
- There are a variety of insulin formulations that can allow for individualized treatment plans.

31.1 Introduction and Background

Diabetes mellitus is a lifelong disorder characterized by alteration in the metabolism of glucose and other energy-yielding fuels due to an absolute or relative insufficiency of insulin. This lack of insulin plays a primary role in the hyperglycemia and other metabolic derangements linked to diabetes. Hyperglycemia, in turn, plays a key role in the microvascular and macrovascular complications of diabetes.

Diabetes mellitus can be classified into at least three subclasses: type 1 diabetes (T1D), once known as insulin-dependent diabetes mellitus; type 2 diabetes (T2D), once known as non-insulin-dependent diabetes mellitus; and secondary diabetes that is linked to another identifiable condition or syndrome. Currently, the majority of children diagnosed with diabetes have T1D, but the rates of T2D in the pediatric population are increasing dramatically. The prevalence of T2D increased by 30.5% from 2001 to 2009, with the highest prevalence noted in American Indian youth [1]. Risk factors for the development of T2D include a strong family history of T2D, overweight (BMI > 85th percentile for age and gender), sedentary lifestyle, and having an African-American, Hispanic, Asian/Pacific Islander, or American Indian background.

T1D occurs when pancreatic β-cells are destroyed in an autoimmune process that is currently the focus of many research studies. Autoimmune-mediated destruction of β-cells ultimately leads to nearly complete absence of endogenous insulin secretion in most patients. Children with T1D are dependent on exogenous insulin in order to prevent progressive metabolic decompensation (i.e., ketoacidosis) and death. T1D usually has a prolonged asymptomatic stage in which pancreatic β-cells are progressively destroyed. Once the critical mass of β-cells falls below a given threshold, children with T1D typically present with acute symptoms of polyuria, polydipsia, polyphagia, and weight loss and, if these symptoms go unrecognized, diabetic ketoacidosis (DKA).

The pathophysiology of T2D differs from T1D. Insulin resistance (IR) and the inability of β-cells to fully compensate by increasing insulin secretion are at the core of T2D in children. Resistance to insulin action places a heavy burden on β-cells, forcing them to increase insulin production, but in youth with prediabetes and T2D, their β-cells are unable to meet the demand for increased insulin secretion. The insulin resistance that develops in lean, healthy children during puberty further exacerbates the insulin demands [2]. Overburdened β-cells can lead to beta cell burnout, further reducing insulin supply relative to demand. This complicated pathogenesis results in hyperglycemia despite considerable retention of endogenous insulin secretion. In fact, it is not uncommon for adolescents with T2D to present acutely ill, with marked hyperglycemia and even ketosis, especially in the context of a stressful intercurrent illness.

Genetic, environmental, and physiologic factors all contribute to the development of insulin resistance in obese adolescents with or without T2D. Acanthosis nigricans, a darkening and thickening of the skin notable in skinfolds and creases (e.g., neck, axillae, and groin), is a cutaneous indication of increased plasma insulin levels. Its presence, especially in adolescents who show other risk factors for the development of T2D, warrants a more thorough evaluation for IR and T2D.

31.2 Diagnosis

Regardless of the type of diabetes, the current guidelines from the American Diabetes Association for the diagnosis of diabetes are shown in Box 31.1 [3].

T1D remains the most common type of diabetes in youth; however, in some circumstances, differentiating the type of diabetes may not be straightforward. When insulin is indicated, differentiating the type of diabetes need not affect management decisions. However, understanding the etiology is helpful in future treatment decisions and counseling.

Box 31.1 Criteria for the Diagnosis of Diabetes

- Fasting plasma glucose ≥126 mg/dL (7.0 mmol/L). Fasting is defined as no caloric intake for at least 8 h[a]
- *OR*
- 2-h plasma glucose ≥200 mg/dL (11.1 mmol/L) during an OGTT. The test should be performed as described by the World Health Organization, using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water[a]
- *OR*
- A1C ≥ 6.5% (48 mmol/mol). The test should be performed in a laboratory using a method that is National Glycohemoglobin Standardization Program certified and standardized to the diabetes control and complications trial assay[a]
- *OR*
- In a patient with classic symptoms of hyperglycemia or hyperglycemic crisis, a random plasma glucose ≥200 mg/dL (11.1 mmol/L)

Adapted from Table 2.1 Standards of Medical Care in Diabetes 2016. Diabetes Care 2016;39(Suppl. 1).
[a]In the absence of unequivocal hyperglycemia, results should be confirmed by repeat testing.

T2D should be considered in youth with a personal history of obesity, insulin resistance, or prediabetes, or a family history of T2D. Each case must be considered carefully, given that T1D can occur in those with a phenotype suggestive of T2D. Autoimmune antibody testing can help the clinician differentiate these two types of diabetes; however, cases of antibody-negative T1D and antibody-positive T2D cloud this as an absolute diagnostic indicator. In patients presumed to have T2D with hyperglycemia requiring insulin therapy, the clinician should avoid stopping insulin therapy until the diagnosis is clear, and the clinical picture indicates adequate residual beta cell function.

Maturity-onset diabetes of the young (MODY) is a monogenic, dominantly inherited form of diabetes, which often presents in youth or early adulthood. MODY should be considered in patients with atypical presentations or courses of diabetes, family history of similar cases, negative diabetes-associated autoantibodies, and lack of a phenotype concerning for T2D. Diabetes diagnosed prior to 6 months of age is also usually a monogenic form of diabetes. Genetic testing for monogenic diabetes is available, and results can guide treatment decisions and counseling.

31.3 Treatment of T1D

The treatment of T1D in children and adolescents presents unique challenges to pediatric health-care providers. The combination of almost complete reliance on exogenous insulin and the physical and psychosocial changes that accompany normal growth and development make day-to-day management of pediatric patients especially difficult. Indeed, recent data from the T1D Exchange indicate that <30% of pediatric patients have hemoglobin A1c (A1c) levels <7.5% and that mean A1c values exceed 9.0% in teenagers [4]. In pediatric patients, successful diabetes management is best accomplished with a multidisciplinary team of clinicians, including pediatric endocrinologists, nurse practitioners, certified diabetes educators, nutritionists, social workers, and/or psychologists, to provide ongoing education and support of self-management efforts on the part of parents and patients.

In newly diagnosed patients, the first few weeks are critically important in the process of teaching self-management skills to the parent and child. Initiation of diabetes management can be accomplished either in the inpatient or outpatient setting. Many children require hospitalization for vomiting, dehydration, and/or moderate-to-severe DKA. In patients who are not ill at presentation, admission to the hospital may also provide the child and parent with a safe and supportive environment in which to adjust to the shock of the diagnosis and learn the survival skills of diabetes management. On the other hand, some parents may prefer initial outpatient management, especially if there are no interfering social issues that would complicate initial treatment. Frequent phone follow-up is an important aspect in the care of all newly diagnosed patients, and we speak with our newly diagnosed families on a daily basis for the first 2–3 weeks after diagnosis. In addition, we routinely schedule follow-up outpatient visits 1–2 and again 6 weeks after diagnosis.

Once out of the newly diagnosed phase, regular follow-up visits approximately every 3 months are recommended [5]. These visits provide the opportunity to evaluate whether the patient has met the treatment goals (see next section), to review diabetes management principles, and to assess child and family functioning. During these visits, measurements of A1c provide a means of evaluating glycemic control. A point of care method (such as the Affinion or DCA Vantage) is strongly recommended

since it allows clinicians to incorporate results into the actual clinic visit. Children and teenagers are familiar with the concepts of "progress reports" or "report cards" in the context of their schooling, and the A1c can stand as a diabetes "report card" to provide feedback on the effectiveness of efforts to maintain or improve glycemic control. Patients and their families should also have access, via telephone, fax, e-mail, or other communication methods, to clinicians in between visits for adjustments in the treatment regimen or for advice/counseling on issues that arise between clinic visits.

31.3.1 Goals

The traditional goals of treatment in children and adolescents with diabetes were to balance insulin, diet, and exercise to promote optimal growth and development while minimizing episodes of hypoglycemia and hyperglycemia. The result from the landmark Diabetes Control and Complications Trial (DCCT) raised the bar with respect to these traditional treatment goals by demonstrating that intensive treatment leading to near-normal glucose and A1c levels significantly reduces the risk for retinopathy and the development of microalbuminuria [6–8]. These findings were supported and extended by the follow-up of the DCCT cohort in the Epidemiology of Diabetes Interventions and Complications (EDIC) study. Current standards of care for both the American Diabetes Association (ADA) and the International Society for Pediatric and Adolescent Diabetes (ISPAD) identify an A1c of <7.5% as the glycemic target for children and adolescents under 19 years of age [3, 9]. Nevertheless, it is important to individualize the treatment plan to meet the specific needs of each child. For instance, some children with frequent episodes of hypoglycemia may need a higher glycemic target. Additionally, intensive treatment places an extra burden on patients and their families, and practical considerations such as acceptability of and compliance with the treatment regimens must be balanced appropriately in order to achieve treatment goals.

31.3.2 Insulin Management

Once so simple, the choice of insulin has become ever more complicated. Current insulin options include standard human regular, neutral protamine Hagedorn (NPH), rapid- and long-acting insulin analogs, premixed combinations of these insulins, and newer ultra-long-acting analogs. Most insulin analogs are available in a standard concentration of 100 U/mL (U-100). A rapid-acting analog is available in a concentration of 200 U/mL (U-200), and long-acting analog is available in a concentration of 300 U/mL (U-300). Regular insulin is also available in a U-500 concentration for patients (usually with T2D) who require very large doses of insulin because they are very insulin resistant. Certain insulins can also be diluted to lower concentrations. Diluted insulin is typically used in the very young child who requires very small doses. Since today's insulin pumps can deliver very small, incremental doses of insulin, use of diluted insulin has become less common.

The three rapid-acting analogs in common use are the following: aspart (NovoLog®), lispro (Humalog®), and glulisine (Apidra®). These rapid-acting insulin analogs have amino acid substitutions on the β-chain, which result in more rapid absorption following subcutaneous injection, with a sharper peak and shorter duration of action when compared to regular insulin. When compared to regular insulin, rapid-acting insulin analogs give better control of postprandial glucose surges and lower rates of late, post-meal hypoglycemia [10]. An ultra-rapid acting insulin was FDA approved for use in adults with type 1 and type 2 diabetes. Fiasp, is insulin aspart that has been reformulated with the addition of niacinamide (vitamin B3) and arginine to decrease absorption time. With this addition, Fiasp enters the blood stream within 2.5 min and can thus be given either immediately prior to a meal or up to 20 min after eating begins [67]. Randomized controlled trials have looked at Fiasp in both adults with T1D on injection and pump therapy and T2D on injection and metformin therapy. In the T1D studies, Fiasp was found to be similar to insulin aspart in terms of risk of hypoglycemia while improving postprandial glucose levels in pump patients [67, 68]. In T2D, Fiasp was again noninferior to insulin aspart but did have a slight increase in rates of hypoglycemia in the first 2 hours after a meal [69]. Fiasp is currently available in U-100 concentration in an insulin pen or vial.

U-200 lispro is more commonly known as Humalog® U-200 Kwikpen. The Kwikpen signifies that this insulin is only available in a pen format. The pen has been designed to deliver the

same dose of insulin as U-100 but in half the volume. This is especially helpful for patients who require a larger dose of preprandial insulin. Both U-100 and U-200 lispro have been shown to have similar pharmacodynamic and pharmacokinetic actions when compared to each other [11].

There are now four long-acting analog preparations: glargine U-100 (Lantus®), glargine U-300 (Toujeo®), detemir (Levemir®), and degludec (Tresiba®). Insulin glargine is engineered to be soluble in the acid pH solution in which it is packaged but relatively insoluble in the neutral pH of subcutaneous interstitial fluid. This leads to precipitation of glargine following subcutaneous injection which delays its absorption into the circulation, creating the first long-acting insulin [10]. Glargine U-300 (Toujeo®) has been shown to have a flatter insulin action profile and a longer duration of action when compared to glargine U-100 [12]. Blood glucose levels should be monitored closely when making the transition between glargine U-100 and glargine U-300 as some patients may need to have their doses increased by 10–15% [13]. Currently glargine U-300 is approved by the Food & Drug Administration (FDA) for use in adults, and glargine U-100 is FDA approved for use in children >6 years old.

The fatty acid side chain in insulin detemir causes it to bind to albumin in the circulation and interstitial fluid, resulting in a prolonged duration of insulin action. Studies show that it too has a flat time-action profile and lower dose-to-dose variability than either NPH or glargine U-100 [14]. Although controversial, insulin detemir may have a shorter duration of action than glargine U100. To date, there are no studies comparing insulin detemir to insulin glargine U-300. Insulin detemir is FDA approved for use in children, ages 2–17 years, and adults.

Insulin degludec is the first ultra-long-acting insulin. It initially forms multihexamers in subcutaneous tissue in order to delay absorption, and like insulin detemir, it also binds to albumin to delay elimination [15]. Because of this design, insulin degludec has been shown to have a half-life of 24 h and a duration of action that exceeds 42 h [16]. In children and adolescents with T1D, insulin degludec has been shown to be equivalent to insulin detemir in terms of its glucose-lowering ability, and it has the ability to do so in a single daily injection [17]. Furthermore, insulin degludec appears to significantly reduce the risk of developing hyperglycemia with ketosis [17]. Two additional features of insulin degludec that make it very appealing as a basal insulin are that (i) mixing is possible with rapid-acting insulin and (ii) its ultra-long-acting profile can allow for less stringent timing of daily administration [18]. It should be noted that insulin degludec is only available in an insulin pen format, and that there are two available concentrations, U-100 and U-200. The U-200 insulin degludec pen allows for a single dose of insulin up to 160 units and may be most appropriate for those who may have significantly increased insulin needs. Currently, insulin degludec is only approved by the FDA for use in adults.

NPH is the only intermediate-acting insulin in the market today. As an insulin suspension, it is imperative that adequate mixing of the components occurs before injection. There is significant dose-to-dose variability in the peak effect of NPH making it less satisfactory for basal insulin replacement, particularly during the overnight period [19].

There are also premixed combinations of both human regular and NPH (e.g., Humulin 70/30®) and of insulin aspart protamine and insulin aspart (NovoLog 70/30®) and insulin lispro protamine and insulin lispro (Humalog 50/50®). These types of premixed insulins may not be as effective in the treatment of T1D as it is difficult to achieve the necessary 24-h insulin coverage with these fixed ratios of insulin. However, we have used these insulins when faced with a challenging patient who cannot or will not take more than two to three injections per day.

Finally, inhaled insulin is now available, although currently only approved for use in adults with diabetes. Insulin human inhalation powder (Afrezza®) is a prandial insulin that comes in 4, 8, and 12 unit cartridges. Larger doses of prandial insulin would require a combination of individual cartridges. The dry powdered formulation of insulin is delivered from the cartridges to the deep lung through the use of a small, breath-powered inhaler [20]. Although this product has not been shown to significantly reduce hemoglobin A1c levels, it is associated with improved fasting blood glucose along with a reduction in hypoglycemia and weight gain [20]. Thus, this insulin could be used in young adult patients for whom multiple injections of insulin are not an option.

31.3.3 Regimens

With so many types of insulin available, the choice of regimen has also become more complicated. The findings of the DCCT established that intensive basal-bolus therapy with multiple daily injections or an insulin pump could be used to optimize blood glucose control [6, 8]. However, insulin only works if the patient takes it, so other factors such as willingness to take four or more injections per day and ability and willingness to count carbohydrates and test blood glucose (BG) levels must be assessed in order to determine the regimen that will provide the best outcomes. Regardless of the regimen that is chosen, no insulin regimen will precisely duplicate normal insulin secretion due to a number of factors including subcutaneous injection affecting absorption rates and a lack of precision in empiric dosing of insulin. Thus, periods of hypoglycemia resulting from excessive plasma insulin concentrations, along with periods of hyperglycemia from inadequate insulin concentrations, will occur even in the most conscientious patients.

31.3.3.1 Basal-Bolus Regimen with Multiple Daily Injections (MDI, 4+ Injections per Day)

In the individual without diabetes, basal plasma insulin levels are overlaid by meal-related spikes in circulating insulin concentrations. Current intensive insulin regimens attempt to simulate this pattern of insulin secretion by employing a basal-bolus approach to insulin replacement, typically with each component being covered by a particular type of insulin. Basal insulin, given once or twice daily with glargine or detemir, or once a day with the newer longer-acting insulins, is paired with food-related boluses of rapid-acting insulin, such as lispro, aspart, or glulisine. Not only is this regimen close to the physiologic model, but it has also been associated with lower rates of nocturnal hypoglycemia compared to NPH-based regimens [19]. Further, this intensive regimen is associated with lower rates of retinopathy in adolescents when compared to more traditional regimens consisting of one to two injections of insulin per day [21]. A drawback to this type of regimen is that the flat time-action profile of basal insulin makes it imperative that patients take their premeal boluses. In this type of regimen, hungry adolescents can expect to take upward of six to eight injections per day for basal and meal/snack bolus coverage. This can quickly become a problem as the difficulty of daily administration of premeal injections accounted for the findings in a study in which adolescents who were randomized to a glargine-based basal-bolus regimen had a higher A1c than those randomized to the insulin pump [22].

It should also be noted that all insulin replacement regimens generally involve administration of fixed basal insulin doses that are delivered by either injections of long-acting insulin analogs or by an insulin pump. Such fixed doses of insulin, in combination with changes in insulin sensitivity due to antecedent exercise in the afternoon and suppressed catecholamine responses during sleep, put patients with type 1 diabetes at "triple jeopardy" for severe nocturnal hypoglycemia [23].

31.3.3.2 Alternative New Onset Regimen

Although many clinicians start the newly diagnosed patient on an intensive basal-bolus regimen with four or more daily injections, our patients are started on three injections per day using a combination of NPH and aspart with breakfast and separate injections of aspart and detemir with dinner. The initial morning dose is divided into 2/3 NPH and 1/3 aspart, and the evening dose is 1/2 aspart and 1/2 detemir. The rationale for using this regimen at the onset of diabetes is that with aggressive control of blood glucose levels, most children enter a "honeymoon" or partial remission period. This partial remission is a result of increased insulin secretion by residual β-cells and improved insulin sensitivity with normalization of blood glucose levels [24].

To achieve rapid improvements in glucose control, most patients are started on a total daily dose of 1 unit per kilogram body weight per day, although smaller doses in the 0.4–0.7 units per kilogram body weight range may be sufficient in younger children and in older patients without marked or prolonged metabolic decompensation. During the 2–3 weeks following discharge, insulin doses are titrated toward target premeal glucose values of 70–130 mg/dL during daily telephone contacts. The patients are initially maintained on an age and weight appropriate, fixed carbohydrate intake with each meal. Follow-up clinic visits are scheduled at approximately 2, 6, 13, 26, 39, and 52 weeks following diagnosis. The concepts of correction doses and insulin to carbohydrate ratios (based on the predinner carbohydrate

intake divided by the predinner dose of rapid-acting insulin analog) are usually introduced during the first two follow-up visits. During the "honeymoon" period, insulin requirements drop sharply. Commonly, the doses of rapid-acting insulin are markedly reduced or even discontinued during this time, and some can even be well managed on an injection of intermediate-acting NPH in the morning and long-acting insulin detemir at dinner. In rare cases, young children recover enough beta cell function to be managed only on basal insulin.

A major reason why this three-injection/day regimen is so effective during the honeymoon phase is that endogenous insulin secretion provides much of the overnight basal insulin requirement, leading to normal fasting blood glucose levels, as well as "smoothing out" blood glucose variations throughout the day. As β-cell function declines during the second half of the first year of treatment, blood glucose levels become more labile, and HbA1c levels begin to rise. It is at this point that the limitations of the three-injection regimen become apparent. These include high pre-supper and much more variable fasting blood glucose levels. High pre-supper glucose values begin to occur despite normal prelunch and midafternoon values, most commonly caused by the consumption of an afternoon snack at the same time as waning effects of the prebreakfast NPH dose. Fasting blood glucose levels become more unpredictable because relatively small dose-to-dose variations in the time-action profile of detemir or glargine can lead to hyper- and hypoglycemia due to the small margin of error in regulating overnight hepatic glucose production. As noted previously, patients are more vulnerable to hypoglycemia in the middle of the night because the normal plasma epinephrine response to low blood glucose levels is markedly blunted during sleep [25], and extra physical activity during the day contributes to low glucose concentrations on the following night.

31.3.4 Insulin Pumps

Educational materials regarding continuous subcutaneous insulin infusion (CSII) pump therapy are provided during initial diabetes education, and there is wide variation in the timing of a family's desire to switch to pump therapy. Some request ordering pumps immediately, others may wait until the "honeymoon period" is ending, and in some patients, the decision to switch to CSII occurs later. Readiness for insulin pump therapy is assessed by clinicians, with both the clinician and family playing a role in the decision to transition to CSII. Very few of our families switch to a true basal-bolus MDI regimen rather than a pump.

CSII via an insulin pump provides what is perhaps the most physiological option for insulin replacement. The proof of concept for CSII was established in the late 1970s, but it is only within the last 15 years that there has been a substantial increase in pump use in pediatrics. Indeed, nearly half of the children and adolescents in the USA and German/Austrian type 1 diabetes registries are currently receiving insulin pump therapy [26]. Insulin pumps use only one type of insulin, most commonly rapid-acting analogs as they have been associated with better blood glucose control, particularly postprandially, and fewer episodes of hypoglycemia than human regular insulin [27]. Current technology allows for the delivery of doses as small as 0.025 U of insulin in some models.

These devices are battery powered and about the size of a small cell phone or pager. The pump employs a reservoir to hold the insulin. In the conventional pump, the insulin reservoir is attached to an infusion set. The infusion set consists of a length of tubing with a small (e.g., 6–12 mm) catheter or steel needle at the end. This small catheter or steel needle is inserted into the subcutaneous tissue, most commonly the abdomen, buttock, or upper leg/hip, by the child or parent. The infusion set should be changed every 2–3 days. There is an alternative to the conventional insulin pump, called a patch pump. In this type of insulin pump, the insulin and the mechanics to deliver it are all encased in a disposable "pod" which is directly attached to the skin, in a similar manner as the infusion set. It must be changed every 2–3 days. Insulin is dosed through this device by way of a wireless link to a handheld device that is manipulated by the patient or family member. In addition to the potential insertion sites mentioned above, the "pod" type of pump is often worn on the upper arm.

All insulin pumps deliver insulin in two ways. The first is the "basal" or background insulin delivery, which is designed to keep blood glucose levels steady in between meals and overnight.

Basal insulin doses can be programmed to change throughout the day and are entered into the pump in units/h. Multiple basal rates can be programmed throughout a 24-h period which allows for variation in insulin delivery to match the daily variations in insulin need. Additionally, use of temporary basal rates (temporary increases or decreases of the programmed basal rates by a percentage or an absolute amount for a specified period of time) are very helpful in modifying doses for illness and exercise.

The second method of insulin delivery is the "bolus" or immediate burst of insulin that the patient delivers at mealtimes to limit meal-related glucose excursions and to correct hyperglycemia. There are two types of bolus doses: the meal bolus, designed to cover the carbohydrate content of a meal, and the correction bolus, designed to return elevated blood glucose levels to the target range. In today's world of "smart" pump technology, the insulin pump's computer is able to calculate a recommended dose of insulin for both the meal and correction bolus, which can be combined at mealtime. Bolus calculators only suggest the dose of insulin based on the estimated carbohydrate content of the meal and premeal blood glucose values; it is then up to the patient or caregiver to determine whether this amount of insulin should be adjusted based on previous or anticipated exercise or on overall blood glucose trends.

When the pump dose calculator is used for meal boluses, the child or caregiver enters the number of carbohydrates that will be consumed in the upcoming meal or snack, and the pump's computer calculates the amount of insulin to be given based on the programmed insulin to carbohydrate ratio (e.g., often 1 U/10 g of carbohydrates in adolescents) for that time of day. Timing of bolus doses is also important. Research has shown that there is significant reduction in postprandial blood glucose levels when the meal bolus is delivered 10–15 min before the meal [28]. However, delivery of the bolus after or during the meal is acceptable and may be particularly useful for very young children, the "picky" eater, or those in the school setting where there is less oversight to ensure that a child actually eats the amount of carbohydrates entered into the pump [29].

For a correction bolus, the pump's computer is programmed with a correction or sensitivity factor (e.g., 1 U drops blood glucose levels by 50 mg/dL) to correct for blood glucose levels that are outside the target range. The pump is also programmed with a target blood glucose which acts as the mathematical goal for the correction equation. The blood glucose value is entered into the pump either manually or by wireless transmission from the glucose meter into the pump. In the case of a value that is above target, additional insulin can be given to lower the high reading based on the preset correction factor. In the case of a value that is below target, insulin is subtracted from a meal or snack bolus in order to compensate for the low reading.

Recent advances in pump therapy have led to the first commercially available hybrid closed-loop insulin pump system. This system integrates a continuous glucose monitor (see ▶ Sect. 31.4.5.2) with an insulin pump to create a device in which the system dynamically adjusts the basal component of insulin delivery in response to data from an indwelling subcutaneous glucose sensor [69]. This automated insulin delivery is combined with manual delivery of prandial boluses to meet total daily insulin requirements. The results of the pivotal trial in adolescents and adults with type 1 diabetes demonstrated that with this system, not only is there an improvement in A1c and the amount of time patients remain with their glucose levels in the target zone of 70–180 mg/dL, but the percentage of time with sensor glucose values below 70 mg/dL (in the hypoglycemic range) was cut nearly in half [70]. There are many companies and institutions that are working on closed-loop systems, and the next several years will likely bring about many further advances in this domain.

It is important to remember that interruption of delivery of rapid-acting insulin analogs from a pump can rapidly lead to increases in blood ketone levels (within hours) with progression to ketoacidosis if unrecognized [30]. Training and reinforcement of diabetes prevention protocols for parents of children treated with insulin pumps can reduce the risk of DKA. Paradoxically, the risk of DKA was lower in youth with type 1 diabetes treated with pumps than insulin injections in the T1D Exchange Registry [31], an observation that likely reflects closer monitoring and better overall control in this population.

In our clinic, patients typically transition to insulin pump therapy when coming out of the

honeymoon period as warranted by glycemic control or when patients and their families express a need for an improvement in quality of life. We recommend early use CSII in the very young infant and toddler as we have demonstrated durable improvement in both A1c and risk of severe hypoglycemia [32]. Regardless of the age of the child, transition to CSII is most successful when children and their families recognize that an insulin pump does not "cure" the diabetes; it serves only as a tool and that their active participation in decision-making is essential to success with this modality.

31.3.5 Adjusting Insulin Doses

31.3.5.1 Self-Monitoring of Blood Glucose (SMBG)

SMBG allows families and clinicians to regularly evaluate the efficacy of the current insulin regimen. Today's glucose meters are small, accurate, and relatively inexpensive. There are many different brands currently commercially available, and most have reliability and accuracy of 5–8% of laboratory measurements [33, 34]. Many will display a result within 5 s and require small amounts of blood. Traditionally, SMBG blood samples are obtained from finger stick, but the smaller blood volume that is now needed for today's meters has allowed the use of alternate sites such as the forearm. However, alternate sites are associated with a lag time effect, especially during times of rapidly changing glucose levels such as with exercise or after meals [35].

We recommend that children test their blood glucose levels at least four times daily, before meals and at bedtime. However, most patients on either insulin pump therapy or basal-bolus therapy test six to ten times daily: premeals, intermittently postprandially, before exercise or driving, at bedtime, and following episodes of hyper- or hypoglycemia [36]. In fact, evidence suggests that "more is better" as the T1D Exchange Registry found a strong inverse correlation between increased frequency of SMBG and improved glycemic control as evidenced by lower hemoglobin A1c levels [37].

The results should be kept in either a written logbook or an electronic form for regular review by patients and parents. There are many computer programs and media "Apps" available to support the recording and review of SMBG data. Typical targets for SMBG include 90–145 mg/dL premeals, <180 mg/dL 2 h after meals, and 80–160 at bedtime [9]. However, these targets may be altered based on individual need. As noted previously, premeal and other SMBG measurements allow for dose-to-dose corrections for measurements that are outside target range. Equally important, regular retrospective reviews of SMBG trends allow the family and clinicians to adjust insulin doses to keep up with the ever-changing insulin needs of children and adolescents. Unfortunately, too few parents of school-aged and adolescents download glucose meters to make these adjustments.

31.3.5.2 Continuous Glucose Monitoring (CGM)

Even when performed correctly, four or more blood tests a day give only a small glimpse of the wide blood glucose fluctuations that occur over a 24-h period in children with diabetes [38]. Consequently, the introduction of continuous glucose monitoring (CGM) has the potential to be the most influential advancement in the management of diabetes in the last 20 years. Currently available CGM devices give patients a steady stream of glucose values, every 5 min. Recent advances in CGM devices have markedly improved sensor accuracy, and the FDA has recently approved a dosing claim without a confirmatory blood glucose test for the DexCom sensor. However, all commercially available units require calibration with SMBG at least twice per day. Nevertheless, uptake and regular use of CGM in youth with T1D has been very limited until very recently. There have been a number of advances in CGM technology that have driven the increased uptake of CGM in pediatrics. These include improved sensor accuracy, improvements in the ease of the use of these devices by patients, integration of pumps and sensors into sensor-augmented insulin pump systems that can automatically shut off basal infusions in the face of low sensor glucose levels [71], and the availability of programs that allow for the data from the CGM device to be "shared" with other users via cloud interface. In the care of pediatrics, the latter advance allows parents/guardians and other caregivers to monitor the CGM data independent of the location of the child. The data from a CGM can be used in many different ways. See Box 31.2 for possible uses of CGM in day-to-day management of T1D.

Box 31.2 Continuous Glucose Monitoring

- *Improved overnight control*
  - Hypoglycemia alarms
  - Retrospective data to optimize overnight basal insulin needs
  - Automatic suspension of basal insulin to prevent or limit the duration of hypoglycemia
- *Improved daytime bolus dosing*
  - Trend arrows and hyperglycemia alarms for real-time adjustments
  - Retrospective data to optimize carbohydrate ratios and correction doses
- *Enhanced understanding of diabetes management teaching*
  - Effects of different foods
  - Effects of exercise
  - Effect of stress
  - Effect of hormonal variation

Several studies including the JDRF CGM randomized control trial (CGM RCT), the guard control study, and others indicated that adults with T1D who had an A1c ≥ 7.0% had a better improvement in their A1c with the use of CGM than with SMBG alone [39, 40]. Even more importantly, this improvement in A1c was not associated with any increase in hypoglycemia. The JDRF CGM Study Group also showed that adult patients with T1D who use CGM and have baseline A1c levels <7.0% are better able to maintain their A1c at this target level than those patients who only used SMBG [41]. The JDRF CGM RCT showed that youth who wore the sensor almost every day achieved the same benefits as adults with respect to changes in A1c [39, 41]. More recent trials also support the need for continuous wearing of the CGM in order to see an impact on the HbA1c and on quality of life, specifically a reduction in the number of school days missed due to diabetes [42, 43]. Unfortunately, many fewer pediatric patients were able to use these early, relatively inaccurate and difficult to use CGM devices consistently enough to receive these benefits over the long run [39]. Patient and family education is another cornerstone of the successful use of these devices in pediatrics. Families must understand the strengths and limitations of CGM and must be part of a comprehensive training program to learn the ins and outs of both the mechanics of CGM and the successful interpretation of both real-time and retrospective data. Smaller, more accurate, and easier to use systems are needed for children with T1D.

31.3.6 Hypoglycemia

Severe hypoglycemia is a significant risk for patients attempting to achieve tight glycemic control. In the DCCT, the risk of severe hypoglycemia was threefold higher in the intensively managed cohort than it was in the conventionally managed one [6]. Although advances in diabetes technology over the past 20 years have not eliminated the risk of hypoglycemia, they have allowed patients to lower HbA1c into the target range without increasing the risk of severe hypoglycemia [44–46]. Most severe hypoglycemic events occur in the overnight hours, likely due in part to sleep-induced defects in counter-regulatory hormone responses to hypoglycemia [25]. Hypoglycemia represents a significant barrier to successful obtainment of tight glycemic control in people with diabetes, and thus, effective management of hypoglycemia has to be at the forefront of any diabetes regimen for children and adolescents.

Regular SMBG in combination with targeted SMBG is the mainstay to detecting hypoglycemia and preventing its progression to severe hypoglycemia. It is important to note that unawareness of hypoglycemic symptoms can be "developmentally appropriate" in the very young child, making it more challenging for caregivers to know when the blood glucose level is low. The normal response to falling plasma glucose levels in nondiabetic individuals includes rapid suppression of insulin secretion followed by release of glucagon and epinephrine if the plasma glucose level does not stabilize following the decrease in insulin release alone. Children with diabetes suffer from defective counter-regulation because the action of exogenously supplied insulin cannot be adjusted in response to falling glucose levels, and they additionally lose the ability to secrete glucagon in response to hypoglycemia. Thus, patients with T1D are dependent upon increases in circulating plasma catecholamines to signal the presence of hypoglycemia. Unfortunately, recurrent episodes of even mild hypoglycemia that occur with intensive treatment lead to blunting of catecholamine response leading to episodes of hypoglycemia unawareness or hypoglycemia-associated autonomic failure.

The American Diabetes Association defines hypoglycemia in the setting of diabetes as any plasma glucose of 70 mg/dL or less [36], whether accompanied by symptoms or not. The severity of hypoglycemic events is defined by their impact on function. Mild-to-moderate events are those where patients are able to treat themselves. Severe events are those in which patients have sufficient cognitive impairment as to be unable to treat themselves and must rely upon the assistance of others.

Typical treatment for mild-to-moderate hypoglycemia is 15 g of fast-acting carbohydrate such as 4 ounces of regular juice or soda or three to four glucose tablets. Ideally the low blood glucose level is confirmed via SMBG; however, children should be advised that if they have symptoms of hypoglycemia, they should treat these right away, even if unable to test their blood glucose level. In the case of severe events, seizure or loss of consciousness may preclude the safe use of oral carbohydrate sources and an intramuscular or subcutaneous injection of glucagon (0.5–1 mg), or IV glucose infusion may be required. Recent studies evaluating intranasal administration of glucagon powder are promising as a treatment alternative to glucagon injections for hypoglycemia [47].

Once an episode of hypoglycemia has been resolved, it is important for children and their families to review the event for precipitating factors such as changes in eating habits and exercise or activity levels. If this review does not yield a clear cause, the blood glucose records for the last few days should be reviewed to determine whether a change in the insulin regimen is needed. Prevention of hypoglycemia should be the goal, and many options exist to aid in the attainment of this goal. In clinical practice, we consider decreasing insulin dosage 24–48 h after a severe hypoglycemic episode in efforts to prevent repeat severe hypoglycemia. Although uncommon, recurrent hypoglycemia in the face of reductions in total daily insulin doses may be presenting symptoms of celiac disease, adrenal insufficiency, or growth hormone deficiency.

31.3.7 Medical Nutrition Therapy

Dietary guidance for children with diabetes is a key component in the diabetes regimen and ideally best provided by a registered dietician who is a member of the multidisciplinary diabetes team and is comfortable working with children. In addition to achieving optimal glycemic control and maximizing growth and development, medical nutrition therapy is also aimed at reducing the risk for other diseases such as obesity, dyslipidemia, and hypertension. Underlying all of these goals is the establishment of sound eating patterns incorporating healthy food choices [48]. Sadly, there is an epidemic of childhood obesity in developed countries. The DCCT showed that an adverse consequence of intensive insulin therapy was a twofold increase in risk of being overweight. Thus, it is important to monitor for any changes in the BMI *z*-score and to promptly attend to these changes.

Carbohydrate counting is by far the most popular way to introduce flexibility into the dietary plan. In patients using basal-bolus insulin therapy with an insulin pump or multiple injections of insulin, bolus doses of insulin are based on the grams of carbohydrates that will be consumed in the meal. Specifically, patients use a ratio that represents the amount of carbohydrates (in grams) that 1 U of insulin will cover. This ratio is very individualized; determined and adjusted by trial and error, and often varies throughout the day; more insulin is usually needed for breakfast than other meals. For this method of insulin coverage to work properly, reasonable accuracy at counting carbohydrates is essential. Patients and their families must be comfortable with reading food labels and quantifying the size of servings, either through measurement or weight. Protein and fat content, while important to an overall healthy meal plan, are not counted as a general rule. However, they can impact the absorption of carbohydrates, and foods such as pizza may thus require an individualized approach. Ultimately, the patient and family determine the size of the meal and its carbohydrate content and then determine an insulin dose to match food intake.

Carbohydrate counting can also be used in a more traditional approach to diabetes therapy in which set insulin doses are matched with consistent carbohydrate targets for meals and snacks. In fact, we use this approach, stressing consistency in the timing and size of meals, as a starting point for newly diagnosed families who are too overwhelmed to learn more advanced nutritional concepts at the very beginning of treating their child's diabetes.

31.3.8 Exercise

Regular exercise and active participation in organized activities have positive implications for both the psychosocial and physical well-being of all children and are especially important for children with diabetes. Exercise and being physically fit are associated with increased sensitivity to insulin and better glucose utilization. Despite its many benefits, exercise in children with diabetes can make it more challenging to regulate glucose levels. Hypoglycemia is a common occurrence which can then result in excessive carbohydrate intake leading to hyperglycemia. This effect is only compounded by the intermittent and spontaneous nature of physical activity in children, especially those not involved in organized sports or activities. Children with T1D who participate in any type of exercise should test their blood glucose values before and after the exercise and potentially during the exercise as well, depending on whether the duration is more than an hour or so. It is also important to test the blood glucose for the delayed effects of exercise, since hypoglycemia can occur up to 7–11 h later [49]. As many children participate in late afternoon or evening activities, this delayed hypoglycemic response puts them at risk for nocturnal hypoglycemia.

When working with children and their families, it is important to discuss the triad of exercise, food intake, and insulin. When exercise is increased, one of the others must be adjusted in order to minimize the risk of hypoglycemia. For patients on injections of insulin, the dose of insulin can be adjusted when activities are planned; otherwise, additional snacks may need to be eaten. Both additional carbohydrates and reductions in insulin may be necessary if the activity is to last longer than 1 h [50]. In patients who use insulin pump therapy, suspension of the basal infusion rate (or simply disconnecting the pump) during exercise can reduce rates of hypoglycemia [51]. Use of lower temporary basal rates during and after exercise can help to prevent hypoglycemia during the activity and later that day or night. Serious athletes may need to reload on carbohydrates following intense and/or prolonged activity in addition to adjusting their insulin doses. Some patients find that raw corn starch can slow the digestion of sugar, providing a more sustained fast-acting carbohydrate effect in combatting exercise-induced hypoglycemia.

Many studies closely looking at methods to combat the impact of exercise on glycemic control have shown that there is an almost infinite number of factors that need to be considered when designing the diabetes management plan. Thus, trial and error are still a key component in the management of exercise and glucose levels in children with diabetes.

31.3.9 Sick Day Management

The T1D Exchange Registry has shown that DKA remains a serious threat to youth with T1D, especially those in poor metabolic control [31]. It is imperative that parents of children with T1D who develop acute symptoms of nausea, abdominal discomfort, and vomiting remember that they need to closely monitor their child for elevations in blood glucose and blood or urine ketone levels that signal the development of ketosis or DKA, with or without the presence of another acute illness like a viral infection. Unfortunately, parents often forget about the threat of DKA until dehydration and acidosis result in a hospital admission of their child to the pediatric intensive care unit.

On sick days, blood glucose levels should be checked every 2 h, and urine or blood tested for increases in ketone levels frequently. It should be stressed to all families from the outset that insulin should never be held; adjustments in the doses are necessary during intercurrent illness, but a complete cessation of all insulin replacement will quickly lead to diabetic ketoacidosis.

It is important to maintain adequate fluid intake during serious illness in order to prevent dehydration and to improve excretion of ketones, if present. Families should keep on hand at all times a variety of different fluids including regular soda, juice, sports drinks, sugar-free drinks, clear soups, popsicles, and gelatin as well as water. In the child tolerating oral rehydration, a fluid "dose" of 1 ounce per year of age per hour serves as a rough guideline; the sugar content of which depends on the serum glucose. For blood glucose values >180–200 mg/dL, sugar-free fluids should be given, and for blood sugar <180 mg/dL, sugar-containing fluids should be used.

Insulin doses will likely need to be adjusted depending on blood glucose levels, ketone levels, and the presence of emesis impairing normal oral intake. In the presence of emesis or significant

alteration to appetite, it is recommended that families reduce the dose of intermediate- or long-acting insulin by 20–50% [52] and may also need to temporarily discontinue it and instead give small doses of rapid-acting insulin every 2–3 h. Small doses of parenteral glucagon can be used to treat mild-to-moderate hypoglycemia in the setting of nausea or vomiting, when the child is unable to ingest fast-acting carbohydrates. Once the ketones have cleared and the child is tolerating an oral diet, the family may resume the normal routine. If vomiting is persistent and ketones remain moderate or large after several supplemental insulin doses, arrangements should be made for hydration and evaluation in the emergency department.

In pump-treated patients, it is critically important that infusion site problems leading to interruption of insulin delivery and ketosis, be differentiated from an intercurrent gastroenteritis or other acute infection. Any elevation of glucose and ketone levels is an indication for changing the infusion site, whereas, with hyperglycemia alone, the effectiveness of a correction bolus in lowering blood glucose levels can be checked before changing the infusion site.

31.3.10 Psychosocial Considerations

Diabetes is an insidious condition; it requires patients and their families to be "on" 24 h per day, 7 days per week. Consequently, it can have a profound negative impact on lifestyle and interpersonal relationships. All of the burdens of diabetes and its care are superimposed on the already challenging transitions through childhood, adolescence, and into early adulthood.

Successful adaptation to diabetes was traditionally measured by the degree to which patients achieved and maintained blood glucose and HbA1c as close to target as possible. Increasingly, however, optimizing quality of life has become another important measure of successful adaptation [53]. It is important to remember that in pediatrics, successful management of a chronic illness not only involves the child with the illness but the family as well. Factors such as socioeconomic status, family structure and coping styles, the presence of maternal depression, and age can all impact a family's ability to cope with diabetes and to achieve successful outcomes [53]. In fact, increased psychological stress is directly correlated with reduced self-efficacy regarding diabetes management and with poor glucose control [54]. Professionals with expertise in the psychosocial challenges of diabetes care, such as psychologists and social workers, are essential members of the multidisciplinary diabetes care team. Regular interaction with these professionals should be encouraged on at least an annual basis, and more formal assessment should be provided for those patients and their families who exhibit psychosocial risk factors and/or have been unable to achieve diabetes care goals. Additionally, it is important to involve these professionals from the time of diagnosis as the actual diagnosis itself causes significant psychological distress in both the child who is diagnosed and the parents and family system [55].

Depression is the most common psychological disorder in children and adolescents with diabetes. Research has shown that symptoms can wax and wane throughout the course of time with diabetes, being higher in the first few years then settling only to rise again after 10 years [56]. The SEARCH for Diabetes in Youth study found that rates of mild depression were 14% in youth with diabetes, and 8.6% of study participants exhibited moderate or severe symptoms [57]. Comprehensive diabetes management should include evaluation for signs of depressions both informally during routine clinic visits and more formally with a standardized screening tool. Managing problems of depression will improve the quality of life and can also have an impact on glycemic control, whereas untreated depression and poor glycemic control can create a vicious cycle of one negatively reinforcing the other.

Research indicates that disordered eating is more common in adolescent girls and young adult women with T1D [58], in association with poor glycemic control [59]. In addition, withholding insulin is a way of purging excess calories that is unique to people with diabetes, hence the name diabulimia. This chronic state of hyperglycemia can lead to dehydration and loss of both body fat and lean muscle mass. It is reported that more than 30% of women with T1D report using insulin restriction for weight control [60]. Women who chronically use this method of purging are at risk for both the acute complication of diabetic ketoacidosis and more chronic complications associated with long-standing poor glycemic con-

trol. The first step in the treatment of this condition is to identify it. Warning signs can include persistently poor glycemic control, especially in combination with extreme focus on body shape and weight, strict and/or low-calorie meal plans, strict exercise regimens, and potentially repeated problems with ketonuria and/or ketoacidosis [58]. Treatment is complex and should involve a multidisciplinary team. Initially glycemic goals should be aimed at promoting the safety of the individual as they begin to address the more complicated psychological issues, with a gradual return to more intensive glycemic targets as the individual situation warrants.

Strategies to address the psychosocial implications of diabetes include family-focused interventions, behavioral contracting, stress management/coping skills, and motivational interviewing [55]. These strategies can be utilized in one-on-one situations, peer groups, or with family systems. It cannot be stressed enough that providers with expertise in the psychosocial domain should be a part of every comprehensive diabetes team.

31.3.11 Associated Autoimmune Conditions

When treating a child with T1D, care should also include screening for several other autoimmune disorders. Autoimmune thyroiditis is the most common comorbid condition associated with T1D. According to the International Society for Pediatric and Adolescent Diabetes, up to 29% of children with diabetes will exhibit antithyroid antibodies [61]. It is recommended that providers consider obtaining both the antithyroid peroxidase and anti-thyroglobulin antibodies at the time of diagnosis with type 1 diabetes [62]. Current recommendations for evaluation in all children with T1D include measurement of thyroid-stimulating hormone levels every 1–2 years or whenever there is a change in a child's growth and development, an unexplained increase in hypoglycemia or signs and symptoms of thyroid disease [62].

According to various sources, celiac disease is found in about 1–15% of children with T1D. Although children may present with classic gastrointestinal symptoms and poor growth [61], most youngsters do not have any symptoms. For some children with T1D, the only signs of celiac disease may be an increase in the frequency of hypoglycemia as well as a persistent reduction in insulin needs in the months leading up to the diagnosis [61]. Current guidelines recommend screening for celiac shortly after diagnosis with either deamidated gliadin antibodies or tissue transglutaminase antibodies [62]. A total IgA should be a part of the assessment in children, as low circulating levels of IgA will give false-negative screening results. In this case, tissue transglutaminase IgG or deamidated gliadin IgG should be measured. Children with a positive screening test for celiac should be referred to a pediatric gastroenterologist, ideally one with expertise in the management of celiac disease, for a confirmatory small bowel biopsy. Once a diagnosis is made, the child must be placed on a gluten-free diet of lifelong duration. Gluten-free foods and resources have become more plentiful in recent years, and children and their families can benefit from nutrition counseling from a health-care professional that is well versed in today's options.

T1D patients are also at risk for Addison's disease, but this is uncommon enough that we do not routinely screen for it. However, when diabetes and thyroid disease coexist, the possibility of adrenal insufficiency should be considered. This may be heralded by decreased insulin requirements, increased pigmentation of the skin and buccal mucosa, salt craving, weakness, and postural hypotension. Rarely, frank Addisonian crisis is the first evidence of adrenal failure. This syndrome generally occurs in the second decade of life or later. The astute clinician should also consider that frequent, unexplained hypoglycemia or a reduction in insulin requirements in the absence of exercise or activity may be a subtle indicator of hypothyroidism, adrenal insufficiency, or celiac disease.

31.4 Screening for Complications

Screening for complications and comorbidities should be incorporated into diabetes care on an annual basis. The American Diabetes Association provides recommendations for screening pediatric patients for hypertension, dyslipidemia, retinopathy, and nephropathy [62].

Currently hypertension is defined as three consecutive blood pressures higher than the 95th percentile based on age, gender, and height, and high-normal blood pressure is defined at a blood

pressure above the 90th percentile for age, gender, and height. Treatment options include dietary changes to eliminate salt, regular and sustained physical activity, and ultimately, if warranted, initiation of pharmacological interventions, with angiotensin-converting enzyme (ACE) inhibitors being the first-line agent. When using an ACE inhibitor, it is important to remember to provide preconception counseling as this class of medication is considered a teratogen.

Children with T1D should be evaluated for a familial history of early cardiovascular disease (cardiac event <55 years old). Children with a positive history should have a fasting lipid panel completed shortly after diagnosis, once glucose levels have normalized. For those children without a family history of early cardiovascular disease, cholesterol screening should begin at puberty or age 10, whichever comes first. Lifestyle changes and maintaining/achieving optimal glucose control are the first-line therapies for dyslipidemia. Specifically, it is recommended that the Step 2 American Heart Association diet be followed for lipid management as this diet has been shown to allow for normal growth and development in children as young as 7 months of age [62]. If after these treatments there is still evidence of dyslipidemia, pharmacological treatment with a statin in children older than 10 years should strongly considered. For the younger age group, it is acceptable to consider referral to a pediatric lipid disorder specialist for further treatment. It should be noted that a recent report from the T1D Exchange Clinic Registry suggests that hypertension and dyslipidemia are often underdiagnosed and undertreated in youth with T1D [63].

In general, retinopathy is not seen in prepubertal children and in those who have had diabetes for less than 5–10 years. Consequently, current guidelines recommend that retinopathy screening with an eye care professional with diabetes experience does not need to begin until the child is at least 10 years old and has had diabetes for more than 3–5 years. Annual follow-up is recommended after this, unless the eye care professional feels that less frequent exams (every 2 years) are acceptable. Eye exams should encompass both an exam through dilated pupils and retinal photography [64]. It should be noted that the frequency of screening eye exams in youth with T1D is controversial, since improvements in metabolic control during the intensive treatment era have made identification of treatable retinal lesions very uncommon in pediatric patients [65].

Screening for renal disease is an essential component of comorbidity management. In fact, the Pittsburgh Epidemiology of Diabetes Complications study showed that in the absence of renal disease, people with T1D had similar mortality risk to that of the general population [66]. Evaluation for microalbuminuria, a potential early indication of nephropathic changes, does not need to begin until a child has had T1D for at least 5 years. Screening microalbuminuria should be done with a spot urine for determination of the albumin-to-creatinine ratio, since 12–24 h urine collections do not improve identification of microalbuminuria and are much more burdensome on the patient and family. It is important to note that exercise can increase albumin excretion; therefore, time of random collections should take this into account. An initial positive result is repeated at least two more times over a 3- to 6-month period, and, if albumin excretion remains persistently elevated, treatment with an ACE inhibitor is warranted.

Case Study

Nicole S. is an 8-year old who presents to the emergency department with the following symptoms: nausea, vomiting and stomach pain for the previous 24 h, deep-sighing respirations, and a "fruity odor" to her breath. She is tachycardic and appears very dehydrated with sunken eyes, dry mucous membranes, and altered skin turgor. She also appears very emaciated, and her mother states she seems to have "disappeared before my eyes." Her weight is 25.4 kg. Her mother states that her weight 1 month ago at her well-child visit was 29.6 kg. She is neurologically intact.

Initial lab results are significant for a pH of 7.15, bicarbonate of 6, glucose of 754 mg/dL (initial fingerstick upon arrival was >600 mg/dL), and urine specific gravity of 1.030, and urine is large for glucose and ketones on dipstick.

This patient is diagnosed with new-onset type 1 diabetes and transferred to the pediatric intensive care unit for management of diabetic ketoacidosis (DKA). With close monitoring, the DKA resolves over the next 16 h, and this patient is ready to transition to subcutaneously injected insulin.

Table 31.1 Case study: blood glucose monitoring

Date	Breakfast	2H PP	Lunch	2 H PP	Dinner	2H PP	HS	2–3A
	125	308	222	164	98	144	107	114
	97	269	213	152	110	129	138	121
	111	195	180	146	121	133	116	135
	102	284	265	123	89	151	124	146

There are many different insulin formulations available. However, only a few regimens will appropriately cover the 24-h insulin replacement needs. In this case, it is determined that the patient should start on basal-bolus therapy with a long-acting and short-acting insulin pen. Insulin glargine is started as the long-acting insulin as its action profile allows for once daily dosing. Insulin aspart or lispro is sufficient for the short-acting insulin.

The first step in determining starting doses is to estimate the Total Daily Dose (TDD) of insulin. This is generally between 0.5 and 1 unit/kg/day of insulin with the higher doses being used for patients who present in DKA, while the lower doses are for those asymptomatic at diagnosis.

1. Basal insulin dose should initially be 50% of the TDD. For this child, her weight is 25.4 kg; therefore, her starting dose of basal insulin should be 13 units daily (we must round the dose to the nearest whole number as basal insulin pens do not allow for ½ unit dosing). This insulin should be given at the same time every day. This family chooses to take this dose at 8 AM every morning
2. Short-acting insulin must be given to cover the carbohydrate content of meals and/or to correct high blood glucose levels (note that these doses can and should be combined when administered before meals and snacks). These initial doses are calculated as follows:
   1. Insulin/carbohydrate ratio – This is calculated with the "500 rule." 500 is divided by the TDD to calculate this ratio. In this case: 500/25 = 20. This means that 1 unit of insulin is expected to cover 20 grams of carbohydrates that the child eats. To calculate the actual dose of insulin, the total carbohydrates to be consumed for the meal must be divided by the insulin/carbohydrate ratio. For example, if this child was eating 60 grams of carbohydrates, 60/20 = 3 units of short-acting insulin should be administered to cover the carbohydrates for that meal.
   2. Correction factor ratio – This is calculated with the "1800 rule." 1800 is divided by the TDD. In this case, 1800/25 = 72. This means that 1 unit of insulin is expected to drop the blood glucose by 72 mg/dL. One must also determine the level to which the blood glucose (BG) should be dropped, better known as the target glucose. This level is typically anywhere between 100 and 150 mg/dL, depending on the age of the child and the time of day the correction is being given. To calculate the actual dose of insulin, the following formula is used: (actual glucose-target glucose)/correction factor = # units of insulin to give. For example, if this child's blood glucose is 347 mg/dL and the target glucose is 125 mg/dl, then the insulin dose would be (347–125)/72 = 3 units for correction of high BG.

Thus, this child's initial daily doses of insulin would be:

- 13 units of insulin glargine daily at 8 AM
- Insulin aspart/lispro with meals – 1 unit per 20 grams of carbohydrates and 1 unit per 72 mg/dL with a target pre-meal BG of 70–130 mg/dL

After several days of frequent blood glucose monitoring, premeal, 2-h postprandial (2H PP), HS, and overnight, the following data are reviewed (Table 31.1).

Review of these levels indicates that the 2H PP after breakfast is running above target range. Given that this appears to be directly meal related, the insulin/carbohydrate ratio for breakfast should be adjusted. This means that this child will now use two different ratios, one for breakfast and one for the rest of the day. The following changes are recommended:

- 13 units of insulin glargine daily at 8 AM
- Insulin aspart/lispro with meals – 1 unit per 18 grams of carbohydrates with breakfast, 1 unit per 20 grams carbohydrates with lunch and dinner, and 1 unit per 72 mg/dL to correct the BG to a target of 125 mg/dL

Review Questions

1. You are seeing a 16-year-old girl on insulin therapy for diabetes, who has been experiencing frequent hypoglycemia prior to lunch. In addition to decreasing her morning short-acting insulin, you tell her that at lunchtime:
   A. If her blood glucose is <70 mg/dl, she should eat a candy bar and recheck in 15 min.
   B. If her blood glucose is <70 mg/dl, she should eat her lunch and recheck in 15 min.
   C. If her blood glucose is <70 mg/dl, she should drink 4 oz of juice and recheck her blood sugar in 15 min. If it has increased past 70 mg/dl, she should eat her lunch. If it is still low, she should drink another 4 oz of juice.
   D. If her blood glucose is <70 mg/dl, she should drink 4 oz of juice with her lunch.
   E. If her blood glucose is <70 mg/dl, she should drink 8 oz of juice, recheck her blood sugar in 15 min, and only eat her lunch after her blood sugar is >200 mg/dl
2. You receive a call from the parent of a 6-year-old boy with type 1 diabetes who is managed using an insulin pump. He woke up complaining of a tummy ache this morning and now has vomited. His blood glucose is 436 mg/dl now and was 138 mg/dl when he went to bed last night. He is playing a video game. They just dipped his urine and it shows large ketones. How should you advise this family?
   A. Send him directly to the emergency room.
   B. Due to the rise in his blood sugar without carbohydrate intake, you assume the insulin pump infusion site has failed, and advise the family to replace the site.
   C. The family should administer a correction dose of insulin.
   D. They should check for ketones to determine how aggressive the acute plan should be.
   E. Encourage him to frequently sip water, and once his stomach is feeling better, he should drink a lot of water.
3. The father of a 10-year-old boy recently diagnosed with type 1 diabetes calls your office for advice. The boy will be participating in a soccer tournament this weekend and will be playing for multiple hours. He usually has a low blood sugar during soccer practices. What strategies might you advise?
   A. He should keep his glucometer, water, a sugar-containing beverage, and snacks in his bag on the side of the field.
   B. If he feels like his blood sugar is low, he should remain in the game until a time out is called.
   C. He should decrease his morning long- and short-acting insulin doses since exercise increases a person's sensitivity to insulin.
   D. He should have snacks available in addition to a sugar-containing beverage and use these to maintain his blood sugar >150 mg/dl.
   E. He should monitor for low blood sugars that evening and consider decreasing his evening insulin doses.

Answers

1. C
2. B, C, D, E
3. A, C, D, E

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Type 2 Diabetes Mellitus in Youth

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Key Points

- There has been a significant rise in the incidence of type 2 diabetes in youth over the past two decades, parallel to the rise in childhood obesity.
- The incidence of new cases of type 2 diabetes in children is greater in minority populations and females and in youth between the ages of 10 and 19 years compared with younger children.
- Insulin and metformin are currently the only two medications that are approved for the treatment of type 2 diabetes in children.
- The rates of comorbidities and complications in youth with type 2 diabetes are high, even when compared to adults with type 2 diabetes and youth with type 1 diabetes.
- The management of type 2 diabetes involves a multidisciplinary approach that is sensitive to the cultural and financial background of the patient and should ideally include a team consisting of a physician, diabetes nurse educator, registered dietitian, and behavioral specialist or social worker.
- There is a crucial need for better preventive and therapeutic strategies for the treatment of type 2 diabetes in youth.

32.1 Introduction and Background

Type 2 diabetes has emerged as a global epidemic and as one of the most burdensome chronic diseases of our time. Although once thought to be exclusively a disease of adulthood, it has increased at an alarming rate in youth, paralleling the rise in obesity. The Centers for Disease Control and Prevention (CDC) recently published estimated projections for prevalence of type 2 diabetes in children based on data from the SEARCH for Diabetes in Youth study, a population-based observational study of diabetes in youth in the United States [1]. In the year 2009, SEARCH estimated that more than 20,000 youth in the United States younger than the age of 20 years had type 2 diabetes. Assuming a 2.3% annual increase, the prevalence of type 2 diabetes in youth less than 20 years of age is expected to quadruple in the next 40 years [2]. Type 2 diabetes and its comorbidities are significant risk factors for the development of cardiovascular and microvascular complications. As the risk of complications increases with the duration of the disease, children represent an extremely high risk population, and youth with type 2 diabetes have higher rates of complications when compared with adults with type 2 diabetes or children with type 1 diabetes [3, 4]. Presently we have limited therapeutic options for the treatment of type 2 diabetes youth and the development of preventative strategies and effective therapeutic modalities is most crucial. Complete characterization of the pathogenesis of the disease remains an important step as better understanding of the disease process will lead to the emergence of better therapeutic and preventative strategies. This chapter describes the various aspects of type 2 diabetes in youth.

32.2 Epidemiology of Type 2 Diabetes in Youth

There has been a worldwide increase in the prevalence of type 2 diabetes in children and adolescents. Since 2000, the SEARCH for Diabetes in Youth study funded by the CDC and the National Institutes of Health has provided us with very important data on the epidemiologic pattern of diabetes in US children. SEARCH estimated that between the years of 2008 and 2009, 5089 people younger than 20 years were newly diagnosed with type 2 diabetes annually. The rate of new cases of type 2 diabetes was greater among older youth between the ages of 10 and 19 years compared with younger children, and higher rates were found in minority populations and females [5]. SEARCH also reported data demonstrating the increase in prevalence of diabetes between 2001 and 2009. The overall prevalence of type 2 diabetes in youth was 0.34 per 1000 (95% CI, 0.31–0.37) in 2001 and 0.46 per 1000 (95% CI, 0.43–0.49) in 2009. This is an alarming relative increase of 35% (95% CI, 21.4–50.0%) [6]. Increases in prevalence have occurred in both males and females, across all age groups between 10 and 19 years of age, and in white, Hispanic, and black youth. However, despite significant increases in prevalence rates of childhood obesity in the United States during the past two decades, the rates of type 2 diabetes mellitus among youth at the population level have not followed a trend similar to that seen in adults.

There remains a mismatch between rates of childhood obesity and type 2 diabetes, which is likely due to the substantial latency between onset of obesity and the related risk for type 2 diabetes [7].

The incidence of type 2 diabetes is increasing in many countries concurrent with the rise in rates of obesity [8], and not just in the United States. Observational reports and case series from Asia and Europe have indicated a similar pattern of increase. Some examples illustrate the global burden of type 2 diabetes in youth. In Japan, an oral glucose tolerance test was conducted to confirm the diagnosis of diabetes in children identified to have glycosuria during urine screening of over 8 million school children in the greater Tokyo area from 1974 to 2002. While the overall annual incidence of type 2 diabetes was 2.63/100,000, the annual incidence after 1981 was significantly higher than that before 1981 (2.76 vs. 1.73/100,000, $P < 0.0001$). Consistent with the pattern observed in the United States, the annual incidence was significantly higher for older children between 13 and 15 years of age when compared with children who were 12 years of age or younger (6.43 vs. 0.78/100,000, $P < 0.0001$) [9]. In the United Kingdom, the prevalence of type 2 diabetes increased over time in all ethnic groups in West Yorkshire between 1999 and 2006, and a significant excess of the disease burden was in South Asians. The overall incidence of type 2 diabetes was 2.5/100, 000 (95% CI, 2.2–2.7/100, 000) and was significantly lower for non-South Asians at 1.8/100, 000 (95% CI, 1.6–2.1/100,000) when compared with 6.9/100,000 (95% CI 5.6–8.1) for South Asians [10]. Case reports and clinic-based studies have also reported an increasing prevalence of type 2 diabetes in children in India [11].

32.3 Pathophysiology

The pathophysiology of type 2 diabetes in youth is distinct from the disease process in type 1 diabetes and is similar to that in adults with type 2 diabetes. Failure of compensatory beta-cell insulin secretion in the presence of increased insulin resistance appears to be the key factor necessary for the development of type 2 diabetes. Impaired glucose tolerance is an intermediate stage in the natural history of type 2 diabetes mellitus and is an independent predictor of the risk of developing type 2 diabetes and cardiovascular disease. In adults the rate of progression of impaired glucose tolerance to diabetes is estimated at 5–8% per year [12]; little is known about the natural history of impaired glucose tolerance in children although available literature suggests that the rate of beta-cell decline is more rapid than in adults [13], with clinicians often missing the impaired glucose tolerance phase. Although it is well established that deterioration in both insulin sensitivity and beta-cell function is a critical step in the natural course of type 2 diabetes, their temporal relationship in the progression of disease is still debated. More studies are needed to understand the sequence of development of these abnormalities to better define those subjects at increased risk and potentially identify individuals who would benefit most from interventions.

Type 2 diabetes is a complex disease characterized by the interaction between many genes and environmental factors. Obesity predisposes to type 2 diabetes because of increased peripheral resistance to insulin-mediated glucose uptake. While obesity is overwhelmingly the most important risk factor for the development of type 2 diabetes, not all obese children go on to develop type 2 diabetes, and some children develop type 2 diabetes at a relatively lower body mass index percentile than others suggesting the involvement of other factors in disease pathogenesis. SEARCH reported that in the United States, among youth with type 2 diabetes, the prevalence of overweight was 10.4% and obesity was 79.4% [14].

32.3.1 Effect of Puberty

Many youth with type 2 diabetes manifest the disease during active puberty. Puberty is associated with a transient physiological reduction in insulin sensitivity even in lean healthy children and insulin resistance can increase up to 50% during puberty. Insulin resistance in puberty is believed to be related in part to an increase in growth hormone levels and is normally associated with a compensatory increase in insulin secretion in children without diabetes.

32.3.2 Genetic Susceptibility

Type 2 diabetes is a heritable condition. The high concordance rates for type 2 diabetes in identical twins [15], the clustering of type 2 diabetes in fami-

lies [16], and the increasing prevalence of type 2 diabetes among certain populations such as Native Americans [17] all lend support to the existence of genetic determinants for type 2 diabetes. In the Framingham offspring study, a cohort study in which participants are primarily Caucasian and at relatively low risk for diabetes, both parental and offspring phenotypes was ascertained by direct examination. The study showed that the risk for type 2 diabetes among offspring with a single diabetic parent was 3.5-fold higher, and for those with two diabetic parents was 6-fold higher compared with offspring without parental diabetes [18]. In the Treatment Options for Type 2 Diabetes in Youth and Adolescents (TODAY) study, a clinical trial of therapeutic strategies for type 2 diabetes in youth, almost 60% of subjects reported at least one parent, full sibling, or half-sibling with diabetes, and almost 90% had an affected grandparent [5]. Linkage analysis studies have led to the identification of specific genes that cause monogenic forms of diabetes such as maturity-onset diabetes of the young (MODY). However, these rare monogenic causes of diabetes are identified in only a small fraction of youth with diabetes. The common form of type 2 diabetes is polygenic and caused by the interaction between a large number of common and rare variants with the environment. Over 80 common genetic variants have been associated with type 2 diabetes using genome-wide association studies [16–25], but each individual variant only contributes a small percentage of the disease risk. All variants that have been identified to date explain less than 15% of the estimated type 2 diabetes heritability [26], and the mechanism of disease risk remains largely unclear.

32.4 Clinical Features

Given the current obesity epidemic, distinguishing between type 1 and type 2 diabetes in children can be difficult. Nevertheless, accurate diagnosis is critical as treatment regimens, educational approaches, dietary advice, and outcomes differ markedly between the two conditions. Most children with type 2 diabetes are overweight or obese at the time of diagnosis and present with glucosuria, usually without ketonuria, absent or mild polyuria and polydipsia, and little or no weight loss [27]. About 40% of children and adolescents with type 2 diabetes have no symptoms on presentation but are discovered on screening based on risk factors such as obesity, the presence of acanthosis nigricans on physical examination, or glycosuria detected during a medical evaluation [28]. Acanthosis nigricans is a clinical finding associated with the insulin resistance and obesity that is very often seen in children with type 2 diabetes. This cutaneous finding is characterized by velvety, hyperpigmented patches most prominent around the neck and other intertriginous regions and is present in as many as 90% of children with type 2 diabetes [29]. Acanthosis nigricans is speculated to be a result of insulin action on cutaneous IGF receptors. In adolescent girls, polycystic ovarian syndrome (PCOS) is associated with insulin resistance, and girls with PCOS are also at increased risk for the development of type 2 diabetes [30].

32.4.1 Diabetic Ketoacidosis

Although most children who present with diabetic ketoacidosis (DKA) at diagnosis are subsequently found to have type 1 diabetes, occasionally children with type 2 diabetes present in DKA, characterized by hyperglycemia, ketosis, and acidosis. In children with type 2 diabetes, a severe clinical presentation of polyuria, polydipsia, weight loss and fatigue, and severe dehydration similar to that seen in DKA with type 1 diabetes can confuse the diagnosis. In a retrospective case review of 69 children between the ages of 9 and 18 years admitted for DKA at a tertiary care center in Northern California, 9 (13.0%, 95% CI [6.1–23.3%]) were found to have clinical and laboratory features consistent with type 2 diabetes on follow-up [31]. Sometimes the distinction of type 2 diabetes from type 1 diabetes cannot be made until months after the presentation in DKA, when insulin requirements decline and the patient can be weaned off insulin. Children in DKA typically require hospitalization with intravenous insulin replacement therapy, rehydration, and close laboratory and clinical monitoring in an intensive care setting.

32.4.2 Hyperosmolar Hyperglycemic State

Hyperosmolar hyperglycemic state (HHS) is a rare and serious acute complication of type 2 diabetes. HHS is usually associated with more severe

hyperglycemia than DKA but is typically not characterized by ketoacidosis. It is characterized by marked hyperglycemia, hyperosmolality, and severe dehydration without ketonuria or acidosis (although mild ketosis may be present in HHS). Rapid and accurate diagnosis of HHS is important because it is associated with high morbidity and mortality if not treated appropriately [32].

32.5 Screening for Type 2 Diabetes

Type 2 diabetes is often asymptomatic at presentation, but screening the entire adolescent population for diabetes is not cost-effective, and targeted screening of high-risk groups is a more reasonable strategy. The American Diabetes Association (ADA) recommends that screening for type 2 diabetes should be considered in children who are overweight or obese and have two or more additional risk factors for diabetes. These risk factors include (1) family history of type 2 diabetes in a first- or second-degree relative; (2) Native American, African-American, Latino, Asian American, or Pacific Islander race/ethnicities; (3) evidence of insulin resistance or conditions associated with insulin resistance (acanthosis nigricans, hypertension, dyslipidemia, polycystic ovary syndrome, or small-for-gestational-age birth weight); and (4) maternal history of diabetes or gestational diabetes mellitus during the child's gestation. The ADA recommends that screening should be initiated at the age of 10 years or at onset of puberty, if puberty occurs at a younger age, and should be repeated every 3 years [33]. Additionally, screening will help identify individuals with pre-diabetes, the term used for youth who are at high risk for future development of diabetes, and includes children with impaired fasting glucose and impaired glucose tolerance. Such individuals may also be at increased risk for cardiovascular disease independent of associated risk factors [34]. As there are data to suggest that the onset of type 2 diabetes can be prevented or delayed in some individuals, screening can lead to a potential therapeutic intervention in youth who are at high risk for the development of the disease [35]. With the limited evidence base that we have related to diabetes prevention in children, lifestyle intervention with the goal to prevent excessive weight gain should be recommended for all children at risk for the development of type 2 diabetes. There is insufficient evidence and lack of long-term safety data in children to recommend the use of metformin or other medications to prevent or delay the onset of diabetes.

32.6 Diagnosis of Type 2 Diabetes in Youth

The ADA criteria for the diagnosis of both type 2 diabetes and prediabetes in children are essentially the same as in adults. The criteria for the diagnosis of type 2 diabetes in youth are the presence of one of the following conditions: (1) fasting plasma glucose ≥126 mg/dl (7.0 mmol/l); (2) 2-h plasma glucose ≥200 mg/dl (11.1 mmol/l) with a 75 g oral glucose tolerance test (OGTT); (3) HbA1C ≥ 6.5%; or (4) a random plasma glucose ≥200 mg/dl in a patient with classic symptoms of hyperglycemia or hyperglycemic crisis. Other than in the case of unequivocal hyperglycemia, the results should be confirmed by repeat testing. Similar to adults, the criteria for the diagnosis of prediabetes in youth are the presence of one of the following: (1) fasting plasma glucose 100 mg/dl (5.6 mmol/l) to 125 mg/dl (6.9 mmol/l); (2) 2-h plasma glucose 140 mg/dl (7.8 mmol/l) to 199 mg/dl (11.0 mmol/l) during a 75 g OGTT; or (3) HbA1C 5.7–6.4%. It is important to remember that for all three tests, the risk is continuous, extending below the lower limit of the range and becoming disproportionately greater at the higher end of the range [33].

It is critical to take race/ethnicity and the presence of anemia and hemoglobinopathies into consideration when using HbA1C to diagnose type 2 diabetes as these factors may influence the value obtained. Also, it is important to understand that the studies that lead to the recommendation that HbA1C be used as a diagnostic criterion were entirely carried out in adult populations, and therefore it remains unclear if the use of the HbA1C or the same HbA1C cut point is appropriate in the pediatric population [36, 37]. The ADA in its guidelines acknowledges the limited data supporting the HbA1C for diagnosing type 2 diabetes in children and adolescents but still recommends the HbA1C for the diagnosis of diabetes because of ease of obtaining it in the non-fasting state. The HbA1C should not be from a point-of-care device and should be done in a laboratory that uses a method that is certified by

the National Glycohemoglobin Standardization Program. Unless there are clear clinical features (e.g., marked hyperglycemia at the time of diagnosis), a test on a second blood sample is generally required for the confirmation of diabetes.

It is important to distinguish between type 1 and type 2 diabetes because the overall treatment and long-term management strategies differ between the two diseases. Unfortunately, no single laboratory result can unequivocally distinguish between type 1 and type 2 diabetes, and the distinction has to be made using a combination of clinical findings, laboratory studies, and medical judgment over time. Young people with type 2 diabetes are generally obese or overweight, typically present during puberty, and show clinical evidence of insulin resistance in the form of acanthosis nigricans. These children typically also have an immediate family history of type 2 diabetes, and many belong to minority racial and ethnic groups. Hyperketonemia and ketonuria are infrequently associated with type 2 diabetes and are easy and rapid laboratory markers, although not very specific. In general, the presence of serum pancreatic autoantibodies is confirmatory of type 1 diabetes, and these are probably the most useful laboratory measures to differentiate between the two conditions. The antibodies most commonly measured are anti-glutamic acid carboxylase (GAD), anti-tyrosine phosphatase (IA-2), and anti-insulin antibodies. Anti-insulin antibodies are useful up to 2 weeks after initiation of insulin therapy. Recently, assay of an antibody to zinc transporter-8 (ZnT8) has been introduced and may increase the diagnostic utility of antibody measurement in type 1 diabetes [38]. The TODAY clinical trial estimated the frequency of markers of pancreatic autoimmunity in youth between the ages of 10 and 17 years clinically diagnosed with type 2 diabetes who were screened for trial participation. Of the 1206 subjects screened, 118 (9.8%) were positive for either GAD-65 or IA-2 autoantibodies; of these, 71 (5.9%) were positive for a single antibody, and 47 were positive (3.9%) for both antibodies. Diabetes autoantibody-positive subjects were more likely to be white (40.7% vs. 19%, $P < 0.0001$) and male (51.7% vs. 35.7%, $P < 0.0007$). The antibody-positive subjects were less likely to display the metabolic syndrome phenotype clinically associated with type 2 diabetes although there was much overlap [39].

The challenge of characterizing the type of diabetes in youth was also described by the SEARCH for Diabetes in Youth study. SEARCH conducted a comprehensive assessment of 2291 subjects who were less than 20 years of age with a physician-established diagnosis of either type 1 or type 2 diabetes. Using the presence of at least one of two diabetes autoantibodies – GAD-65 and IA-2 antibodies – and a surrogate measure of insulin sensitivity validated against hyperinsulinemic-euglycemic clamps as markers, they classified youth into four categories: autoimmune plus insulin-sensitive, autoimmune plus insulin-resistant, non-autoimmune plus insulin-sensitive, and non-autoimmune plus insulin-resistant. Most of the subjects who fell into the non-autoimmune plus insulin-resistant categories (15.9%) had characteristics that aligned with a clinical phenotype of type 2 diabetes. The group classified as autoimmune plus insulin-resistant (19.5%) had similar prevalence and titers of diabetes autoantibodies and similar distribution of human leukocyte antigen risk genotypes as those in the autoimmune plus insulin-sensitive group, suggesting that it included obese youth with type 1 diabetes [40].

C-peptide levels as a measure of insulin secretion are generally higher in children with type 2 diabetes when compared with type 1 diabetes. However, this measure is only reliable when the patient has a stable metabolic status. C-peptide levels decrease during states of acute decompensation, even in youth with type 2 diabetes. Therefore there is overlap in values of C-peptide levels during the acute presentation of both type 1 and type 2 diabetes [41].

32.7 Treatment of Type 2 Diabetes in Youth

As in adults, treatment goals for youth with type 2 diabetes include achieving and maintaining near-normal glycemia and preventing and reducing the risk of long-term vascular complications, as well the appropriate management of comorbidities including hypertension, dyslipidemia, and non-alcoholic fatty liver disease. The ADA recommends a multidisciplinary diabetes management team, including a physician, diabetes nurse educator, registered dietitian, and behavioral specialist or social worker, for optimal management of type 2 diabetes [42]. The type of

treatment initiated at the time of diagnosis will vary according to clinical presentation. Recently, an American Academy of Pediatrics subcommittee, with the support of the ADA, the Pediatric Endocrine Society, the American Academy of Family Physicians, and the Academy of Nutrition and Dietetics, developed practice guidelines for the management of type 2 diabetes in children and adolescents [43]. This guideline supported lifestyle change and metformin as mainstays of diabetes management and insulin therapy for children who present in acute decompensation or do not reach goal glycemic control with lifestyle and metformin.

The TODAY study is a landmark multicenter clinical trial that evaluated therapeutic options for type 2 diabetes in youth between the ages of 10 and 17 years. All participants were initially treated with metformin alone and received standard diabetes education focused on healthy eating and exercise to attain a glycated hemoglobin level of less than 8%. Subsequently 699 children were randomized to continue treatment with 1000 mg twice a day of metformin alone, metformin combined with 4 mg twice a day of rosiglitazone, or an intensive lifestyle intervention program that focused on weight loss. The mean follow-up period for the study was 3.8 years, and the primary outcome was loss of glycemic control (defined as a HbA1C of greater than or equal to 8%) for at least 6 months or sustained metabolic decompensation requiring insulin for 3 months or more. The study showed that metformin plus rosiglitazone was superior to metformin alone ($P = 0.006$) and that treatment with metformin plus lifestyle intervention was not significantly different from treatment with metformin alone, with failure rates of 51.7%, 38.6%, and 46.6% in the metformin, metformin plus rosiglitazone, and metformin plus lifestyle intervention arms, respectively. Overall 45.6% of study participants experienced treatment failure irrespective of treatment regimen, and the median time to treatment failure was 11.5 months. Treatment failure rates in youth were higher than in comparable adult studies, and the failure of the lifestyle arm to make major improvement in metabolic control was different from previous studies in adults with pre-diabetes or early diabetes [44, 45]. This study has set the stage for a relook at how young people with type 2 diabetes should be managed.

It is essential to initiate insulin therapy for youth with type 2 diabetes who are ketotic at initial presentation or who are in diabetic ketoacidosis. Insulin therapy should also be initiated in youth in whom the distinction between type 1 diabetes and type 2 diabetes is unclear. In most cases, insulin therapy should be initiated in children who have a random blood glucose concentration that is greater than or equal to 250 mg/dl or in whom the HbA1C is greater than 9%. Initiation of insulin therapy in such children, at least on a short-term basis, allows for quicker restoration of glycemic control. Many patients can be weaned gradually from insulin therapy and subsequently managed for some period of time with metformin and lifestyle modification. Despite the surprising outcome of the TODAY study, given the limited medical options for the treatment of type 2 diabetes in young people, clinical consensus remains that in all patients, providers should initiate a lifestyle modification program aimed at weight loss, which incorporates both nutritional modification and physical activity, as well as start metformin therapy.

32.7.1 Lifestyle Modification

Non-pharmacological measures of diet and physical exercise aimed at weight reduction must remain as initial steps in the management of youth with type 2 diabetes. Although difficult to achieve and maintain long term, weight loss improves glycemic control and the underlying insulin resistance and should be an essential part of the successful management of type 2 diabetes in children. The current literature is inconclusive about a single best meal strategy for patients with diabetes, and there are very few studies addressing this issue in children. Practice guidelines suggest that youth with type 2 diabetes follow the Academy of Nutrition and Dietetics pediatric weight management evidence-based nutrition practice guidelines [46]. Children should be referred to a registered dietitian who has experience in the nutritional needs of youth with type 2 diabetes. Some of the stronger recommendations of the guidelines include the following:

1. Interventions to reduce weight should be multicomponent and include diet, physical activity, nutritional counseling, and parent or caregiver participation.

2. A nutrition prescription should be formulated as part of the dietary intervention in a multicomponent pediatric weight management program.
3. Dietary factors that may be associated with an increased risk of being overweight are increased total dietary fat intake and increased intake of calorically sweetened beverages.
4. Dietary factors that may be associated with a decreased risk of being overweight are increased fruit and vegetable intake.
5. A balanced micronutrient diet that contains no fewer than 900 kcal per day is recommended to improve weight status in children 6–12 years of age who are medically monitored.
6. A balanced micronutrient diet that contains no fewer than 1200 kcal per day is recommended to improve weight status in adolescents 13–18 years of age who are medically monitored.

Regardless of the type of meal plan prescribed, nutritional education must be provided to the patient and the family to maximize adherence and to obtain positive results. This education should encourage patients to follow some basic healthy eating patterns, such as eating regular meals and snacks and not eating while watching television or while using the computer or other electronics [47]. Common dietary recommendations should also stress choosing calorie-free beverages (and probably not artificially sweetened substitutes), except for milk, reducing portion sizes, and reducing fast food consumption [48]. A family-centered approach to nutrition and lifestyle modification is crucial in children with type 2 diabetes, and nutrition recommendations should be culturally appropriate and sensitive to the financial structure and needs of the family.

In terms of a weight goal for youth with type 2 diabetes, the ideal goal for weight reduction is to reach a BMI that is less than or equal to the 85th percentile for age and gender. In children who are still actively growing, maintenance of weight without further weight gain will lead to a reduction in BMI over time. However, most often children with type 2 diabetes are in mid to late puberty and severely obese, and therefore weight reduction is recommended.

Physical activity is an essential component of weight management for the treatment of type 2 diabetes in youth. Providers should encourage children to engage in moderate-to-vigorous exercise for at least 60 min daily [49] and to limit screen time, which contributes to a sedentary lifestyle, to less than 2 hours per day [50].

32.7.2 Pharmacological Agents

Metformin and insulin are presently the only two agents approved by the US Food and Drug Administration (US FDA) for the treatment of type 2 diabetes in children. Other agents that are used in adults are not presently FDA approved in children either because they have failed noninferiority testing against metformin or because they lack adequate safety and efficacy data in the pediatric population.

32.7.2.1 Metformin

Metformin is the first-line pharmacologic therapy for treatment of type 2 diabetes in children. The guidelines recommend that metformin be initiated along with lifestyle intervention in all children with type 2 diabetes (unless insulin is required) due to the low success rate of lifestyle intervention alone in pediatric patients. Metformin is a biguanide that largely acts by decreasing hepatic glucose output by inhibiting gluconeogenesis. In addition, metformin increases insulin-mediated glucose utilization in peripheral tissues. This is done by altering energy metabolism in the cell, primarily by mitochondrial inhibition. The molecular mechanism of action of metformin is not completely understood but is thought to be mediated through activation of the enzyme 5′ adenosine monophosphate kinase, which in addition to modulating insulin signaling is also thought to have positive downstream effects on lipid metabolism [51].

Metformin can cause some weight loss on initiation of treatment but is overall considered a weight-neutral drug. The most bothersome side effects seen with metformin therapy are gastrointestinal and include abdominal pain, bloating, change in appetite, and diarrhea. These adverse effects are often transient and disappear with continuation of the medication. Metformin is generally better tolerated when taken with meals. To minimize gastrointestinal side effects seen with

metformin therapy, the dose should be started at 500 mg once a day and increased by 500 mg every week to reach a maximum daily dose of 2000 mg a day in divided doses. Overall, metformin is considered to be a very safe drug but is contraindicated in children with liver disease, impaired renal function, or in cases of cardiopulmonary dysfunction because of the small risk of lactic acidosis [52]. It should be discontinued during acute illness or before imaging studies requiring a contrast agent.

Metformin generally is effective in the initial phases of diabetes treatment, but unfortunately in many cases, monotherapy is not effective, and so the addition of insulin may be required. In the TODAY study, metformin alone provided durable glycemic control in approximately only half of the subjects [13], again suggesting that many youth with type 2 diabetes are likely to require combination treatment within a few years of diagnosis.

32.7.2.2 Insulin

Insulin therapy is recommended during the acute presentation of type 2 diabetes or in cases of severe hyperglycemia as described earlier. In addition, a high proportion of children with type 2 diabetes will ultimately go on to require insulin therapy. Similar to the management of type 1 diabetes, both long-acting agents, such as insulin glargine and insulin detemir, and rapid-acting agents, such as insulin lispro and aspart, should be considered based on the individual patient, severity of disease, the ability to perform self-blood glucose monitoring, and the risk of hypoglycemia. Insulin pump therapy can also be considered in children with significant insulin needs.

32.7.2.3 Thiazolidinediones

Thiazolidinediones are a group of drugs that act primarily by increasing hepatic and peripheral insulin sensitivity and by preserving insulin secretion. Thiazolidinediones are thought to act by binding and activating the nuclear transcription factor PPAR-γ (peroxisome proliferator-activated receptor-gamma), thereby regulating transcription of several genes involved in glucose and lipid metabolism [53]. Pioglitazone also exerts some PPAR-α effects that might account for some differences in pharmacological effects compared to the other thiazolidinediones. Rosiglitazone was used in the TODAY trial and in combination with metformin provided better glycemic control than metformin monotherapy and metformin in conjunction with lifestyle intervention, but at the cost of weight gain [13]. Thiazolidinediones have subsequently fallen out of favor because of their side effect profile and are presently not FDA approved for use in children.

32.7.2.4 Sulfonylureas

Sulfonylureas are oral therapeutic agents that act as insulin secretagogues. This drug class was studied in a 6-week, single-blinded, multinational study that randomized 285 pediatric subjects to receive either glimepiride at a dose that ranged between 1 and 8 mg daily or metformin at a dose between 500 mg and 1000 mg twice daily for 24 weeks. The primary end point was mean change in HbA1C from baseline to week 24. The safety profile of glimepiride was assessed by the incidence of hypoglycemia and other adverse events. The results of the study showed that glimepiride reduced HbA1C similarly to metformin but at the cost of greater weight gain, and there was comparable safety over the 24 weeks of the study [54]. None of the sulfonylureas are FDA approved for use in children.

32.7.2.5 Other Drugs

There are limited pediatric data regarding other drugs used in adults such as glucagon-like-peptide-1 (GLP-1) analogs, dipeptidyl-peptidase-4 (DPP-4) inhibitors, meglitinide derivatives, amylin analogs, and sodium-glucose transport protein-2 (SGLT2) inhibitors, and they are not recommended in children. In spite of the rising prevalence of type 2 diabetes in children, most pivotal clinical pediatric trials that are necessary for FDA approval of drugs are facing the challenge of enrolling an adequate number of pediatric patients, despite global recruitment efforts [55].

32.7.3 Bariatric Surgery

Bariatric surgical procedures are effective interventions for treating type 2 diabetes in adults who are extremely obese and refractory to medical therapy. Improvement in metabolic control is often evident within days to weeks following Roux-en-Y gastric bypass surgery, most likely reflecting an alteration in metabolism that is independent of weight loss [56]. At present, there is a limited but increasing body of literature to support the use of

bariatric surgery for carefully selected adolescents with extreme obesity. Diabetic adolescents with extreme obesity experience significant weight loss and similar rapid remission of type 2 diabetes mellitus after Roux-en-Y gastric bypass as adults with improvements in insulin resistance, beta-cell function, and cardiovascular risk factors [57].

32.7.4 Treatment Monitoring

Guidelines from the ADA recommend that the target HbA1C should be less than 7%. Patients should have individualized short-term goals with the ultimate goal of reaching the target HbA1C. Diabetes therapy should be intensified if possible when the HbA1C is above target. In patients who are on insulin therapy, HbA1C measurement should be done every 3 months, and finger stick blood glucose monitoring should be performed at least three times daily. For other patients using non-insulin therapy and in those with stable HbA1C values that are within target range, HbA1C measurement may only be required every 6 months. In such patients, fasting and 2-h postprandial finger stick blood glucose levels after the largest meal of the day, a few times a week, can provide valuable information on the range of glycemic excursion.

32.8 Comorbidities of Type 2 Diabetes in Youth

In many cases, comorbidities are already present at the time of diagnosis in youth with type 2 diabetes. Youth with type 2 diabetes are at increased risk for other obesity-associated disorders including hypertension, dyslipidemia, and non-alcoholic fatty liver disease. These increase the already high risk for long-term vascular and other complications. Therefore it is crucial to appropriately manage them in conjunction with the diabetes.

32.8.1 Hypertension

Hypertension increases the risk of future cardiovascular disease risk and renal disease in a child with type 2 diabetes. The TODAY study found that at enrollment, 11.6% of participants had hypertension with a mean age of 14 ± 2 years and mean duration of diabetes of 7.8 ± 5.8 months. The prevalence of hypertension increased to 33.8% by the end of the study, with an average follow-up of 3.9 years [58]. The SEARCH study found that hypertension was present in 65% of 95 youth with type 2 diabetes of mean disease duration of 2.1 (SD 1.6) years who were enrolled in the study between 2001 and 2003 [59]. Blood pressure should be measured accurately and reviewed at each visit. Both systolic and diastolic blood pressures should be below the 90th percentile for age, gender, and height (or below 120/80, whichever is lower) according to the National Heart, Lung, and Blood Institute standards. The initial treatment of elevated blood pressure should consist of efforts at lifestyle changes aimed a weight loss reduction and limitation of dietary salt. If there is persistent elevation of blood pressure after 6 months of lifestyle modifications, pharmacological management should be initiated. Angiotensin-converting enzyme (ACE) inhibitors are generally recommended for initial treatment of hypertension because they reduce the risk of progressive renal disease. In cases when adequate control of hypertension is not achieved, referral to a specialist trained in the treatment of hypertension in youth is recommended [60].

32.8.2 Dyslipidemia

In the TODAY study, at baseline, 4.5% of participants had low-density lipoprotein cholesterol (LDL-C) levels that were greater than 130 mg/dl or were already receiving lipid-lowering therapy. After 36 months of follow-up, the prevalence of youth with elevated LDL-C levels or on lipid-lowering agents increased to 10.7%. At baseline, 21% of youth had elevated triglyceride levels, and this number rose to 23.3% after 3 years [61]. The SEARCH study found that 65% of 41 youth with type 2 diabetes had hypertriglyceridemia and 60% of 38 youth had a low high-density lipoprotein cholesterol level [62]. Current recommendations by the ADA and American Heart Association are that at the initial evaluation, all patients with type 2 diabetes should have a baseline complete fasting lipid profile after initial glycemic control has been established, with follow-up testing based on these findings, or every 2 years thereafter if initial results are normal [63]. The guidelines suggest that if the initial LDL-C concentration is greater than or equal to 130 mg/dl, dietary counseling by a nutritionist is necessary. LDL-C measurements should be repeated after 6 months, and if levels

are still between 130 and 160 mg/dl, statin therapy should be initiated, with a goal LDL-C of less than 130 mg/dl and an ideal target of less than 100 mg/dl. Lovastatin, simvastatin, pravastatin, rosuvastatin, and atorvastatin are the statins approved by the US FDA for use in children. Treatment should be initiated with the lowest recommended dose for each statin and titrated based on LDL-C measurements and side effects. As an example, atorvastatin can be initiated at 5 mg, and the dose titrated upward as needed to the maximum daily dose. The maximum daily dose for atorvastatin in adults is 80 mg per day, but a dose of only up to 20 mg per day has been examined in pediatric clinic trials. Similarly, fluvastatin can be initiated at 20 mg and titrated up to a maximum dose of 80 mg per day [64]. Some side effects that are common to many of the statins include muscle injury and hepatic and renal dysfunction. It is also very important to note that statins are teratogenic and therefore should not be used in the absence of effective contraception in adolescent girls who are sexually active. For triglycerides, if the initial concentrations are between 150 and 600 mg/dl, patients should be given appropriate dietary guidance. If levels are between 700 and 1000 mg/dl at the initial or follow-up visit, fibrate or niacin therapy should be considered if the patient is older than 10 years because of the increased risk of pancreatitis at these concentrations. Although there are limited data on the use of fibric acid derivatives in children, agents such as gemfibrozil and fenofibrate are used in cases of severe hypertriglyceridemia. Gemfibrozil is typically administered at a dose of 1200 mg a day, and fenofibrate can be initiated at 40 mg and titrated upward to close to 200 mg based on the preparation used. Niacin is rarely used because of multiple intolerable side effects that include flushing, abdominal pain, vomiting, headache, and elevated serum aminotransferase levels [65].

32.8.3 Nonalcoholic Fatty Liver Disease

Nonalcoholic fatty liver disease (NAFLD) refers to the presence of hepatic steatosis with or without inflammation and fibrosis in the absence of other causes for hepatic steatosis. NAFLD may progress to cirrhosis and liver failure. In the TODAY study among the 927 youth at initial screening with a median duration of type 2 diabetes of 2 months, 6.5% had elevated aminotransferase levels that were 1.5 to 2.5 times the upper limits of normal [66]. Screening for NAFLD should be done by obtaining aminotransferase levels at the time of diagnosis of type 2 diabetes and annually afterward if found to be normal. For the management of suspected NAFLD, a trial of gradual weight reduction is recommended. If hepatic abnormalities persist after weight reduction, referral to a pediatric gastroenterologist is necessary, and a liver biopsy is generally required to confirm the diagnosis. Some of the drugs that are being tested in clinical trials for the treatment of NAFLD include vitamin E, metformin, and pioglitazone, a thiazolidinedione. So far, none of these agents have been shown to be sufficiently superior to lifestyle intervention alone to warrant specific recommendations for clinical use [67, 68]. A well- conducted randomized control trial that included 178 youth with NAFLD demonstrated that there was no benefit from either vitamin E or metformin on the primary outcome of reducing serum aminotransferase levels [69]. However, the same study showed that over time there was better histologic resolution of steatohepatitis in those treated with vitamin E when compared with metformin or placebo. Some of this improvement may have been due to weight loss, because all patients were given advice on lifestyle intervention throughout the study, and the mean BMI z-score decreased slightly in each group. There are no well-designed studies of pioglitazone use for NAFLD in children.

32.8.4 Depression

Depression is a significant comorbidity of type 2 diabetes in children and can complicate the medical management of diabetes and adherence to treatment. The identification of depression with the use of appropriate screening tools and referral to a specialist when necessary should be essential components of type 2 diabetes management in youth.

32.9 Complications of Type 2 Diabetes in Youth

Type 2 diabetes is a major risk factor for the future development of cardiovascular disease. The microvascular complications of type 2 diabetes include retinopathy, nephropathy, and both peripheral and autonomic neuropathy.

32.9.1 Retinopathy

Diabetic retinopathy can lead to impaired vision and visual loss. Results from both the TODAY and SEARCH studies indicate that there is a substantial prevalence of diabetic retinopathy in youth with type 2 diabetes. In the TODAY study, eye examinations with retinal photographs were taken in 517 subjects, after they had diabetes for an average of 4.9 years. Retinopathy was present in 13.7% of participants. The prevalence of retinopathy increased with age, duration of diabetes, and mean HbA1C [70]. Similarly, SEARCH evaluated the prevalence of retinopathy in 423 children with type 2 diabetes with mean disease duration of 6.8 years using non-mydriatic retinal photography of both eyes. The prevalence of diabetic retinopathy was 17% for subjects with type 1 diabetes and 42% for subjects with type 2 diabetes. Average HbA1C was significantly higher in subjects with diabetic retinopathy compared to subjects without diabetic retinopathy (mean 9.4% vs. 8.6%, $P = 0.015$) [71]. The ADA provides recommendations for the identification and management of diabetic retinopathy in adolescents with type 2 diabetes. Patients should have an initial dilated and comprehensive eye examination performed by an ophthalmologist or optometrist shortly after diabetes diagnosis. Subsequent examinations by an ophthalmologist should be repeated annually, and less frequent examinations may be considered after one or more normal eye examinations. Providers should promptly refer patients with any retinal changes to an ophthalmologist who is knowledgeable and experienced in the diagnosis and treatment of diabetic retinopathy.

32.9.2 Nephropathy

Diabetic nephropathy is a complication of type 2 diabetes and a major cause of chronic renal disease in adults. Moderately increased albuminuria, formerly called "microalbuminuria," is defined as urinary albumin excretion between 30 and 300 mg/day or between 30 and 300 mg/g creatinine on a random urine sample, and severely increased albuminuria, formerly called "macroalbuminuria," is defined as urinary albumin excretion above 300 mg/day or above 300 mg/g creatinine on a random urine sample. Moderately increased albuminuria precedes the development of severely increased albuminuria and is considered to be a finding that predicts high risk for future nephropathy. In the TODAY cohort, moderately increased albuminuria was found in 6.3% of participants at baseline and rose to 16.6% by end of study, regardless of treatment arm. Glycemic control was the only significant predictor of risk of microalbuminuria [58].

The SEARCH study also evaluated the presence of increased albumin-to-creatinine ratio in 2885 children with type 1 diabetes with a mean duration of diabetes of 3.7 years and 375 youth with type 2 diabetes with a mean duration of diabetes of 1.9 years. The prevalence of elevated albumin-to-creatinine ratio was 2.3 times higher in youth with type 2 diabetes (22.2%, 95% CI 18.3–26.7) when compared with youth with type 1 diabetes (9.2%, 95% CI, 8.2–10.3) [72].

According to the ADA, screening for albuminuria with a random spot urine sample for albumin-to-creatinine ratio should begin at the time of type 2 diabetes diagnosis and be repeated annually. Albumin excretion is influenced by various factors including exercise and can vary from day to day. Therefore an abnormal value should be repeated. Orthostatic proteinuria is not uncommon in adolescents and is usually considered benign. Therefore all patients with documented albuminuria should have a first morning urine void immediately on waking to determine the albuminuria is benign. Persistent albuminuria requires referral to a pediatric nephrologist for further management. Initial treatment of persistent albuminuria is with an ACE inhibitor and should be initiated, even if the blood pressure is not elevated. Enalapril, fosinopril, and lisinopril are among the ACE inhibitors that are FDA approved for use in children. Typically, treatment is initiated with 5 mg of enalapril or 2.5 mg of lisinopril a day and can be titrated upward as needed. In the case of side effects such as persistent cough that are associated with ACE inhibitors, an angiotensin receptor blocker (ARB) such as losartan can be used at an initial dose of 25 mg a day. Both ACE inhibitors and ARBs are teratogenic, and adolescent girls should be counseled on effective contraception before use. Further monitoring of urinary albumin levels should be at 3 to 6 month intervals to assess both the patient's response to therapy and the disease progression with the goal to achieve as normal an albumin-to-creatinine ratio as possible.

32.9.3 Neuropathy

Diabetic neuropathy includes both peripheral and autonomic neuropathy. The prevalence of

nephropathy has not been systematically studied in youth with type 2 diabetes. However, similar to other complications, the risk of diabetic neuropathy in adults has been shown to increase with increasing duration of disease and is related to glycemic control. Youth with type 2 diabetes should be screened for peripheral neuropathy by performing a 10 g monofilament exam over the plantar surface of the foot to detect loss of sensation as well as undergo simple examination of vibration perception using a 128 Hz tuning fork over the interphalangeal joint of the right hallux at least annually. Patients should be referred to a neurologist if abnormalities are detected on exam.

32.9.4 Macrovascular Complications

Macrovascular complications include coronary artery, peripheral, and cerebral vascular disease and are major sources of morbidity and mortality in adults with type 2 diabetes [73]. Currently, we lack formal evidence on the incidence of these complications in children, but they are thought to be exceedingly rare. However, results from adult studies indicate that the combination of poor glycemic control and additional risk factors such as hyperlipidemia and hypertension increases the risk of these complications. The TODAY study performed echocardiograms in 455 youth with a mean age of 18 years and a mean duration of diabetes of 4.5 years. The results showed that 16.2% of participants had adverse measures of cardiac structure and function that were positively related to BMI and blood pressure but were not related to treatment of their diabetes [74]. These findings offer preliminary evidence of the risk of cardiac complications in youth with type 2 diabetes, and further long-term studies are needed to better evaluate macrovascular complications.

32.10 Conclusions

Type 2 diabetes, although still relatively uncommon in childhood, is increasing at an alarming rate in youth and is associated with significant comorbidities and diabetes complications. These youth are at future risk for the development of long-term macrovascular and microvascular complications, which pose an enormous public health challenge. There is limited literature on disease pathogenesis in children, and therapeutic options are few and not widely effective. Further studies are crucial to better understand the underlying basis of the disease in youth and for the development of effective preventative and therapeutic strategies.

Case Study

An Adolescent with Diabetic Ketoacidosis

A 17-year-old young man, whose parents are from Central America, presented with diabetic ketoacidosis. He has a history of polyuria and polydipsia for about 2 weeks before presentation. His mother, who has obesity, said she has problems with "blood sugar." On presentation, he was disoriented and had a venous pH of 7.1, blood glucose of 633 mg/dl, and ketonuria. His HbA1c was 11.2%. He was 173 cm tall and weighed 145 Kg. He was treated successfully with insulin and rehydration and was discharged from the hospital receiving 80 units of insulin glargine daily and a short-acting insulin analog before each meal.

You see him again in one month. At that time, the single most important factor to determine whether he has type 2 diabetes and not type 1 diabetes is:

A. Ability to wean off insulin in a month
B. Absence of anti-GAD and IA2 antibodies
C. Absence of anti-insulin antibodies
D. High serum level of C-peptide
E. Presence of known type 2 diabetes-related genes

Discussion

Correct answer: B.

Absence of antibodies to the two most commonly measured anti-pancreatic antibodies (GAD and IA2) is the best single confirmation that this is not type 1 diabetes, the most common form of diabetes in young people. Individuals who present in diabetic ketoacidosis, but have type 2 diabetes, may take longer than 1 month to be weaned fully from insulin, or this may never be possible if beta-cell reserves are limited. Anti-insulin antibodies are likely to be present in an individual who has been taking insulin for a month. High levels of serum C-peptide are seen in type 2 diabetes, but an individual with slowly progressive type 1 diabetes may also release C-peptide, and the absolute values may overlap. Currently known type 2 diabetes-related genes explain only a small part of the genetic basis of type 2 diabetes.

Review Questions

1. You are caring for a 15-year-old girl with newly diagnosed type 2 diabetes and a HbA1c of 8.5%. Her mother who also has type 2 diabetes asks if her daughter can use the same drugs that have worked so successfully for her diabetes and include a GLP-1 analog and metformin. You tell her:
   A. We will start with metformin alone because the GLP-1 analog is not approved by the FDA for use in children.
   B. We will start with metformin alone because this medication is often sufficient as treatment for some period of time in young people with type 2 diabetes.
   C. We will start with metformin and then if necessary add insulin because these two drugs are approved by the FDA for use in children.
   D. We will start with metformin and also try to intensively modify her lifestyle in order to help her improve her diabetes control.
   E. All of the above.
2. An obese 14-year-old boy with type 2 diabetes is at increased risk of:
   A. Autoimmune thyroiditis, hyperlipidemia, and depression
   B. Depression, cardiac arrhythmia, and hypertension
   C. Hyperlipidemia, nonalcoholic fatty liver, and depression
   D. Hypertension, arthritis, and nonalcoholic fatty liver disease
   E. Hypertension, nonalcoholic fatty liver, and autoimmune thyroiditis
3. The most important factor in the development of type 2 diabetes in young people is:
   A. Excess growth hormone secretion
   B. Failure of glucagon secretion
   C. Insulin resistance and relative lack of insulin secretion
   D. Insulin resistance
   E. Obesity

Answers

1. E
2. C
3. C

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Disorders of Lipid Metabolism

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Key Points

- Screening for abnormal lipids with a non-fasting lipid panel should occur as part of universal screening at ages 9–11 and 17–21 years in order to identify children and adolescents with primary lipid disorders at risk for early CVD.
- Secondary causes of hyperlipidemia, such as obesity, are the most common etiology of hyperlipidemia, especially when the lipid abnormality is mild to moderate. Frequently, more severe lipid abnormalities are from either a primary cause (i.e., polygenic or heterozygous FH) or a mix of a primary with a secondary condition such as obesity. Homozygous primary causes of hyperlipidemia are exceedingly rare.
- Heterozygous FH should be suspected if LDL is >190 mg/dl or >160 mg/dl with a family history of early CVD. FH always requires dietary restriction of cholesterol and often requires pharmacologic management, generally in the form of statin therapy in addition to lifestyle modification.
- Lifestyle modification is the first line of treatment for all abnormal lipid states, with the focus on dietary restriction of total and saturated fat as well as total cholesterol. Reducing high glycemic index foods in cases of hypertriglyceridemia is recommended as well as regular exercise to maintain normal insulin sensitivity and cardiovascular fitness.
- Lipemic serum should alert one to severe hyperlipidemia, most commonly hypertriglyceridemia. Lifestyle modification with dietary management is the cornerstone of treatment for children; medication, including fibrates or omega-3 fatty acids, is often needed in adulthood to avoid pancreatitis.

33.1 Introduction

Cardiovascular disease is typically thought of as an adult disorder; however, its predecessor, atherosclerosis, typically begins during childhood. An abnormal lipid profile is one contributing factor to atherosclerosis that may increase the risk of future premature cardiovascular events. Normal lipid physiology is based on lipid production and metabolism. Metabolism occurs by three interconnected pathways: exogenous, endogenous, and reverse cholesterol transport. Abnormalities in these processes result in lipid disorders, which can be acquired or genetic. Acquired lipid disorders are secondary to etiologies such as obesity, type 1 and 2 diabetes, metabolic syndrome, or chronic renal failure. Genetic mutations are inherited abnormalities that in some cases, such as heterozygous familial hypercholesterolemia, have been shown to cause abnormal changes in lipid profiles, endothelial dysfunction, and carotid intima thickness as early as infancy.

In 2011 the National Heart, Lung, and Blood Institute (NHLBI) released new controversial screening guidelines for non-fasting cholesterol levels in all children at 9–11 years old and again at 17–21 years old in order to identify genetic and acquired causes of lipid disorders. With an estimate of 21% of children and adolescents in the United States having high total cholesterol, low HDL cholesterol, or high non-HDL cholesterol [1], screening allows for identification of both mild abnormalities treated with lifestyle modification as well as more severe hyperlipidemia requiring both dietary and pharmacologic management. This chapter reviews the diagnosis, presentation, and management of disorders of HDL, LDL, and triglyceride metabolism.

33.2 Normal Physiology

In order to understand the pathology of lipid disorders, a brief review of the normal physiological processes of lipid metabolism and production will be provided. Lipids are metabolized via three intersecting pathways (Fig. 33.1): exogenous (or dietary), endogenous (or hepatic), and reverse cholesterol transport.

The exogenous pathway (Fig. 33.1: green arrows) involves dietary triglycerides (TG) and cholesterol, which comes from animal sources such as meat, fish, eggs, and dairy. After a meal, dietary TG are hydrolyzed in the intestine to free fatty acids and monoglycerides, which are absorbed by enterocytes and either resynthesized to form TG or assembled with ApoB-48 into chylomicrons. Chylomicrons (CM) enter the plasma,

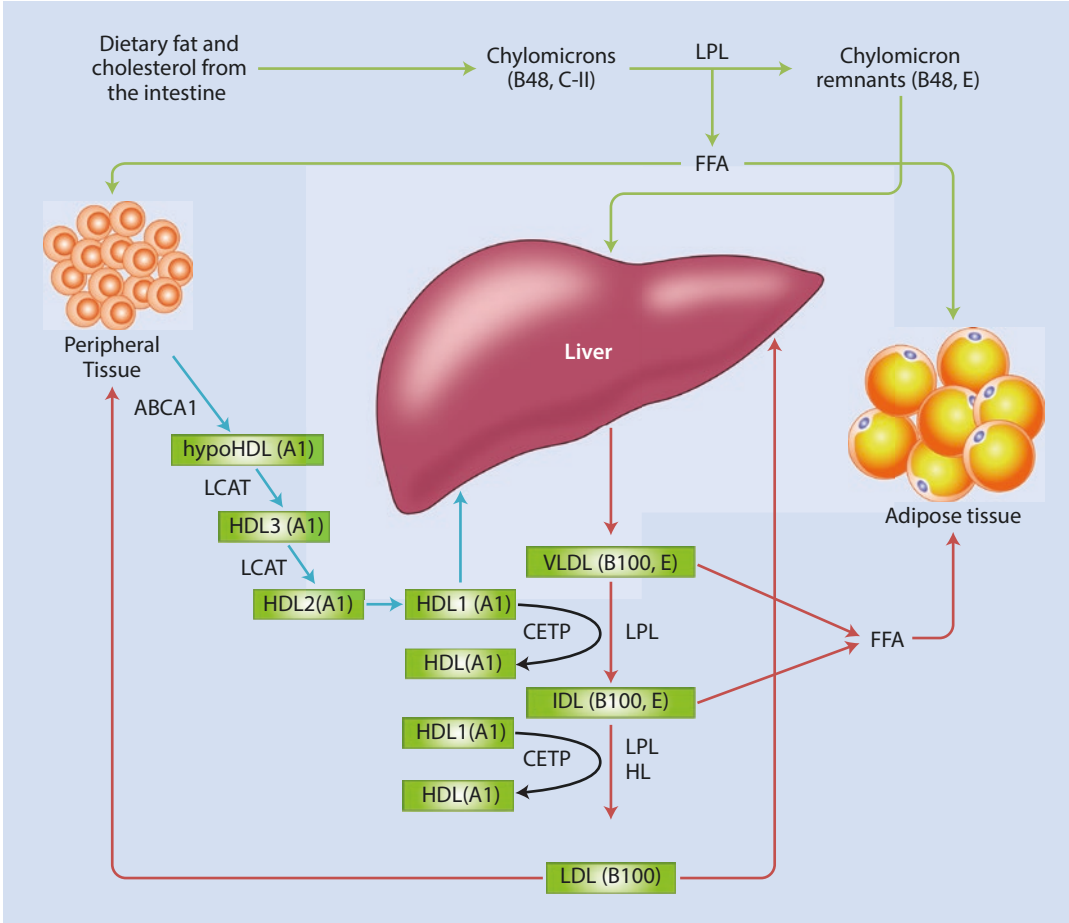

Fig. 33.1 Normal lipid physiology. The exogenous pathway is shown with *green arrows*. Dietary TG are hydrolyzed in the intestine to free fatty acids and monoglycerides, which are absorbed by enterocytes and either resynthesized to form TG or assembled with ApoB-48 into chylomicrons (CM). CM enter the plasma and its TG are hydrolyzed by lipoprotein lipase (LPL). The resulting CM remnant has acquired ApoE, which helps deliver TG and cholesterol to the liver. In the endogenous pathway, shown with *red arrows*, very-low-density lipoprotein (VLDL) synthesized in the liver is released into the plasma where it undergoes conversion to intermediate- and then low-density lipoprotein (IDL and LDL). VLDL, IDL, and LDL are taken up by the liver or by peripheral tissues to deliver cholesterol and fat-soluble vitamins. Reverse cholesterol transport, shown with *blue arrows*, involves the acquisition of cholesterol by HDL and delivery back to the liver via specific HDL receptors. Nascent HDL particles, also referred to as HDL3, acquire free cholesterol through the ATP-binding cassette transporter 1. Enrichment with CE via activation of lecithin-cholesterol acyltransferase by ApoA1 leads to mature HDL1, which can transfer CE via CETP to VLDL and IDL to form LDL, or it can be hydrolyzed by hepatic lipase to smaller HDL particles that are more easily excreted

and its TG are hydrolyzed by lipoprotein lipase (LPL) with its cofactor ApoC-II, resulting in the release of free fatty acids for uptake by tissues, including adipose tissue. The resulting CM remnant has acquired ApoE, which binds both remnant receptors and LDL receptors to deliver TG and cholesterol to the liver. LPL activity and clearance of remnants by ApoE binding liver receptors are the major regulators of post-prandial TG levels.

In the endogenous pathway (Fig. 33.1: red arrows), VLDL synthesized in the liver is released into the plasma where it undergoes conversion to IDL and then LDL [2]. TG and cholesterol are assembled around ApoB-100 into very-low-density lipoproteins (VLDL) in the liver and released into the plasma. VLDL particles are approximately 80% TG and 20% cholesterol esters (CE). After further hydrolysis by LPL and acceptance of CE from high-density

lipoproteins (HDL) by cholesterol ester transport protein (CETP), VLDL particles become intermediate-density lipoproteins (IDL). IDL is further hydrolyzed by LPL and hepatic lipase and accepts transfer of more CE from HDL by CETP to become low-density lipoproteins (LDL). VLDL and IDL remnants are taken up by the liver through ApoE binding to both remnant and LDL receptors, while LDL binds only to LDL receptors through ApoB-100. VLDL, IDL, and LDL can also be taken up by peripheral tissues to deliver cholesterol and fat-soluble vitamins [3]. Regulation of this pathway includes insulin activation of LPL to promote lipolysis of VLDL-containing TG. Fasting TG levels reflect VLDL synthesis, metabolism, and clearance of remnants. LDL levels are the result of LDL synthesis and clearance of LDL by its receptor.

Endogenous cholesterol is produced in a number of tissues including the gonads, adrenals, brain, and intestine, with about 20% of cholesterol production occurring in the liver. Cholesterol synthesis begins with three molecules of acetate that form 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA), and then via HMG-CoA reductase, mevalonic acid is synthesized. The formation of mevalonic acid is the rate-limiting step in cholesterol synthesis, and HMG-CoA reductase is the target of cholesterol-lowering therapy known as statins. Through a series of steps involving conversion of mevalonic acid to farnesyl pyrophosphate and lanosterol, cholesterol is formed. Cholesterol can be used to synthesize bile acids and is secreted into the intestine or excreted as free cholesterol in the bile. About half of the cholesterol that enters the intestine is reabsorbed and recirculated to the liver, while the other half is excreted in feces. Hepatic cholesterol stores originate from three sources: dietary cholesterol delivered by CM remnants, endocytosis of LDL through the binding of ApoB-100 to the LDL receptor, and de novo cholesterol synthesis from HMG-CoA. Clearance of LDL is increased by upregulation of LDL receptors when dietary cholesterol is restricted or de novo synthesis is inhibited by statins.

Reverse cholesterol transport (Fig. 33.1: blue arrows) involves the acquisition of cholesterol by HDL and delivery back to the liver via specific HDL receptors. Nascent HDL particles, also referred to as HDL3, are disc-shaped molecules synthesized from cholesterol and phospholipids in the liver and intestines that acquire free cholesterol through the ATP-binding cassette transporter 1 (ABCA1). ApoA1 on the surface of HDL3 activates lecithin-cholesterol acyltransferase (LCAT), which converts free cholesterol into CE thus transforming HDL3 into larger particles known as HDL2. Further enrichment with CE forms the larger HDL1 particle. This mature HDL particle can bind to its receptor through ApoA1and deliver CE to the liver (reverse cholesterol transport) or to cholesterol-requiring tissues such as the gonads and adrenals. HDL1 can transfer CE via CETP to VLDL and IDL particles to form LDL, or it can be hydrolyzed by hepatic lipase to smaller HDL particles that are more easily excreted. HDL levels are therefore determined by its synthesis and clearance: insulin increases HDL levels by inhibiting hepatic lipase and thus reducing HDL metabolism and subsequent clearance.

33.3 General Cholesterol Screening

Screening recommendations have been recently revised through the NHLBI guidelines on hypercholesterolemia in order to identify more children with primary hyperlipidemias associated with a risk of early cardiovascular disease (CVD). Screening is recommended from ages 2 to 8 years only in high-risk individuals, which includes patients with type 1 or type 2 diabetes mellitus, Kawasaki disease with current coronary artery aneurysm, chronic renal failure/post-renal transplant/end-stage renal disease, or post-orthotopic heart transplant [1]. Patients with a family history of hypercholesterolemia and early CVD should also be screened before the age of 8 years. Universal cholesterol screening is recommended in individuals between the ages of 9 and 11 years and again between the ages of 17 and 21 years in order to identify and treat children and adolescents with severe hyperlipidemia such as familial hypercholesterolemia who have not been identified through family history. Non-fasting cholesterol levels are satisfactory for screening, but if total cholesterol (TC) ≥200 mg/dl OR non-HDL-cholesterol (TC-HDL) ≥145 mg/dl, a repeat fasting lipid profile should be drawn [4]. Guidelines for plasma lipid profile interpretation for children and adolescents are outlined in Table 33.1. A summary of the major lipid disorders is found in Table 33.2.

Table 33.1 Guidelines for plasma lipid profile interpretation for children and adolescents

	Normal/acceptable	Borderline	Abnormal
Non-fasting lipids			
Total cholesterol	<170 mg/dL	170–199 mg/dL	≥200 mg/dL
Non-HDL cholesterol	<145 mg/dL		≥145 mg/dL
HDL	>40 mg/dL		≤40 mg/dL
Fasting lipids			
Total cholesterol	<170 mg/dL	170–199 mg/dL	≥200 mg/dL
LDL	<110 mg/dL	110–129 mg/dL	≥130 mg/dL
HDL	>45 mg/dL	40–45 mg/dL	<40 mg/dL
Apolipoprotein B (ApoB)	<90 mg/dL	90–109 mg/dL	≥110 mg/dL
Apolipoprotein A1 (ApoA1)	>120 mg/dL	115–120 mg/dL	<115 mg/dL

Modified from NHLBI

Table 33.2 Summary of common lipid disorders in children and adolescents (Modified from NHLBI guidelines for hyperlipidemia treatment)

Primary lipid disorder	Lipid/lipoprotein abnormality	Comments
Familial combined hyperlipidemia	Type IIa - ↑ LDL	Family members may have different types of FCHL
	Type IIb -↓HDL, ↑LDL,VLDL, TG	Family Hx frequently positive for CVE*
	Type IV - ↑VLDL, ↑TG, ↓ HDL	Associated with T2DM and metabolic syndrome in adults
Polygenic hypercholesterolemia	↑ LDL	Most common type of hypercholesterolemia Result of multiple different mutations Low risk of CVE* Dietary/lifestyle modification often successful
Heterozygous familial hypercholesterolemia	↑ LDL	Autosomal dominant LDL >190 mg/dl confers an ~80% risk for FH Frequently requires pharmacologic treatment despite dietary modification Moderate risk of CVE*
Homozygous familial hypercholesterolemia	↑↑ LDL	Variable expression based on gene mutations Xanthomas, xanthelasmas, and corneal arcus Despite lifestyle modification and pharmacologic treatment, difficult to control
Familial defective apolipoprotein B-100	↑ LDL	Autosomal dominant Features similar to FH May require pharmacologic treatment despite lifestyle modifications Moderate risk of CVE*

Table 33.2 (continued)

Primary lipid disorder	Lipid/lipoprotein abnormality	Comments
Familial hypertriglyceridemia	↑ VLDL, ↑ TG	Most commonly due to Apo B100 deficiency Autosomal dominant Risk of pancreatitis with TG levels >1000 mg/dl Low risk of CVE* Pharmacologic intervention if TG > 1000 mg/dl despite lifestyle modifications
Familial hypobetalipoproteinemia	↓LDL, ↓total cholesterol	Rare Autosomal codominant Reduced risk of CVE*; however liver and neurologic dysfunction can be present Treatment: Fat-soluble vitamins if with neurologic findings and low-fat diet if with liver dysfunction
Familial hypoalphalipoproteinemia	↓ HDL	HDL typically <30 mg/dl Mildly increased risk of CVE* Treatment: Lifestyle modifications +/– statin therapy

* CVE cardiovascular event

33.4 Hypertriglyceridemia

33.4.1 Introduction and Background

Hypertriglyceridemia is defined as fasting plasma TG levels that are greater than or equal to the 95th percentile for age and gender [5]. Based on NHLBI screening guidelines, this corresponds to fasting TG ≥ 100 mg/dl in children between the ages of 0 and 9 years and fasting TG ≥ 130 mg/dl in children between the ages of 10 and 19 years [6]. Hypertriglyceridemia can be divided into primary and secondary forms. Primary forms of hypertriglyceridemia are hereditary, while secondary forms result from diseases that can lead to elevated TG, such as obesity, type 1 and type 2 diabetes, hypothyroidism, metabolic syndrome, renal disease, nonalcoholic fatty liver disease, and chronic alcohol use [4, 5]. Medications that alter lipid metabolism and lead to elevated TG would be considered a secondary cause as well. These include antiretroviral therapy, selective estrogen receptor modulators, beta blockers, some chemotherapeutic agents, isotretinoin, oral contraceptives, some antipsychotics, and systemic glucocorticoids [3, 4, 7].

There are multiple classifications of hypertriglyceridemia based on etiology and risk of pancreatitis [3, 8]. The Endocrine Society classifies hypertriglyceridemia into mild (150–200 mg/dl), moderate (200–999 mg/dl), severe (1000–1999 mg/dl), and very severe (≥ 2000 mg/dl). The American Academy of Pediatrics suggested criteria based on age, with hypertriglyceridemia defined as triglyceride levels >100 mg/dl in children under 10 years of age and >130 mg/dl in children 10–18 years of age.

33.4.2 Etiology

Elevated TG are a result of either increased production from the liver and intestine or decreased catabolism in the periphery. For a list of primary and secondary causes of hypertriglyceridemia, see Table 33.3.

33.4.2.1 Primary Causes

The most severe cause of primary hypertriglyceridemia is familial chylomicronemia (also known as hyperlipoproteinemia type 1 by the Frederickson classification), which is a deficiency of lipoprotein lipase (LPL), the enzyme that catalyzes the hydrolysis of TG carried by chylomicrons and VLDL in the peripheral circulation. The disease is inherited in an autosomal recessive pattern with 100% penetrance. The gene for LPL is located on chromosome 8p22, and to date over 163 mutations have been

Table 33.3 Causes of hypertriglyceridemia

Primary	Secondary
LPL deficiency	Obesity
Apo C-II deficiency	Type 1 diabetes
Apo AV deficiency	Type 2 diabetes
GPIHBP1 deficiency	Uncontrolled hypothyroidism
Apo E deficiency	Lipodystrophy
Lipase maturation factor 1 deficiency	Nonalcoholic fatty liver disease

identified, 90% of which are loss of function mutations. The disease is rare, with an occurrence of 1 in 500,000–1,000,000 for homozygous LPL deficiency and associated with severe hypertriglyceridemia (typically TG > 5000 mg/dl). Heterozygous forms have wide variability in TG levels and are more prevalent, likely occurring in 1 of every 500 individuals [9].

LPL binds glycosylphosphatidylinositol-anchored high-density lipoprotein-binding protein 1 (GPIHBP1), which serves as a platform for lipolysis. The gene for GPIHBP1 is located on chromosome 8q24.3, and deficiency is inherited in an autosomal recessive pattern. Genetic mutations in *GPIHBP1* lead to a deficiency of this binding protein and have been associated with hypertriglyceridemia. Homozygous mutations lead to TG levels similar to those found in LPL deficiency (>1000 mg/dl), while heterozygous mutations have not been shown to cause abnormalities in TG levels [10].

Apolipoprotein C-II is a component of chylomicrons, VLDL, LDL, and HDL and is required for activation of LPL. A deficiency in ApoC-II leads to hypertriglyceridemia due to elevated TG-containing particles (chylomicrons and VLDL) as well as decreased LDL and decreased HDL [11]. The gene for ApoC-II is located on chromosome 19q13.32. Deficiency is inherited in an autosomal recessive pattern; heterozygotes have normal TG levels despite low levels of ApoC-II. Interestingly, elevated concentrations of ApoC-II also hinder LPL activity and result in hypertriglyceridemia [11].

ApoA-V via interaction with GPIHBP1 and LPL stimulates LPL activity [12]. A deficiency in ApoA-V caused by genetic mutations in the *Apo A5* gene located on chromosome 11q23 leads to hypertriglyceridemia.

Type III hyperlipoproteinemia or dysbetalipoproteinemia, also known as chylomicron remnant disease, is an autosomal recessive disorder characterized by a mutation in the *Apo E* gene, located on chromosome 19q13.32, leading to impaired uptake of Apo E-containing lipoproteins (CM remnant, VLDL/IDL remnants) by the liver. ApoE is expressed in three isoforms (ApoE2, ApoE3, and ApoE4) which have variable binding affinity to the remnant receptor and the LDL receptor. Some of these isoforms, in the presence of another genetic defect or cause of altered lipid metabolism such as insulin resistance or deficiency, lead to reduced clearance of remnants [3] and hypertriglyceridemia, hypercholesterolemia, and low HDL. These patients are at high risk for pancreatitis and possible early CVD. Other causes of severe hypertriglyceridemia include mutations in lipase maturation factor 1 (LMF1), which is required for transport and activation of LPL [3]. The gene encoding LMF1 is on chromosome 16p13.3, and deficiencies are inherited in an autosomal recessive pattern.

A more common etiology of hypertriglyceridemia that runs in families is familial hypertriglyceridemia, with a prevalence of 5–10%. It has an autosomal dominant pattern and is thought to be polygenic in origin, with some cases displaying heterozygous mutations in LPL or overproduction of VLDL [5]. Triglyceride levels tend to be moderately elevated, and a positive family history of hypertriglyceridemia and/or pancreatitis tends to be present.

33.4.2.2 Secondary Hypertriglyceridemia

The most common secondary cause of mild-moderate (200–500 mg/dl) hypertriglyceridemia is obesity-associated insulin resistance. The prevalence of obesity in the pediatric population has increased parallel to the increase in obesity rates in adults. The most recent data from the CDC indicate that the prevalence of obesity is 17.7% in children aged 2–19 years. Poor diet habits include a deficit in fruit and vegetable intake, increased intake of processed foods and fast foods, as well as

an increase in portion sizes. This has not only led to rising BMIs and waist circumferences, but to elevations in TG, total cholesterol and LDL, and decreases in HDL as well. The term dyslipidemia is used to describe hypertriglyceridemia along with low HDL levels and is commonly seen in cases of obesity. In addition, the consumption of excessive amounts of sucrose and fructose has been linked to elevated triglycerides as fructose metabolism bypasses a key rate-determining step in glycolysis and produces glycerol-3-phosphate, which is the backbone of triacylglycerol [13]. Insulin is a powerful regulator of LPL activity. Therefore in conditions of insulin deficiency such as type 1 or type 2 diabetes mellitus, or insulin resistance associated with obesity, decreased stimulation of LPL can lead to elevated TG. In addition to hypertriglyceridemia, a higher proportion of small dense LDL particles and a change in the ApoA-I/ApoA-II ratio in HDL are features of both type 2 diabetes and the metabolic syndrome [3].

Thyroid hormone has been shown to increase LPL activity via upregulation of ApoA-V activity [14]; thus, hypothyroidism is associated with elevated triglycerides. Hypertriglyceridemia is the most common dyslipidemia in patients with chronic kidney disease, thought to be secondary to defective clearance of TG from the circulation in addition to a decrease in LPL activity, although the reason for the latter is not understood. Nonalcoholic fatty liver disease is heralded by the accumulation of TG in the hepatocytes. This is associated with obesity-related insulin resistance, and the mechanism is similar to that observed in states of type 2 diabetes, metabolic syndrome, and obesity [15]. Alcohol consumption has been found to decrease LPL activity and induce TG accumulation through increased fatty acid synthesis and decreased oxidation in the liver, resulting in alcoholic fatty liver disease [16].

Lipodystrophy syndromes are common causes of hypertriglyceridemia, with over 70% of people with congenital lipodystrophy exhibiting elevated TG [3]. Lipodystrophy syndromes are a wide spectrum of congenital and acquired disorders that are characterized by a complete or partial lack of adipose tissue. The most common form of lipodystrophy is seen in HIV patients and is caused by antiretroviral therapy. The mechanism of hypertriglyceridemia is thought to be a decrease in adipose tissue leading to increased VLDL synthesis and decreased clearance.

33.4.3 Clinical Presentation

Symptoms and signs of LPL deficiency tend to occur in infancy or early childhood, while those of ApoC-II deficiency tend to occur in adolescence or adulthood [17]. Other genetic causes of hypertriglyceridemia such as familial hypertriglyceridemia manifest themselves in youth, while secondary causes can arise at any age, depending on the underlying disease.

Physical findings of hypertriglyceridemia include eruptive xanthomas, which are foam cell-filled yellow palpable lesions 2–5 mm in diameter that appear diffusely on extensor surfaces of the limbs, buttocks, and shoulders (Fig. 33.2). These are generally present when triglyceride levels are >2000 mg/dl. Xanthomas occur secondary to phagocytosis of chylomicrons by macrophages. They are generally single lesions and are not painful [17]. Lipemia retinalis is the milky appearance of retinal vessels observed generally at triglyceride levels >3000 mg/dl (Fig. 33.3) [8, 9]. This is reversible and tends not to impact vision. Hepatomegaly and splenomegaly due to infiltration of macrophage foam cells can occur as well. Infants may present with poor feeding and GI bleeding, in addition to eruptive xanthomas.

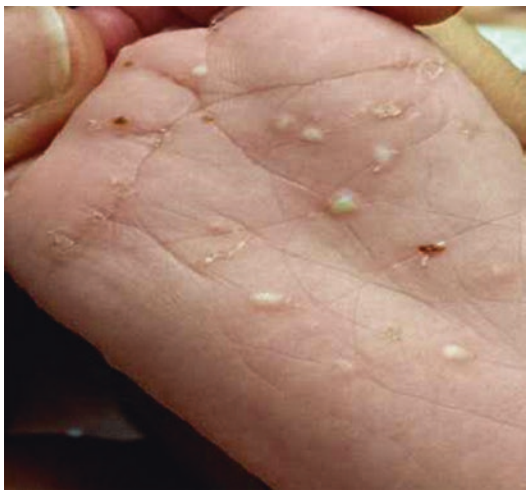

Fig. 33.2 Xanthoma. Xanthomas on the palmar aspect of the foot in a 2-year-old boy with homozygous LPL deficiency

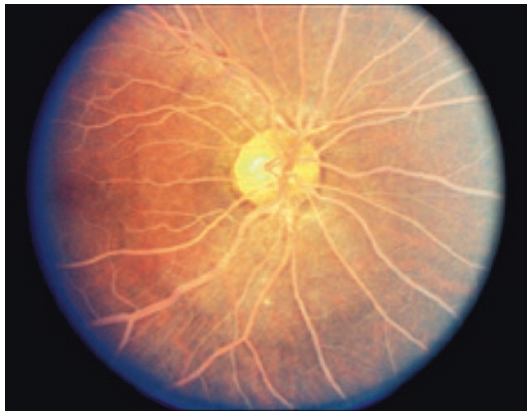

Fig. 33.3 Lipemia retinalis. White and pink retinal vessels due to excessive triglyceride accumulation (Reprinted with permission from OpenStax-CNX. Herbert L. Fred, MDHendrik A. van Dijk, Images of Memorable Cases: Case 45. OpenStax CNX. Dec 3, 2008 ► http://cnx.org/contents/11786e68-a0c1-4675-a4fa-3437db52ae69@3)

33.4.4 Outcomes and Possible Complications

While cardiovascular disease is a concerning outcome of hypertriglyceridemia [18], the most serious clinical consequence of severe hypertriglyceridemia is pancreatitis, the risk of which increases with triglyceride levels >1000 mg/dl. Of note, marked increases in triglyceride levels can impair accurate measurement of amylase, hemoglobin, and electrolyte levels, so one must not rule out pancreatitis in the setting of abdominal pain and high TG, even when lipase and amylase levels are low [9]. The reason for the development of pancreatitis is still unclear but is thought to be in part due to TG hydrolysis within the pancreas leading to toxic accumulation of free fatty acids, as well as CM-induced ischemia [3].

33.4.5 Diagnosis

The presence of lipemic plasma should prompt one to order fasting lipids, including a TG and ApoB level. When presented with a case of hypertriglyceridemia, confirmation with a fasting level is imperative. A careful history of medical problems, medication use, and family history are important to diagnose both primary and secondary causes of hypertriglyceridemia. Thyroid function tests, fasting glucose, hemoglobin A1C, and liver function tests are useful to rule out common secondary causes of hypertriglyceridemia.

Diagnosis of genetic causes of hypertriglyceridemia can be made via genetic analysis for LPL and/or GHIBP1 mutations as well as mutations in ApoE and ApoC-II. Lab results suggesting LPL deficiency include TG > 1000 mg/dl, elevated VLDL with total cholesterol that is 1/10th of the triglyceride levels, and a low HDL. Plasma is lipemic even after an overnight fast in cases where TG levels are >1000 mg/dl. While LDL levels tend to be within normal limits or even low, they cannot be calculated using the "Friedewald equation" if TG ≥ 400 mg/dl, as the assumptions about VLDL are no longer valid. Direct measurement by ultracentrifugation or LDL immunoprecipitation can be performed. Assays that measure LPL activity, through treatment of plasma with heparin, an activator of LPL in the presence of Apo C-II, can also assist in diagnosis of LPL deficiency. Absence of activity indicates LPL deficiency [17]. These assays are no longer commonly performed. The level of ApoB-100 can help distinguish the hypertriglyceridemia-only variant of familial combined hyperlipidemia and some cases of familial hypertriglyceridemia from LPL deficiency as the latter has normal levels, while the former is associated with elevated levels of ApoB-100. Labs suggestive of ApoC-II deficiency include markedly elevated triglyceride levels in addition to low levels of LDL and HDL. Dysbetalipoproteinemia typically has elevated levels of both TG and often LDL due to impaired remnant binding and interference with LDL binding to its receptor. Familial hypertriglyceridemia tends to exhibit moderate elevations in TG with elevated VLDL.

33.4.6 Treatment

While there are no studies to date that link pediatric hypertriglyceridemia with cardiovascular disease risk, a number of studies in adults have reported an increased risk of cardiovascular disease with elevated TG levels [18]. Therefore, treatment is geared toward preventing pancreatitis and also reducing the risk of cardiovascular disease. Table 33.4 lists recommendations for treatment and referral of patients with elevated triglycerides [22]. In the pediatric age group, lifestyle modification continues to be the cornerstone of treatment, with medications reserved for the most severe

Table 33.4 Recommendations for treatment and referral for abnormal triglycerides

TG	Management
≥500 mg/dl	Restrict fat to <15% of total energy intake, increase fiber, and decrease foods with high glycemic index Repeat TG in 3–6 months Omega-3 fatty acids Consider fibrate therapy in a teenager if TG > 1000 mg/dl Referral to a lipid specialist
≥200–499 mg/dl	Restrict fat to <15% of total energy intake, increase fiber, and decrease foods with high glycemic index Repeat TG in 3–6 months Consider omega-3 fatty acids or fibrate therapy Consider referral to a lipid specialist
≥100–199 mg/dl	Restrict fat to <15% of total energy intake, increase fiber, and decrease foods with high glycemic index Repeat TG in 3–6 months

cases. Dietary modification involves restriction of fat to <15% of total energy intake, or no more than 20 g of total fat per day, as well as an increase in fiber intake and a reduction in foods with a high glycemic index [7]. Evidence suggests that high fructose-containing foods lead to higher TG and VLDL production and that a diet low in fructose can improve TG considerably [3]. Increased exercise reduces TG levels through muscle TG oxidation for fuel and improvements in insulin sensitivity. A hypocaloric diet combined with aerobic exercise can reduce TG by 20–50% [8, 19].

Restriction of long-chain fatty acids from the diet (e.g., breast milk and regular formulas) and substituting medium-chain triglyceride formula for infants with primary hypertriglyceridemia such as LPL deficiency is very effective, as medium-chain fats enter the circulation without being incorporated into chylomicrons [9]. This has been used successfully in cases of both LPL [20] and GPIHBP1 deficiencies [21].

Pharmacologic therapy is added when triglyceride levels cannot be reduced as much as necessary through dietary restriction to keep pancreatitis risk low (levels <1000 mg/dl). Fibrates such as fenofibrate or gemfibrozil work via stimulation of PPARα in the liver, which leads to a reduction in VLDL secretion. In addition, through stimulation of LPL gene expression and inhibition of Apo C-III gene expression, fibrates increase triglyceride lipolysis [8]. Fibrates have been shown to reduce TG levels by 30–50% in adults [5]. They are generally well-tolerated without major side effects; however, minor side effects such as GI discomfort have been reported. In rare cases, severe side effects such as liver dysfunction and myopathy may occur [7]. There are limited studies on the use of fibrates in children [22].

High doses of newer statins such as atorvastatin or rosuvastatin as a second-line therapy have been shown to improve triglycerides in adults when levels are <500 mg/dl [5, 8]. The VOYAGER study assessed the impact of statins on TG levels in adults and found that rosuvastatin had a greater reduction in TG than simvastatin and low-dose atorvastatin [23]. At higher but equal doses, the reduction was similar between rosuvastatin and atorvastatin but still greater than with simvastatin. Reductions amounted to 15–31%. Statins have not been found to be effective for primary hypertriglyceridemia secondary to LPL deficiency and are, therefore, not recommended [17].

Niacin has been shown to reduce plasma TG levels in adults by 30–45% via inhibition of hepatic TG synthesis. Doses to achieve this result approach 3 g daily and are associated with adverse effects such as flushing, gastrointestinal discomfort, pruritus, or lightheadedness [5, 8], so they are not used in children. Orlistat has been used in some pediatric cases of hypertriglyceridemia in conjunction with a low-fat diet with some success [21, 24]. Orlistat is a pancreatic lipase inhibitor that reduces intestinal dietary fat absorption. Side effects of the medication include

steatorrhea, fecal incontinence, flatulence, and abdominal pain. While side effects are not severe, they can interfere with patient compliance.

Omega-3 fatty acids have been shown to reduce TG by up to 45% at doses of up to 4 g daily in adult studies by inhibiting hepatic TG and VLDL synthesis [19]. Studies in children and adolescents with hypertriglyceridemia found that doses of 500–1000 mg of omega-3 fatty acids led to a clinically but not statistically significant decrease in TG levels when combined with dietary modification [18, 25], suggesting that higher doses of omega-3 fatty acids may be needed in children. The mechanism by which omega-3 fatty acids leads to an improvement in TG levels is still unclear but may involve impairment of its synthesis through enzyme inhibition and/or a decrease in substrate availability by increasing fatty acid oxidation in the liver [19, 26]. Side effects of omega-3 fatty acids include diarrhea, nausea, eructation, and abdominal pain. Omega-3 fatty acids are ineffective in the treatment of LPL deficiency and are not recommended, although cases have been reported of successful treatment, one of which was in an 8-year-old female [27]. Caution should be used when treating patients with hypercholesterolemia and hypertriglyceridemia, as omega-3 fatty acids have been shown to increase LDL levels [19, 26].

In cases of hypertriglyceridemia-induced pancreatitis, fasting in addition to insulin and dextrose via continuous IV infusion can reduce triglyceride levels within 48 h. Recently, LPL gene therapy has been developed. In phase I and II clinical trials, it has been shown to increase LPL activity and significantly decrease TG levels with no treatment-related adverse events to date [28]. This treatment is currently being studied in those with homozygous LPL deficiency. During episodes of hypertriglyceridemia-induced pancreatitis and in those with homozygous forms of LPL deficiency only, plasmapheresis has been used with success, although TG levels quickly rise without more long-term therapy [29]. ApoC-III inhibition by an antisense inhibitor ISI304801 was shown to reduce triglyceride levels in patients with mild to severe hypertriglyceridemia by up to 80% in phase 2 trials [30]. The medication is currently undergoing phase 3 studies in adults.

If secondary causes of hypertriglyceridemia are present, treatment of the underlying cause generally improves triglyceride levels. Good diabetes management with proper insulin administration can improve triglyceride levels in patients with type 1 diabetes, while weight loss and medical management to improve insulin sensitivity are effective in type 2 diabetes. Replacement with levothyroxine restores normal metabolism and reverses hyperlipidemia in cases of hypothyroidism.

33.5 Hypercholesterolemia

33.5.1 Introduction and Background

Hypercholesterolemia is a heterogeneous group of lipid disorders that are characterized by abnormalities in LDL and, in some cases, HDL and TG. Polygenic hypercholesterolemia is thought to be the most common etiology of genetically inherited hyperlipidemia; however, actual estimates of prevalence are difficult to ascertain given the wide range of mutations and severity of lipid abnormalities that are included in the diagnosis. Heterozygous familial hypercholesterolemia (FH) is one of the most common types of genetic dyslipidemias with gene frequency estimated at 1:500 in the general population [31]. Heterozygous FH is inherited in an autosomal dominant pattern. Certain populations, including Afrikaners, French Canadians, Lebanese, and Finns, have been shown to have an increased risk for FH [31]. In contrast to the heterozygous FH, homozygous FH is extremely rare with estimates of 1:1,000,000 in the general population [32]. It is inherited in an autosomal codominant pattern. Familial combined hyperlipidemia (FCHL) is a heterogeneous group of lipid abnormalities that has been linked to multiple loci including 1q21-23, a gene which encodes for a transcription factor critical in lipid and glucose metabolism, *USF1* [33, 34]. While sometimes difficult to distinguish FCHL from metabolic syndrome, epidemiological data estimate a prevalence of 0.5–2% in the adult population with a higher prevalence in families with a history of premature cardiovascular disease [33]. Familial defective ApoB-100, an autosomal dominant condition, is due to a missense mutation in the LDL receptor-binding domain of ApoB-100 that results in elevated serum LDL due to reduced hepatic LDL removal and catabolism [35]. The prevalence of familial defective ApoB-100 is estimated at 1 in 500 individuals [35].

Table 33.5 Causes of elevated LDL-C

Primary	Secondary
Heterozygous familial hyperlipidemia Mutations typically in LDL-R	Medications Cyclosporines Retinoids Select antiretroviral agents (i.e., protease inhibitors)
Homozygous familial hyperlipidemia Most commonly mutations of two different alleles for genes coding LDL-R, however, can have two mutations of same allele	Chronic kidney disease
Familial combined hyperlipidemia Gene mutations causing a variety of abnormalities in TG, LDL, and HDL levels	Hypothyroidism
Polygenic hyperlipidemia Hypothesized multiple minor mutations or polymorphisms in genes encoding LDL-R, ApoB, and/or PCSK9	Nephrotic syndrome
Familial defective apolipoprotein B Gene mutation in ApoB which impairs binding of LDL to LDL-R	

33.5.2 Etiology

Causes of elevated LDL can be divided into both primary and secondary (see Table 33.5).

33.5.2.1 Primary Hypercholesterolemia

FH is a primary dyslipidemia caused by genetic anomalies that affect the LDL receptor (LDL-R) pathway. It is typically characterized by mutations in the LDL-R gene (*LDLR*), that are estimated to account for >80% of heterozygous FH. Over 1500 mutations in *LDLR* have been described [36]. LDL receptors are responsible for binding LDL through ApoB-100 leading to endocytosis of the LDL particle. Mutations in *LDLR* can decrease the production of LDL-R, affect the expression of LDL-R on cell surfaces or the ability of the receptor to bind LDL, and activate endocytosis. PCSK9, the gene for which is encoded on chromosome 1, aids in the degradation of the LDL-R [35]. A gain of function mutation in *PCSK9* reduces LDL-R recycling to the cell membrane, causing an increase in LDL levels, which phenotypically is similar to mutations in *LDLR*. Mutations in ApoB-100 gene (*APOB*), as seen in familial defective ApoB-100, impair the binding of LDL to the LDL-R, resulting in elevation of serum LDL levels.

In homozygous FH, the severity of the lipid abnormality is related to the genetic mutation involved. Most commonly, mutations of two different alleles for the gene coding the LDL-R are involved; however, occasionally familial homozygotes can have two mutations of the same allele (a true homozygote). The type of mutation dictates the degree of LDL elevation; typically in homozygous FH, the LDL elevation is >300 mg/dl and HDL is low.

Given that most children are clinically asymptomatic and most types of FH are inherited, family history is an essential component in screening, but relying on family history has failed to identify many children with FH. Screening questions should ascertain whether there is a family history of premature CVD (including myocardial infarction, percutaneous catheter interventional procedure, coronary artery bypass grafting, or stroke) in mother, father, siblings, uncles, aunts, and grandparents (men < 55 years old, women < 65 years old); elevated cholesterol in mother, father, and/or siblings; and/or known genetic or suspected inheritable hyperlipidemia. However, given that screening family history is inadequate, the 2011 guidelines recommend universal screening by measuring TC and HDL levels in order to identify these children with no known family history.

The proposed mechanism of polygenic hypercholesterolemia hypothesizes multiple minor mutations or polymorphisms in the genes encoding LDL-R, ApoB, and/or PCSK9, which cumulatively result in abnormalities of lipid metabolism [37].

Typically the mutations result in mild to moderate cholesterol abnormalities (LDL 130–160 mg/dl) without frank early CVD compared to more moderate to severe LDL elevations (180–300 mg/dl) in heterozygous FH.

FCHL is thought to be due to a number of gene mutations (oligogenic) that result in a variety of abnormalities in TG, LDL, and HDL levels, sometimes within the same family [38]. Type IIa is characterized by isolated LDL elevation, whereas elevated VLDL and TG with low HDL is described in type IV. Type IIB is characterized by an elevated TG, VLDL, and LDL with low HDL. Unlike FH, FCHL may not be associated with an elevation in LDL until adulthood, so it can be missed during pediatric screening. FCHL has been shown to be associated with increased levels of ApoB as a result of overproduction of VLDL. Therefore, measuring ApoB levels may be a better screening test in pediatric patients with a family history of early CVD and moderate hypertriglyceridemia with or without elevated LDL.

Perhaps the least well-understood risk factor for cardiovascular disease is lipoprotein(a) (Lp(a)). Lp(a) is an atherogenic and heterogeneous lipoprotein that is linked to cardiovascular disease and stroke. Lp(a) excess is inherited as an autosomal codominant trait and is expressed in children. Lp(a) particles are modified, newly synthesized LDL with an extra lipoprotein named ApoA covalently linked to ApoB-100. ApoA has significant homology to plasminogen, so this likely explains the thrombosis risk associated with elevated Lp(a) levels. Lp(a) has been shown to be expressed in children who are greater than 13 months old at levels equivalent to those found in their parents [39]. Furthermore, pediatric studies have shown that elevation in Lp(a) in pediatric patients correlates with increased rates of cardiovascular events in their parents [40].

33.5.2.2 Secondary Hypercholesterolemia

Secondary hyperlipidemia needs to be ruled out so that potential causes can be appropriately treated. Multiple etiologies exist, including medication side effects, renal disease, endocrine/metabolic issues, inflammatory processes, or hepatic dysfunction. Medications such as cyclosporines have been shown to increase LDL and lipoproteins, retinoids have been shown to increase the LDL to HDL ratio, and short-term use of thiazide diuretics has been shown to increase LDL and TG, although these findings have not been substantiated by long-term studies. Antiretroviral regimens, especially protease inhibitors, have also been associated with abnormalities in lipid and glucose metabolism, often as part of a lipodystrophy syndrome. Renal diseases can result in lipid abnormalities, with nephrotic syndrome typically being the most profound. It is postulated that elevations in TC and, more specifically, LDL occur as a compensatory response to low oncotic pressure in patients with nephrotic syndrome [35]. Chronic kidney disease can also result in elevated TC and LDL; however this is less common than in nephrotic syndrome. Untreated or inadequately treated hypothyroidism can result in elevations in LDL and/or TG; therefore, thyroid function tests should routinely be performed in patients with clinically significant hyperlipidemia.

33.5.3 Clinical Presentation

The effects of the genetic mutations in heterozygous FH are completely expressed in infancy, as demonstrated by Kwiterovich et al. who showed elevated LDL levels in cord blood samples of infants that remained elevated on follow-up assessment unless dietary modification occurred [41]. Subclinical early atherosclerotic lesions have been demonstrated in children by the age of 5 years. Endothelial function is impaired, as has been shown by decreased dilatation of the brachial artery in children with hyperlipidemia after intense post-ischemic flow of arterial blood [42]. In addition, children with hyperlipidemia due to FH may have increased carotid intima-media thickness [31]. Physical exam is typically normal in children except in homozygous FH, in which physical exam may reveal xanthomas, which are cholesterol deposits found in tendons – most commonly in the Achilles tendon or finger extensor tendons. Xanthelasmas, cholesterol deposits found around the eyes, and corneal arcus, a white ring around the cornea due to lipid deposition, are classic findings in severe heterozygous FH and homozygous FH associated with LDL levels >300 mg/dl.

33.5.4 Diagnosis

Cholesterol screening should be considered as early as age 2 years in families with early CVD associated with hypercholesterolemia. LDL ≥ 190 mg/dl in patients under 20 years old confers an approximately 80% risk of FH [43]. Genetic testing in general is not needed for diagnostic or clinical management but may be useful if the diagnosis is uncertain. Negative testing of common mutations does not rule out FH, as approximately 20–40% of clinically definite heterozygous FH have had negative genetic testing [44]. Labs to rule out secondary causes of hypercholesterolemia include thyroid function tests, BUN, and creatinine. Mild elevations in LDL (130–150 mg/dl) are typically due to a diet very rich in fat and cholesterol or polygenic hypercholesterolemia. Lp(a) can be associated with abnormalities in lipid profiles (specifically elevations in LDL); however, in some cases the lipid profile may be normal. Therefore, in the patient with a family history of extensive premature cardiovascular events and a normal lipid profile, it is reasonable to measure the Lp(a) level, although no formal recommendations have been made for such screening.

Multiple assays are available for screening for Lp(a) levels. It is important to understand the assay that is ordered as this can affect interpretation. Assays include measurements of only the cholesterol carried by the Lp(a) protein (analogous to the LDL-C), Lp(a) protein mass, and Lp(a) particle mass, the latter of which is comprised of the protein, lipid, and carbohydrate components. Most commonly, Lp(a) particle mass is measured with abnormal levels being >30 mg/dL [39].

33.5.5 Outcomes and Possible Complications

Patients with heterozygous FH have an increased risk for cardiovascular events before the age of 50 years, with estimates of 25% of untreated women and 50% of untreated men having an event. Individuals with homozygous FH typically have cardiovascular events in the first two decades of life due to rapidly accelerated atherosclerosis. FCHL is associated with type 2 diabetes and the development of metabolic syndrome in adults [47], and there is a moderate risk for early cardiovascular disease. FCHL, which may be more difficult to diagnose in childhood, has a significant risk for future cardiovascular events, with approximately 11.3% of acute myocardial infraction survivors <60 years old meeting diagnostic criteria for FCHL [37]. One study estimates that 40% of all myocardial infarct survivors are diagnosed with FCHL if no age limits are used [48].

Multiple pediatric studies have also shown an increased risk for acute ischemic strokes with elevations in Lp(a) as well as a poor response to statin therapy in patients with elevated Lp(a) and elevated LDL [45, 46].

33.5.6 Treatment

Dietary modification is an essential component for treatment of hyperlipidemia regardless of etiology (see Table 33.6). Dietary restriction of cholesterol upregulates the LDL-R, which increases binding and clearance of LDL particles, thus reducing LDL levels 10–20% regardless of

Table 33.6 Summary of recommendations for the cardiovascular health integrated lifestyle diets (CHILD)

	CHILD-1	CHILD-2
Fat intake Saturated fat Trans fat Monounsaturated fat	<30% 8–10% Avoid N/A	25–30% ≤7% Avoid ~10%
Cholesterol	<300 mg/dl	<200 mg/dl
Miscellaneous	1 h/day of vigorous activity, <2 h/day of screen time	1 h/day of vigorous activity, <2 h/day of screen time

etiology or severity of hyperlipidemia. Dietary restriction also stimulates de novo cholesterol synthesis, the target of statin medications; thus, statin treatment is much more effective in conjunction with dietary cholesterol restriction. Nutritionist/dietitian consultation is necessary in initiation and maintenance of a dietary plan to successfully treat familial hyperlipidemia. The Cardiovascular Health Integrated Lifestyle Diet (CHILD)-1, which emphasizes restricting dietary fat and cholesterol, should be initial therapy. The CHILD-1 diet involves restriction of total fat to <30% of daily caloric intake, saturated fat to 8–10% of daily intake, and modest restriction of cholesterol intake to <300 mg per day. It also recommends the avoidance of trans fat. The CHILD-1 diet can be advanced to the CHILD-2 diet after 3–6 months. Goals of CHILD-2 are to restrict fat intake to 25–30% of daily caloric intake, saturated fat intake to ≤7% of daily intake, and monounsaturated fat to ~10% of total intake and to avoid all trans saturated fat. Total cholesterol intake should be kept to <200 mg/day [49]. This level of restriction will invariably decrease total cholesterol levels up to 10%. In patients with moderate to severe hypercholesterolemia (LDL > 160 mg/dl), begin with the CHILD-2 diet because the cholesterol restriction in CHILD-1 is minimal. Plant stanol or sterol esters can be considered for adjunctive therapy. Plant sterols work by inhibiting incorporation of cholesterol into the lipid micelles and by preventing intestinal resorption of cholesterol by blocking cholesterol receptors. Limited data about the efficacy of plant sterols as a spread has suggested a modest decrease in LDL by about 7.5% [6].

Despite dietary modification and adherence to CHILD-2 in familial hyperlipidemia, pharmacologic management is typically necessary if LDL reductions after dietary restriction do not result in LDL levels at target. Therapy is recommended for those with a family history of CVD and a fasting LDL ≥ 160 mg/dl (likely heterozygous FH) despite dietary restriction or no family history of CVD and fasting LDL ≥ 190 mg/dl (very likely heterozygous FH) despite dietary restriction. The first-line medication in patients over the age of 10 years is an HMG-CoA reductase inhibitor or "statin." Pharmacologic therapy in general is not recommended in children <10 years unless there is severe primary hyperlipidemia (homozygous FH, evident cardiovascular disease, or a high-risk condition such as type 1 or type 2 diabetes mellitus, chronic renal disease, end-stage renal disease, post-renal transplant, post-orthotopic heart transplant, or Kawasaki disease with current coronary artery aneurysms) [50]. HMG-CoA reductase is the enzyme that converts three molecules of acetate into mevalonate, which is the rate-limiting step in cholesterol biosynthesis. By decreasing hepatic cholesterol synthesis, hepatic cellular LDL receptors are upregulated, resulting in increased clearance of LDL from plasma, but statin efficacy is blunted if dietary cholesterol is not restricted. Significant decreases in LDL up to 40% have been demonstrated with the use of statins [51–54]. Specific agents that have been associated with a reduction of LDL to approximately 40% are atorvastatin, simvastatin, and rosuvastatin at doses of 10–20 mg, 5–20 mg, and 40 mg daily, respectively (see Table 33.7) [52, 54–57]. Statins have also been shown to decrease TG and increase HDL, although these effects are modest.

While data are limited for long-term side effects of statins in children, short-term side effects have been minimal. The major concerning side effects of statins are hepatic dysfunction and myopathy. Given the lack of long-term data on the safety of statins in pediatric patients, statins should be initiated at the lowest dose, and baseline liver function tests and muscle enzyme levels should be measured (AST, ALT, and CK) prior to initiation. During treatment, ALT, AST, and CK should be followed at least every 6 months to ensure no serious adverse effects are developing. Complaints of myalgia should be taken seriously; serum CK should be measured, and the statin should be stopped until myositis is ruled out. Although rare, if severe myositis occurs, individuals can develop myoglobinuria and renal failure. Concerns have also been raised about effects on sexual development in pediatric patients as steroid hormones are derived from cholesterol. Studies have shown both increases and decreases in dehydroepiandrosterone sulfate in boys and girls; however, there has not been any association of abnormal sexual development in multiple studies [54, 55].

Another class of medication that was frequently used in children for elevated LDL is bile acid sequestrants. Bile acid sequestrants bind to intestinal bile and prevent its resorption, thereby interrupting enterohepatic recirculation. With less bile acid resorption, more cholesterol

Table 33.7 HMG-CoA reductase inhibitors efficacy based on select studies in children and adolescents

Medication	Dose	Efficacy in LDL reduction	Approximate monthly cost in the USA[a]
Atorvastatin	10 mg with increase to 20 mg daily	↓ 40%	$68
Pravastatin	5–20 mg daily	↓ 23–32%	$36
Lovastatin	10–40 mg daily	↓ 21–36%	$67
Simvastatin	40 mg daily	↓ 40%	$68
Rosuvastatin	5–20 mg daily	↓ 35–45%	$205
Fluvastatin	80 mg daily	↓ 27%	$235

[a]Cost varies by dose of medication and estimates are based on use of generic medications where available (Modified from Consumer Reports Best Buy Drugs. Uploaded from ► https://www.consumerreports.org/health/resources/pdf/best-buy-drugs/StatinsUpdate-FINAL.pdf. Accessed on July 11, 2016)

is converted into bile acids, thus decreasing the hepatic cholesterol pool. This causes an upregulation of LDL-R and thus decreased plasma LDL. Today, bile acid sequestrants are often used as adjunctive therapy to statins if dietary restriction and statins do not result in target LDL. Bile acid sequestrants typically have a more modest effect in decreasing plasma LDL (10–20%) [58]. Cholestyramine and colestipol are two agents not commonly used any more due to significant gastrointestinal side effects: doses were 8 gm/day and 10 gm/day, respectively [59]. Colesevelam is a newer bile acid sequestrant with fewer side effects and is approved for children aged 10 years and older. It is well-tolerated and has been shown to decrease LDL levels by 10–15% [60]. It can be used as monotherapy or in combination with statins. Achieving goal LDL levels can be especially challenging in homozygous FH. Complete restriction of cholesterol-containing foods and adjunctive therapy to statins with colesevelam is necessary and even then may not result in achievement of goal levels of LDL.

Ezetimibe is another medication that historically has been a second-line agent for elevated cholesterol, in particular for elevated LDL-C. Ezetimibe inhibits cholesterol absorption by targeting the cholesterol transport protein Niemann-Pick C1 like 1 protein at the jejunal enterocyte brush border. Ezetimibe is an effective LDL-C-lowering agent with estimates of an additional decrease of 10–25% when used as an adjunct to statin and ~25–40% when used as monotherapy [61–63]. However, controversy of its efficacy in prevention of cardiovascular events has arisen after an adult study showed no difference in carotid intima-media thickness in patients with heterozygous FH who were treated with simvastatin plus ezetimibe when compared to simvastatin plus placebo despite a statistically significant decrease in the LDL-C (~25%) [61]. Data in the pediatric population regarding its efficacy and use as a primary or secondary agent are sparse with relatively short-term follow-up. These studies have shown similar reductions in LDL-C to adult studies [62, 63]. Ezetimibe is generally a well-tolerated medication, and no significant side effects have been reported in pediatric studies, although adult studies have noted occasional occurrence of musculoskeletal symptoms similar to what can occur on statins. One study showed an increased risk for cancer; however, subsequent studies have not supported these findings [64].

Interestingly, adult studies have not yet shown a conclusive effect of reducing Lp(a) levels on reduction in cardiovascular events, which is one reason that adult and pediatric guidelines do not recommend routine Lp(a) screening. Given that Lp(a) levels are dominated by genetic influences, dietary modification does

Table 33.8 Recommendations for treatment and referral for abnormal LDL

LDL	Management
≥190 mg/dl	CHILD-2 diet Repeat LDL in 3–6 months Consider statin therapy if repeat LDL ≥160 mg/dl Referral to a lipid specialist
≥160–189 mg/dl with family history of CVD *or* 1 high-level risk factor *or* 2 moderate-level risk factors	CHILD-2 diet Repeat LDL in 3–6 months Consider statin therapy if repeat LDL ≥ 160 mg/dl Referral to a lipid specialist
≥130–150 mg/dl with family history of CVD *or* 2 high-level risk factors *or* 2 moderate- and 1 high-level risk factors	CHILD-1 diet Repeat LDL in 3–6 months Consider statin therapy if repeat LDL ≥ 160 mg/dl
≥130–189 mg/dl with no family history of CVD or risk factors	CHILD-1 diet Repeat LDL in 3–6 months Consider genetic testing for LDL 160–189 mg/dl

Family history of CVD: family history of a first-degree relative with MI, CVA, angina, or coronary artery bypass or sudden cardiac death before the age of 55 years in a male and 65 years in a female
High-risk conditions include type 1 and 2 diabetes mellitus, chronic renal disease, heart transplant, and Kawasaki disease with aneurysms. High-level risk factors include hypertension requiring medication, smoking, and morbid obesity
Moderate-risk conditions include chronic inflammatory disease, systemic lupus erythematosus, juvenile inflammatory arthritis, nephrotic syndrome, HIV, and Kawasaki disease with resolved aneurysms. Moderate-level risk factors include hypertension not on medication, obesity, and low HDL

not lower levels but may decrease the particle mass by lowering the amount of linked LDL [39]. Aspirin and niacin decrease Lp(a) in adults [65]; however pediatric data are lacking. Statins have a variable effect on reduction of Lp(a) in adults that may be isoform-dependent [65]. A summary of recommendations for treatment and referral for elevated levels of LDL is listed in Table 33.8.

33.6 Hypobetalipoproteinemia

33.6.1 Introduction and Background

Hypolipoproteinemia refers to a group of disorders that are characterized by low plasma levels of LDL (<50 mg/dl) and/or low levels of TC (<70 mg/dl). Low LDL levels are generally due to increased LDL uptake or decreased formation. Both primary and secondary causes exist, with primary hypolipoproteinemias being overall quite rare (see Table 33.9).

Table 33.9 Causes of hypobetalipoproteinemia

Primary	Secondary
MTTP deficiency (abetalipoproteinemia)	Liver disease
Apo B deficiency (FHBL)	Chronic pancreatitis
PCSK9 deficiency (FHBL)	Kidney disease
Chylomicron retention disease	Profound hyperthyroidism
FHBL 2 (angiopoietin-like 3 deficiency)	Malabsorptive states

33.6.2 Etiology

Abetalipoproteinemia is an extremely rare autosomal recessive condition due to a mutation in the gene encoding microsomal triglyceride transfer protein (MTTP) [66]. MTTP is a chaperone protein required for the formation of both VLDL

and chylomicrons through transfer of lipids onto ApoB. The *MTTP* gene is located on chromosome 4q22-24. Lack of formation of VLDL and chylomicrons causes a deficiency of both IDL and LDL cholesterol production.

Familial hypobetalipoproteinemia (FHBL) is a rare autosomal codominant disorder caused by a mutation in the gene encoding ApoB (*APOB*), which is located on chromosome 2p23-24. This causes a truncated ApoB and therefore reduced lipoprotein lipid content [67]. ApoB cofactors are necessary for proper LDL, VLDL, and CM formation. Heterozygous single mutations, heterozygous compound mutations, and homozygous mutations have been described, with the latter two clinically mimicking abetalipoproteinemia. FHBL is also caused by loss of function mutations in *PCSK9*, the gene product of which targets the LDL receptor for degradation. The *PCSK9* gene is located on chromosome 1p32.2. Loss of function yields a larger number of LDL receptors on the hepatocyte cell surface, resulting in more LDL being taken up by the liver, thus lowering plasma LDL levels [68]. PCSK9 inhibitors were recently approved as a treatment for FH to due to its LDL-lowering capabilities [69].

CM retention disease is caused by mutations in *SAR1B* which codes for GTPases that are important for allowing chylomicrons to travel through the secretory pathways and fuse to the Golgi apparatus. Mutations of this gene, located on chromosome 5q31.3, lead to an inability to secrete CM resulting in their accumulation within intestinal enterocytes [70]. Familial combined hypolipidemia (FHBL2) is due to mutations in the gene encoding angiopoietin-like 3, whose role is partial inhibition of LPL and endothelial lipase, both of which are involved in degradation of VLDL and HDL. The *ANGPTL3* gene is located on chromosome 1p31, and both compound heterozygous and homozygous mutations have been described [71].

Secondary causes of hypobetalipoproteinemias are chronic pancreatitis, malabsorptive states, and profound hyperthyroidism. Hyperthyroidism leads to excessive LPL activity through its activation by thyroid hormone, resulting in low levels of VLDL, LDL, and total cholesterol. Chronic pancreatitis and malabsorptive states can lead to poor absorption of fat, leading to low cholesterol and LDL levels.

33.6.3 Clinical Presentation

The clinical presentation of hypobetalipoproteinemia depends on the etiology of the disease.

The most observed feature of abetalipoproteinemia and CM retention disease is fat malabsorption shortly after birth that leads to failure to thrive. Symptoms of malabsorption include steatorrhea, vomiting, and abdominal distention. With the lack of absorption of fat-soluble vitamins, neurological sequelae ensue in the first to second decades of life [72]. Neurologic findings include ataxia and muscle weakness, as well as loss of proprioception, vibration, and deep tendon reflexes. Loss of night and/or color vision can also occur, which may lead to blindness.

Compound heterozygotes and homozygotes for FHBL have presenting features similar to those with MTTP mutations. Heterozygous FHBL is characterized by the development of fatty liver disease secondary to reduced TG export from the liver, but no symptoms of malabsorption or neurologic sequelae are present. FHBL2 cases have not been associated with fatty liver, neurologic findings, or symptoms of malabsorption [73].

33.6.4 Diagnosis

Diagnosis of hypobetalipoproteinemia is made with a lipid panel that shows a markedly low LDL level (<50 mg/dl). This is generally found on routine lipid screening or during evaluation of clinical features concerning for lipid disorders. People with abetalipoproteinemia and heterozygous compound or homozygous FHBL will have low levels of LDL, TC, TG, and ApoB. Those with heterozygous FHBL will have 1/3–1/4 of normal levels of LDL and ApoB. Those with CM retention disease will have low levels of LDL, TC, and HDL, but TG will be normal. FHBL2 is considered in cases of low LDL, TG, and HDL. Genetic testing for mutations in *MTTP*, *ANGPTL3*, *SAR1B*, *APOB*, and *PCSK9* can be performed for complete diagnosis. Western blots can be used to assess for truncated ApoB proteins if the ApoB protein is more than 30% of the size of the normal protein [58].

33.6.5 Outcomes and Possible Complications

Low levels of LDL and total cholesterol are associated with a reduced risk of cardiovascular disease. However, in some cases of primary hypolipoproteinemia, liver and neurologic dysfunction can be present.

33.6.6 Treatment

Low levels of LDL and TC have been linked to a reduced risk of cardiovascular disease, and in cases of heterozygous states of hypolipoproteinemia, no treatment is needed. However, in more severe cases where hepatic steatosis and neurologic sequelae are present, supplementation with fat-soluble vitamins is needed to improve neurologic findings, and a low-fat diet is required to reduce the fatty deposition in the liver [67].

33.7 Disorders of HDL Metabolism

33.7.1 Introduction and Background

Causes of low levels of HDL are difficult to diagnose and manage. The risk of cardiovascular disease rises with a low HDL; thus, treatment modalities to increase HDL are made in an effort to reduce risk of cardiovascular morbidity. Both primary and secondary causes of low HDL exist, with secondary causes being much more common than primary genetic disorders of HDL metabolism (see Table 33.10). One genetic mutation has been shown to lead to markedly elevated HDL levels and will also be discussed here.

Table 33.10 Causes of HDL deficiency

Primary	Secondary
Apo A1 deficiency	Smoking
ABCA deficiency (Tangiers disease)	Obesity
LCAT deficiency (familial/fisheye disease)	Type 1 or type 2 diabetes

33.7.2 Etiology

Primary causes of low HDL include genetic mutations leading to a deficiency of any of the apolipoproteins associated with HDL or the enzymes involved in producing it. ApoA1 deficiency is secondary to mutations of the *Apo AI* gene, located on chromosome 11q23.3. Lack of ApoA1 impacts the structure of the HDL molecule and does not allow for proper HDL assembly. Deficiency of ApoA1 has also been found in conjunction with deficiencies of other apolipoproteins involved in HDL metabolism, such as ApoA-III, ApoC-II, and ApoE [69].

Tangier disease is a rare but severe form of HDL deficiency, named after the island in Chesapeake Bay, Virginia, where the first few patients were described. It has an autosomal recessive clinical phenotype and is caused by a mutation in the ATP-binding cassette transporter 1 (*ABCA1*) gene located on chromosome 9q22-3 [70]. The ABCA1 protein is involved in the transport of cholesterol and phospholipids to ApoA1 to form nascent HDL (HDL3); thus, a defect resulting in low or absent ABCA1 leads to a lack of HDL production.

Lecithin-cholesterol acyl transferase (LCAT) is the enzyme that catalyzes the conversion of free cholesterol and lecithin to cholesterol esters that help form HDL2. The *LCAT* gene is located on chromosome 16 q21-22. A deficiency of LCAT is seen in two disease states, both of which are autosomal recessive and result in markedly low levels of HDL. The first, familial LCAT deficiency, involves total loss of the LCAT enzyme, and the second, fish eye disease, is characterized by decreased LCAT enzyme production. LCAT deficiency leads to altered concentrations of both cholesterol esters and lecithin, leading to abnormal structure of the HDL particles. There are about 50 patients with LCAT deficiency described in the literature.

Secondary causes of low HDL levels include obesity, smoking [71], states of insulin deficiency such as type 1 diabetes, and states of insulin resistance such as type 2 diabetes. Insulin is a potent activator of LCAT, which promotes the formation of large, cholesterol-rich HDL particles. Insulin inhibits hepatic lipase, which hydrolyzes HDL phospholipids to form smaller HDL particles. Thus, in states of insulin resistance or deficiency,

the conformation of the HDL particle is altered, and cholesterol ester content is decreased, thus predisposing the HDL particle to renal catabolism and excretion and lower HDL levels [72].

Cholesterol ester transfer protein (CETP) is involved in the transfer of cholesterol esters from HDL particles onto VLDL, IDL, and LDL in exchange for TG [69]. The gene for CETP is located on chromosome 16q21, and genetic mutations have been found that cause a deficiency of CETP, which is heralded by marked elevations in HDL, as well as elevated ApoA1, low LDL, and low ApoB.

33.7.3 Clinical Presentation

ApoA1 deficiency manifests as tendinous xanthomas caused by lipid-laden macrophages that usually present on the Achilles tendon and extensor tendons of the hands. Cerebellar ataxia due to decreased fat-soluble vitamin absorption can occur as well. Premature cardiovascular disease [60] may occur, although there are a number of cases reported where no premature cardiovascular disease was observed. Clinical manifestations of Tangier disease are secondary to lipid deposition in various tissues, leading to orange tonsils, hepatosplenomegaly, corneal opacities, neuropathy, anemia, and thrombocytopenia [70]. Clinical features of LCAT deficiency include corneal opacification secondary to accumulation of cholesterol and phospholipids, anemia, and proteinuria, with kidney failure being the most common cause of death [74]. There are no clinical features specific to CETP deficiency.

33.7.4 Diagnosis

Diagnosis of disorders of HDL metabolism generally begins upon finding a markedly low HDL level (<20 mg/dl) on routine lipid testing in childhood or upon workup for some of the secondary manifestations of low HDL in adulthood. The differential of low HDL should be divided into primary and secondary causes, with secondary causes ruled out through a careful history and laboratory evaluation when indicated. Primary causes can be ruled out with genetic testing looking for mutations in genes coding for ApoA1, LCAT, and ABCA1. CETP deficiency is considered in cases of a markedly elevated HDL (>70 mg/dl).

33.7.5 Outcomes and Possible Complications

The risk of heart disease increases in secondary and primary causes of low HDL. In ApoA1 deficiency and Tangier disease, the risk of premature cardiovascular disease increases. No increased risk of premature cardiovascular disease has been observed in cases of LCAT deficiency [69].

33.7.6 Treatment

Treatment of low HDL involves, in cases of secondary causes, smoking cessation and/or treatment of the underlying disease. Insulin administration in cases of type 1 diabetes can restore HDL levels to within the normal range. Dietary modification, weight loss, and especially exercise improve HDL levels in obesity. Lifestyle modification, in addition to blood sugar control in cases of type 2 diabetes, improves HDL levels.

In cases of primary causes, dietary modification with or without statin therapy to achieve LDL levels below 70 mg/dl is warranted to reduce cardiovascular morbidity and mortality [75]. Statin therapy improves HDL levels but by only 5–10% in most trials. Niacin can improve HDL levels by 15–35% and fibrates by up to 20%. The benefits of using these medications are that they not only improve HDL levels but also have positive effects on LDL and TG levels and therefore have a beneficial effect on lowering cardiovascular risk [76].

Treatment of LCAT deficiency is focused on preservation of renal function by pharmacologic management using angiotensin receptor blockers, ACE inhibitors, and/or possibly steroid therapy. Lifestyle modification is also important to reduce LDL levels and cardiovascular morbidity.

To date, the neurologic sequelae of Tangier's disease have not responded to medication, but hepatomegaly has been treated successfully with a low-fat diet. CETP inhibitors such as anacetrapib, evacetrapib, and dalcetrapib have been used in a number of trials in adults to increase HDL levels. CETP inhibitors block the transfer of cholesterol

esters from HDL to other molecules resulting in HDL levels remaining elevated. Studies have shown variable results with most showing an increase in HDL by up to 25% when used alone and an increase in 60–130% when used in conjunction with statin therapy. Results are mixed with respect to cardiovascular outcomes in adults [77, 78]. No studies to date have assessed the impact of CETP inhibitors in children or adolescents. CETP deficiency has no harmful sequelae or risk of cardiovascular disease, so treatment is not warranted.

Case Study #1

A 14-day-old baby born at 41 weeks with birth weight 8 lbs 9 oz after an uncomplicated pregnancy was doing well until 7 days of age, when his mother noticed "blisters" in his perianal region that kept worsening despite different topical therapies. He became fussy and started vomiting his formula. In his PCP's office, he was noted to have increased work of breathing and had a questionable seizure in the office. He was sent to the ER for further evaluation. In the ER he was found to be febrile. Lipemic serum was observed, and consequently triglycerides were requested. The initial level was >20,000 mg/dl with a total cholesterol of 1206 mg/dl. He was placed NPO and started on an insulin drip with dextrose. Repeat triglycerides the next morning were 5000 mg/dl, with a total cholesterol of 178 mg/dl. Amylase and lipase were normal.

The baby's father reports no history of abnormal triglycerides or cholesterol on his side of the family. The baby's mother has no cholesterol or triglyceride abnormalities but is unsure of her family history as she is adopted.

The baby was discharged home on a medium-chain triglyceride formula. During follow-up with the endocrinologist a few weeks later, the baby was found to be thriving. Fasting lipids showed triglycerides of 430 mg/dl with total cholesterol of 150 mg/dl.

Discussion/Management

The most likely diagnosis is homozygous LPL deficiency. Lipemic serum should always alert one to hypertriglyceridemia, and lipids should be obtained immediately. It is less likely to be familial hypertriglyceridemia, as this tends to be associated with a positive family history of hypertriglyceridemia or pancreatitis and the TG levels are not as high. IV fluids and NPO status will improve the pancreatic inflammation and fasting will lower triglycerides. In cases where the triglycerides are well over 1000 mg/dl, the use of insulin to increase LPL activity and lower TG may be needed. Insulin should always be given with glucose to avoid hypoglycemia. Long-term management of this baby involves a specialized formula that contains mostly medium-chain triglycerides, as these will bypass the steps toward CM formation and reduce post-prandial TG levels. As the baby starts to add other foods to the diet, counseling of the family is critical to maintain low-saturated fat, low-simple sugar intake, as well as long-chain fat restriction to keep triglycerides below 1000 mg/dl and reduce the risk of pancreatitis. Fibrates can be added much later in childhood or adulthood for further TG reduction.

Case Study #2

KF is a 17-year-old girl referred to pediatric endocrinology for evaluation of hyperlipidemia. She has no complaints and denies headaches, polyuria, excessive fatigue, snoring, or waking suddenly in her sleep. She has had no recent changes in her weight. She takes no medications and has no medication allergies. The PCP recently ordered a fasting lipid profile that revealed the following: total cholesterol 344 mg/dl, triglycerides 63 mg/dl, HDL 64 mg/dl, and LDL 267 mg/dl. Her family history is notable for the father having hyperlipidemia, for which he had been treated with a statin since his 30s. Paternal grandfather also has hyperlipidemia that was noted "later in life," for which he was also treated with pharmacologic therapy. There are no first-degree relatives with cardiovascular events before the age of 55 years old. Physical examination revealed a well-appearing talkative athletic adolescent in no apparent distress. BMI is at the 61st percentile. On examination of the eyes, there are no xanthelasmas, and fundi show no hemorrhage or exudates. Thyroid is palpable, soft, and smooth. She has Tanner stage 5 development. Skin examination reveals no xanthomas on visual inspection or on palpation of the Achilles tendons or finger extensor tendons. She received dietary counseling by a registered dietician to reduce cholesterol intake to <200 mg per day.

Follow-up occurred 3 months after initial evaluation and a repeat

fasting lipid panel revealed: total cholesterol 300 mg/dl, triglycerides 89 mg/dl, HDL 95 mg/dl, and LDL 187 mg/dl.

Discussion/Management

KF has heterozygous FH given the elevated LDL (>190 mg/dl), normal HDL and triglycerides, and family history of hyperlipidemia. There is no history of early CVD, but this is not terribly unusual. Heterozygous FH is an autosomal dominant disorder that she has inherited from her father. Initial intervention of dietary modification via cholesterol restriction was very successful, reducing her LDL levels by 30%. The expected decrease in LDL with dietary intake <200 mg cholesterol per day is 10–20%, so her cholesterol intake was likely even lower. The success of dietary restriction on her LDL levels was emphasized with her and her family. Initiation of medication would be indicated for KF if LDL remained >160 mg/dl as she had a positive family history of early CVD. At this time medication is not indicated; however it is likely that keeping her LDL <190 mg/dl will be difficult to maintain with dietary restriction alone, so pharmacologic therapy will be needed in the future. An HMG-CoA reductase inhibitor or "statin" beginning at a low dose at bedtime would be the next step; however, given that statins are teratogenic, appropriate counseling with consideration of implementation of contraceptive measures is indicated. Screening labs (AST, ALT, and CK) would be checked after initiation of medication and at least every 6 months while on statin therapy.

Review Questions

1. A 13-year-old female is brought to the office by her mother for evaluation of abnormal lipids. She has total serum cholesterol of 310 mg/dl, total triglycerides of 120 mg/dl, HDL of 45 mg/dl, and LDL of 190 mg/dl. Her BMI is at the 75th percentile for age and gender. Her mother and maternal grandparents have high cholesterol; mom takes statin therapy but is unsure about whether or not her grandparents take medication. Her paternal grandfather had heart surgery a few years ago, but she is unsure how old he was at the time of the surgery. The father has high cholesterol but controls it with diet. Your recommendations for initial treatment for this patient include:
   A. Initiation of HMG-CoA reductase inhibitor therapy
   B. Initiation of a bile acid sequestrant
   C. Initiation of the CHILD-2 diet
   D. Initiation of omega-3 fatty acid treatment
2. A 10-year-old male with no significant past medical history is found to have fasting triglycerides of 840 mg/dl with a total cholesterol of 185 mg/dl, HDL of 42 mg/dl, and LDL unable to be calculated. His BMI is at the 85th percentile, and his mother reports that she has high triglycerides and had three episodes of pancreatitis in her teens and early 20s. The most likely cause of the patient's abnormal labs is:
   A. Familial hypercholesterolemia
   B. Familial hypertriglyceridemia
   C. LPL deficiency
   D. ApoC-II deficiency
   E. Dysbetalipoproteinemia
3. A 12-year-old obese patient returns for follow-up of his obesity. He gained 3 lbs, and his BMI increased to the 98th percentile since his last visit 1 month ago. His diet is rich in fast foods and simple carbs. Physical exam is significant for acanthosis nigricans at the neck and axilla, and his sexual maturity rating is 2 for genitalia and pubic hair. At his initial visit you had asked for labs to screen for comorbidities. His fasting glucose was 90 mg/dl and HbA1C 5.9%. Lipid profile showed cholesterol 190 mg/dl, LDL 130 mg/dl, HDL 30 mg/dl, and triglycerides 150 mg/dl. What is the next best step in management of this patient?
   A. CHILD-1 diet
   B. Initiation of omega-3 fatty acids
   C. Initiation of fibrates
   D. Initiation of statins

Answers

1. C
2. B
3. A

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Other Endocrine Disorders

Autoimmune Endocrine Disorders

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Key Points

- Type 1 diabetes serves as a model for autoimmune endocrine disorders.
- Autoimmune polyendocrine syndrome type 1 (APS-1) also known as autoimmune polyendocrinopathy candidiasis and ectodermal dystrophy (APECED) is an autosomal recessively inherited disorder caused by mutations of the *AIRE* gene resulting in multiple autoimmune diseases.
- Autoimmune polyendocrine syndrome type 2 (APS-2) is a common disorder associated with type 1 diabetes, primary adrenal insufficiency, and hypothyroidism.

34.1 Introduction and Background Information

It is hypothesized that autoimmune endocrine diseases progress through a series of stages starting with genetic susceptibility followed by an environmental trigger that initiates the autoimmune process and ultimately culminating in overt clinical disease. Type 1 diabetes serves as a model disorder for autoimmune endocrine disorders. Initial disease schema proposed by Dr. George Eisenbarth [1] has recently been updated to include the possibility of waxing and waning of the disease and persistence of glandular function after onset of disease [2]. The model proposes several progressive stages for the development of type 1 diabetes: genetic predisposition, overt immunological abnormalities, progressive β-cell dysfunction, and overt diabetes (Fig. 34.1).

34.1.1 Genetic Risk

There are multiple genes that have been associated with the risk for autoimmune disease [3]. Genes may influence the risk for the development of autoimmunity and/or the risk for the progression from autoimmunity to glandular dysfunction. Data derived from prospective follow-up of high-risk individuals are beginning to tease out these

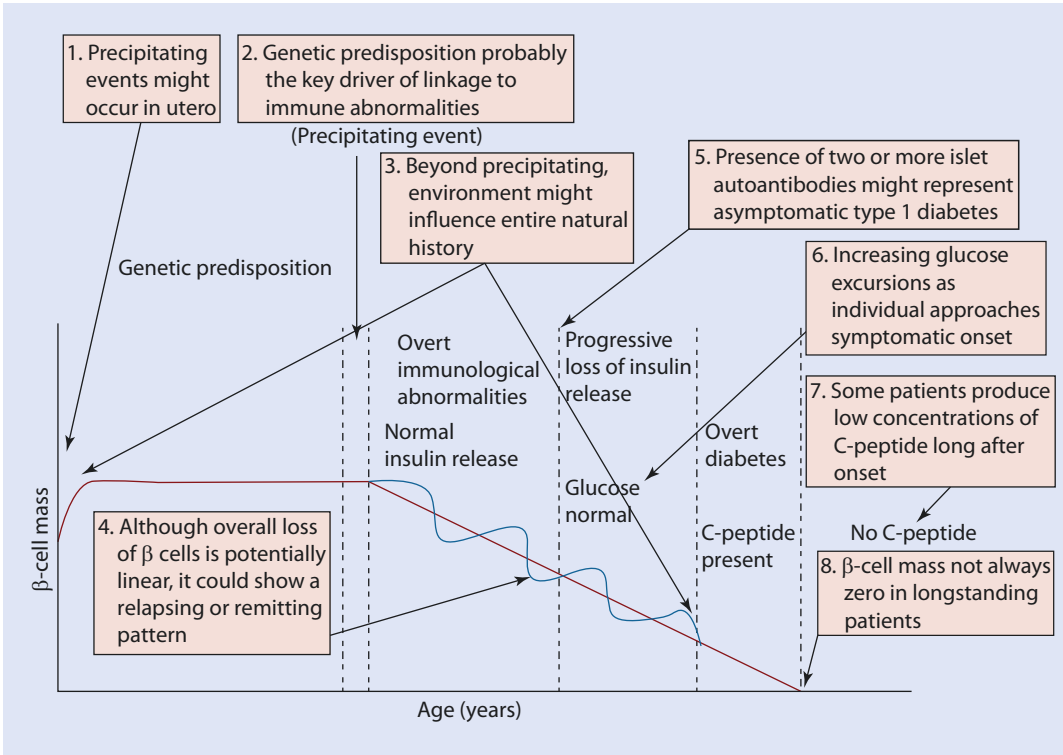

Fig. 34.1 Model for the development of type 1 diabetes. Highlighted are updates in the understanding of the pathophysiology of type 1 diabetes (From Atkinson et al. [2]. Reprinted with permission from Elsevier)

relationships. The most consistent risk has been shown with the genes that make up the human leukocyte antigens (HLA) found on chromosome 6. In subjects followed for type 1 diabetes, HLA alleles have been differentially associated with the first positive diabetes-related autoantibody. HLA DR4-DQ 8 has been associated with insulin autoantibodies as the first antibody, and HLA-DR3-DQ 2 has been associated with GAD autoantibodies. These data suggest that these HLA alleles may be important for the initiation of the autoimmune process. HLA may also influence the progression from autoimmunity to disease. For example, HLA-DRB1*15:01-DQA1*01:02-DQB1*06:02 has been shown to be protective for the progression of autoimmunity from a single positive autoantibody to multiple positive autoantibodies and dysglycemia [4]. It has been shown that subjects that are HLA identical for the DR3/4 locus as their sibling with diabetes have a risk for developing diabetes-related autoimmunity of approximately 75% and diabetes risk of approximately 50% after 5 years of follow-up [5]. Thus, the risk for development of autoimmune disease can be additive.

HLA alleles partially explain the disease associations observed in APS-2. DR3-DQ2 and DR4-DQ8 are associated with autoimmune hypothyroidism.

Genes outside of the HLA region have also been implicated in the risk for autoimmune diseases. Some of these genes increase an individual's likelihood of developing any autoimmune disease, while other genes are associated with specific autoimmune conditions (e.g., the variable number of tandem repeats VNTR of the insulin gene). Specific genes may be associated with progression from autoimmunity to β-cell dysfunction (e.g., PTPN22) [6].

34.1.2 Environmental Factors

Despite the strong genetic influence in development of autoimmune diseases, not all with genetic risk develop disease. Environmental triggers have been hypothesized to be important in the development of autoimmune disease. The classic example of this is celiac disease, where the environmental trigger, gluten, is known. In type 1 diabetes, multiple environmental triggers have been proposed [7]. Environmental triggers may influence both steps of the process: initial development of autoimmunity and progression from autoimmunity glandular dysfunction. Earlier introduction of cereals and gluten (<4 months) has been associated with risk for type 1 diabetes [8, 9] and celiac disease [10]. Increased weight at 12 months has been associated with risk for diabetes-related autoimmunity but not progression from autoimmunity to type 1 diabetes [11]. Emerging data suggests that enteroviral infections may be associated with type 1 diabetes [7, 12]. There may be protective environmental factors such as breast feeding [13]. Other active areas of research include the influence of the intestinal microbiota and development of autoimmunity and disease [14]. It is likely that extensive genetic-genetic and genetic-environmental interactions influence not only the development autoimmunity but also progression of autoimmunity to disease.

34.1.3 Markers of Autoimmunity

While the autoimmune process is thought to be mostly T-cell mediated, the autoimmune process is marked by the presence of autoantibodies (antibodies against self-antigens) (Table 34.1). Diabetes-related autoantibodies include antibodies against insulin (IAA), GAD65, IA-2, and ZnT8. These autoantibodies are used in the research and clinical setting to identify patients at an increased risk for an autoimmune disease and to confirm autoimmunity as the underlying cause of the disease in an affected individual. Autoantibodies can be detected in the serum prior to the development clinical disease. Studies in subjects with diabetes-related autoantibodies show that the risk for the development of diabetes increases with increasing number of diabetes-related autoantibodies [15], the persistence of the autoantibodies on multiple tests [16], and autoantibody level and affinity of autoantibodies for the antigens [17, 18]. Long-term follow-up of subjects at risk for type 1 diabetes suggests that some of those with single autoantibodies can progress to multiple autoantibodies and diabetes [19].

The presence of disease-related autoantibodies can precede the development of overt disease by many years. For example, in patients with type 1 diabetes and antibodies associated with thyroid disease, thyroid disease developed over 10–20 years in 80% of the subjects with positive

Table 34.1 Autoimmune endocrine disorders

Disease	Autoimmune markers	Diagnosis of disease
Type 1 diabetes	Insulin autoantibodies (IAA) GAD65 autoantibodies IA-2 autoantibodies ZnT8 autoantibodies	Glucose Hemoglobin A1c
Hypothyroidism	Thyroid peroxidase Thyroglobulin autoantibodies	TSH Thyroid hormone levels
Hyperthyroidism	Thyroid-stimulating immunoglobulin	TSH Thyroid hormone levels
Adrenal insufficiency	21-Hydroxylase autoantibodies	ACTH Cortisol PRA Electrolytes Dynamic testing with cosyntropin
Gonadal failure	21-Hydroxylase autoantibodies	Primary or secondary amenorrhea Elevated FSH/LH, low estradiol or testosterone
Celiac disease	Tissue transglutaminase autoantibodies	Small intestinal biopsy
Pernicious anemia	Intrinsic factor autoantibodies Parietal cell antibodies	Vitamin B12 deficiency Gastric biopsy

antibodies [20]. Insulin autoantibodies are often the first to develop. Antibodies to IA-1 and ZnT8 are rarely the first to develop. Therefore, the autoantibodies are a marker of risk for disease, but the disease may develop over many years.

Regulatory T-cells are thought to be important in the pathogenesis of disease, and regulatory T-cell gene signatures are associated with type 1 diabetes [21]. Additional analyses of T-cell function and development of T-cell assays will be important for monitoring for risk of autoimmunity and progression of disease.

34.1.4 Hormone Dysfunction

Once the autoimmune process is initiated, progressive failure of the affected gland occurs. In type 1 diabetes, markers of insulin release, insulin resistance, and glucose metabolism are associated with progression to dysglycemia once autoimmunity has occurred [22]. Hemoglobin A1c tends to rise within the normal range as diabetes develops in subjects with diabetes-related autoimmunity [23]. When followed with serial oral glucose tolerance tests, subjects are often noted to have impaired glucose tolerance, diabetes diagnosed on the basis of 2-h glucose alone, and then overt fasting hyperglycemia. However, some will have a normal hemoglobin A1c at the time of diabetes diagnosis based on oral glucose tolerance testing [24]. In subjects with 21-hydroxylase autoantibodies, progressive deterioration in adrenal secretion of cortisol and aldosterone is noted [25]. In thyroid disease, patients may initially present with compensated hypothyroidism and be relatively asymptomatic (elevated TSH but normal thyroid hormone levels) and progress to overt hypothyroidism.

Once sufficient tissue is destroyed, patients present with overt disease. At times, the presentation can be catastrophic and life-threatening such as with diabetic ketoacidosis (DKA) as the initial presentation for type 1 diabetes and adrenal crisis as the initial presentation of Addison's disease. C-peptide decline continues after diagnosis of diabetes in a biphasic fashion [26]. However, some patients with long-standing diabetes continue to secrete C-peptide, opening the door to treatment options even long after onset of overt disease.

34.2 Autoimmune Polyendocrine Syndrome Type 1 (APS-1)/Autoimmune Polyendocrinopathy Candidiasis and Ectodermal Dystrophy (APECED)

34.2.1 Etiology

APS-1 is an (mostly) autosomal recessive disorder historically defined by the presence of two of the following three conditions: hypoparathyroidism, adrenal insufficiency, and candidiasis [27]. The disorder is rare but has an increased frequency in certain populations such as Iranian Jews (1:9000), Sardinians (1:14,000), and the Finns (1:25,000) [28].

Mutations in the gene (located at 21q22.3) that encodes the AIRE protein are responsible for APS-1. It is inherited in a mostly autosomal recessive manner. However, a dominant negative mutation has been identified and associated with an atypical presentation of APS-1 [29]. The gene is a transcription factor which influences transcription by interacting with chromatin, not directly the DNA. It is expressed to a high degree in medullary thymic epithelial cells. These cells play an important role in T-cell maturation. It is hypothesized that the AIRE is important for the expression of self-antigens within the thymus and that this expression is important for the deletion of autoreactive T-cells (negative selection). Therefore, autoreactive T-cells are released into the periphery and can precipitate the autoimmune destruction of the organ to which the T-cells respond.

34.2.2 Clinical Presentation

Patients often present in infancy with chronic mucocutaneous candidiasis. Additional autoimmune diseases develop over time. Hypoparathyroidism often presents in early childhood at a median of 6 years of age. Adrenal insufficiency develops at a median of 10 years of age. The time from first disease component to the second component that would classify a patient as APS-1 can range from 2 to 20 years, which can profoundly delay the diagnosis of this complicated disorder. Autoimmunity affecting other organs can develop over time, and patients need to be monitored carefully for these disorders. Additional autoimmune endocrine disorders can occur including diabetes mellitus, hypothyroidism, and male and female hypogonadism [30–32]. Table 34.2 shows common autoimmune disorders associated with APS-1 and prevalence at various ages.

The autoimmunity associated with APS-1 is not limited to the endocrine disorders. Gastrointestinal symptoms are common and can include diarrhea and constipation. This has been hypothesized to be associated with autoimmune attack of the cells in the duodenum that produce cholecystokinin and serotonin and has been associated with autoantibodies against tryptophan hydroxylase. Patients develop autoimmune hepatitis, pernicious anemia, severe obstipation, and diarrhea. More rarely, patients develop autoimmune hypophysitis with resultant pituitary hormone deficiency, autoimmune disease affecting the lung, rheumatoid arthritis, and nephritis. Asplenia can also be present putting the patient at risk for the development of severe bacterial illness associated with pneumococcal infection. Therefore, subjects need to be carefully monitored for other organ system involvements [30–33].

The candidiasis associated with APS-1 is usually limited to the skin and mucosa. The candidiasis can be difficult to control, and treatment with antifungals may be required on a continuous basis. Patients may present with candidal esophagitis, which requires endoscopy to diagnose. Additionally, candida that is poorly responsive to treatment is a risk for carcinoma of the esophagus with a high morbidity and mortality. Therefore, aggressive control of candidal infections is recommended [33]. The candidiasis is associated with impaired T helper 17 cell response which is thought to increase the local production of antifungal chemokines and antimicrobial peptides [34]. This may be related to autoantibodies against key cytokines.

Patients with APS-1 may also manifest with ocular disease. Approximately 20% of patients develop keratoconjunctivitis. Keratoconjunctivitis often presents in childhood and puts the patient at risk for blindness. Ectodermal dystrophies include enamel hypoplasia, nail dystrophy, and calcium salt deposits in the tympanic membrane. The underlying cause of these abnormalities is not known.

Table 34.2 Autoimmune polyglandular syndrome type 1 (APS-1): disease associations

Component	Time of onset	Disease markers
Mucocutaneous candidiasis	Infancy	Symptoms and physical examination findings consistent with candidiasis
Hypoparathyroidism	Childhood	Low calcium with an inappropriately low or normal parathyroid hormone
Adrenal insufficiency	Childhood/adolescence	Elevated ACTH Decreased cortisol at baseline and in response to stimulation
Hypothyroidism	Adulthood	Elevated TSH, low thyroid hormone levels
Type 1 diabetes	Adulthood	Elevated glucose
Gonadal failure	Females: 20–30s Males: late manifestation	Elevated FSH/LH and low estradiol or testosterone
Autoimmune hepatitis	Prior to age 20 years	Elevated liver function tests Biopsy consistent with hepatitis
Intestinal malabsorption	Throughout lifespan	Constipation and/or diarrhea May complicated medical management of additional autoimmune disease
Celiac disease		Screened with tissue transglutaminase (TTG) IgA antibodies Confirmed on small intestinal biopsy
Pernicious anemia		Antibodies against parietal cells or intrinsic factor B12 deficiency
Asplenia	Throughout lifespan	Howell-Jolly bodies on peripheral blood smear
Ectodermal dystrophy	Childhood	Nail dystrophy Abnormalities of dental enamel Calcification of tympanic membranes
Keratoconjunctivitis	Childhood/adolescence	Diagnosed on eye examination

Data from Husebye et al. [30], p. 519

34.2.3 Diagnostic Evaluation

The diagnosis can be made on a clinical, immunologic, or genetic basis. Clinically, the disorder can be diagnosed when at least two of the three major disease components are present (candidiasis, adrenal insufficiency, and/or hypoparathyroidism). In subjects with a sibling with APS-1, the presence of one of the autoimmune or ectodermal components is diagnostic. However, when these criteria alone are used, a large proportion of subjects with genetically diagnosed APS-1 may be missed. Therefore, a high index of suspicion in addition to understanding the other components of the disorder can aid in the diagnosis of APS-1. Autoantibodies against interferon alpha and omega have been found in almost 100% of patients with APS-1 and are present prior to the development of autoimmune disease [35, 36]. These autoantibodies are rarely identified in healthy controls. Therefore, some have proposed the use of the autoantibodies to screen subjects with autoimmune disorders suspicious for APS-1. A positive result would be considered diagnostic of APS-1. These autoantibodies have the additional advantage of being present throughout the disease course. They have been identified in very young children prior to the development of the classic diagnostic criteria, and they have been identified in subjects with long-standing disease. Some authors propose screening for these autoantibodies and

following up positive results with genetic analysis of the AIRE gene [35]. Subjects with APS-1 require careful and close monitoring for the development of additional autoimmune diseases. Table 34.2 shows a proposed schema for follow-up and screening of patients with APS-1.

34.2.4 Outcomes and Possible Complications

Successful treatment of APS-1 requires close attention to detail and is largely dependent upon the underlying disorders that are present. Diseases such as autoimmune hepatitis and autoimmune pulmonary disease are associated with a particularly poor prognosis.

Given the chronic nature of their condition, the multiple organ systems that can be involved, and the need for frequent hospitalization and intensive treatment, subjects with APS-1 are at a high risk for associated psychiatric disease including depression and anxiety. Screening for such disorders is an important component of the care of patients with APS-1.

34.2.5 Treatment

The treatment of APS-1 is dictated by the clinical features for each patient. Generally, autoimmune endocrine disorders are treated by replacing the missing hormone. Chronic candidal infection may require treatment with systemic antifungals. Patients identified with asplenia require immunization and antibiotics to prevent overwhelming pneumococcal infection. Additional disease components are treated as they are identified. Diseases such as autoimmune hepatitis and autoimmune pulmonary disease may require treatment with systemic immunosuppressive medications.

34.3 Autoimmune Polyendocrine Syndrome Type 2 (APS2)

34.3.1 Etiology

The association of multiple autoimmune endocrine disorders was initially described by Schmidt as the coexistence of Addison's disease with type 1 diabetes and/or autoimmune hypothyroidism. Other autoimmune associations have been described including APS-3 (autoimmune hypothyroidism and another autoimmune disease not including type 1 diabetes or Addison's disease) and APS-4 (two or more organ-specific autoimmune diseases). These distinctions likely do not have clinical significance, and therefore, for the purposes of this discussion, we will use APS-2 to refer to any two organ-specific autoimmune diseases in one individual. Diseases both within and outside the endocrine system are associated with APS-2 including autoimmune thyroid disease (hypo- and hyperthyroidism), type 1 diabetes, Addison's disease, celiac disease, alopecia, vitiligo, autoimmune hypoparathyroidism, primary hypogonadism, myasthenia gravis, and pernicious anemia. Therefore, with the presence of one autoimmune endocrine disorder, practitioners need to be aware of the increased risk for additional diseases and screen with comprehensive history and physical and laboratory testing when indicated.

Patients with type 1 diabetes are at a high risk for the development of thyroid autoimmunity (20%) and disease (5–20%), depending upon duration of follow-up. Hypothyroidism is more common than hyperthyroidism. The presence of thyroid-related autoantibodies is associated with a progression to thyroid disease [20]. Autoimmunity associated with celiac disease is seen in approximately 10% of patients with type 1 diabetes. Approximately 30–50% of these patients have abnormalities on small intestinal biopsies that are consistent with celiac disease [37]. Specific HLA genotypes are associated with an increased risk for celiac disease in the population with T1D [38]. Genes outside the MHC such as *CTLA4* and *IL2RA* have been found in higher proportion of patients with CD and T1D than with either disease alone [39]. Adrenal autoimmunity is increased in patients with type 1 diabetes, such that approximately 1.5% of patients with type 1 diabetes are positive for 21-hydroxylase autoantibodies. Followed over time, approximately 30–40% of these patients go on to become adrenally insufficient [25, 40]. In this population, the risk for adrenal insufficiency is influenced by genes outside of the MHC, level of 21-hydroxylase autoantibody, gender, and presence of associated autoimmune conditions [25]. Patients with celiac disease are at an increased risk for the development of autoimmune thyroid disease, most commonly

hypothyroidism [41, 42]. Patients with autoimmune thyroid disease are also at risk for the development of celiac disease [43].

34.3.2 Clinical Presentation

Patients with APS-2 typically present with symptoms of an autoimmune endocrine disorder (type 1 diabetes, Addison's disease, or autoimmune thyroid disease) and are then found to have an additional autoimmune disease on the basis of more subtle symptoms of routine screening tests (e.g., testing TSH in a patient with type 1 diabetes). Clues to the development of additional autoimmune diseases can be identified on careful history, physical examination, and evaluation of the growth chart. Patients with type 1 diabetes developing adrenal insufficiency may present with decreasing insulin requirement, decreasing A1c, and increasing hypoglycemia. Patients developing hypothyroidism may have a decreased linear growth velocity. Celiac disease may manifest with weight loss and decreasing insulin requirements.

34.3.3 Diagnostic Evaluation

Given the increased rate of autoimmune diseases in patients with one autoimmune endocrine disease, careful screening is required for additional diseases. Current recommendations in patients with type 1 diabetes suggest annual screening for thyroid disease with at least measurement of a thyroid-stimulating hormone (TSH) level. Screening for celiac disease is recommended at onset of type 1 diabetes and with the presence of symptoms of celiac disease. There are no current recommendations for laboratory screening for Addison's disease in the populations with type 1 diabetes [44, 45]. Practice guidelines acknowledge the relationship between celiac disease and thyroid disease and suggest screening for celiac disease in patients with other autoimmune conditions that are associated with celiac disease such as hypothyroidism or a family history of celiac disease. Practitioners should also consider screening for thyroid disease in patients with celiac disease.

Screening for autoimmune diseases includes a careful history and physical examination to identify symptoms or signs of the underlying autoimmune condition. In pediatrics, we have the advantage of monitoring growth and development. Any abnormalities of growth or pubertal development in groups at high risk for the development of underlying autoimmune disease should serve as a red flag and warrant further evaluation including laboratory testing. Patients at high risk for the development of autoimmune disease include those with a previously diagnosed autoimmune disease such as type 1 diabetes, celiac disease, or thyroid disease and patients with chromosomal disorders associated with autoimmunity including Down and Turner's syndromes. The specific testing undertaken depends upon the underlying autoimmune disease and can include measurement of autoantibody levels, chemistry, and hormone levels. Depending upon these tests, additional testing including small intestinal biopsy (for celiac disease) and stimulation testing (for Addison's disease) may be necessary.

34.3.4 Outcomes and Possible Complications

Successful treatment of APS-2 requires close attention to detail and is largely dependent upon the underlying disorders that are present.

34.3.5 Treatment

Treatment focuses on treating the underlying autoimmune disease identified. Knowing the associations of these diseases allows for careful assessment for additional underlying autoimmune diseases, which has important clinical implications. For example, treatment of patients with undiagnosed adrenal insufficiency and hypothyroidism with levothyroxine may unmask the adrenal insufficiency and precipitate an adrenal crisis.

Treating the underlying autoimmune process to prevent the development of active disease is an area of active research in the setting of type 1 diabetes [46, 47]. Treatment has been targeted at each of the stages of autoimmune disease development, including genetic risk, presence of autoimmunity prior to development abnormalities of glucose metabolism, and presence of autoimmunity, impaired fasting glucose or impaired glucose tolerance, and early type 1 diabetes. The goals for the

treatment vary depending upon the stage of progression to diabetes. Treatment consortiums such as the TrialNet for type 1 diabetes conduct clinical trials in prevention of type 1 diabetes or preservation of C-peptide in patients newly diagnosed with type 1 diabetes [48].

The very earliest stages of disease are found in infants and young children. Therefore, a primary consideration is the safety of the treatment. Treatment trials include the use of hydrolyzed formulas at discontinuation of breast feeding [49], treatment with docosahexaenoic acid (and other components of fish oil), and treatment with oral insulin [50]. These trials are difficult to implement and monitor because the majority of people at high genetic risk for disease will never go on to develop disease. Therefore, many patients will be treated who will never develop disease. Additionally, disease develops over months to years. For this reason, many of these trials use markers of the autoimmune process as treatment end points.

People who have diabetes-related autoantibodies are already at an increased risk for the development of disease. Fewer subjects are needed to see treatment effect, and treatments can be slightly more toxic compared with interventions directed at antibody negative subjects. Large-scale trials have suggested that treatment with oral insulin in subjects who are first-degree relatives of patients with type 1 diabetes and have high levels of insulin autoantibodies may be effective in delaying the development of diabetes by approximately 4 years [51]. Follow-up confirmatory studies are underway. Additionally, treatment with glutamic acid decarboxylase 65 (GAD65) has been suggested to preserve C-peptide production in patients with newly diagnosed type 1 diabetes, without significant side effects [52]. Current trials are underway in patients with newly diagnosed type 1 diabetes and patients with positive GAD65 autoantibodies identified and followed through the TrialNet study.

Patients who are newly diagnosed with type 1 diabetes have been treated with immune-modulating drugs with the intent to preserve C-peptide function. Long-term studies of patients with type 1 diabetes have shown that persistent production of C-peptide is associated with decreased risk for long-term complications of type 1 diabetes. Patients stand to directly benefit from the sustained production of C-peptide. Taken together, treatments that have a higher toxicity are tolerated in patients newly diagnosed with type 1 diabetes compared with treatment directed at antibody-positive subjects without diabetes. At this stage, treatments are generally targeted toward the immune system with the goal preservation of C-peptide production. Anti-CD3 is a short-term T-cell-depleting therapy. Its use in humans was suggested by studies in animal models of type 1 diabetes. It has been used in clinical trials of patients with newly diagnosed type 1 diabetes and has demonstrated preservation of C-peptide production for up to 18 months. However, after the initial preservation of C-peptide production, the autoimmune process reemerges, and C-peptide production begins to decline again [53, 54]. Similarly, treatment with anti-CD 20 (a B-cell-specific antibody) has shown preservation of C-peptide production for approximately 1 year after treatment [55]. The treatments appear to temporarily halt the autoimmune process, but do not alter the underlying autoimmunity. It is possible that multiple or combination treatments will be required over a lifetime to permanently maintain C-peptide production.

34.4 Immunodysregulation Polyendocrinopathy Enteropathy X-Linked Syndrome (IPEX): A Brief Review

IPEX is a rare autoimmune endocrine disorder inherited in an X-linked fashion and associated with autoimmunity and immunodeficiency [56]. The underlying genetic defect is in the FOXp3 gene. FOXp3 is important for the development of regulatory T-cells. Without this gene, CD25+/CD4+ genes do not develop. These cells are regulators of CD4 effector T-cells in the periphery. Without these cells, fulminant autoimmunity can develop. Boys generally present as neonates with early onset type 1 diabetes and severe enteropathy resulting in diarrhea and profound failure to thrive. Patients are also at risk for severe dermatitis, hypo- or hyperthyroidism, anemia, thrombocytopenia, neutropenia, hepatitis, and kidney disease. As the genetic cause of the disease has been identified, it is now clear that the clinical phenotype is wider than previously appreciated and includes later onset of disease (into childhood) with a less fulmi-

nant presentation [57]. FOXp3 has been reported to be associated with recurrent intrauterine fetal demise of male infants [58]. As this is a rare condition, treatments are largely based on anecdotal evidence and have included immunosuppressive medications such as sirolimus and bone marrow transplantation. A report of two patients with low-intensity non-myeloablative conditioning hematopoietic cell transplantation showed stable engraftment of the transplanted cells [59]. Additional series shows an improvement in the disease even with chimeric T-cell populations, suggesting that less toxic preparatory regimens can be considered [60, 61].

Case Study

JF presented with type 1 diabetes at age 10 years. She was symptomatic at the onset of diabetes with polyuria, polydipsia, and a 5 kg weight loss. Initial laboratory testing was significant for an A1c of 10.5%, positive insulin, ZnT8 and GAD65 autoantibodies. Treatment with insulin in a basal bolus regimen was associated with improvement in her symptoms, weight gain, and decrease in her hemoglobin A1c to 8%. After 2 years of diagnosis, she was noted to have a decreased linear growth velocity and no pubertal development. Laboratory testing was obtained which was significant for a markedly elevated TSH of 200 uIU/mL, and she was diagnosed with hypothyroidism. Treatment was begun with levothyroxine at 75 mcg by mouth once daily. Approximately 1 week after treatment was initiated, JF developed pre-syncope and increased frequency of hypoglycemia. In retrospect, she had symptoms of salt craving and increased pigmentation. Laboratory testing was obtained and revealed an elevated ACTH and PRA with low random cortisol. Further testing confirmed an autoimmune process with positive 21-hydroxylase autoantibodies. Treatment with fludrocortisone and hydrocortisone was initiated.

34.5 Summary

Autoimmune endocrine disorders are common disorders in pediatric endocrinology and can coexist in recognized syndromes. Classification of subjects into specific syndromes allows for patient education related to disease and genetic risk, and providers can appropriately monitor their patients for disease. APS-1 is an autoimmune endocrine disorder that is inherited in an autosomal recessive manner. Patients are at risk for the development of multiple autoimmune diseases, and the disease is characterized by the presence of mucocutaneous candidiasis, hypoparathyroidism, and adrenal insufficiency. The disease has a high morbidity and mortality, and multiple organ systems may be involved in the autoimmune process. APS-2 is inherited in a polygenic manner. It is more common in women than man and has a strong HLA association. Other more rare autoimmune endocrine syndromes include the IPEX syndrome. Prompt recognition of this syndrome may allow for lifesaving bone marrow transplantation.

Patients with a single autoimmune endocrine disorder are at an increased risk for the development of additional diseases and warrant close follow-up. Patients should be screened with a thorough history and physical examination for signs or symptoms of autoimmune diseases. Routine screening with laboratory tests may be indicated for certain disorders.

Review Questions

1. What is the genetic basis for APS-1?
   A. *AIRE*
   B. *CYP21A*
   C. *FOXP3*
   D. HLA class II
2. Which autoantibody is present in nearly 100% of patients with APS-1?
   A. 21-hydroxylase autoantibody
   B. Insulin autoantibody
   C. Interferon-alpha autoantibody
   D. Thyroid peroxidase autoantibody
3. What treatment has shown the most promise for prolonging the survival of patients with IPEX?
   A. Cytoxan
   B. Insulin
   C. Hematopoietic stem cell transplantation
   D. Pancreatic enzyme replacement

Answers

1. A
2. C
3. C

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Multiple Endocrine Neoplasia Syndromes

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Key Points

- While children with MEN1 most commonly manifest clinical disease after reaching 10 years of age, there are multiple reports of the syndrome affecting children as young as 5 years.
- The most common initial presenting feature of MEN1 is primary hyperparathyroidism with hypercalcemia, followed by pituitary prolactinoma, and insulinoma with hypoglycemia.
- Medullary thyroid carcinoma and its management dominate the landscape in the management of children with MEN2A and MEN2B, with the specific RET mutation determining level of risk and subsequently the timing of prophylactic thyroidectomy. Surgery is indicated before 1 year of age in the highest-risk group.
- Children with a family history indicating a risk of MEN1 or MEN2 should undergo *MEN1* or *RET* sequencing, respectively, as such testing allows for the targeted use of biochemical and radiographic screening in MEN1, while crucially determining the optimal age for thyroidectomy in MEN2.

35.1 Overview

The multiple endocrine neoplasia (MEN) syndromes constitute an assorted group of familial disorders that include neoplasias (hyperplasia, adenomas, and carcinomas) of endocrine glands. The MEN syndromes include MEN type 1, MEN type 2A and the related familial medullary thyroid carcinoma (FMTC), MEN type 2B, and the rare MEN type 4. The MEN syndromes share similar modes of pathogenesis consisting of germline mutations in discrete genetic loci, propagated via autosomal dominant inheritance. Clinical manifestations vary widely within and between their respective patterns of tumorigenesis. Prior to the identification of the responsible gene mutations, all offspring of affected individuals were considered at risk and were thus subjected to regular biochemical and radiographic best-practice screening. Today, mutation carrier status assessment eliminates the need for prospective screening in any child not harboring the specific familial mutation.

Other syndromes featuring endocrine gland neoplasia, such as von Hippel-Lindau disease, Carney complex, the PTEN hamartoma tumor syndromes, and the rare MEN4 [1], are beyond the scope of this chapter, which is limited to discussion of MEN syndrome types 1 and 2 as they pertain to children and adolescents.

35.2 Multiple Endocrine Neoplasia Type 1

35.2.1 Introduction and Background

Approximately 20 individuals affected by multiple adenomas of various endocrine glands had been described in Europe and the United States when, in 1954, Paul Wermer's paper entitled *Genetic Aspects of Adenomatosis of Endocrine Glands* appeared in the American Journal of Medicine [2]. The presentations of tumors variably affecting the adenohypophysis, the pancreatic islets, and parathyroid glands in a man and four of his nine adult offspring were described, and while the possibility had previously been suggested that a familial disorder may account for the syndrome, Dr. Wermer was the first to propose a single genetic defect with autosomal dominant transmission and high penetrance. Over the 40 years which followed, Wermer syndrome, subsequently renamed multiple endocrine neoplasia (MEN) type 1 [3], proved to fulfill perfectly the assertions made by Dr. Wermer in 1954.

The estimated prevalence of MEN1 ranges from one in 10,000 to one in 50,000 of the general population [4, 5] with an estimated incidence of up to 0.25% from random postmortem studies [6]. MEN1 is a familial syndrome of neoplastic transformation of combinations of endocrine glands, featuring the principle triad of parathyroid hyperplasia, pancreatic neuroendocrine tumors, and pituitary adenomas, recalled by the mnemonic "the 3 Ps" [2, 7]. Nonsecreting carcinoid tumors and adrenocortical tumors, including carcinomas, and non-endocrine tumors, such as lipomas, facial angiofibromas, meningiomas, and skin collagenomas, are occasionally present [8–11]. Any of the three chief components may be

the initially presenting manifestation, and the syndrome may be diagnosed in the presence of any two of the three types of endocrine tumors. The specific constellation of tissue involvement is variable between kindreds, between individuals in an affected family, and even between identical twins [12].

35.3 Primary Hyperparathyroidism

35.3.1 Overview

Primary hyperparathyroidism (pHPT) manifests in approximately 95% of individuals with MEN1 by the fifth decade of life, thus exhibiting the highest penetrance of the MEN1 tumors [4, 13–15]. MEN1-related pHPT is the result of a process of multiglandular, asymmetric, and asynchronous hyperplasia [16], differentiating it from the usually solitary adenoma found in sporadic pHPT.

35.3.2 Clinical Presentation

Primary hyperparathyroidism is usually the initial manifestation of MEN1. It was detected in 75% (120/160) of patients less than 21 years of age in a large, multicenter French study of MEN1 subjects published by the Groupe d'étude des Tumeurs Endocrines (GTE) [11]. Ninety percent of these (110 of 120) were at least 10 years of age, with a mean age at diagnosis of 16 years. Similarly, pHPT was detected at a mean age of 19 years during prospective biochemical screening of adolescents at risk for MEN1 in a Swedish cohort [17]. In the GTE, three children with asymptomatic pHPT were detected before 6 years of age [11], recalling a previous report of MEN1-associated pHPT in a 5-year-old child [18].

The degree of hypercalcemia may be relatively mild [19], and classic symptoms (polyuria, constipation, myalgias, abdominal pain, etc.) may be absent. In adults, loss of bone mineral density tends to be more severe, and onset of renal manifestations such as urolithiasis tends to occur earlier in MEN1-related pHPT in comparison with sporadic pHPT [20–22]. Clinical symptoms, including urolithiasis, fatigue, or bone pain, were present in 17% of the GTE cohort, with a mean age of symptom onset of 15 years [11].

35.3.3 Diagnosis

Primary hyperparathyroidism is diagnosed by the combination of elevated albumin-corrected total serum calcium and an elevated plasma parathyroid hormone (PTH) measured by either an intact PTH assay or a PTH 1–84 assay [23]. Secondary hyperparathyroidism (e.g., PTH elevation subsequent to vitamin D deficiency) and tertiary hyperparathyroidism in the setting of chronic renal failure must be excluded. In cases of hypercalcemia with an inappropriately normal PTH level, familial hypocalciuric hypercalcemia (FHH) must also be considered. When pHPT is confirmed biochemically, parathyroid scintigraphy and/or an ultrasound of the parathyroid glands should be performed but may be relatively insensitive for the detection of multigland hyperplasia [24].

35.3.4 Therapy

As with sporadic pHPT, MEN1-related pHPT is treated surgically, the indications for which, in adults, include symptomatic hypercalcemia or serum calcium more than 1.0 mg/dL above the upper limit of normal, nephrolithiasis or marked hypercalciuria (>400 mg/day), impaired renal function (GFR < 60 ml/min), osteoporosis, or young age (less than 50 years) [25]. Reflecting the characteristic four-gland parathyroid hyperplasia and the predisposition for thymoma in MEN1, the surgical approach consists of bilateral neck exploration with total or subtotal parathyroidectomy and transcervical thymectomy. Two surgical strategies are described [26, 27], consisting of (a) total parathyroidectomy with autograft, in which all identified parathyroid glands are removed, with implantation of a parathyroid autograft to the nondominant forearm and (b) subtotal parathyroidectomy, in which all but one-half gland is removed, leaving a small residual in the neck with its native vasculature. Postoperative hypocalcemia resulting from hypoparathyroidism may complicate either approach but is generally avoided in subtotal parathyroidectomy when as little as 50 mg of parathyroid tissue is left intact [27]. In total parathyroidectomy with autograft, permanent hypoparathyroidism due to autograft failure results in approximately one-third of patients [28]. Regardless of surgical strategy, rates of recurrent hypercalcemia are high – greater

than 50% by 10 years in long-term follow-up series [26, 29], a reflection of the inexorable parathyroid hyperplasia of MEN1.

35.4 Pancreatic Neuroendocrine Tumors

35.4.1 Overview

Neoplastic transformation of pancreatic ductal pluripotent stem cells is reported to occur in 30–80% of adult patients with MEN1. Because of the risk of malignant transformation and metastasis, pancreatic neuroendocrine tumors (PNTs) represent the most important threat to survival in individuals with MEN1 [15]. PNTs are classified according to the primary hormone secreted, if any, and the resulting clinical syndrome. "Nonsecreting" pancreatic tumors (NSPT), which may secrete chromogranin A or pancreatic polypeptide (PP), are otherwise clinically silent and present with mass effects or with metastatic disease, unless detected early via radiographic screening. Among functional PNTs in adults, gastrinoma with Zollinger-Ellison syndrome (ZES) is usually reported as the most common, followed by insulinoma; however in the GTE cohort of patients under 21 years of age, this trend was unambiguously reversed, with 20 out of 160 diagnosed with insulinoma and only 3 presenting with a gastrinoma [11]. NSPTs have been reported in a child as young 12 years of age [30]. Tumors secreting somatostatin, vasoactive intestinal polypeptide, glucagon, and adrenocorticotropic hormone (ACTH) are far less common [31].

35.4.2 Clinical Presentation

Pancreatic neuroendocrine tumors occasionally present as the initial manifestation of MEN1 in children [11, 18]. The mean age at diagnosis of PNT in the Swedish series was 25 years, with a youngest presentation at 16 years [17]; however in the larger GTE cohort, the youngest was a 5-year-old with an insulinoma, who presented with signs of neuroglycopenia [11, 31]. As noted above, insulinoma was diagnosed in 12.5% of subjects (20 of 160) under 21 years of age in the GTE cohort and was the initial manifestation of MEN1 in 10% of all subjects [11], suggesting a greater relative prevalence of this tumor in children and adolescents with MEN1, as compared with adults.

The symptoms and signs of MEN1-related insulinoma are identical to those of sporadic cases, with recurrent and often severe postabsorptive and fasting hypoglycemia, fulfilling Whipple's triad. None of the insulinomas reported in the GTE cohort were malignant; however metastatic insulinoma in an adolescent male has been reported [32] (see below).

Gastrinoma with ZES develops in about one-third of adults with MEN1 and presents roughly 10 years earlier than sporadic ZES [33, 34]. In a large NIH series of ZES, the average age of diagnosis was 33 years, and the youngest patient was 12 years old [35]. Three of the 160 MEN1 subjects in the GTE cohort developed ZES, the youngest of whom was a 6-year-old girl presenting with diarrhea and esophagitis, followed by duodenal and antral ulcers leading to the diagnosis at age 7 [11]. As in adults, in whom gastrinomas as small as 1–2 mm often arise within the duodenal wall and tend to metastasize to local nodes [36], the child in the GTE study had a 2 mm prepyloric gastrinoma with a retropancreatic metastatic node and required reoperation in her adolescent years for hepatic metastases [11].

35.4.3 Diagnosis

Insulinoma should be suspected in a child at risk for MEN1 with symptoms of hypoglycemia such as weakness and adrenergic activation or with symptoms of neuroglycopenia, including confusion, disorientation, loss of consciousness, or seizure. During a carefully monitored fast, the finding of a plasma insulin concentration above 3 μU/mL (18 pmol/L) and C-peptide above 0.60 ng/mL (200 pmol/L), concurrent with plasma glucose less than 45 mg/dL (2.5 mmol/L), confirms hyperinsulinism [37].

Classic symptoms of a gastrinoma include diarrhea, epigastric pain, and peptic ulcer disease or hyperplastic gastric folds on endoscopy. A fasting plasma gastrin level above 200 pg/mL (114 pmol/L) with fasting gastric pH below 5.0 is diagnostic of hypergastrinemia. When the fasting gastrin is under 200 pg/mL, the secretin stimulation test may provide confirmation (increase in serum gastrin of >200 pg/ml above baseline after administration of

1–2 μg/kg of secretin IV) [34]. Gastric carcinoid tumors are common in adults with MEN1-related hypergastrinemia, while enterochromaffin-like cell hyperplasia was reported as essentially universal in such patients [38].

PNTs may secrete multiple hormones; the presence of a NSPT or preclinical tumor formation may be detected by the analysis of plasma PP and gastrin response to ingestion of a mixed meal [39]; however measurement of unstimulated levels of the neuroendocrine hormones glucagon, chromogranin A, and PP has low diagnostic utility [40].

35.4.4 Therapy

As reliable pharmacologic suppression of insulinoma secretory activity is not currently available, localization and surgical excision are the only effective treatment [41, 42], while adjuvant somatostatin analog therapy may be useful in controlling secretory activity of unresectable disease [43]. All of the 20 insulinoma cases reported from the GTE cohort were operated; 17 (85%) were immediately successful, while 3 cases required reoperation [11].

Following imaging for tumor localization, surgery is indicated in patients with PTNs because of the risk for malignant transformation and metastasis. Imaging studies include a combination of T1-weighted magnetic resonance imaging (MRI) and endoscopic ultrasonography [44], somatostatin-receptor imaging [45], or Gallium 68-DOTATATE PET/CT [46]. Surgery may include a combination of duodenotomy and subtotal pancreatectomy or, with the aid of intraoperative ultrasonography, focused enucleation of any identifiable PNTs [31, 33, 47]. Long-term risks of subtotal pancreatectomy include insulin-dependent diabetes mellitus and exocrine pancreas dysfunction.

Because very small, 1–2 mm gastrinomas are commonly hidden within the duodenal wall or within the pancreas, surgical attempts at cure of MEN1-related ZES have historically failed. Medical management of ZES however, irrespective of the potential necessity for surgical resection of bulky or metastatic disease, is extremely effective. The commonest cause of death related to MEN1 classically – duodenal or gastric ulceration and perforation, with massive bleeding – has been virtually eliminated by the mitigation of gastric acid hypersecretion with proton pump inhibitors (PPI) such as omeprazole and lansoprazole.

35.5 Tumors of the Anterior Pituitary

35.5.1 Overview

MEN1-associated anterior pituitary adenomas occur with a general order of incidence comparable to sporadic pituitary tumors: prolactin (PRL)-secreting adenomas are most common, followed by nonsecreting, co-secreting, growth hormone (GH)-secreting, and adrenocorticotropic hormone (ACTH)-secreting adenomas [48]. A pituitary adenoma was the second most frequent tumor in the GTE cohort of young persons, diagnosed in 55 of 160 subjects (34%), and accounting for the initial clinical manifestation of MEN1 in 21% of patients [11]. There were 38 PRL-secreting adenomas (of which 4 co-secreted either GH or ACTH), 14 nonsecreting, 1 GH-secreting, and 1 ACTH-secreting, while one tumor could not be further characterized [11].

35.5.2 Clinical Presentation

Similar to PNTs, the clinical features of pituitary adenomas depend upon hormone hypersecretion, if any, and upon the presence of mass effects. They are very rare before 10 years of age; however an aggressive PRL/GH co-secreting macroadenoma in a 5-year-old child with MEN1 has been reported [49]. As in sporadic cases, pituitary adenomas in adults with MEN1 demonstrate a 2–3:1 female/male ratio [48, 50]. Three-fourths of cases diagnosed before 21 years of age in the GTE cohort occurred in females [11]. Clinical symptoms were present in 55% of GTE cases and included amenorrhea/galactorrhea (44%), headache (13%), visual impairment (9%), Cushing's disease (4%), and acromegaly (2%) [11]. Delayed puberty or hypogonadism, hypopituitarism, and central diabetes insipidus are other possible presentations [51]. GH-secreting adenomas produce acromegalic changes (such as coarsened facial features, hyperhidrosis, interphalangeal synovitis, and headaches) and, if epiphyseal growth plates are open, accelerated linear growth with gigantism. Features of ACTH-secreting adenoma, Cushing's

disease, in children include growth failure and weight gain [51, 52] in addition to classic Cushingoid changes. In the GTE cohort, larger, more locally aggressive pituitary tumors tended to occur in adolescent males [11]. MEN1-associated pituitary tumors in adults are frequently multicentric, large, and may be locally invasive [48].

35.5.3 Diagnosis

A small pituitary adenoma may be seen on MRI as a hypointense lesion on post-gadolinium images [53]. Biochemical screening for a pituitary adenoma in a child or adolescent at risk should include investigation for PRL and GH excess. A plasma PRL concentration of greater than 200 ng/mL (8696 pmol/L) strongly suggests the presence of a prolactinoma, although lower PRL levels in the absence of drugs which raise PRL should also raise concerns for a prolactinoma [51]. A GH-secreting tumor should be suspected when the plasma insulin-like growth factor I (IGF-I) level is above the age- and sex-matched reference range, while confirmation is defined by a failure of plasma GH to suppress to less than 1 mcg/L after an oral glucose challenge [54]. In suspected cases of Cushing's disease, the diagnosis requires unambiguously abnormal results on at least two different first-line tests, which include late-night salivary cortisol, urinary excretion of free cortisol in a 24-h collection, and the overnight low-dose (1 mg) dexamethasone suppression test [55], with a non-suppressed ACTH level.

35.5.4 Therapy

Because dopamine agonist (DA) therapy is usually effective in suppressing PRL hypersecretion and can lead to markedly reduced tumor volume, it is the first line of treatment for a prolactinoma. Tumor resistance to DA therapy in adolescents is not uncommon and may require relatively high dosing [56, 57]. Cabergoline is preferred over bromocriptine due to better tolerability, greater effectiveness at normalizing PRL and at reducing tumor volume, and more convenient twice-weekly administration. It is dosed variably at 0.25–3.5 mg per week [58]. Transsphenoidal or occasionally transcranial surgery at a high-volume center is indicated for the treatment of acromegaly/pituitary gigantism and Cushing's disease and for large non-secreting adenomas [52, 53]. Macroprolactinomas which impinge upon the optic chiasm and/or are unresponsive to dopamine agonist therapy and those causing hydrocephalus may also require operative management.

35.6 Genetics of MEN1

MEN1 is caused by a heritable germline loss-of-function mutation of the *MEN1* gene, located on chromosome 11q13. *MEN1* encodes a 610 amino acid nuclear protein, menin, which acts as an anti-oncogene by regulating several cell-cycle functions including DNA replication, repair, and transcription [16, 59–61]. The syndrome is expressed via loss of heterozygosity, in which a somatic mutation, "a second hit," affects the wild-type normal allele inherited from the non-affected parent. More than 80% of probands with a family history of MEN1 are identified as harboring a germline mutation, whereas in individuals with simplex MEN1 syndrome (i.e., a single occurrence in a family), a mutation of *MEN1* is identified in about 65% [16]. With possible rare exceptions [62], genotype-phenotype correlation is not observed [16, 48, 63].

MEN1 gene mutation analysis should be obtained in any child suspected of having MEN1 on clinical grounds and in all children with a family history of the syndrome [64]. By virtue of autosomal dominant transmission, every child of a parent affected by MEN1 has a 50% chance of inheriting the mutation. The risk of MEN1 in relatives of a proband with no family history will depend upon the genetic status of the proband's parents. DNA analysis and genetic counseling should be offered to all patients and closely related family members at risk for MEN1. Gene analysis should further be obtained in any child or adolescent with pHPT or with a PNT and should be considered in any with a pituitary adenoma.

35.7 Screening of Children and Adolescents at Risk of Developing MEN1

As noted above, the genetic status of any child with a family history of MEN1 must be determined in order to identify those at risk and in need of prospective biochemical and radiographic

Table 35.1 Recommended MEN1 screening outline

	Biochemical (age)	Radiographic (age)
Parathyroid	Total calcium, intact PTH (5–8 yrs)	–
Pancreas	Fasting glucose (5 yrs)	EUS or MRI every other year (10 yrs)
	Fasting gastrin (10 yrs)	
Pituitary	PRL, IGF-I (5–8 yrs)	MRI every other year (5–8 yrs)

All biochemical screening is recommended yearly
EUS endoscopic ultrasound, *IGF-I* insulin-like growth factor I, *MRI* magnetic resonance imaging, *PTH* parathyroid hormone, *PRL* prolactin
Adapted from Goudet et al. [11]

screening. Such screening is unnecessary in any child whose *MEN1* analysis is negative for the mutation identified in affected relatives. Several groups have published recommendations for MEN1 screening in children [5, 11, 16, 63]. Screening should be carried out in any child with a known *MEN1* mutation, as well as in any child of a parent with clinical MEN1 without an identified mutation (Table 35.1).

The recommended age for initiating annual biochemical screening has decreased as numerous reports of children as young as 5 years of age, affected by various MEN1-related tumors, have accumulated [11, 18, 31, 49]. The relatively low risk of tumorigenesis before the second decade, however, suggests that delay of screening until 5–10 years of age and of proceeding with a stepwise approach is reasonable. Screening for insulinoma is recommended to begin at 5 years of age with the annual measurement of fasting plasma glucose; the fasting insulin is not a useful screening test. Clinicians must watch for indications of hypoglycemia, while parents and young patients should also be familiarized with the classic symptoms [11]. Annual screening for hyperparathyroidism should begin at 5–8 years of age with measurement of plasma total calcium with intact PTH. Pituitary tumor screening should also begin at 5–8 years with measurement of plasma IGF-I and prolactin, as well as pituitary MRI every third year. Screening for non-insulinoma PNTs should begin by age 10 years [11], with the annual measurement of fasting plasma gastrin, and with T1-weighted contrast-enhanced pancreatic MRI or endoscopic ultrasound, every other year. The reader is referred to Table 6 in Goudet et al. [11] for a direct comparison of various screening recommendations.

35.8 Multiple Endocrine Neoplasia Type 2

35.8.1 Introduction

In 1959, 5 years after the publication of Dr. Wermer's paper and while a resident in internal medicine at the State University of New York, Syracuse, John Sipple was consulted on a hypertensive man who had been operated for a hemorrhagic cerebral arteriovenous malformation [65]. The patient died in a matter of days, and on autopsy, Dr. Sipple was impressed by the striking finding of bilateral pheochromocytomas, bilateral thyroid carcinomas, and a parathyroid adenoma. He commenced a meticulous review of published cases of pheochromocytoma in search of other patients with associated thyroid carcinoma. Among 537 cases of pheochromocytoma, five also had thyroid cancer [65]. He published *The association of pheochromocytoma with carcinoma of the thyroid gland*, in the American Journal of Medicine in 1961 [66]. Sipple syndrome, as the entity has been known, was named MEN type 2 in 1968 [3].

Progressive advances in our understanding of MEN2 have revolutionized clinical care of patients with the syndrome. Recognition of its clinical components and autosomal dominant pattern of inheritance was followed by the development of improved biochemical methods of preclinical screening [67]. The characterization of activating point mutations of the *RET* proto-oncogene as the initiating molecular defect for MEN2 and for the familial medullary thyroid carcinoma variant [68, 69] allowed for recognition of the strong genotype-phenotype correlation with specific *RET* codon involvement [70]. This enabled prediction of medullary thyroid carcinoma (MTC) tumor

aggressiveness and risk for early tumorigenesis, which transformed clinical practice. Genetic testing in members of families affected by MEN2 is therefore a tool not merely for recognition and screening but also as an important guide to disease management of MTC in particular, surpassing the utility of calcitonin-based stimulation testing.

MEN2 includes two distinct syndromes – MEN2A with its three subtypes and MEN2B [71]. Classic MEN2A, the most common of the MEN2 syndromes, consists of MTC, pheochromocytoma, and parathyroid hyperplasia [72]. The MEN2A subtypes include:

- MEN2A with cutaneous lichen amyloidosis (MEN2A-CLA), characterized by a pruritic rash caused by deposition of keratin-like peptide in the dermal-epidermal junction [73, 74] and accounting for about 5% of MEN2A cases
- MEN2A with Hirschsprung disease (MEN2A-HD), heralded in infancy with underweight, abdominal distension, and constipation or obstipation [75] and accounting for about 8% of MEN2A cases [76]
- Familial medullary thyroid carcinoma (FMTC), a distinct entity involving only MTC without other associated endocrinopathies [77] and accounting for 10–20% of cases of MEN2 [75]

MEN2B, which accounts for about 5% of all MEN2 cases, includes MTC associated with pheochromocytoma; parathyroid hyperplasia is almost universally absent. MEN2B is further defined by peculiar phenotypic features including a Marfanoid habitus (without cardiac, palate, or lens anomalies), mucosal and conjunctival neuromas [78–80], and diffuse transmural intestinal ganglioneuromas [81]. The often unsubtle presence of oral mucosal neuromas may be present in infancy, allowing for the possibility of early diagnosis of the syndrome [82]. The incidences of the various clinical manifestations of MEN2 are summarized in Table 35.2.

Table 35.2 Incidence of clinical manifestations associated with MEN2A, 2B, and FMTC

	MEN2A (%)	MEN2B (%)	FMTC (%)
MTC	95	100	100
Parathyroid hyperplasia	10–25	<5	0
Pheochromocytoma	~50	~50	0
Mucosal ganglioneuromas	0	~100	0

35.9 Medullary Thyroid Carcinoma

Medullary thyroid carcinoma (MTC) is a bilateral, multifocal cancer originating in the calcitonin-secreting parafollicular C cells, distributed primarily in the upper third of the thyroid lobes. MTC develops in more than 90% of individuals with MEN2, often as the initial manifestation of disease [83, 84]. The MEN2 syndromes account for about 25% of all MTC cases. Metastatic MTC is the most common cause of death in the MEN2 syndromes [85] and represents the end result of a process which begins with C cell hyperplasia progressing to microscopic carcinoma, macroscopic carcinoma, locoregional lymph node metastases, and finally distant metastatic disease over a time course of months to years [83–86]. The specific *RET* codon mutation determines the risk of early tumorigenesis, which can be extremely early: MTC has been diagnosed in a 9-week-old infant with MEN2B [87]. Similarly, in MEN2A, C cell hyperplasia has been demonstrated in a child as early as 20 months of age [88], MTC as early as 3 years of age [89], and metastatic disease as early as 6 years of age [90]. MTC associated with MEN2B is more aggressive than both sporadic MTC and MEN2A-associated MTC [91].

Total thyroidectomy offers the only possibility of cure for MTC and is recommended for all children with MEN2. Surgery performed before disease that is clinically apparent has been termed "prophylactic" thyroidectomy; however in most cases C cell hyperplasia or MTC is identified in surgical pathology [71]. The appropriate age for thyroidectomy is determined based upon the particular *RET* mutation involved and upon clinical data including the basal or stimulated calcitonin level [71]. Although consensus is lacking in regard to timing of surgery, guidelines updated by the American Thyroid Association in 2015 offer a

thorough discussion of the problem with expert recommendations [71]. The guidelines take into consideration the *RET* mutation risk divided into highest-, high-, and moderate-risk categories, informed further by serum calcitonin and carcinoembryonic antigen levels. Surgery is recommended before 1 year of age in the highest-risk group, by 5 years in the high-risk group, and before age 10 in the moderate-risk group [71]. The adequacy of the initial operation determines clinical success [5, 71]. In light of the rarity of MEN2, its complicated diagnostic considerations, clinical and surgical demands, postoperative decision-making, and the need for ongoing outcome data, it is recommended that early thyroidectomy for children occur in experienced tertiary care settings [71].

35.10 Pheochromocytoma

Pheochromocytoma occurs in about one-half of patients with MEN2, with a mean age at presentation of 25–32 years [92, 93]. It has been reported, however, in a child with MEN2A as young as 8 years of age [94], and hypertensive encephalopathy secondary to pheochromocytoma has been described in a 13-year-old child with MEN2A [95]. It is infrequently detected before the onset of MTC [85, 92, 93]. Similar to MTC, pheochromocytoma in MEN2 is usually multicentric and is found in the context of diffuse adrenomedullary hyperplasia [72]. About half of MEN2-related pheochromocytomas are bilateral [96], and while extra-adrenal tumors occur, they are rare. Malignant pheochromocytoma is also relatively rare in MEN2, with an incidence in the range of 0–8% [96, 97].

The classic history of facial pallor, paroxysmal hypertension, and headache is not reliably present in MEN2-related pheochromocytoma. Clinical manifestations may be subtle, and more than one-half of individuals are asymptomatic and normotensive [98]. Symptoms may include paroxysms of anxiety, palpitations or tachycardia, headaches, and diaphoresis. Most children with pheochromocytoma who are hypertensive have sustained rather than paroxysmal hypertension [99]. Initial biochemical findings include elevated plasma levels of the epinephrine metabolite metanephrine, with an increase in urinary excretion of metanephrines and catecholamines.

35.11 Primary Hyperparathyroidism

Approximately 20% of persons affected by MEN2A eventually develop hyperparathyroidism [5], as a result of parathyroid adenomatous formation within a background of hyperplasia involving multiple glands [100]. The diagnosis of pHPT in MEN2 is as noted in the discussion of MEN1 above, hinging on hypercalcemia with a concurrently elevated (or high normal) intact PTH level. Pheochromocytoma is a rare cause of hypercalcemia but should be considered and excluded as well in patients with MEN2 [86].

35.12 Diagnostic Guidelines: Screening for the Presence of MEN2

35.12.1 Genetics of MEN2 Syndromes

Discussion of screening paradigms for the presence of MEN2 must be prefaced by an understanding of the molecular genetics of the disorder, since current standards of care are based on the outcome of DNA testing. The MEN2 syndromes are either inherited as an autosomal dominant disorder, or they occur as new germline mutations in the absence of a family history. The gene locus for MEN2 was linked in 1987 to the centromeric region of chromosome 10 [101, 102]. Single activating point mutations within the *RET* proto-oncogene were found to associate with the range of clinical phenotypes of the MEN2 syndromes [70]. The *RET* gene, at chromosome 10q11.2, encodes RET, a transmembrane receptor tyrosine kinase which transduces growth and differentiation signals in several developing tissues, and plays a critical role in neural crest development and in neural crest-derived tumors [80, 103]. MEN2 is caused by gain-of-function missense mutations in the RET extracellular cysteine-rich region involved in receptor dimerization and in the intracellular tyrosine kinase domain [70]. Codon 634 mutations are present in about 80% of individuals affected with the classic MEN2A syndrome, although a small percentage of families harboring this mutation have FMTC only. MEN2B is most commonly associated with a germline

mutation of codon 918 and less commonly with a mutation of codon 883 [104]. The specific *RET* codon mutation present correlates with the phenotypic expression of MEN2 [70, 105].

35.12.2 Screening for the Presence of MEN2

Prior to the era of molecular genetic screening, provocative testing of calcitonin release using pentagastrin stimulation was the preferred procedure for MTC screening [86]. The test is administered by giving pentagastrin 0.5 mcg/kg as an intravenous bolus over 5–10 s, followed by plasma calcitonin measurement at 2 and 5 min. Widely available DNA testing has surpassed calcitonin stimulation in importance, though biochemical screening does retain utility in the assessment for C cell hyperplasia and MTC [71].

The use of DNA testing in at-risk family members allows detection of the MEN2 syndrome before development of C cell abnormalities, allowing for potentially curative or truly prophylactic thyroidectomy. DNA testing also eliminates the need for repeated biochemical testing in those that do not harbor a *RET* mutation. And finally, DNA testing eliminates the false-positive rate of the pentagastrin test, which is estimated at 3–5% and which may have resulted in unnecessary thyroidectomy [104]. Screening for *RET* germline mutations can be performed at any age from birth onward, since only a small blood sample is required. Genotyping should thus ideally be performed before the age at which thyroidectomy would be recommended if a mutation were discovered [106]. Multiple centers now offer genomic DNA analysis for *RET* mutations (see endnote). Perhaps the greatest difficulty occurs in the rare situation where germline transmission of MTC is proven, but no *RET* mutation is identified, in which case it may be necessary to identify a research laboratory that will analyze regions of the *RET* gene outside the most commonly mutated regions [107].

35.12.3 Screening: Pheochromocytoma and Hyperparathyroidism

Annual screenings for pheochromocytoma consisting of blood pressure measurement and biochemical testing should be initiated in all children with a known RET mutation beginning at 5 years of age [108]. Measurement of plasma-fractionated metanephrines offers the highest sensitivity and is generally easier to obtain in children than 24-h urine collection. However the relatively low specificity of plasma metanephrines necessitates measuring fractionated metanephrines and catecholamines in a 24-h urine collection, for confirmation [109]. When the diagnosis is confirmed biochemically, imaging with adrenal computed tomography (CT) or MRI is indicated for localization. The presence of pheochromocytoma must be ruled out prior to any operative procedure to avoid a hypertensive crisis in the perioperative period. When both MTC and pheochromocytoma are present, the adrenal tumor should be resected before thyroidectomy is undertaken.

Screening for hyperparathyroidism in patients with MEN2A includes measurement of serum calcium yearly, beginning at 11–16 years, depending upon the RET mutation present [71]. An elevated serum albumin-corrected total calcium should be repeated for confirmation, with an intact PTH. Treatment is as discussed above for MEN 1-related primary hyperparathyroidism.

35.13 Management of MEN2 Kindreds: Incorporating Genetic Data

The availability of the highly sensitive and specific DNA-based screening for identification of the MEN2 syndromes spares half of patients at risk – those without a demonstrable genetic mutation – from further specialized medical follow-up. Because pheochromocytoma is rarely malignant in the MEN2 syndromes, genetic identification of a *RET* mutation does not dictate prophylactic adrenalectomy. Hyperparathyroidism occurs in a minority of patients and also does not have malignant potential. Recommendations for screening for these components of the syndrome therefore remain unchanged by the genetic advances, with the exception that only those with mutations demonstrated by DNA-based testing need to undergo recurrent screening.

Most influenced by the advent of DNA-based diagnostic testing is the management of MTC. The opportunity to improve the outcome for those with MTC lies in the performance of a safe and comprehensive initial surgical procedure [71, 88, 89].

Multiple studies have demonstrated that stage of disease at diagnosis most accurately predicts the length of patient survival [71, 88]. For those with MEN2B, due to the more aggressive form of disease, prophylactic thyroidectomy is recommended as early as possible [88]. Genetic diagnosis of the MEN2 syndromes is readily available commercially.

35.14 Conclusion

The multiple endocrine neoplasia syndromes in their various forms are capable of producing significant morbidity in children and adolescents. Molecular genetic analysis allows conclusive identification of children at risk, making possible the elimination of needless and costly surveillance for half of offspring of affected parents. Extensive experience with affected and at-risk children, as accumulated and published by the GTE and other groups, has yielded fuller awareness of the early natural history of the MEN syndromes while allowing for improved biochemical and radiographic screening protocols. The goal of early disease detection before the onset of morbidity has become increasingly possible. The treatment of MEN-related tumors should be undertaken in centers with experience in the management of these challenging syndromes.

MEN1 Case Study

A previously well 16-year-old male was referred for evaluation of a 4-month history of episodic tremors, sweats, and palpitations. Episodes tended to occur in the morning hours, were sometimes accompanied by disorientation, and were relieved with eating. He had gained 15 pounds in the 2 months prior to presentation. He was taking no medications, and no family members were taking oral hypoglycemic drugs. A paternal uncle had been diagnosed with a prolactinoma at 30 years of age; there was no family history of further tumors nor of hypercalcemia. On examination he was moderately overweight and had stage IV sexual maturity.

Laboratory testing revealed:

- Fasting blood glucose 41 mg/dL, 2.3 mmol/L (60–99 mg/dL, 3.4–5.6 mmol/L)
- Plasma insulin 38 mIU/L, 264 pmol/L (0–17 mIU/L, 0–118 pmol/L)
- C-peptide 5.0 ng/mL, 1655 pmol/L (0.9–4.3 ng/mL, 298–1423 pmol/L)
- Albumin-corrected serum total calcium 10.9 mg/dL, 2.73 mmol/L (8.6–10.4 mg/dL, 2.15–2.60 mmol/L)
- Intact PTH 72.1 pg/mL, 7.6 pmol/L (11.0–67.0 pg/mL, 1.2–7.1 pmol/L)
- Serum prolactin 19.5 ng/mL, 415 mU/L (3.0–15.0 ng/mL, 63–319 mU/L)
- Serum testosterone 216 ng/dL, 7.5 nmol/L (100–1200 ng/dL, 3.5–41.7 nmol/L)

Serum gastrin was not measured preoperatively.

Abdominal MRI revealed a macrolobulated distal pancreatic neoplasm measuring 10 x 8 x 7 cm, with heterogeneous T1 hypointensity and intermediate to hyperintense T2 signal. Several hepatic lesions ranging from 2 to 11 mm were also noted; the larger lesions demonstrated T2 hyperintensity and rim enhancement. Brain MRI demonstrated a 5 mm anterior pituitary T1-hyperintense microadenoma. Ultrasonography of the neck revealed a subcentimeter left parathyroid mass, a suspected adenoma. Genetic analysis of *MEN1* was recommended but was declined.

Distal pancreatectomy and splenectomy with wedge biopsy of the left lateral liver were performed, confirming a pancreatic neuroendocrine tumor with liver metastases, staining positive for insulin, with minor positive staining for gastrin (TNM tumor classification T2 N0 M1). Immediately following surgery, episodes of hypoglycemia and of dyspepsia ceased.

Indium In-111 pentetreotide (OctreoScan®) revealed minimal uptake of octreotide within hepatic metastases. Monthly Sandostatin® LAR (octreotide acetate) injections were started after a short initial course of subcutaneous octreotide injections.

No further episodes of hypoglycemia occurred over the 24 months following surgery, while the liver metastases remained stable on Sandostatin® LAR. Mild hypercalcemia and hyperprolactinemia remained stable, and the pituitary microadenoma also remained stable in size. After 2 years of treatment, the patient elected to discontinue octreotide because of abdominal discomfort and lightheadedness which were attributed to somatostatin analog treatment. Within several months however, new growth of the liver metastatic disease was noted. Treatment was restarted with lanreotide (Somatuline® Depot), and the patient remains under close follow-up.

For information on genetic screening for MEN types 1 and 2, see ► www.genetests.com.

Review Questions

1. Which of the three tumor types forming the classic triad of MEN1 is most commonly the initial presentation in the pediatric age group?
2. What is the second most commonly encountered category of tumors in children and adolescents, and what is the most common subtype?
3. What PNT tumor subtype, relatively rare in adults with MEN1, was the most common PNT tumor among the GTE cohort of patients? What was the second most commonly diagnosed PNT in this study?
4. C cell hyperplasia or MTC occurs in virtually all children with MEN type 2A and type 2B and has been reported extremely early in both types, necessitating prophylactic thyroidectomy. In which type, MEN 2A or 2B, does MTC most characteristically exhibit aggressive behavior?
5. Which MEN2A and 2B tumor occurs in about one-half of patients and is uncommonly diagnosed prior to adulthood?
6. What physical characteristic of MEN2B may appear early in life and allows for ready differentiation from MEN2A?
7. A child with no family history of endocrine tumors presents with hypercalcemia and an intact PTH level in the upper one-third of the normal range. What gene should be sequenced?

Answers

1. pHPT was detected in 75% of the GTE cohort of patients under 21 years of age, presenting at a mean age of 16 years. It was the most common initially diagnosed tumor of MEN1 in the study; however it was the initially presenting tumor in only 56% of cases. Three children with asymptomatic pHPT presented under age 6, indicating how early parathyroid hyperplasia may develop in MEN1.
2. Pituitary adenoma is the second most common tumor type diagnosed in children with MEN1, led by prolactinoma. Prolactinoma is the second most commonly diagnosed discrete MEN1 tumor in the pediatric age group, after pHPT.
3. Insulinoma was the most common PNT diagnosed in the GTE cohort, representing the initial manifestation of MEN1 in 10% of cases. NSPT was the second most common PNT in the GTE cohort.
4. MTC tends to be more aggressive, metastasizing early in MEN2B, as compared with sporadic MTC and MEN2A-associated MTC.
5. Pheochromocytoma occurs in MEN2A and 2B with roughly equal incidence, about 50%.
6. Mucosal ganglioneuromas are distinct in appearance and may be seen in very young children affected with MEN2B. They do not occur in MEN2A.
7. A child presenting with pHPT without a family history may likely represent a proband case of MEN1 and should be screened for a mutation in *MEN1*, the menin gene.

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Care of Gender Nonconforming/ Transgender Youth

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Key Points

- Gender identity exists as a continuum between maleness and femaleness and is influenced by a complex interplay of biologic, cultural, and environmental factors.
- Knowledge and sensitivity regarding care of gender nonconforming/transgender individuals are important in achieving optimal medical and psychosocial outcomes.
- Multidisciplinary team approach of medical, psychological, social, school advocacy, and, if needed, legal services can provide optimal care for gender nonconforming children.
- Medical and surgical treatment of gender dysphoria can decrease mental health morbidity but carry important health and fertility implications that are yet to be fully delineated.

36.1 Introduction and Background

Recent literature and clinical experience have demonstrated that gender identity, described as the authentic inner sense of self as male, female, or other non-binary category, exists as a continuum between maleness and femaleness. Furthermore, there is increasing evidence that gender identity is not merely a psychosocial construct but rather an entity governed by complex interactions among biology, environment, and culture. Gender identity is distinct from sex, the latter of which is categorized based on chromosomal and apparent physical characteristics such as genitalia. The general practice has been to assign sex at birth—and therefore, gender of rearing—based on phenotype, but growing awareness of potential discordance between the body and gender identity has transformed our understanding of what truly governs gender identity [1].

Transgender may be defined as a condition where one's gender identity is not aligned with one's physical sex characteristics. Gender identity may be non-binary. Some individuals describe themselves as gender expansive, gender fluid, gender queer, or agender [2]. "Cisgender" is a term that is occasionally used to describe an individual whose gender identity is in alignment with one's physical sex characteristics. It is important to recognize that gender identity is distinct from sexual identity (sexual orientation). A person with any gender identity can have any sexual orientation.

In 2013, the American Psychiatric Association (APA) removed the diagnosis of "gender identity disorder (GID)" and replaced it with "gender dysphoria," described as a marked incongruence between one's experienced/expressed gender and assigned gender of at least 6 months duration, which causes clinically significant distress or impairment in social, occupational, or other important areas of functioning [3]. This change in terminology underscores the concept that a transgender identity, in and of itself, is no longer considered pathological and, instead, directs clinical concern to the dysphoria that may exist as a consequence of being transgender/gender nonconforming.

The epidemiology of gender nonconforming individuals is difficult to assess, as previous studies may have underreported prevalence and incidence due to still-existent stigma surrounding gender identities that may not align with physical sex characteristics. One large population-based household sample in Massachusetts estimates the incidence to be 0.5% or one in 200 (95% confidence interval 0.3–0.6%) [4]. This is a much higher estimate than previously reported, and similar population studies in the UK, Belgium, the Netherlands, and New Zealand have found the prevalence to be between 0.5% and 1.2% [5].

36.2 Etiology

Gender identity is not simply a psychosocial construct but likely reflects a complex interplay of biology, culture, and environment. Evidence for biological determinants of gender identity comes from three biomedical disciplines: genetics, endocrinology, and neurology.

36.2.1 Genetics

Evidence implicating genetics as a contributing factor to gender identity development is derived

from studies of twins. A literature review in 2012 focused on twins with what was then known as GID. Of 23 pairs of monozygotic female or male twins, 9 pairs (39.1%) were concordant for GID, while there was no concordance for GID in 21 same-sex dizygotic female and male twin pairs or in 7 opposite-sex twin pairs [6]. While these studies suggest that DNA does play a role in gender identity development, the fact that concordance for being transgender was only 39.1% in this study of identical twins may reflect epigenetic changes or the fact that individuals may "come out" as transgender at any age, even late in life.

36.2.2 Endocrinology

While most transgender individuals do not have a difference in sex development (DSD), studies of patients with DSDs support a role of hormones—prenatal (and perhaps postnatal) androgens, in particular—in gender identity development. However, the hormonal environment is not likely to be the only determinant of gender identity, given that most transgender individuals do not have any discernable evidence of hormonal abnormalities (though there may have been some variations in prenatal hormone secretion or sensitivity that are not readily apparent from examination of the genitalia).

Individuals with virilizing congenital adrenal hyperplasia (CAH) represent a population in which the early hormonal environment of 46,XX individuals is largely androgenic, due to various defects in the steroidogenesis pathway. Review of the literature has shown that in a case series of 250 46,XX adult individuals with virilizing CAH caused by 21-hydroxylase deficiency that were raised female, nearly 95% were identified as females. However, 5.2% had significant gender dysphoria or cross-gender identification, which is approximately ten times higher than the estimated prevalence of female-to-male transgender individuals in the general population. There was, however, no correlation with degree of genital virilization and gender identity outcome [7]. A more recent study in youth with virilizing 21-hydroxylase-deficient CAH demonstrated that 12.8% of 46,XX individuals demonstrated cross-gender identification independent of gender behavior [8]. A variety of other hormonal and non-hormonal DSDs, particularly in 46,XY individuals, support some role of prenatal/postnatal androgens in gender identity development; however, an individual with complete androgen insensitivity syndrome associated with an unambiguous female phenotype and an *androgen receptor* gene mutation resulting in a premature stop codon was found to have a male gender identity, indicating that androgen signaling may not be essential for male gender identity development [1, 9–11].

36.2.3 Neurobiology

Compelling studies in transgender individuals have shown that some sexually dimorphic brain structures (both gray and white matter) are more closely aligned with a person's gender identity than with their physical sex characteristics (reviewed in reference [1]) and that this is apparent even before treatment with cross-sex hormones.

A recent study analyzed sexually dimorphic regions in over 1400 brains by MRI and found extensive overlap between females and males. In fact, only a minority of individuals had internal consistency such that the males or females had exclusively the male or female versions, respectively, of these sexually dimorphic regions. Instead, the majority of these brains were described as mosaics having some features more common in females and some more common in males [12]. While this report did not directly address gender identity, the findings may be viewed in support of the concept that gender identity exists on a continuum rather than in binary categories.

Functional positron emission tomography (PET) studies have been done to assess changes in regional cerebral blood flow in response to pheromone compounds. A Swedish study performed PET imaging in response to smelling progesterone derivative 4,16-androstadien-3-one (AND) and estrogen-like compound estra-1,3,5(10),16-tetraen-3-ol (EST). These compounds have been shown to activate hypothalamic blood flow in a sexually dimorphic fashion. A group of 12 male-to-female transgender women, prior to any treatment with estrogen, had hypothalamic activation

with AND. This was the same pattern as seen in the non-transgender ("cisgender") women controls. Cisgender men demonstrated hypothalamic activation with EST [13]. A study in adolescents with gender dysphoria revealed similar sex-atypical functional brain characteristics before any hormone treatment, indicating that such variations occur early in brain development [14].

36.3 Clinical Presentation

The principal clinical manifestation for transgender children and youth is often gender dysphoria. Gender dysphoria is manifested in a variety of ways, including a strong desire to be treated as the experienced gender and not the assigned gender, to be rid of one's sex characteristics, or a strong conviction that one has feelings and reactions typical of the experienced gender [5].

Children, as young as the age of 2 years, may present with gender incongruence, behaving in traditional gender roles opposite to their assigned gender, asking to be called by a different name more suitable with their gender identity, or simply stating they are a "boy" or a "girl," opposite to their assigned gender [2]. Not all children presenting with gender incongruence will persist throughout their lifetimes. Persistence rates in prospective studies ranged between 2% and 27%, with the main factor associated with persistence into adolescence and adulthood being the intensity of gender dysphoria in childhood [15]. Other studies have shown that children that experienced increased dysphoria in adolescence, starting between 10 and 13 years of age, were more likely to have a stable transgender identity [16].

Many children, who do not persist with gender incongruence, explore gender at its margins in a developmental progression toward their later gay identity, at which point the gender incongruence may dissipate or disappear [17].

With the onset of either female or male endogenous puberty, transgender adolescents may present with significant distress and worsening of gender dysphoria. The development of an adult version of a body that the young person experiences as "wrong" may represent the permanence of gender incongruence and can trigger negative psychosocial consequences [18]. These psychosocial outcomes may include depression, anxiety, social isolation, self-harming behaviors, illicit drug use, high-risk sexual behavior, and suicidality. Compared with cisgender controls, transgender youth have been shown to have a twofold to threefold increased risk of depression (50.6% vs. 20.6%; RR, 3.95), anxiety disorders (26.7% vs. 10.0%; RR, 3.27), suicidal ideation (31.1% vs. 11.1%; RR, 3.61), suicide attempts (17.2% vs. 6.1%; RR, 3.2), self-harm without lethal intent (16.7% vs. 4.4%; RR, 3.2), and the need for both inpatient and outpatient mental health treatment (22.8% and 45.6% vs. 11.1% and 16.1%, respectively; RR, 2.36, 4.36) [19]. Children and adolescents presenting with gender dysphoria may also have increased rates of autism spectrum disorder (ASD), with a rate of 7.8% reported in transgender children and adolescents, ten times higher than the prevalence of 0.6–1% of ASD in the general population [20].

36.4 Diagnostic Evaluation

Primary care providers and mental health professionals are often the first point of contact for gender nonconforming individuals. It is important for these providers to recognize the possibility of gender dysphoria and refer patients to clinical services with experience in the treatment of gender nonconforming people—ideally, a multidisciplinary care team that includes mental health, medical, social work, education/advocacy, and, if needed, legal support. It is essential that determination of gender dysphoria in a youth/adolescent be made by a qualified mental health professional.

36.5 Treatment

A letter of support from a mental health provider is often obtained prior to proceeding with medical intervention. It is the medical provider's responsibility to ensure that patients and their families understand not only the benefits but also the potential risks of medical interventions as well as to establish a strategy for laboratory surveillance of potential adverse effects. The World Professional Association for Transgender Health

(WPATH) and the Endocrine Society have published clinical practice guidelines which are periodically updated [21, 22]. The Endocrine Society clinical practice guidelines are co-sponsored by the Pediatric Endocrine Society and a number of other professional endocrine societies.

36.5.1 Prepuberty

For prepubertal children who are insistent, consistent, and persistent with significant gender dysphoria, a gender-affirmative approach is thought to have the best outcome [17]. In some cases, these children may insist on living as their experienced gender (social transition). Establishing care with a mental health gender specialist can assist in providing optimal support to the child. At this stage, there are no medical therapies indicated, as there are no significant levels of pubertal sex hormones. Additionally, the preemptive treatment with puberty blockers (see below) prior to development of Tanner stage 2 is not advised [22].

36.5.2 Early Puberty

When gender nonconforming children enter Tanner stage 2 of puberty, there is often an increase in gender dysphoria. It is at this point that medical treatment should be considered, as long as there is adequate psychosocial support. Pioneering work by the Dutch has demonstrated that putting puberty "on hold" in early pubertal, gender dysphoric youth can provide additional time to explore one's gender identity working with a gender specialist and can prevent the development of otherwise irreversible secondary sex characteristics [23]. Gonadotropin-releasing hormone (GnRH) agonists such as leuprolide and histrelin, fully reversible interventions, are the mainstay for blocking the progression of puberty in gender dysphoric youth. Confirmatory blood testing with ultrasensitive assays can be used in conjunction with the physical examination if the individual appears to be at the earliest stage of puberty. It should be noted that the emotional impact of early puberty is considered to be of diagnostic value in the determination of gender dysphoria [23]. Initiation of and timing of pubertal blockers should be considered on a case-by-case basis.

36.5.3 Late Puberty and Postpuberty

Studies have shown that gender dysphoric youth blocked at Tanner 2 will often seek phenotypic transition with cross-sex hormones when they are older [24]. While the Endocrine Society guidelines recommend that cross-sex hormone therapy be started at about age 16, there are compelling reasons to consider starting such treatment at an earlier age. In addition to potential mental distress and social isolation by being kept in a prepubertal state until age 16 years, being hypogonadal during this time could have adverse consequences on bone mineral acquisition (see below).

In the case of male-to-female transition during adolescence, GnRH analogs are continued in conjunction with estrogen, as the typical doses of estrogen alone used are not thought to be sufficient to adequately suppress the hypothalamic-pituitary-testicular axis [22]. For female-to-male transition, GnRH analogs are continued until adult levels of testosterone are reached, which are typically sufficient to suppress the hypothalamic-pituitary-ovarian axis. Breakthrough bleeding occasionally occurs in such individuals and often responds to treatment with a GnRH analog in addition to testosterone. In those individuals previously blocked at Tanner 2, cross-sex hormones are typically increased to adult ranges over the course of 2–3 years. Not infrequently, transgender youth present for medical services having already nearly or fully completed endogenous puberty. In such cases, cross-sex hormones can be titrated to adult levels over 2–3 months. GnRH analogs are expensive, currently not labeled for pubertal suppression in gender dysphoric youth, and are often denied by insurance companies. Recommended treatment regimens, including alternate approaches, are described in ▶ Box 36.1. Recommendations for physical examination and laboratory surveillance during treatment with pubertal blockers and with cross-sex hormones are summarized in ◘ Table 36.1.

Box 36.1 Hormonal Interventions for Transgender Adolescents (All Currently Off-Label for Gender Nonconforming/Transgender Youth)

1. Inhibitors of gonadal sex steroid secretion or action
   1. GnRH analogs: inhibition of the hypothalamic-pituitary-gonadal (HPG) axis (FTM and MTF)
      1. Leuprolide acetate IM (1- or 3-month preparations) or SC (1-, 3-, 4-, or 6-month preparations) at doses sufficient to suppress pituitary gonadotropins and gonadal sex steroids
      2. Histrelin acetate 50 mg SC implant (once-yearly dosing, though may have longer effectiveness)
      3. Other options: goserelin acetate SC implant (4- or 12-week preparations); nafarelin acetate intranasal (multiple daily doses) also available, but no reported use in this population
   2. Alternative approaches
      1. Medroxyprogesterone acetate orally (up to 40 mg/day) or IM (150 mg q 3 months): inhibition of HPG axis and direct inhibition of gonadal steroidogenesis (FTM and MTF)
      2. Spironolactone (25–50 mg/day with gradual increase to 100–300 mg/day orally, divided into BID dosing) Inhibition of testosterone synthesis and action (MTF)
      3. Cyproterone acetate (gradual increase up to 100 mg/day orally; not available in the United States) Inhibition of testosterone synthesis and action (MTF)
      4. Finasteride (2.5–5 mg/day orally) Inhibition of Type II 5 α-reductase, blocking conversion of testosterone to 5 α-dihydrotestosterone (MTF)
2. Cross-sex hormones
   1. MTF: estrogen (17 β-estradiol)
      1. Transdermal: twice-weekly patches [6.25 mcg (achieved by cutting a 25 mcg patch) with gradual increase to full adult dose (typically, 100–200 mcg patch)]
      2. Oral/sublingual: daily (0.25 mg with gradual increase to full adult dose of 2–6 mg/day)
      3. Parenteral IM (synthetic esters of 17 β-estradiol): estradiol valerate (5–20 mg increasing up to 30–40 mg q 2 weeks) or estradiol cypionate (2–10 mg q 1 week)
   2. FTM: Testosterone
      1. Parenteral IM or SC (synthetic esters of testosterone): testosterone cypionate or enanthate (12.5 mg q week or 25 mg q 2 weeks, with gradual increase to 50–100 mg q week or 100–200 mg q 2 weeks)
      2. Transdermal (consider once full adult testosterone dose has been achieved parenterally): patch (2–6 mg/day) or 1.62% gel (20.25–81 mg/day)

From Rosenthal [1]. Reprinted with permission from the Endocrine Society
MTF male-to-female, *FTM* female-to-male

Gender-affirming surgery, which may include gonadectomy, genital surgery, and plastic surgery for facial contouring, is often postponed until 18 years of age [22]. In some cases, mastectomy may be indicated before age 18 years. As with medical interventions, a mental health gender specialist should be involved in decisions regarding such surgical procedures.

36.6 Outcomes and Possible Complications

There are currently minimal outcomes data regarding the use of pubertal blockers and cross-sex hormones in gender dysphoric youth. While some studies have been reported in transgender adults [1, 22], the long-term risks of altering the hormonal milieu are unknown. Thus, long-term follow-up is essential.

36.6.1 Mental Health

Medical intervention, as part of a multidisciplinary gender team approach in transgender adolescents, has been shown to have favorable mental health outcomes. A prospective Dutch study showed that after an average of nearly 2 years of GnRH agonist treatment, psychological functioning of adolescents diagnosed with gender identity disorder (a term that has now been replaced with "gender

Table 36.1 Monitoring during pubertal suppression and during cross-sex hormone treatment

Measure	Frequency
1. *Pubertal suppression*	
1. Physical exam: height, weight, Tanner staging	T 0 & q 3 mo
2. Hormonal studies: ultrasensitive LH, FSH, estradiol/testosterone	T 0 & q 3 mo
3. Metabolic: Ca, phos, alk phos, 25-OH vitamin D (see also Ref. [3])	T 0 & q 1 yr
4. Bone mineral density: DXA	T 0 & q 1 yr
5. Bone age	T 0 & q 1 yr
2. *Cross-sex hormone treatment in previously suppressed patients or in late pubertal patients not previously suppressed*	
1. Physical exam: height, weight, Tanner staging, BP (for FTM, in particular); monitor for adverse reactions	T 0 & q 3 mo[a]
2. Hormonal studies: ultrasensitive LH, FSH, estradiol/testosterone	T 0 & q 3 mo[a]
If MTF: also monitor prolactin	T 0 & q 1 yr
3. Metabolic: Ca, phos, alk phos, 25-OH vitamin D, complete blood count, renal and liver function, fasting lipids, glucose, insulin, glycosylated hemoglobin	T 0 & q 3 mo[a]
If MTF on spironolactone: serum electrolytes (potassium)	T 0 & q 3 mo[a]
4. Bone mineral density: DXA (if puberty previously suppressed)	T 0 & q 1 yr[b]
5. Bone age (if puberty previously suppressed)	T 0 & q 1 yr[b]

From Rosenthal [1]. Reprinted with permission from the Endocrine Society
Modified from Hembree et al. [22]
MTF male-to-female, *FTM* female-to-male
[a]q 3–12 mo after first yr
[b]Until puberty is completed

dysphoria" in DSM-5) had improved in many respects. Adolescents showed fewer behavioral and emotional problems, reported fewer depressive symptoms, feelings of anxiety and anger remained stable (but did not decrease), and their general functioning improved [24]. A longer-term evaluation of the same cohort demonstrated that gender dysphoria and body image difficulties persisted through puberty suppression but remitted after the administration of cross-sex hormones and gender reassignment surgery. Psychological functioning improved steadily over time, resulting in rates of clinical problems that were indistinguishable from general population samples, and quality of life, satisfaction with life, and subjective happiness were comparable to same-age peers [25].

In prepubertal transgender children, where medical intervention is not indicated, it has been shown that socially transitioned children, who were supported in their identities, demonstrated substantially lower rates of depression and anxiety as compared with children with gender identity disorder reported in previous studies. Rates of depression resembled population averages, with only slightly elevated rates of anxiety symptoms. These findings suggest that familial support in general, or specifically via the decision to allow their children to socially transition, may be associated with better mental health outcomes among transgender children [26].

Parental support is also beneficial in older age groups, as shown by a report from Ontario, Canada, that assessed the impact of the degree of parental support on mental health outcomes of transgender youth and young adults aged 16–24 years. This report demonstrated that satisfaction with life and self-esteem was significantly greater in transgender youth whose parents were "very supportive"

vs. those whose parents were "somewhat to not at all supportive," with depression and suicide attempts significantly decreased among those with supportive parents [27, 28].

36.6.2 Bone Health

As peak bone mass, a major determinant of future adult fracture risk, is achieved during puberty and young adulthood, medical treatment of gender nonconforming youth can affect bone health. Some clinicians routinely obtain measures of bone density at baseline and annually while GnRH analogs are being used alone and subsequently after initiation of treatment with cross-sex hormones. Any individual treated with a pubertal blocker should have careful monitoring of Vitamin D status (with supplementation if necessary), adequate intake of dietary calcium, and adequate weight-bearing exercise.

Bone density has been studied in individuals with delayed and early puberty. Delayed menarche beyond the age of 15 years carries a 1.5-fold increase in fracture risk. In delayed male puberty, the effects on bone health are less clear [29]. There has been evidence that age of onset of puberty does predict bone mass in young adulthood, although long-term follow-up is lacking [30]. In patients with central precocious puberty treated with GnRH agonists, there is as dip in bone mineral density (BMD) at onset of puberty suppression, but levels rebound to control values after discontinuation of GnRH agonists, which in turn allows resumption of endogenous puberty [31].

Few studies have looked at BMD in transgender individuals, but several have indicated that transgender women have a propensity toward lower BMD at baseline prior to any cross hormone therapy [32, 33]. In transgender youth, only one group has published data regarding BMD in adolescents receiving therapy for pubertal suppression and cross hormones, and there have not been any long-term follow-up studies regarding overall effect on BMD. However, the same lower BMD (by Z-score) was found at baseline in transgender girls, which subsequently fell further with GnRH agonists. These subjects were only followed until age 22, and at that point, BMD Z-scores had not recovered to control levels, although cross hormones were not started until age 16 [34]. A separate study also looked at bone turnover markers and BMD in transgender adolescents and reported similar findings for BMD at baseline, with initiation of GnRH agonists, as well as changes in BMD with cross hormone therapy, but found no clear value in assessing bone turnover markers [35].

36.6.3 Fertility

Transgender individuals often wish to preserve potential for fertility. Treatment with GnRH agonists in central precocious puberty does not impair future fertility [31]. However, if the use of GnRH agonists in early-pubertal gender dysphoric children is later combined with cross-sex hormones, fertility will be compromised due to arrested gonadal maturation. Currently, experimental harvesting of prepubertal gonadal tissue, cryopreservation, and in vitro maturation are being explored in other medical contexts [36, 37] and may be of benefit for fertility preservation in early pubertal transgender youth. Research to obtain artificial gametes through stem cells is also ongoing and could be a feasible option in the future, for those individuals who cannot or have not stored their own gametes [38].

In late pubertal or postpubertal transgender youth with mature gonads, fertility may be preserved via cryopreservation of sperm or oocytes, before initiating cross-sex hormonal therapy. Prolonged estrogen treatment results in reduction of testicular volume and has a suppressive effect on sperm motility and density, but these effects can be reversible. Testosterone treatment in most cases results in reversible amenorrhea, though ovarian follicles are not depleted. As such, hormonal interventions for transitioning do not exclude a possible pregnancy in female-to-male transgender men. In fact, it is important to counsel female-to-male transgender adolescents that may engage in receptive vaginal sex that testosterone is not a reliable contraceptive. In cases where transgender men decide to retain their ovaries and uterus, they may regain fertility after discontinuing androgen therapy [38].

The first live birth after living uterine transplantation was reported in 2014 in a woman with congenital absence of the uterus (Rokitansky syndrome). The uterus was donated by a 61-year-old woman who had already had two successful pregnancies [39]. This type of procedure opens the possibility for assisted gestation for male-to-female transgender women, though not without significant challenges, including the need for immunosuppressive therapy [40].

36.6.4 Brain

There are theoretical risks to the brain when using GnRH agonists during adolescence, as puberty is thought to be associated with a further organizational period of brain development. GnRH agonist treatment in transgender adolescents did not appear to affect executive function, a cognitive milestone associated with pubertal development [41]. More research is needed to better assess potential adverse effects of GnRH agonists on the brain in transgender youth.

Case Studies

The approach to each patient should be tailored, particularly taking into consideration pubertal status and degree of family, psychosocial, and school support.

Case 1
A 16-year-old assigned female at birth presents to the clinic with significant depression, self-harming behaviors, and a history of suicide attempts. The patient identifies as a transgender male and prefers masculine pronouns. Susan reports never having felt comfortable as a girl, refusing to wear dresses or play with dolls as a young child. Anxiety and depression began at the initiation of puberty and intensified as puberty progressed. Two years ago, he cut his hair short and asked to be called John. He is extremely distressed about his breasts and wears a binder. Additionally, he experiences significant dysphoria around his menstrual cycles, opting to stay home from school when they occur. His mother is supportive but his father is not. School has also been difficult, and he has fallen victim to bullying by his peers.

Case 2
A 9-year-old assigned male at birth presents to the clinic. Parents report that since the age of 24 months, Jake preferred girl's clothing and insisted on using hair barrettes, wearing girl's underwear, and stated consistently that he was a girl and not a boy. At age 4 years, Jake asked to wear his hair long "like mommy." Jake started presenting socially as female in kindergarten, changing his name to Julie, and wearing girl's clothes to school. Parents report that school and extended family are all supportive. Both parents and Julie are in clinic today for assessment of pubertal development. They are all concerned about onset of male puberty and are anxious to start treatment with a pubertal blocker if any signs of male puberty are present.

36.7 Summary

As public awareness and scientific knowledge about gender nonconformity grow, pediatric endocrinologists and other medical providers (e.g., primary care, family practice, and adolescent medicine specialists) will be increasingly tasked with caring for gender nonconforming youth, many of whom will ultimately desire medical or surgical transition. Identifying appropriate services, ideally in a multidisciplinary setting, is an important first step. Understanding that gender identity likely represents a complex interplay of biology, culture, and environment, and is influenced by more than just karyotype, hormones, and gender of rearing, can lead to increased understanding of the challenges faced by transgender individuals and hopefully lead to increased acceptance and improved quality of life. While rigorous studies have shown that gender-affirming care is important in achieving positive mental health outcomes, there is a relative paucity of outcomes data, and long-term research is necessary to optimize medical and psychological care of gender nonconforming youth.

Review Questions

1. Amanda, a 5-year-old assigned female at birth, and her parents are seen in clinic for the first time. Amanda's parents describe that since the age of 3, she has insisted that she is a boy, refusing to wear dresses, and been interested only in traditional boy toys. For the last 2 months, she has been repeatedly asking to be called Tom and to cut her hair short. She also is asking to join the boys' soccer team. What would you advise the parents?
   A. This is just a phase. Parents are advised to continue explaining to Amanda that she is a girl, to prevent any further confusion.
   B. It is not known if Amanda's feelings will persist. Nevertheless, it is highly recommended for the family to consult with a mental health/gender specialist,

who can support them through this process and help determine if a social transition is recommended.

C. Gonadotropin agonists should be initiated now to prevent female puberty from starting in the future.

D. Further workup is indicated to rule out high levels of androgens as a cause for this child's behavior.

2. Dakota is a 14-year, 6-month-old assigned male at birth whose gender identity is non-binary, with preferred pronouns of they/them. At the age of 12, Dakota began treatment with a GnRH agonist and is in clinic for follow-up. Dakota states that they wish to continue on GnRH agonists to prevent any pubertal changes. What would you advise?

A. Dakota can continue on gonadotropin agonists alone, with yearly DEXA scans to monitor bone mineral density.

B. Dakota should start bisphosphonates and calcium supplementation if continuing on only GnRH agonists.

C. Dakota should not continue with GnRH agonists alone at this age, due to potential risks for bone health. A decision should be made to either discontinue GnRH agonists and resume endogenous male puberty or initiate phenotypic transition with cross-sex hormone treatment.

D. As Dakota's gender identity is non-binary, Dakota should stop GnRH agonists and continue with male puberty.

Answers

1. B
2. C

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Management of Endocrine Emergencies

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37.1 Diabetic Ketoacidosis

Key Points

- Diabetic ketoacidosis (DKA) is the most common endocrine emergency.
- Common presenting symptoms and signs of DKA include polyuria, polydipsia, weight loss, dehydration, and Kussmaul breathing.
- Hyperglycemia (blood glucose >200), HCO_3 <15, and venous pH <7.30 are consistent with DKA in the presence of classic symptoms and signs.
- Management of DKA includes cautious attention to intravenous fluid administration and insulin dosing.

37.1.1 Introduction

Diabetic ketoacidosis (DKA) is the most common endocrine emergency. DKA occurs when there is insufficient insulin available for cellular uptake of glucose. Free fatty acids are converted to ketone bodies for alternative cellular fuel [1]. Excess buildup of acidic ketone bodies, primarily beta-hydroxybutyrate and acetoacetate, causes metabolic acidosis [2]. Though more common in patients with type 1 diabetes, DKA can also be seen in patients with type 2 diabetes, due to actual or relative insulin deficiency and dysregulated glucagon secretion, often precipitated by the stress of illness or infection. Approximately 30% of new-onset type 1 diabetes patients present initially in DKA. In patients with an established diagnosis of type 1 diabetes, DKA is most commonly precipitated by missed insulin doses or illness [1]. Prompt treatment with hydration and insulin is key to the management of DKA.

37.1.2 Clinical Presentation

Polyuria, polydipsia, and weight loss are the cardinal symptoms of new-onset diabetes mellitus. DKA should be suspected in patients with new-onset diabetes mellitus as well as in established patients with type 1 diabetes who complain of abdominal pain, nausea, or vomiting. Clinical signs include ill appearance, dehydration, tachycardia, and rapid breathing, also called Kussmaul respirations. Kussmaul breathing increases minute ventilation to optimize exhalation of carbon dioxide and thus compensates for the metabolic acidosis from accumulated ketone bodies. There is often a fruity odor to the breath from exhaled ketone bodies. Worsening DKA can progress to altered mental status and coma if untreated.

Risk factors for initial presentation as DKA include younger age (<4 years), lower socioeconomic status, lack of private insurance, and no first-degree relative with type 1 diabetes.

37.1.3 Differential Diagnosis

The differential diagnosis of a patient with diabetes mellitus presenting with abdominal pain, nausea, vomiting, rapid breathing, and/or altered consciousness is broad. See Table 37.1 for a summary of differential diagnoses.

37.1.4 Diagnostic Evaluation

DKA is characterized by hyperglycemia (and glucosuria), hyperketonemia (and ketonuria), and metabolic acidosis.

Testing for DKA

1. Point-of-care blood glucose; confirm with laboratory serum glucose.
2. Venous (or arterial) blood gas.
3. Serum electrolytes, blood urea nitrogen (BUN), and creatinine. Look for elevated anion gap. The anion gap can be calculated as follows: (sodium + potassium) – (chloride + bicarbonate).
4. Urine dipstick for glucosuria and ketonuria.
5. Serum acetone and beta-hydroxybutyrate are optional.
6. Electrocardiogram (EKG) if there is hyper- or hypokalemia.
7. Head computed tomography (CT) scan if there is altered sensorium concerning for cerebral edema (after giving mannitol or hypertonic saline).

Table 37.1 Differential diagnoses for diabetic ketoacidosis (DKA) categorized by presenting feature

Presenting symptom	Differential diagnoses for DKA
Polyuria/polydipsia	Diabetes insipidus
	Electrolyte derangement (hypercalcemia, hypokalemia)
	Hyperglycemia without DKA
	Urinary tract infection
Abdominal pain/anorexia/emesis	Appendicitis
	Constipation
	Gastroenteritis
	Ovarian or testicular pathology
	Urinary tract infection
Respiratory distress/tachypnea	Anaphylaxis
	Asthma
	Heart failure
	Inborn error of metabolism/hyperammonemia
	Carbohydrate metabolism defect (e.g., *galactosemia)*
	Disorders of fatty acid oxidation (e.g., *MCHAD)*
	Organic acidemias (e.g., *methylmalonic acidemia)*
	Primary lactic acidemias
	Urea cycle defects (e.g., *OTC deficiency)*
	Increased intracranial pressure (Cushing's triad)
	Respiratory tract infection
	Sepsis
	Toxic ingestion (e.g., salicylates, methanol)
	Uremia
Altered cognition	Central nervous system trauma
	Encephalitis/meningitis
	Hyperosmolar coma
	Hypoglycemia
	Inborn error of metabolism/hyperammonemia
	Seizure
	Septic shock
	Stroke
	Toxin

Abbreviations: *MCHAD* medium-chain acyl-CoA dehydrogenase deficiency, *OTC* ornithine transcarbamylase

37.1.5 Management

37.1.5.1 Administration of Intravenous (IV) Fluid

Patients who present in DKA have varying degrees of dehydration. The insertion of two larger peripheral catheters is recommended over central venous access due to the high risk of thrombosis in pediatric DKA [3, 4]. Volume should be expanded as required to restore adequate peripheral circulation [5, 6]. Isotonic fluid, such as normal saline, should be administered intravenously. Consensus guidelines recommend administering aliquots of 10–20 ml/kg over 1–2 h to be repeated as necessary [5, 6]. Once the patient's circulation has been stabilized, the remaining fluid deficit should be calculated and replaced, along with maintenance fluids, as a continuous infusion spread over the next 36–48 h [5, 6]. For ease of quick calculation, using the calculation of 1.5× maintenance rate is often adequate for an estimated 10% dehydration. Historically, there has been concern that rapid IV rehydration leads to osmotic shifts and subsequent cerebral edema [7–10]. However, the available literature is inconclusive regarding the potential risk for cerebral edema with specific rehydration techniques. Well-controlled retrospective studies identify no increased risk of cerebral edema based on fluid management [11–13]. Given inconclusive data, the fluid administration described here is based on the widely accepted International Society of Pediatric and Adolescent Diabetes, Pediatric Endocrine Society, and American Diabetes Association guidelines [2, 5, 6].

37.1.5.2 Insulin Therapy

Once volume status is stable, insulin therapy should be initiated. Our criteria for IV insulin infusion are a venous pH <7.25 or HCO_3 <15. An initial insulin bolus is not recommended. Consensus guidelines recommend an infusion of 0.1 units/kg/h of regular insulin for rapid resolution of acidosis, but little evidence exists indicating that this dose is superior to lower doses [5, 6]. During continuous insulin infusion, glucose levels should be monitored hourly with point-of-care testing. Serum electrolytes, glucose, BUN, creatinine, and phosphorus are monitored every 2–4 h depending on the severity of DKA. Often, hyperglycemia will correct more rapidly than acidosis. Acidosis requires continued insulin therapy even when a patient has reached euglycemia. To prevent hypoglycemia with continuous insulin infusion, intravenous glucose should be administered once a patient's serum glucose declines to approximately 300 mg/dl (17 mmol/L). Many experienced centers employ a "two-bag" method for providing escalating concentrations of glucose to a patient who is being treated for DKA with a continuous insulin infusion. See ◘ Fig. 37.1 for an example of such a protocol. Also, if the patient's DKA is not severe and/or likely to resolve within the next 24 h, long-acting basal insulin can be administered simultaneously with the infusion, as a onetime subcutaneous dose per the patient's home dosing or weight-based calculation (0.3–0.4 units/kg) for new-onset type 1 patients (see ◘ Fig. 37.1), which helps with the transition off of the infusion once the acidosis resolves and decreases rebound hyperglycemia. This should be done in consultation with endocrinology (◘ Table 37.2).

37.1.5.3 Correction of Electrolyte Imbalance

Numerous electrolyte imbalances occur in acute DKA, but fortunately many of them resolve with insulin and rehydration. Potassium is the most "volatile" electrolyte during DKA treatment. Extracellular acidosis causes upregulation of potassium proton pumps in the cellular membrane in order to transport hydrogen protons into the cells in exchange for potassium, causing elevated extracellular potassium. This extracellular potassium leads to increased serum potassium, which is ultimately excreted in the urine. Thus, serum potassium levels may be high, normal, or low at any given point of DKA. However, total body potassium tends to be low secondary to obligatory losses from osmotic diuresis. Consensus guidelines recommend adding 40 mEq/L of IV potassium when the serum potassium is within the normal range in a patient with DKA who has demonstrated successful urination [5, 6]. If the patient presents with hyperkalemia, potassium should be withheld from fluids until the serum potassium normalizes. If the patient presents with hypokalemia, replacement potassium should be initiated right away with administration of insulin [5, 6]. Insulin administration will cause potassium to shift from the extracellular to the intracellular space with a rapid decrease in serum potassium levels. In patients in severe DKA and prolonged potassium losses, more than 40 mEq/L of potassium may be necessary to avoid hypokalemia.

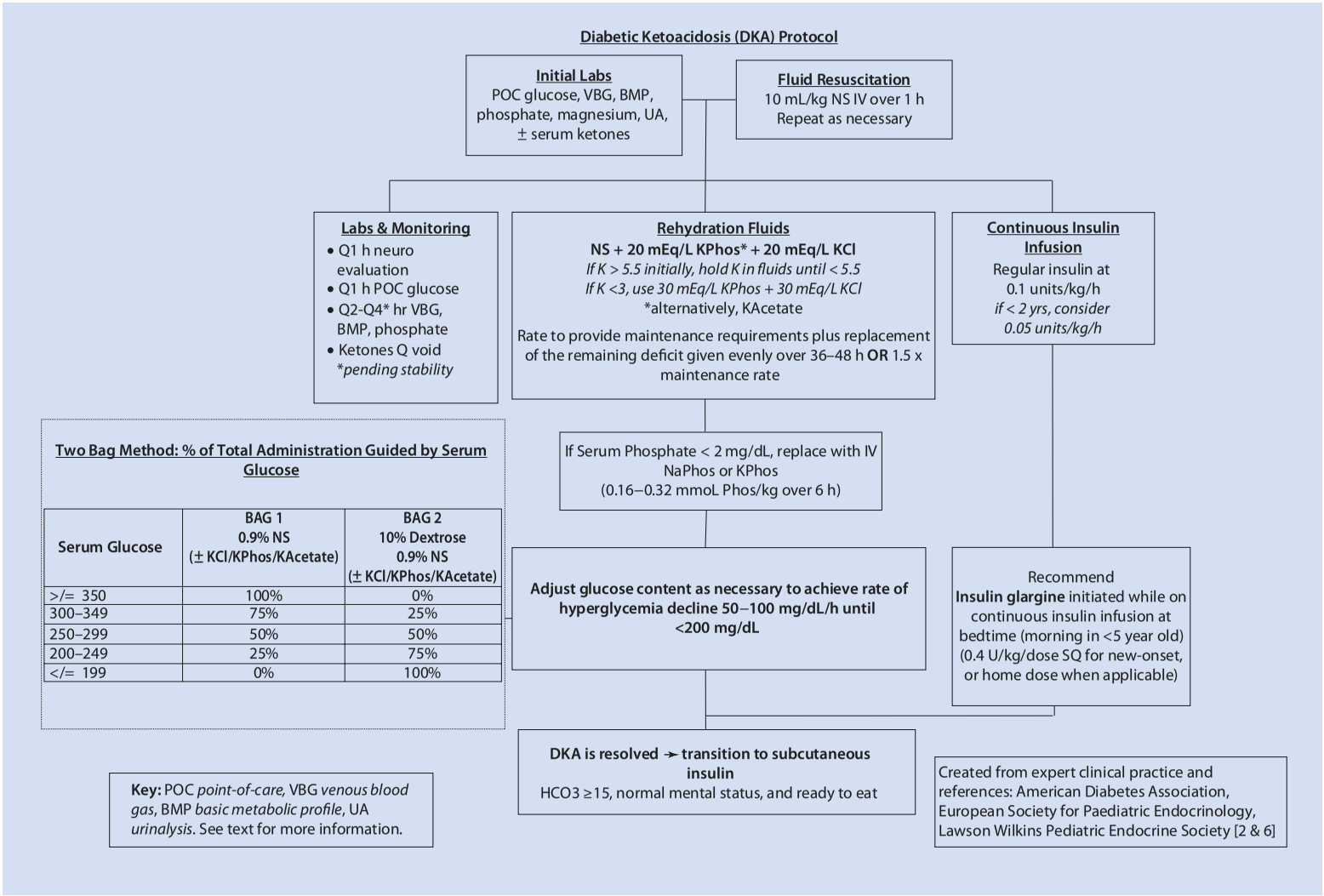

Fig. 37.1 Diabetic ketoacidosis (DKA) protocol

Table 37.2 Severity of DKA based on biochemical criteria

	Mild	Moderate	Severe
Blood glucose (mg/dl)	>250	>250	>250
Arterial pH[a]	<7.3	<7.2	<7.1
HCO_3^{-a}	<15	<10	<5
Anion gap	>10	>12	>12

[a]International Society for Pediatric and Adolescent Diabetes [5]

Similar trends occur with phosphorus in DKA. Thus guidelines recommend considering replacement of phosphorus in the form of 20 mEq/L of IV potassium phosphate, with close monitoring [5, 6]. Thus the recommended 40 mEq/L of potassium could be administered as 20 mEq/L of potassium phosphate and 20 mEq/L of potassium chloride or acetate.

Hyperglycemia causes an osmotic shift of fluid from the intracellular to the extracellular space, leading to pseudohyponatremia. The actual sodium content can be calculated by adjusting for hyperglycemia as follows: corrected sodium = measured sodium + 2[(plasma glucose in mmol/L − 5.6)/5.6], or measured sodium + 2[(plasma glucose in mg/dl − 100)/100], or the measured serum sodium is decreased by 1.6 mmol/L(=Meq/L) for every 100 mg/dl blood glucose over 100 mg/dl [5]. Fluid resuscitation with isotonic saline such as normal saline and standard insulin management of DKA will correct any remaining mild hyponatremia.

37.1.6 Adverse Events

Cerebral edema occurs at a rate of 0.5–1% of pediatric patients who present in DKA [11, 14]. The exact cause (or causes) of cerebral edema during DKA is (are) unknown and widely debated [15]. As discussed above, some have postulated that the rate and composition of fluid resuscitation contributes to cerebral edema [7, 8, 10]. Others postulate that insulin administration can precipitate cerebral edema [16, 17]. Although no consensus on etiology exists, risk factors for cerebral edema in DKA include younger age, new onset of diabetes, as well as longer duration and more severe signs and symptoms before initiation of treatment [1]. Once cerebral edema occurs, mortality is around 20% and approximately 25% of survivors have permanent neurologic sequelae [1, 17]. Symptoms and signs of cerebral edema include headache, change in consciousness, unequal pupils, emesis, incontinence, hypertension, and bradycardia [1]. Morbidity and mortality from cerebral edema can be minimized if treated promptly by reducing the rate of IV fluid administration and administering mannitol 0.25–1 g/kg IV per dose or 3% hypertonic saline, 5–10 mL/kg IV over 10–30 min [1, 5]. Treatment can be repeated every 2–4 h if symptoms persist [1]. It is important to note that imaging should not delay administration of hypertonic therapy when concerned for cerebral edema.

Hyperosmolar hyperglycemic state (HHS) is a complication of diabetes mellitus classically characterized by severe hyperglycemia (plasma glucose >600 mg/dl), hyperosmolality (serum osmolality >320 mosm/kg), and dehydration, without significant ketoacidosis (or ketonuria), although mild ketoacidosis may be associated. HHS is more common in type 2 diabetes mellitus, in which residual insulin action results in less lipolysis, and hence less ketosis, than is seen with DKA [5]. Manifestations vary and can include tachycardia, hypotension, hyper- or hypothermia, depressed level of consciousness, seizure activity, and/or focal neurological symptoms, among others [5]. Although rare in children, this syndrome is increasingly recognized in adolescents with type 2 diabetes mellitus [5]. HHS is treated similarly to DKA, with a few differences. Volume repletion should be performed quickly and aggressively using normal saline, 20 ml/kg boluses to be repeated as needed, followed by two times maintenance of 0.45–0.75% NS IV infusion. Individuals in a hyperosmolar hyperglycemic state are particularly prone to vascular collapse and venous thrombosis, making fluid resuscitation extremely important. Regarding insulin treatment, HHS necessitates less insulin than DKA, and therefore consensus recommendations advise holding insulin until glucose ceases to decline from fluid resuscitation, and then administering 0.025–0.05 units/kg/hr of insulin as opposed to 0.1 units/kg/hr as recommended for DKA [5].

37.1.7 Disposition

Ideally, care for a child or adolescent with DKA or HHS should occur at a multidisciplinary center experienced with the treatment of diabetes mellitus in pediatric patients. If the initial presentation is

not at such a facility, support from an experienced center should be obtained and arrangements made for safe transfer, if possible [5]. Moderate and severe DKA or HHS require intensive care monitoring for frequent electrolyte measurements and close neurologic assessments. These patients must remain on the insulin infusion until transition criteria are met for subcutaneous insulin. In our center, a bicarbonate level of 15 and above is considered suitable for transition to subcutaneous insulin and accordingly allows for transition to a step-down unit. Mild DKA requires less frequent monitoring and has negligible neurologic concerns; therefore, depending on the medical center, a non-intensive care hospital unit experienced with DKA management may be appropriate.

37.2 Adrenal Crisis

Key Points

- An adrenal crisis is characterized by shock associated with severe glucocorticoid deficit.
- Common presenting signs of adrenal crisis include tachycardia, hypotension, and hypoglycemia.
- Fluid resuscitation and hydrocortisone are the mainstays of treatment for adrenal crisis.

37.2.1 Introduction

Adrenal crisis is an important cause of shock in children [18]. An adrenal crisis is a state of cardiovascular decompensation caused by a severe deficit of glucocorticoid with or without concurrent mineralocorticoid hormone deficit. Adrenal crisis occurs when the demand for glucocorticoid is not met. Such an imbalance commonly occurs when a patient with primary adrenal insufficiency (including Addison's disease and congenital adrenal hyperplasia) or secondary adrenal insufficiency (hypopituitarism) develops a routine illness, and glucocorticoid dosing is not increased as recommended for such times. Additionally, illness in a patient in whom the hypothalamic-pituitary axis has been suppressed from chronic exogenous steroid use (resulting in secondary adrenal insufficiency) can bring about an adrenal crisis. Rarely, it can be an initial presentation of undiagnosed primary or central adrenal insufficiency or due to under- or missed dosing of glucocorticoid therapy.

37.2.2 Clinical Presentation

Patients with adrenal crisis can present with long-standing insidious symptoms or acute symptoms depending on underlying etiology [19]. If the crisis develops from a progressive loss of adrenal function, preceding symptoms can include depression, fatigability, weakness, anorexia, nausea, abdominal pain, constipation or diarrhea, and weight loss or failure to thrive [19, 20]. Primary adrenal insufficiency with insidious onset classically presents with a history of salt craving and orthostatic hypotension due to mineralocorticoid deficiency. Salt craving in children can be elicited by asking about consumption of table salt or other high-salt-containing foods. Salt craving does not occur in secondary adrenal insufficiency because the renin-angiotensin-aldosterone system remains intact [20]. Insufficient cortisol synthesis in primary adrenal insufficiency causes an upregulation of proopiomelanocortin, the precursor of both adrenocorticotropic hormone (ACTH) and melanocortin, which can lead to increased skin pigmentation in sun-exposed regions of the body as well as flexor surfaces and mucosal membranes [19, 21, 22]. Providers typically describe a "bronzed" appearance of the skin as if the patient has been sun tanning.

Some patients with adrenal insufficiency have no insidious symptoms, and the crisis develops acutely after a sudden physiologic stress. A history of sudden stress such as trauma, hemorrhage, acute infection, or a medical procedure may be elicited [19]. If the patient has developed acute primary insufficiency from adrenal hemorrhage associated with bacterial sepsis (as in Waterhouse-Friderichsen syndrome), a petechial rash may be present. This rash classically starts over the trunk and progresses to frank ecchymoses.

Iatrogenic adrenal insufficiency is the most common cause of pediatric adrenal insufficiency [23]. Any individual who has been receiving supraphysiologic doses of glucocorticoids for more than 3 weeks may have suppressed adrenal function and is at risk for an adrenal crisis in the event of physiologic stress [24–26]. In addition to glucocorticoids, many other medications affect

Table 37.3 Common drugs that can cause adrenal insufficiency

Mechanism	Drug
Direct inhibition of cortisol synthesis (via p-450-dependent enzymes)	Ketoconazole
	Fluconazole
	Etomidate
Activation of cortisol metabolism	Phenobarbital
	Phenytoin
	Rifampin
Suppression of corticotropin-releasing hormone and corticotropin synthesis	All glucocorticoids
	Systemic, rarely topical, and inhaled therapies
	Megestrol
	Opioids (chronic use)
Peripheral resistance to glucocorticoids	Imipramine
	Chlorpromazine

Recreated from Bornstein, 2009; Ref. [27]

Table 37.4 Differential diagnoses of adrenal crisis categorized by presenting feature

Presenting symptom	Differential diagnoses
Hemodynamic collapse	Shock
	Anaphylactic
	Cardiogenic (heart failure)
	Hypovolemic (hemorrhage/trauma)
	Neurogenic (spinal cord injury)
	Septic
Hyponatremia	Cerebral salt wasting
	Syndrome of inappropriate antidiuretic hormone (SIADH)
Hypoglycemia	Hyperinsulinism
	Iatrogenic/ingestion (e.g., sulfonylureas, propranolol)
	Inborn error of metabolism
	Ketotic hypoglycemia
	Sepsis

cortisol synthesis or function and may exacerbate or potentiate an adrenal crisis. Table 37.3 lists common medications of concern for pediatric patients [27].

Several syndromes may be associated with adrenal insufficiency. Adrenoleukodystrophy is an X-linked syndrome in which progressive cerebral demyelination leads to neurological symptoms and late-onset primary adrenal insufficiency. A diagnosis of adrenoleukodystrophy should be considered in a young male with cognitive regression and adrenal insufficiency [19]. Another cause of adrenal insufficiency is autoimmune-mediated destruction of the adrenal glands, which can present in conjunction with other autoimmune diseases. Type 1 and type 2 autoimmune polyendocrine syndromes may include adrenal insufficiency, hypoparathyroidism, hypothyroidism, pernicious anemia, gonadal failure, alopecia, and vitiligo [19]. Finally, adrenal insufficiency can be associated with congenital adrenal hyperplasia (CAH), which presents with chronically elevated androgen production, or adrenal hypoplasia congenita, which does not present with elevated androgens. CAH results in female patients having ambiguous genitalia or, in milder forms, increased virilization for age in both males and females [19]. Adrenal hypoplasia congenita is classically associated with hypogonadism [19]. Both entities are likely to present in infancy due to crises but are rarely not identified until later in life.

Regardless of the course of symptom onset, patients presenting with an adrenal crisis will have similar signs on exam. Patients with adrenal crisis present with tachycardia and hypotension, as well as hypoglycemia with or without hyponatremia. Depending on the severity of hypoglycemia and hyponatremia, they can have severely altered cognition or even seizure activity [19].

37.2.3 Diagnostic Evaluation

The differential diagnosis of adrenal insufficiency includes any disease that presents with hemodynamic collapse. Adrenal crisis can be associated with hyponatremia, hyperkalemia, and hypoglycemia which can differentiate it from other forms of shock. See Table 37.4 for a narrowed differential diagnosis list.

In the acute setting, diagnostic hormone concentration results are not often available. If an adrenal crisis is suspected, the provider should obtain a serum cortisol, ACTH, glucose, and electrolyte panel and administer glucocorticoid therapy immediately without waiting for laboratory results [18]. Replacement glucocorticoid therapy must be administered emergently to prevent morbidity and in some cases death. A cortisol level <18 mcg/dl during cardiovascular collapse is diagnostic of adrenal insufficiency and should be treated as such [28]. An ACTH level drawn before glucocorticoid treatment can help determine the etiology of the adrenal insufficiency (as an elevated ACTH level suggests primary adrenal disease). Hypoglycemia, mild to frank hyponatremia, and (variably) hyperkalemia support the diagnosis. Antibody evaluation and ACTH stimulation testing can be performed once the patient is stabilized and off glucocorticoid therapy.

37.2.4 Management and Disposition

Normal saline boluses of 20 ml/kg should be administered until perfusion improves [18, 29].

Concurrently, hydrocortisone sodium succinate 100 mg/m^2 should be administered immediately via IV push, which will help restore intravascular tension [21]. If intravenous (or intraosseous) access is not available, intramuscular injection or rectal suppositories are alternatives [18, 30]. In the acute setting, the patient's body surface area (BSA) may be difficult to determine. Estimated doses of hydrocortisone sodium succinate based on age are as follows [30] (Table 37.5):

Uncertain age or BSA should be treated with a higher hydrocortisone dose, as excess hydrocortisone has negligible side effects, but underdosing will lead to continued hemodynamic compromise. The initial hydrocortisone bolus should be followed by administration of the same dose run as a continuous infusion over the next 24 h or divided into every 6 or 8 h doses. Thereafter, continued "stress dosing" of glucocorticoid therapy will be necessary until the patient has recovered from the underlying illness. Standard stress dosing for a moderate to severe illness is 50 mg/m^2/day of hydrocortisone divided into every 6–8-h dosing.

Table 37.5 Estimated doses of hydrocortisone sodium succinate based on age

Age	Hydrocortisone dose
0–3 years	25 mg IV
3–12 years	50 mg IV
>12 years	100 mg IV

Hypoglycemia must be corrected. If the serum glucose level is <70 mg/dl or the patient has symptomatic hypoglycemia, we recommend administering an IV bolus of dextrose (see hypoglycemia management), which can be repeated as necessary [31].

Hyperkalemia should be further assessed with an EKG to evaluate for peaked T waves and other abnormalities. Emergency treatment for significant hyperkalemia includes calcium for myocardial stabilization, bicarbonate, and insulin and glucose infusion, although these are rarely needed [31, 32].

Finally, concurrent illnesses such as bacterial sepsis should be treated as necessary.

Once stabilized, the patient will need close monitoring of neurologic as well as hemodynamic status, usually in the intensive care unit. Adrenal crisis precipitating events such as sepsis often require intensive care unit management too.

37.3 Diabetes Insipidus and Acute Hypernatremia

Key Points

- Diabetes insipidus (DI) is caused by insufficient secretion or action of vasopressin (also known as antidiuretic hormone [ADH]).
- Common presenting signs of DI include polyuria, polydipsia, dehydration, and hypernatremia.
- Treatment of DI includes fluid resuscitation and desmopressin replacement (DDAVP) when appropriate (i.e., central DI).

37.3.1 Introduction

Diabetes insipidus (DI) is the pathologic state caused by insufficient arginine vasopressin secretion or action. Vasopressin (also known as antidiuretic hormone, ADH) is a key regulatory hormone

for maintenance of intravascular volume. In the kidney, vasopressin binds to V2 vasopressin receptors which leads to upregulation of aquaporin channels, allowing water to flow down the osmotic gradient from the tubular lumen into the hypertonic medullary interstitium, thus concentrating urine and increasing intravascular volume [33]. Vasopressin also causes vasoconstriction via V1 vasopressin receptors located on vascular smooth muscle [33, 34]. DI is either a problem with the production of ADH, known as central DI, or with the kidneys' response to ADH, referred to as nephrogenic DI. A lack of vasopressin, as in central DI, causes water loss through inappropriately dilute urine, as well as decreased intravascular tone. Nephrogenic DI, caused by aberrant function of the renal vasopressin receptors, is unresponsive or hyporesponsive to vasopressin replacement. Despite these differences, the acute presentation and management of the decompensated state of DI – i.e., dehydration and hypernatremia – are the same for both central and nephrogenic DI.

37.3.2 Clinical Presentation

Patients presenting with acute decompensation from DI usually have a history of chronic or acute polyuria and polydipsia. They or their parents may report extreme water intake, including eating ice chips or even drinking from the toilet bowl, as well as new-onset nocturia and urinary incontinence. Younger infants typically present with more nonspecific symptoms and signs, such as irritability, vomiting (despite a vigorous suck), and recurrent fevers [35, 36].

All patients with DI who are unable to maintain hydration by increasing water intake will become progressively dehydrated and hypernatremic due to excessive free water loss through urine. Hypernatremia and dehydration can be severe; the patient may present with altered mental status, seizure activity, shock, or even death [36].

Patients may also present with complaints associated with the underlying pathology. Between 18% and 50% of children with central DI are found to have an underlying tumor such as a craniopharyngioma or germ cell tumor [36–38]. Another 12–16% of patients with new-onset central DI have an infiltrative disease such as Langerhans cell histiocytosis [36–38]. With these intracranial processes, patients may report headaches or, in some cases, visual disturbances [37]. They may also exhibit additional signs of pituitary or hypothalamic dysfunction, such as short stature or delayed or precocious puberty, in addition to DI. If Langerhans cell histiocytosis is the cause, other manifestations such as fever, weight loss, rash, lymphadenopathy, ear drainage, pulmonary infiltrates, hepatosplenomegaly, and/or bone lesions may be present [39].

Congenital central DI can occur in conjunction with syndromes displaying midline cerebral and cranial anatomic malformations (e.g., septo-optic dysplasia with agenesis of the corpus callosum, cleft lip and palate) [37, 40, 41]. Familial central DI, caused by mutations in the AVP gene (named for arginine vasopressin) with an autosomal dominant or recessive inheritance pattern, can present throughout childhood and may be accompanied by a positive family history [36, 42].

Rarely, central DI may develop secondary to an abrupt CNS insult such as trauma, hemorrhage, or neurosurgery, especially transsphenoidal pituitary surgery.

Hereditary nephrogenic DI is most commonly the result of an X-linked genetic defect causing an inactivating mutation of the renal vasopressin V2 receptor [43] but may have an autosomal recessive or dominant inheritance pattern if associated with mutations in the aquaporin 2 (AQP2) gene [36, 43]. Nephrogenic DI may also be secondary to medication administration. Medications commonly associated with nephrogenic DI [36, 44] are:

- Alkylating chemotherapeutic agents
- Aminoglycosides
- Amphotericin B
- Lithium

Also note that diuretics or other medications which alter urine solute load can affect the concentrating ability of the kidney, but this is not usually a result of vasopressin receptor malfunction.

37.3.3 Diagnostic Evaluation

Any patient who presents with polyuria, polydipsia, and hypernatremia should be suspected of having decompensated DI. See Table 37.6 for a list of differential diagnoses categorized by each of these features.

A patient presenting with concerns for decompensated DI should be evaluated emergently with

Table 37.6 Differential diagnoses for diabetes insipidus categorized by presenting feature

Presenting symptom	Differential diagnoses
Polyuria	Diabetic ketoacidosis
	Fluid overload
	Hypercalcemia
	Hypokalemia
	Osmotic diuresis, including glucosuria of DKA
	Postobstructive diuresis
	Urinary tract infection
Polydipsia	Dehydration
	Gastroenteritis
	Increased insensible losses
	Osmotic diuresis, including glucosuria of DKA
	Psychogenic polydipsia
Hypernatremia	Dehydration
	Gastroenteritis
	Increased insensible losses
	Osmotic diuresis
	Primary hypodipsia
	Iatrogenic (hypertonic fluid administration)
	Salt poisoning

a serum sodium, serum osmolality, and urine osmolality. If a measured serum osmolality is not rapidly available, serum osmolality can be calculated using the following equation: serum osmolality (mosm/kg or mmol/kg) = (2 × sodium [mEq/L or mmol/L]) + (glucose (mg/dl)/18) + (BUN (mg/dl)/2.8) [45]. A serum osmolality >295–300 mosm/kg and a serum sodium >145 mEq/L, with a concurrent urine osmolality <300 mosm/kg, are diagnostic of complete DI [45, 46]. Serum osmolality >290–300 mosm/kg or hypernatremia, in conjunction with urine osmolality in the more moderate range of 300–600 mosm/kg, raises concern for partial DI, and further diagnostics, such as a water deprivation test, are necessary [46]. Patients with intact thirst can, in the face of the severe polyuria, compensate by drinking enough water and will not have a raised serum osmolality or hypernatremia but will have dilute urine. In order to differentiate excessive urine production from psychogenic polydipsia, a water deprivation test will be necessary. Urine specific gravity correlates with urine osmolality, but it is not as reliable as a direct measurement of urine osmolality [47]. Nevertheless, because specific gravity is often quickest to obtain in the clinical setting, its crude estimate of osmolality may be helpful. A urine specific gravity >1.030 correlates with a urine osmolality >600 mosm/kg and thus usually rules out diabetes insipidus, while a specific gravity <1.010 usually correlates with an osmolality <350 mosm/kg and thus is highly suggestive of DI in a hypernatremic patient. Values ranging between 1.010 and 1.030 may represent partial DI [48]. Further testing can include a trial dose of desmopressin (DDAVP) [44]. A standard dose of DDAVP (4 mcg IV/SC or 10 mcg intranasal) should be given while the patient is hyperosmotic with relatively dilute urine (specific gravity <1.030) [44]. A urine osmolality increase less than 50% after the test dose is highly suggestive of nephrogenic DI, whereas an increase greater than 50% indicates central DI [46].

Additional diagnostics may be necessary, including BUN and creatinine levels to evaluate for renal injury associated with severe dehydration, as well as brain imaging to uncover an underlying pathology (e.g., to rule out an intracranial tumor) in any child with new-onset central DI [45]. If readily available and time permits (e.g., intracranial bleeding from trauma is not a concern), magnetic resonance imaging is the "gold standard" method for evaluating the hypothalamic-pituitary axis [45]. The imaging protocol should include sagittal and coronal thin (≤2–3 mm) slices, T1- and T2-weighted images, and contrast. Additional sequences of the remainder of the brain should be performed to evaluate for comorbid pathology. Otherwise, a head CT scan with multi-slice helical coronal and sagittal images can provide adequate evaluation of the suprasellar region [45].

37.3.4 Management and Disposition

Initial treatment of the dehydration and hypernatremia is aimed at stabilizing the patient's intravascular volume. 20 ml/kg boluses of

normal saline should be administered as necessary. After the patient's intravascular volume is stabilized, IV fluids must be administered cautiously, so as to gradually decrease the serum sodium and replete the free water deficit. A widely used calculation for free water deficit is free water deficit (L) = 0.6 × weight (kg) × ([serum sodium (mEq/L)/140] – 1) [49]. Once the free water deficit is calculated and any previous fluid volume given for resuscitation accounted for (i.e., subtracted from the total deficit), replacement fluid should be started, with the infusion rate calculated to replete the remaining deficit over 24–48 h. Normal saline or 0.45 normal saline (both with 5% dextrose) are good choices because they provide excess free water relative to the patient's hypernatremic serum. Additionally, urinary losses must be monitored closely and replaced periodically with 0.5–1 ml of hypotonic IV fluid (0.3% saline) for every 1 ml of urine volume. One should aim for a rate of sodium decline no greater than 0.5 mEq/L/h [50, 51]. A rate of decline >0.5 mEq/L/h has been associated with an increased incidence of cerebral edema and seizure activity compared to a rate of decline <0.5 mEq/L/h [51, 52]. The patient's serum sodium should be measured hourly initially and can be gradually spaced to every 4 h. Adjustments to the infusion rate and/or saline content will need to be made on an individualized basis because each patient's recovery can be very unpredictable. The patient must be monitored closely for signs and symptoms of cerebral edema including headache and altered cognition. Because of the close monitoring required, patients with significant hypernatremia and dehydration are typically managed in the intensive care unit.

Use of desmopressin replacement (DDAVP) should also be individualized. If a patient is known to have DI and has had a predictable response to DDAVP administration, his or her prescribed dose should be given as soon as possible. If the presentation is unequivocally central DI, a vasopressin infusion can be started in the intensive care unit. Once the patient improves, the IV infusion can be stopped, and oral, intranasal, or subcutaneous DDAVP administered. However, infants with central DI are typically treated with free water replacement, rather than DDAVP, due to their unpredictable responses and higher risk of developing hyponatremia, but it may be reasonable to try very small doses of oral or subcutaneous DDAVP. Transitional and long-term treatment of nephrogenic DI will need to address exacerbating factors (such as medications), and fluid intake must be optimized. Thiazide diuretics are occasionally prescribed for sodium retention and should be done in consultation with nephrology.

37.4 Thyroid Storm

Key Points

- Thyroid storm is a rare state of clinical decompensation from thyrotoxicosis.
- Presenting symptoms of thyroid storm include tachycardia, hyperthermia, agitation, and abdominal pain.
- The mainstay of treatment is stabilization of the airway and circulation, management of hyperthermia, use of beta-blockers, and the judicious use of resuscitation fluids.

37.4.1 Introduction

Thyroid storm is the state of clinical decompensation associated with severe thyrotoxicosis. Although hyperthyroidism is relatively common in the pediatric population (occurring in 1:1000–1:10,000 children), thyroid storm is fortunately exquisitely rare [53–55]. Despite its rarity, thyroid storm is a medical emergency with significant mortality, and providers must be aware of its presenting features, diagnosis, and management.

37.4.2 Clinical Presentation

Thyroid storm presents with extreme features of hyperthyroidism. Symptoms and signs of thyroid storm are listed below and represent a hypermetabolic state. On physical exam, a goiter and exophthalmos are tip-offs to preexisting hyperthyroidism in a patient who does not have a known diagnosis of hyperthyroidism.

Symptoms and Signs of Thyroid Storm [56]

- *Hyperpyrexia*
- *Altered central nervous system*
  - Agitation
  - Lethargy
  - Delirium
  - Psychosis
  - Seizure
  - Coma
- *Gastrointestinal dysfunction*
  - Abdominal pain
  - Nausea, vomiting
  - Diarrhea
  - Unexplained jaundice
- *Tachycardia*
- *Congestive heart failure*
  - Pedal edema
  - Bibasilar rales
  - Pulmonary edema
- *Arrhythmia*

A system-based thyroid storm score, developed by Burch and Wartofsky, is widely used in the adult population [56]. Although these clinical criteria have not been evaluated in the pediatric population, most pediatric patients with thyroid storm are adolescents, so it seems acceptable to use this scoring system for older pediatric patients. All points are added together to obtain a summative score which in turn indicates an unlikely, possible, or probable diagnosis of thyroid storm [56].

In neonates, a maternal history of Graves' disease is significant. Thyroid-stimulating immunoglobulin (TSI) can be transmitted across the placenta to the fetus causing neonatal Graves' disease. This can occur despite adequate control of maternal hyperthyroidism, including mothers with a past history of Graves' disease treated with radioactive iodine therapy who continue to have high TSI titers in their blood [57]. Affected fetuses often have intrauterine growth restriction and are born small for gestational age [55, 58]. The affected newborn may show signs of thyrotoxicosis at birth. Alternatively, if the mother received antithyroid medication, the clinical manifestations of thyrotoxicosis may be delayed until several days after birth, once the antithyroid medication clears the neonatal circulation [55].

Providers should also ask about any potential precipitating event. In those with new-onset hyperthyroidism, as well as in those with an established diagnosis, thyroid storm may be triggered by infection, trauma, surgery, or parturition (to name just a few precipitants of this condition). Abrupt cessation of antithyroid therapy or ingestion of excess thyroid hormone can also trigger thyroid storm [53, 59].

37.4.3 Diagnostic Evaluation

The differential diagnosis of thyroid storm is broad. A high index of suspicion is necessary to make the diagnosis. ◘ Table 37.7 lists the differential diagnoses for some of the common presenting features.

◘ **Table 37.7** Differential diagnoses of thyroid storm categorized by presenting features

Presenting symptom	Differential diagnoses
Tachycardia with compromise	Autonomic dysfunction from intracranial injury (e.g., neuroblastoma, brain injury)
	Ingestion (sympathomimetics, anticholinergics, cocaine)
	Myocarditis
	Pheochromocytoma
	Primary arrhythmia
	Sepsis
Hyperpyrexia	Central nervous system insult (trauma, stroke)
	Diabetes insipidus
	Exertional heat stroke
	Ingestion (sympathomimetics, anticholinergics, cocaine)
	Malignant hyperthermia
	Neuroleptic malignant syndrome
	Pheochromocytoma
	Sepsis
Anxiety/agitation	Carbon monoxide poisoning
	Ingestion (alcohol intoxication or withdrawal, caffeine, sympathomimetics, anticholinergics, hallucinogens, amphetamines)
	Primary psychiatric disorder (mania, psychosis, anxiety)
	Trauma

Patients with primary or autoimmune mediated hyperthyroidism have elevated total and free thyroxine (T4) and triiodothyronine (T3) levels (usually by several fold), along with a suppressed thyroid stimulating hormone (TSH). Thyroid-stimulating immunoglobulin (also known as activating thyrotropin receptor antibody) is elevated in most patients with Graves' disease.

Electrolytes (including calcium), glucose, BUN, creatinine, and liver function tests should be drawn to evaluate for potential renal and hepatic end-organ dysfunction. An EKG is necessary when there is concern for a dysrhythmia or for certain electrolyte abnormalities (e.g., significant hypercalcemia). If cardiovascular compromise is severe, echocardiography should be performed as soon as possible. If sepsis is suspected, a complete blood count as well as blood and other appropriate cultures should be obtained. If ingestion is a possibility, a toxicology evaluation should be performed as well. Finally, a patient with severe altered consciousness may warrant immediate brain imaging.

37.4.4 Management and Disposition

Initial treatment of thyroid storm is aimed at stabilizing the patient's airway, circulation, and body temperature. Patients in thyroid storm are usually net fluid positive; therefore, resuscitation fluids must be administered cautiously.

Beta-blockers are the agents of choice for cardiovascular compromise secondary to thyroid storm [55, 60]. Propranolol has demonstrated efficacy and is most commonly used. Atenolol, a more cardio-selective beta-blocker, is used in patients with asthma. Additionally, IV esmolol (which is also a cardio-selective beta-1 blocker) can be used for its rapid onset of action and can be titrated to normalize heart rate and blood pressure [55, 60]. The blockade should be begun at a standard dose for age and titrated to normalize the heart rate.

Rapid control of the hyperthyroid state is achieved by starting potassium iodide (supersaturated K, "SSKI") or Lugol's solution. Iodides such as potassium iodide cause an abrupt cessation of both hormone synthesis and release [56, 61, 62] and are typically given after at least an hour of starting an antithyroid medication. Both methimazole and propylthiouracil (PTU) inhibit thyroid hormone synthesis. Propylthiouracil has the added benefit of inhibiting peripheral conversion of T4 to T3; however, it carries a black box warning for severe hepatotoxicity so is not recommended in the pediatric population [61, 62]. Therefore, the current standard of care is to start methimazole. See Table 37.8 for detailed age- and weight-based dosing of standard antithyroid medications [60–62]. Adult guidelines indicate that higher doses may be necessary and therefore should be considered in pediatric patients.

Patients in thyroid storm should be admitted to the intensive care unit for continued monitoring, stabilization, and management.

Table 37.8 Antithyroid medication administration recommendations [60–62].

Antithyroid medications	Suggested initial dosing
Methimazole	– Neonatal: 0.2–0.5 mg/kg orally divided into two to three doses/day
	– Children: 0.25–1 mg/kg orally divided daily or twice daily
	Approximate ranges:
	– *Infant: 1.25 mg orally daily*
	– *Ages 1–5 years: 2.5–5 mg orally daily*
	– *Ages 5–10 years: 5–10 mg orally daily*
	– *Ages 10–18 years: 10–20 mg orally daily*
	– *Ages 18+ years: 20–25 mg orally every 6 h*
Potassium iodide (supersaturated K "SSKI") (1–2 h after synthesis blockade)	– Infants: 50–100 mg (one to two drops) orally every 6–8 h – Children/adolescents: 250 mg (five drops or 0.25 ml) orally every 6 h
Lugol's solution (potassium iodide and iodine) (1–2 h after synthesis blockade)	– Children/adolescents: Four to eight drops/dose every 8 h

37.5 Hypoglycemia

Key Points

- Hypoglycemia can present with hunger, irritability, seizures, or coma.
- Hypoglycemia is caused by increased glucose utilization or decreased glucose production.
- Hypoglycemia should be corrected with oral glucose or IV dextrose.

37.5.1 Introduction

Hypoglycemia is a common pediatric emergency with significant morbidity and mortality. The reported prevalence in pediatric emergency departments ranges from 6.5 to 29 per 100,000 visits [63, 64]. Hypoglycemia can cause seizures, coma, permanent neurologic impairment, and, rarely, death. Timely diagnosis can prevent significant neurocognitive sequelae and be helpful in discovering the underlying etiology.

37.5.2 Clinical Presentation

The most common presentation of hypoglycemia is hunger, followed by altered neurologic function. A neonate or young infant may be irritable, jittery, and feed poorly [65]. An older patient may be anxious, confused, and/or tremulous and complain of palpitations. Severe hypoglycemia can cause seizures or coma in patients of all ages [63, 66]. In addition to these neuro-adrenergic manifestations, the patient may have cholinergic-related complaints such as flushing, diaphoresis, emesis, and gastrointestinal distress [63, 66]. Additional features depend upon the underlying etiology – e.g., fever and signs of local infection with sepsis or mottled skin with shock. If hypoglycemia is due to an inborn error of metabolism that produces metabolic acidosis, the patient's respiratory effort will increase to compensate.

Important information to ascertain include the patient's age, duration of symptoms, timing of last oral intake, prior episodes of hypoglycemia, chronic diagnoses, and medications (including the possibility of an accidental ingestion or intentional overdose [e.g., of a beta-blocker, sulfonylurea, or insulin]).

It can be helpful to evaluate hypoglycemia based on the age and fasting status of the individual. The most common cause of fasting hypoglycemia in toddlers is ketotic hypoglycemia, which is associated with poor oral intake or prolonged fasting and is usually in the setting of a concurrent acute illness [67–69]. These children have no identifiable underlying pathology in glucose homeostasis or their endocrine regulatory axes. On the other hand, neonatal hypoglycemia which occurs regardless of recent oral intake is likely a result of hyperinsulinism, an inborn error of metabolism, or a defect in a counter-regulatory hormone pathway.

37.5.3 Diagnostic Evaluation

See Table 37.9 for a list of common underlying diagnoses associated with pediatric hypoglycemia [21, 63, 64, 69, 70]. These are categorized by the underlying mechanism of either increased glucose utilization or decreased glucose production.

The diagnosis of hypoglycemia is made with a serum glucose level (obtained in the setting of

Table 37.9 Differential diagnoses for pediatric hypoglycemia [21, 63, 64, 69, 70]

Glucose metabolism	Differential diagnoses
Increased glucose utilization	Burn injury
	Hyperinsulinism
	Ingestion
	Insulin
	Oral hypoglycemic agents
	Beta-blockers
	Sepsis/shock
Decreased glucose production	Counter-regulatory hormone deficiency (e.g., growth hormone, cortisol)
	Hepatic injury (Reye syndrome)
	Inborn error of metabolism
	Ingestion
	Beta-blockers
	Ethanol
	Ketotic hypoglycemia

symptoms and signs of hypoglycemia). Because rapid glucose determination is standard of care when assessing an acutely ill patient, this is frequently performed by emergency medical services personnel prior to reaching the emergency department. If it was not done or needs to be verified, a bedside point-of-care glucose should be obtained promptly. If low, a confirmatory serum glucose should be obtained.

Historically, there has been much debate regarding the biochemical definition of pediatric hypoglycemia, and accordingly, several cutoff levels are provided by various sources [58, 66, 69, 71]. In general, serum glucose less than 70 mg/dl is considered low blood glucose. When the serum glucose decreases below 65–70 mg/dl, counter-regulatory systems are activated, and symptoms and signs of hypoglycemia begin to appear [66, 69]. When the glucose has declined below 50–55 mg/dl, all counter-regulatory systems are fully employed, and the patient is usually symptomatic.

Once hypoglycemia has been identified, additional diagnostic studies should be obtained to ascertain the underlying etiology and evaluate for any associated comorbidities. When possible, a panel of labs (commonly referred to as a "critical sample") should be obtained prior to administering glucose. An appropriate laboratory panel obtained while the patient is hypoglycemic <50 mg/dl can effectively rule in or rule out many differential diagnoses. The panel should include serum glucose, insulin, c-peptide, growth hormone, cortisol, free fatty acid, beta-hydroxybutyrate, ammonia, and lactate levels, as well as urinary ketones [58, 66, 69, 71]. Note, if highly suspicious for hyperinsulinism, a glucagon challenge should be administered while the patient is hypoglycemic (and assumedly stable). A rise in glucose ≥30 mg/dl after administration of 1 mg of glucagon IV or IM is consistent with hyperinsulinism.

37.5.4 Management and Disposition

Hypoglycemia must be promptly corrected with IV dextrose (or oral glucose [e.g., juice, glucose tablets] for milder cases). The recommended initial IV dose for a child is 5–10 ml/kg of 10% dextrose or 2–4 ml/kg of 25% dextrose. A neonate should receive 2 ml/kg of 10% dextrose. (If 25% dextrose solution is ever needed in a neonate, it should be administered through a central line due to its hypertonicity [31]). The glucose level should then be rechecked, and an infusion of 10% dextrose-containing fluid started at a standard maintenance rate. If the serum glucose has normalized (and remains normal), no further dextrose boluses are needed.

If the underlying diagnosis is known, specific therapy can be prescribed. For example, hyperinsulinemia can be treated in addition with glucagon (neonate, infant, and child <20 kg, 0.02–0.03 mg/kg/dose; child or adolescent ≥20 kg, 1 mg/dose IV/IO/IM/SC) [31]. Adrenal insufficiency should be treated with stress hydrocortisone dosing (as described in the adrenal crisis section) [21].

Once stabilized, disposition must be individualized. Some forms of hypoglycemia may be mild and respond to treatment quickly (e.g., ketotic hypoglycemia). Without additional comorbidities, most of these patients can be treated with dextrose-containing infusions and are easily managed on a standard hospital unit. Other forms of hypoglycemia may present with severe acidosis, neurologic impairment, and/or respiratory compromise. These patients require close monitoring, with frequent adjustments in therapy, and need to be admitted to an intensive care unit for continued care.

37.6 Case Studies and Questions

Case #1

An 8-year-old male presents with 2 weeks of polyuria, polydipsia, and a 7 lb. weight loss. His respiratory rate is 30 breaths/minute and his heart rate is 130 beats/minute. On physical exam, he is found to have dry mucous membranes and a fruity odor to his breath.

Review Questions

1. Which of the following labs are indicated for the patient in Case #1?
   A. Point-of-care glucose
   B. Urine dipstick
   C. Serum electrolytes
   D. Blood gas
   E. All of the above

2. While treating the boy in Case #1, he becomes sleepy and difficult to arouse. Your next step in his management would include which of the following?
   A. Discontinue his glucose-containing fluid infusion
   B. Consider administering mannitol or hypertonic saline and then ordering a head CT to evaluate for cerebral edema
   C. Increase his insulin infusion
   D. Repeat a CBC
   E. Order an ECG
3. Hyperosmolar hyperglycemic state is characterized by all of the following *EXCEPT*
   A. Hyperglycemia
   B. Hyperosmolality
   C. Significantly elevated serum acetone
   D. Dehydration
   E. All of the above are characteristics of HHS

Answers

1. E
2. B
3. C

Case #2

A 7-year-old male presents to the emergency department with tachycardia and hypotension. His parents report recent anorexia, constipation, weight loss, change in behavior, and a decline in school performance. He is not currently on any medications and has had no recent major illnesses. On exam, the patient is noted to have bronzed skin on his face, neck, arms, and legs.

Review Questions

1. Which cause of adrenal insufficiency is most likely in the patient in Case #2?
   A. Congenital adrenal hyperplasia
   B. Chronic exogenous steroid use
   C. Adrenoleukodystrophy
   D. Autoimmune polyendocrine syndrome
   E. Waterhouse-Friderichsen syndrome
2. Which of the following labs are *NOT* indicated in the acute care setting when adrenal crisis is suspected?
   A. Serum cortisol
   B. Antibody testing
   C. ACTH (adrenocorticotropic hormone)
   D. Glucose
   E. Electrolytes

Answers

1. C
2. B

Case #3

A 4-year-old boy presents with complaints of extreme thirst and frequent headaches. His parents report that he has been drinking from the toilet bowl and wetting his bed at night. On physical exam, he is tachycardiac and has dry mucous membranes but is neurologically intact. His labs are concerning for an elevated sodium level.

Review Questions

1. For the patient in Case #3, which of the following tests should *NOT* be ordered acutely?
   A. Serum sodium
   B. Cerebral spinal fluid evaluation
   C. Urine osmolality
   D. Serum osmolality
   E. All of the above should be ordered acutely
2. Which of the following labs would be expected in a patient with decompensated DI?
   A. Elevated serum osmolality
   B. Elevated urine osmolality
   C. Hyponatremia
   D. Elevated urine specific gravity
   E. Hyperkalemia
3. Which of the following underlying disease is known to be associated with DI?
   A. Graves' disease
   B. Melanoma
   C. Langerhans cell histiocytosis
   D. Hyperinsulinemia
   E. Congenital adrenal hyperplasia

Answers

1. B
2. A
3. C

Case #4

A 17-year-old female presents to the emergency department with agitation. Her heart rate is 163 beats/minute and temperature 39.8° C. She complains of severe abdominal pain as well.

Review Questions

1. If the patient in Case #4 is found to have Graves' disease, which of the following labs would you expect to find on presentation?
   A. Undetectable thyroid-stimulating immunoglobulin
   B. Suppressed thyroid-stimulating hormone
   C. Suppressed free thyroxine (T4)
   D. Undetectable triiodothyronine (T3)
   E. You would expect to find all of these lab values
2. The preferred agent to treat this patient's cardiovascular compromise is
   A. Intravenous isotonic fluid
   B. Digoxin
   C. Nitroprusside
   D. Esmolol
   E. Thyroxine
3. The most rapid control of hyperthyroidism can be achieved with
   A. Potassium iodide
   B. Propylthiouracil
   C. Vasopressin
   D. Methimazole
   E. Insulin infusion

Answers

1. B
2. D
3. A

Case #5

A 4-day-old infant presents to the emergency department with irritability, jitteriness, and poor feeding. EMS reported a point-of-care glucose of 36 mg/dl. He was treated with 8 ml of 10% dextrose en route. A repeat point-of-care glucose on arrival in the emergency department is 80 mg/dl.

Review Questions

1. Likely causes for hypoglycemia in the infant above include all of the following *EXCEPT*
   A. Hyperinsulinism
   B. Inborn error of metabolism
   C. Insulinoma
   D. Counter-regulatory hormone pathway defect
   E. All of the above are common causes of hypoglycemia in neonates
2. Which of the following are appropriate treatments for hypoglycemia in an older child?
   A. Apple juice
   B. 5 ml/kg of 10% dextrose solution
   C. 4 ml/kg of 25% dextrose solution
   D. Glucose tablets
   E. All of the above may be acceptable treatments for a hypoglycemic child

Answers

1. C
2. E

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The Endocrine Response to Critical Illness

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Key Points

- There are two distinct endocrinologic responses to critical illness: acute and chronic. The acute response is a fully adaptive to physiologic stress, whereas the chronic response leads to prolonged insulin resistance and severe protein catabolism associated with significant morbidity and mortality.
- Operational definitions and treatment guidelines for critical illness-related corticosteroid insufficiency (or "relative" adrenal insufficiency) in children are lacking. ACTH stimulation testing continues to be guided by adult data, and future pediatric RCTs are needed.
- "Sick euthyroid syndrome," "low T3 syndrome," and hypothyroxinemia of "non-thyroidal illness" (NTI) are synonymous. Low total T3 is the most common laboratory abnormality in children with hypothyroxinemia of NTI.
- Uncontrolled hyperglycemia in critical illness is associated with worse outcomes. However, attempting to control blood glucose too tightly with insulin infusion has not been shown to improve outcomes and carries the risk of hypoglycemia. Therefore, current consensus guidelines recommend an intermediate blood glucose target range of 140–180 mg/dL.

38.1 Paradigm of Endocrine Response to Acute Versus Chronic Critical Illness

The traditional view of the hormonal response to critical illness, including severe infection, trauma, and hemodynamic collapse, has described a singular set of changes. However, clinical research has uncovered two distinct sets of responses to severe illness: an acute response at the onset and a chronic response stimulated by the continuation of extreme stress (Fig. 38.1) [1].

The acute response involves changes in every hormonal axis and is considered fully adaptive, helping the body to manage physiologic stress. Acute stress stimulates the hypothalamic-pituitary-adrenal (HPA) axis, causes peripheral inactivation of the hypothalamic-pituitary-thyroid (HPT) and hypothalamic-pituitary-gonadal (HPG) axes, induces insulin resistance, and increases secretion of growth hormone (GH) and prolactin (PRL).

The chronic response, on the other hand, is characterized by central suppression of the HPT, HPG, and GH axes, as well as decreased production of PRL. These changes are associated with continued insulin resistance and a state of profound protein catabolism that is associated with significant morbidity and mortality but has not, to date, been reversible. Whether this chronic response or its components are adaptive remains uncertain. The advent of modern critical care has allowed humans to survive prolonged catastrophic illness that previously would have been universally fatal. From an evolutionary standpoint, it can be argued that the hormonal response to this unnatural scenario may not be adaptive, as any response would not have yielded a meaningful survival advantage. In fact, a close examination of these hormonal responses to chronic critical illness demonstrates changes that may be considered quite maladaptive [1, 2].

38.2 Adrenal Axis

The adrenocortical response is arguably the single most important hormonal response to critical illness: lacking it, patients may quickly succumb to their illness, as documented by Brown-Sequard in 1956 [3]. The initial adrenal response has traditionally been characterized by high serum ACTH and subsequent cortisol concentrations up to six times normal. Recent evidence, however, has shown that ACTH levels are often only transiently increased or not increased at all [4, 5], and cortisol concentrations may be elevated acutely not due to increased production but rather secondary to decreased breakdown [6, 7], which has important diagnostic and treatment implications. Cortisol suppresses inducible nitric oxide synthetase (iNOS) and thereby substantially enhances vascular tone and increases blood pressure; iNOS is otherwise upregulated in sepsis with resultant hypotension. Cortisol also enhances the inotropic and vasopressor response to catecholamines and angiotensin II, which additionally support blood pressure. A high level of cortisol also induces profound insulin resistance, which accelerates glycogenolysis and mobilizes precursors for gluconeogenesis by increasing

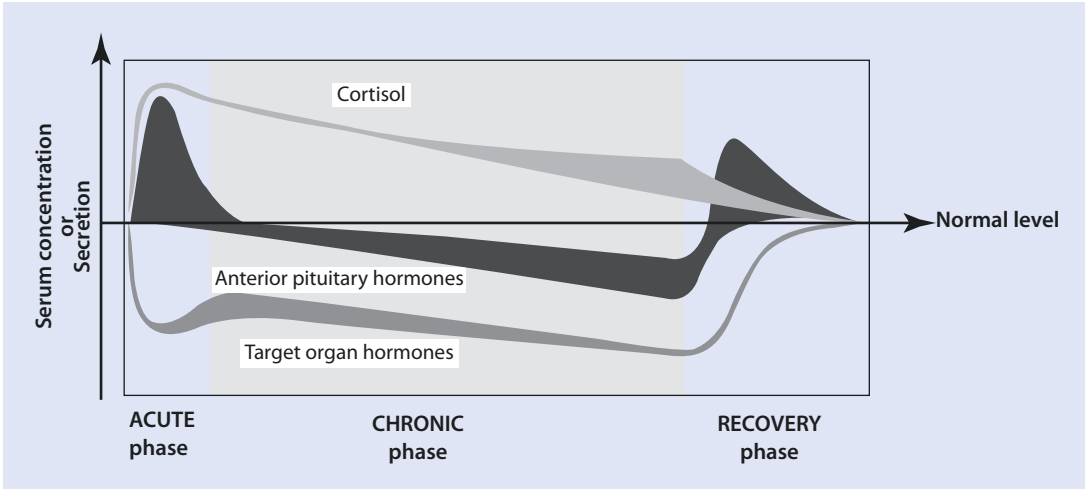

Fig. 38.1 Endocrine changes in critical illness. At the onset of illness, anterior pituitary hormones surge with an associated peripheral inactivation of target organ hormones. Once the chronic response has been engaged, the sensitivity to pituitary hormones is restored, but both remain low due to failure of the pituitary to resume normal secretory activity (From Van den Berghe et al. [1]. Reprinted with permission from Oxford University Press)

protein catabolism and lipolysis. The result is a shunting of energy resources away from peripheral organs and toward the brain and heart. Cortisol also enhances free water clearance. Finally, cortisol suppresses elements of the immune system, which may be important in preventing an over-exuberant, and potentially destructive, immune response [8].

Once the patient enters the chronic phase of critical illness, ACTH levels remain low, and cortisol concentrations remain elevated. Potential mediators of these effects include atrial natriuretic peptide, substance P, and endothelin as well as the negative feedback on ACTH from the increased cortisol levels. Production of other adrenal steroids, including mineralocorticoids and androgens, is relatively suppressed during the chronic phase. This change appears to constitute an overall shift by the adrenal axis toward prioritizing hypercortisolism, whether by upregulating production or by downregulating cortisol metabolism.

The potential disadvantages of this hormonal constellation include increased protein catabolism that leads to myopathy and impaired wound healing, as well as continued immune suppression at a time when the patient is increasingly susceptible to infectious complications. On the other hand, potential benefits may derive from positive hemodynamic effects, both direct and indirect, mediated through the adrenal medulla. In particular, epinephrine is produced via methylation of norepinephrine by phenylethanolamine N-methyltransferase (PNMT), whose mRNA expression and enzymatic activity are induced by initial high intra-adrenal concentrations of cortisol (estimated at 50 times above circulating concentrations) and, independent of intra-adrenal cortisol levels, by continued stress of critical illness [9–11].

38.2.1 Diagnosis

The evaluation of suspected adrenal insufficiency is discussed in another chapter. Since absolute adrenal insufficiency is uncommon, generally the question in the critical care setting is whether cortisol activity is sufficient for the severity of illness a patient is experiencing. Several clinical studies have implicated such "relative" adrenal insufficiency or "critical illness-related corticosteroid insufficiency" (CIRCI) as a risk factor for poor outcomes, primarily in the setting of septic shock [12, 13]. In this context, relative adrenal insufficiency is often defined diagnostically by the landmark, but unreplicated, study by Annane et al. as failure of the serum cortisol concentration to increase by more than 9 mcg/dL 1 h after stimulation with high-dose (250 mcg) ACTH. Critically ill patients with baseline cortisol levels that are low (<10 mcg/dL) or very high (>34 mcg/dL) may also be at higher risk [12, 14, 15]. However, there is little consensus on precise diagnostic criteria for CIRCI particularly as the pathophysiology appears to be multifactorial including relative failure at

any level of the HPA axis as well as peripheral tissue resistance to cortisol [7]. Specific official diagnostic and treatment guidelines for pediatric patients are lacking [16].

38.2.2 Treatment

Data are conflicting as to whether treatment of CIRCI or empiric therapy in the critical care setting improves clinical outcomes. Several small studies and meta-analyses have suggested a benefit, and a large prospective, randomized, controlled trial (RCT) demonstrated decreased mortality in patients with severe septic shock and CIRCI who were treated with hydrocortisone early in their clinical course [17]. On the other hand, a larger RCT demonstrated no significant benefit of hydrocortisone in septic shock, even in patients with CIRCI [18]. Recent evidence from an RCT has also demonstrated no statistically significant effect of hydrocortisone treatment on the development of shock among adults with severe sepsis, regardless of whether they had CIRCI or not [19]. Whether a true benefit exists, and in precisely which patients, remain uncertain. In a different clinical setting, continuous hydrocortisone infusion decreased ICU length of stay and the risk of hospital-acquired pneumonia in adult patients who were admitted to the ICU for multiple trauma and had CIRCI [20]. Thus, there may be a role for treatment of CIRCI in certain clinical contexts, but a further large prospective RCT in children will ultimately be required in order to settle this issue.

Notably, a currently controversial area of clinical use of hydrocortisone is in preterm and very low birth weight (VLBW) infants who frequently have hypotension after birth. It is known that cortisol levels increase as gestation progresses, and VLBW infants are often found to have lower cortisol levels which may lead to decreased effectiveness of intravenous catecholamines [21]. The latest Cochrane Review on this subject found only four studies with which to explore the use of hydrocortisone either as a primary or secondary medication for hypotensive preterm infants, and the outcomes were equivocal. The authors concluded that hydrocortisone may be as effective as dopamine for primary treatment of hypotension in this group, but because long-term safety data are lacking, they do not routinely recommend its use [22].

An important caveat that must be considered in the interpretation of any clinical trial involving the adrenal axis is the use of etomidate, even as a single-dose induction agent for intubation. It blocks synthesis of cortisol and will reliably suppress circulating cortisol concentrations for 24–48 h [23] although its use has not been consistently shown to lead to inferior clinical outcomes [24–26]. In light of these documented effects on cortisol production, we recommend empiric administration of hydrocortisone at stress dosing (50 mg/m^2/day) for a discrete 48-h course after use of etomidate in patients with septic shock. Other commonly used medications in the intensive care setting have also been found to potentially suppress plasma cortisol levels, including opioids, propofol, and dexmedetomidine [27, 28].

38.3 Thyroid Axis

Assessing thyroid function in critically ill patients, as in healthy patients, can be extremely challenging. Ultimately, the clinician would like to quantify the systemic actions of circulating thyroid hormones and then determine whether that level of activity is appropriate to the clinical scenario. As there is currently no direct measure of peripheral thyroid hormone functional activity, we generally depend on the patient's serum thyroid-stimulating hormone (TSH) concentration to provide an index of whether the brain is "satisfied" with available concentrations of thyroxine (T4) and triiodothyronine (T3). In the critically ill patient, however, this approach is no longer reliable, as the TSH level may remain normal despite a low serum level of T3, and possibly of T4 as well. This constellation of thyroid function tests has been variously termed the "sick euthyroid syndrome," "low T3 syndrome," and hypothyroxinemia of "non-thyroidal illness" (NTI).

The most characteristic features of NTI are the drop in serum T3 and the concomitant failure of TSH to rise in response. Within hours of the onset of acute illness or trauma, circulating T3 concentrations decline significantly, and the magnitude of the drop in T3 within the first 24 hours reflects the severity of illness [29]. The decrease in T3 is due both to decreased conversion of T4 to T3 by the outer-ring deiodinases (types I and II – see Fig. 38.2) [30] and to increased turnover of thyroid hormones [31]. T4 uptake by the liver is also suppressed, reducing available substrate for conversion to T3 [32].

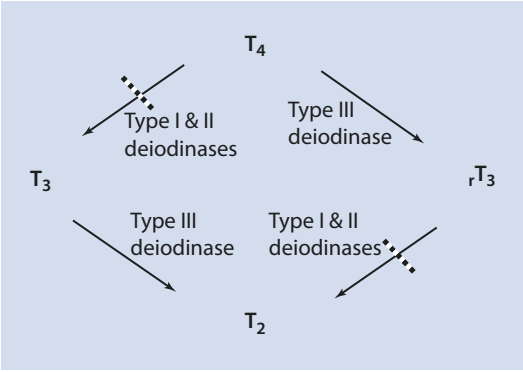

Fig. 38.2 Thyroid hormone metabolism. --- indicates downregulation of type I and II deiodinases in critical illness leading to a decreased T3, increased rT3, and having no direct effect on T4

Under normal conditions, 35–40% of the T4 produced by the thyroid is eventually deiodinated to T3 in the liver and kidney, which accounts for almost all (80–90%) circulating T3 (the remainder is produced directly by the thyroid) [33]. As type I and II deiodinases are suppressed in the acute phase of illness, excess T4 is converted by type III deiodinase into biologically inactive reverse T3 (rT3), and circulating T3 is likewise degraded to inert T2. Recent evidence also suggests that type III deiodinase itself is upregulated during critical illness, which may further decrease T3 and increase rT3 [34]. The peripheral inactivation of thyroid hormones that characterizes the acute phase of critical illness is likely adaptive for the patient in that it decreases overall metabolic rate in a period of physiologic stress, allowing available resources to be allocated to critical functions in the brain, heart, and immune system. Recent adult and pediatric RCTs have lent credence to this hypothesis in finding that delaying initiation of parenteral nutrition (PN) in critically ill patients, thereby restricting early macronutrient delivery, is associated with fewer infections and faster recovery [35, 36]. Subgroup analysis from the adult RCT found that delayed initiation of PN further reduced the T3-to-rT3 ratio, statistically explaining part of the outcome benefit, indirectly providing evidence of NTI's adaptive role in acute critical illness [37].

In the chronic phase of critical illness, TSH concentrations begin to decline into the lower third of the normal range despite low circulating T3 and, in some cases, low T4. Diminished TSH pulsatility and hypothalamic thyrotropin-releasing hormone (TRH) production correlate with low serum T3 [38, 39], which argues for a hypothalamic etiology of the euthyroid sick syndrome in the chronic phase of critical illness. This is supported by the ability of TRH infusion to reestablish normal TSH pulsatility and to increase T3 and T4 concentrations [40]. In prolonged critical illness, these changes in the thyroid axis may become maladaptive, as normal levels of T3 are required for protein synthesis, lipolysis, fuel utilization by muscle, and GH secretion and responsiveness.

38.3.1 Diagnosis

The clinical effects of low serum T3 and T4 may be difficult to discern in the critically ill patient, and the indications for therapy are therefore difficult to define. Physiologic effects of a deficiency of thyroid hormones include elevated systemic vascular resistance (by up to 50%), decreased cardiac output, hyponatremia, hypoglycemia, hypercholesterolemia, induction of a hypocoagulable state, hypothermia, and decreased metabolic rate [41]. From a pulmonary standpoint, there is also a pronounced reduction in hypoxic and hypercapnic ventilatory drive [42] as well as compromised skeletal muscle strength [43], diffusing capacity [44], and clearance of sedation medications [45]. Though individual responses vary, these factors are rarely significant enough to warrant thyroid hormone therapy for the changes of non-thyroidal illness alone, in the absence of true hypothyroidism.

When considering whether to initiate thyroid hormone replacement therapy, evaluation should include a full assessment of thyroid function, including TSH, total T4, total T3, and an index of thyroid-binding globulin (TBG).[1] Depending on the complexity of the clinical scenario, an rT3 level may be helpful, although it is often not available in a timely fashion. Although assays are widely available to measure free T4 or free T3 directly, these assays are of variable accuracy in the setting of altered thyroid hormone binding to

1 This test is known as thyroid-binding globulin index (TBGI), thyroid hormone-binding resin (THBR), T3 resin uptake (T3RU), and free thyroxine index (FTI). The units and normal ranges of each version of the test are unique.

TBG, which is common in critically ill patients; thus such values should be interpreted cautiously in the critical care setting. If a direct measurement of free T4 or free T3 is desired in this setting, it should be performed in an experienced laboratory by equilibrium dialysis, considered the gold-standard technique.

Low total T3 is the most common laboratory abnormality in children with hypothyroxinemia of NTI. Free T3, however, when measured by equilibrium dialysis and radioimmunoassay, was found to be normal in 21 of 25 (84%) adults with NTI [46]. Thus, the observed difference may be due more to alterations in binding than to absolute drops in hormonal concentrations.

Measurement of TSH should be performed using a third-generation assay and interpreted in the context of the patient's overall clinical condition. During the acute phase of illness, TSH may be slightly elevated; during the chronic phase, it is usually normal or slightly low. TSH generally is also elevated during a period of clinical recovery, driving a resurgence of T4 output from the thyroid. The most reliable way to distinguish the etiology of an elevated TSH is to repeat the thyroid function tests 5–7 days later. If the abnormalities are associated with clinical recovery, T4, T3, and TSH will each have migrated closer to the normal range, while in primary hypothyroidism the TSH will remain similarly or more elevated.

Measuring an index of TBG (e.g., TBGI, THBR, T3RU) is helpful in NTI. An elevated index, indicating a paucity of available TBG-binding sites, has been consistently associated with NTI and not hypothyroidism. As noted, in the critical care setting, we favor measurement of total T3 and T4 along with an index of TBG over direct measurement of free T3 or free T4.

Reverse T3 measurement in NTI may be helpful early on in the course, as excess T4 is converted to rT3 rather than to T3. In principle, an elevated rT3 should distinguish NTI from true hypothyroidism, in which all thyroid hormones—including rT3—are expected to be low. After NTI has been established for several days, however, T4 production may be decreased; since less substrate is available for conversion to rT3, the serum concentration of rT3 may be misleadingly low. Furthermore, rT3 may be decreased in patients with renal failure and AIDS and occasionally elevated in patients with mild hypothyroidism. Therefore, rT3 cannot be used to reliably diagnose NTI [47].

Another significant consideration prior to instituting therapy is concomitant medications [48]. Many of those commonly used in the ICU setting have profound effects on various aspects of the HPT axis and are listed below.

Drugs that Influence Thyroid Function

- *Drugs that Decrease TSH Secretion*
  - Dopamine
  - Glucocorticoids
  - Octreotide
- *Drugs that Alter Thyroid Hormone Secretion*
  - Decreased Thyroid Hormone Secretion
    - Lithium
    - Iodide
    - Amiodarone
    - Aminoglutethimide
  - Increased Thyroid Hormone Secretion
    - Iodide
    - Amiodarone
- *Drugs that Decrease T4 Absorption*
  - Colestipol
  - Cholestyramine
  - Aluminum hydroxide
  - Ferrous sulfate
  - Sucralfate
- *Drugs that Alter T4 and T3 Transport in Serum*
  - Increased Serum TBG Concentration
    - Estrogens
    - Tamoxifen
    - Heroin
    - Methadone
    - Mitotane
    - Fluorouracil
  - Decreased Serum TBG Concentration
    - Androgens
    - Anabolic steroids (e.g., danazol)
    - Slow-release nicotinic acid
    - Glucocorticoids
  - Displacement from Protein-Binding Sites
    - Furosemide
    - Fenclofenac
    - Mefenamic acid
    - Salicylates
- *Drugs that Alter T4 and T3 Metabolism*
  - Increased Hepatic Metabolism
    - Phenobarbital
    - Rifampin
    - Phenytoin
    - Carbamazepine
  - Decreased T4 5′-monodeiodinase Activity
    - Propylthiouracil
    - Amiodarone
    - Beta-adrenergic-antagonist drugs
    - Glucocorticoids
- *Cytokines*
  - Interferon alfa
  - Interleukin-2

38.3.2 Treatment

38.3.2.1 Non-thyroidal Illness

Direct supplementation with thyroid hormone in the setting of NTI currently does not appear beneficial in non-cardiac adult patients, as demonstrated in two RCTs [49, 50]. It cannot, therefore, be recommended as standard therapy for NTI in children with non-cardiac critical illness. However, therapy may be justified if T3 is extremely low and important clinical sequelae of hypothyroxinemia are apparent (e.g., severely elevated systemic vascular resistance, severe hyponatremia, or uncontrolled coagulopathy), although no data are currently available regarding this issue.

38.3.2.2 Postoperative Cardiac Patients

T3 has inotropic and chronotropic effects on the myocardium that may be beneficial, as demonstrated by clinical data obtained from T3 infusions in brain-dead organ donors [51]. This data has prompted several RCTs in postoperative cardiac patients. In two adult trials, no benefit was demonstrated with an intravenous loading dose of T3 followed by a T3 infusion [52, 53]. RCTs of T3 treatment in infants after cardiac surgery have shown modest benefits including decreased time to negative fluid balance and reduced need for postoperative intensive care, but data on a direct improvement in cardiac function are conflicting [54–56]. The largest RCT to date in this population demonstrated no improvement in time to extubation or cardiac function in patients treated with T3 postoperatively, although a benefit was seen in the youngest patients (<5 months) [57]. Thus, T3 treatment in children after cardiac surgery appears to have little risk and may have modest benefits in very young infants.

38.3.2.3 Choice of Therapeutic Agent and Dose

Levothyroxine (L-T4) is the mainstay of thyroid hormone replacement strategies, although in the setting of critical illness, it may cause rT3 to rise without any increase in T3 [50]. For patients with pre-existing hypothyroidism not associated with NTI, T4 therapy should be continued at the usual dose while in the ICU. Due to incomplete absorption of T4 when administered orally, 75% of the enteral dose should be given intravenously to patients who cannot take the medication enterally [58, 59].

In the unique situation of severe hypothyroidism, a continuous intravenous infusion of T3 has the theoretical advantage of being titratable to the desired T3 concentration and effect. The risks of such an infusion include fatal arrhythmia in the case of overdosage, and that a prolonged infusion is expected to fully suppress TSH, creating an iatrogenic risk of myxedema coma upon cessation of therapy. Therefore, general recommendations even for the treatment of myxedema coma do not recommend therapy with T3 alone, but rather a combination of T3 and T4 [59, 60].

In the chronic phase of critical illness, continuous infusion of TRH has been demonstrated to restore normal TSH pulsatility and increase T4 and T3 concentrations [40]. In principle, it is a safer option than direct thyroid hormone replacement, as the negative feedback of thyroid hormones on the pituitary thyrotropes is maintained, thus precluding overstimulation of the thyroid axis. However, TRH is not widely available, and no clinical outcome data have yet been reported to support this approach.

38.4 Growth Hormone Axis

The response of the GH axis to stress also follows a biphasic acute and chronic response to critical illness. In the acute phase, GH increases by a factor of three to five above baseline, initially associated with a rise in IGF-I. After several days of critical illness, baseline GH continues to be elevated, with pulsatile secretion at a frequency similar to that of healthy controls, but with a lower pulse amplitude. During the chronic phase, the GH/IGF-I ratio may be elevated well above normal, consistent with what has been described as a state of GH insensitivity [61]. However, despite elevated baseline levels of GH, the integrated production of GH over time is decreased during chronic illness. In addition, IGF-I is known to be much more responsive to pulsatile GH production, which is altered in this state [62]. Furthermore, during the chronic phase (but not the acute phase), normal GH pulsatility and concentrations of IGF-I can be restored by continuous infusion of GH secretagogues [63]. These data suggest that the alterations in the GH axis during chronic critical illness are primarily central in origin, and not due to GH insensitivity.

Other important regulators of GH secretion in the ICU setting include thyroid hormone, glucocorticoids, and dopamine. In the setting of true hypothyroidism or hypothyroxinemia of non-thyroidal illness, GH has markedly decreased pulsatility. This is predominantly a pituitary effect, as thyroid hormones are needed for GH gene transcription, translation, and secretion, although there are documented hypothalamic and peripheral effects as well [64, 65]. Glucocorticoids acutely stimulate GH secretion but beyond 12 hours lead to a prolonged suppression of GH concentrations. Glucocorticoids also raise the serum concentration of IGF-I but inhibit its biological activity. Glucocorticoid deficiency, on the other hand, impairs the GH response to GHRH [66–69]. Dopamine infusions suppress GH production at the level of the pituitary, even at doses as low as 5 mcg/kg/min. Rebound elevations in GH concentrations occur within 20 minutes of discontinuation of dopamine, however, and persist for at least a day [70, 71].

38.4.1 Diagnosis

The diagnosis of true GH insufficiency during critical illness is extremely difficult due to the dissociation of IGF-I levels from GH secretion and to the lack of predictability of random GH levels. Stimulation testing is often impractical, and may be uninformative, given that the individual is already stressed. Documentation of an elevated GH concentration, however, will reliably rule out GH insufficiency.

38.4.2 Treatment

For patients with documented GH insufficiency, replacement of GH should be continued while in the ICU. Similarly, initiation of GH therapy is appropriate in the neonatal intensive care unit (NICU) when a new diagnosis of GH deficiency is made. The usual replacement dose for a neonate is 0.15–0.22 mg/kg/week divided into daily subcutaneous doses. In any other clinical scenario involving critical illness, the use of GH is contraindicated, as the only large prospective, randomized, controlled trial demonstrated significantly increased mortality in adult ICU patients treated with GH [72]. This issue will be discussed further in the final section of this chapter.

38.5 Gonadal Axis

Hypogonadotropic hypogonadism has been demonstrated in virtually all studies of sex hormones in critically ill adults. In adults responding acutely to a variety of severe stresses (e.g., sepsis, trauma, burns, starvation), there is an initial surge in LH, while FSH and inhibin remain in the normal range, and testosterone and estradiol rapidly decrease [73, 74]. This short-term response of decreasing secretion of anabolic androgens may be adaptive as a means of decreasing energy consumption and allowing substrates to be utilized for other functions [2]. As the patient enters the chronic phase of critical illness, she or he becomes hypogonadotropic, and the low sex steroid levels persist [75, 76]. This likely represents the common pattern of peripheral hormonal suppression and pituitary activation in the acute phase of illness, followed by hypothalamic-based pituitary and peripheral suppression in the chronic phase. In a small study of critically ill men, intravenous pulsatile infusion of GnRH only partially overcame their hypogonadotropic hypogonadism pointing toward the interactions between the multiple endocrinologic axes disturbed during critical illness and their interdependence on one another [77]. Very little data exist for children with respect to gonadal axis changes during critical illness. In one recent prospective observational study, prepubertal children undergoing major cardiac surgery were found to have postoperative increases in several urinary sex hormone metabolites, with the increases largest for estradiol and progesterone [78]. Notably, however, these levels were 24-hour collections pre- and post-surgery and therefore did not capture any changes in levels after the first 24 hours postoperatively.

38.5.1 Diagnosis

Laboratory evaluation of hypogonadism should be approached skeptically in the setting of critical illness. High or "inappropriately normal" concentrations of gonadotropins may be encountered early in the clinical course and do not necessarily indicate primary gonadal failure. Likewise, low gonadotropins are uninformative even in the acute phase, as they may be suppressed by elevated prolactin, dopamine infusion, or an early progression into the chronic phase of critical illness.

38.5.2 Treatment

Several sex hormone replacement trials have been attempted, largely in an attempt to treat the catabolic state of critical illness. These studies will be addressed in the final section of this chapter. For patients on sex hormone replacement therapy prior to critical illness, we recommend discontinuing it for the duration of the ICU stay, or until the child has begun to show signs of significant clinical recovery.

38.6 Potential for Therapeutic Hormonal Interventions to Treat the Protein Catabolism of Critical Illness

Critically ill infants and children are under severe catabolic stress. Protein loss is the hallmark of the metabolic stress response, and its extent is determined by the severity of illness [79, 80]. If protein loss persists, it is associated with increased morbidity and mortality [81]. Limiting protein degradation and maximizing protein accretion are of particular importance in children because of their limited protein reserves and their requirement for growth and development. Infants on extracorporeal life support (ECLS, also known as ECMO) are among the most profoundly ill children in pediatric critical care and quantitatively demonstrate extremely high rates of protein loss [82]. Children who have suffered severe burns have also been studied extensively in this regard and have been shown to remain catabolic for at least 1 year after their injury [83].

The hormonal changes detailed earlier in the chapter—in particular, suppressed GH, elevated cortisol, and insulin resistance—contribute to the catabolism of critical illness. They induce a milieu of protein catabolism, carbohydrate intolerance, and paradoxical fat sparing. A number of anabolic hormonal therapies, including GH, IGF-I, insulin, and androgens, have been administered in the past in an effort to counteract these maladaptive changes.

GH (in the form of human pituitary extracts) was first used in an animal model of traumatic injury in 1941 to improve nitrogen retention and reduce weight loss [84]. Recombinant GH has since been investigated in a number of small clinical trials over a period of approximately 25 years. Small RCTs in a variety of ICU adult patients, using a variety of GH doses, documented improvements in indices of protein turnover and clinical outcome [85–88]. One moderate-sized pediatric study ($N = 72$) demonstrated decreased protein catabolism in burned children treated from ICU discharge until 1 year from burn [89]. The adult studies prompted a large multicenter RCT in adults admitted to the ICU for 5–7 days. In this trial, which used a daily dose of 0.1 mg/kg, the relative risk of mortality in patients receiving GH was increased to 1.9–2.4 compared to those receiving placebo [72]. No clear rationale for the increased mortality has yet been demonstrated, although hyperglycemia and associated immune compromise are suspected mediators. The FDA has since warned against the use of GH in patients with acute critical illness, and all related clinical trials outside of the burn population have effectively ceased [90].

Other attempts to augment the suppressed somatotrope axis have focused on delivering IGF-I, with or without GH. In normal adult subjects, IGF-I therapy has multiple effects that are potentially desirable in critically ill patients, including (a) increasing glucose uptake three fold; (b) reducing hepatic glucose output by 60–70%; (c) lowering blood glucose acutely (IV, not SQ); (d) lowering insulin, c-peptide, glucagon, free fatty acids, and ketones; and (e) decreasing proteolysis and thereby decreasing plasma amino acid concentrations [91]. These effects persist in the fed state, with glucocorticoid-induced catabolism [92], and were confirmed in a nonrandomized fashion in stable, post-burn adults [93]. Unfortunately, three RCTs failed to demonstrate a significant effect in ill postoperative adult patients [94–96]. In stable post-burn children, one RCT demonstrated significant effects in mitigating the extent of protein catabolism using IGF-I in combination with IGF-BP3 [97]. IGF-I has also been shown to be safe in a Phase I clinical trial in critically ill adults [98].

Anabolic sex steroid therapy in the critically ill with burns or trauma has produced several reports of positive results in adults [99–101] and one in burned children [89]. Oxandrolone, a non-aromatizable androgen, has been the therapeutic agent of choice due its decreased virilizing potency and hepatotoxicity as compared to testosterone. Data from pediatric burn patients have demon-

strated a doubling of the fractional synthetic rate of protein and a substantial improvement in net protein balance [89]. An RCT in adult burn patients showed decreased length of stay in those treated with oxandrolone compared to placebo [102]. This finding was replicated in a large pediatric RCT that evaluated oxandrolone treatment in the acute phase of post-burn care. This trial demonstrated that oxandrolone decreased length of stay, reversed loss of weight and lean body mass, and markedly improved muscle strength several months after discharge [103]. Furthermore, a single-center RCT evaluated 5-year outcomes of safety and efficacy of oxandrolone use in severely burned pediatric patients and found improved long-term outcomes in height, bone mineral content, cardiac work, and muscle strength in the oxandrolone-treated group [104]. Although these data strongly support the utility of oxandrolone following burn injuries, whether it has a similar role in ameliorating the hypercatabolic state of other critical illnesses remains to be seen.

Insulin therapy has been studied in a wide variety of clinical situations in order to reduce protein breakdown and to stimulate protein synthesis and growth. In small numbers of healthy volunteers, insulin has suppressed proteolysis by 59–91%, in a dose-dependent manner [105–108]. In burned adults, protein synthesis was stimulated, and wound healing was accelerated [109–112]. A single study in children demonstrated an 80% suppression of proteolysis in four stable, premature neonates but noted a significant rise in plasma lactate during the insulin infusion [112]. A small (n = 12) randomized, prospective crossover trial of parenterally fed critically ill neonates on extracorporeal life support also demonstrated that insulin did indeed decrease proteolysis but only improved net protein balance in those who received adequate dietary protein [113].

Aside from its potential broader role in preventing hypercatabolism, use of insulin infusions in critical care has been very actively investigated as a means of addressing the hyperglycemia that is common in critically ill patients. A great deal of evidence links uncontrolled hyperglycemia in critically ill adults and children with a variety of adverse outcomes including morbidity, increased length of stay, and death [114–117]. Two large RCTs demonstrated that tight glucose control using an intravenous insulin infusion significantly reduced mortality by 29% in adult diabetics after myocardial infarction and by 34% in adult postoperative surgical ICU patients [118, 119]. Although these early findings were promising, subsequent trials and meta-analyses of intensive glycemic control in critically ill adults have produced inconsistent results and have not shown a convincing mortality benefit [120–125]. Furthermore, the most recent large RCT demonstrated increased mortality in adults treated with intensive insulin management to near-normoglycemia, compared to those managed less aggressively [126]. Pediatric RCTs of intensive glycemic control in the ICU have found mixed results for main clinical outcomes in the intensively treated group but have uniformly been found to be associated with severe hypoglycemia [127, 128]. Thus, overall the available evidence is still inconclusive regarding the benefits of intensive glycemic control in all critically ill patients. Instead, there is likely benefit in specific patient groups: probable in cardiac surgical patients, improbable in traumatic brain injury patients [129], and still unclear in other forms of pediatric and adult critical illness.

In contrast to the uncertainty regarding its possible benefits, evidence is unequivocal that intensive glycemic control carries an increased risk of hypoglycemia. Hypoglycemia in the intensive care setting is associated with complications of seizure, brain damage, and death in both adults and children [130, 131]. Therefore, the conflicting data from trials of intensive glycemic control may partly derive from increasing risks related to hypoglycemia in trials that treated to lower blood glucose targets. In summary, although uncontrolled hyperglycemia in critical illness is clearly associated with worse outcome, attempting to control blood glucose too tightly with insulin infusion does not definitively improve outcomes and carries the risk of hypoglycemia. Therefore, current consensus guidelines recommend an intermediate blood glucose target range of 140–180 mg/dL [114]. Further studies are necessary to assess the true benefits, risks, and appropriate degree of intensive glycemic control in critically ill children, and they are currently underway in the US and Europe.

During the chronic phase of critical illness, the hypothalamus is suppressed despite normal responsiveness of the remainder of the components of each hormonal axis. This is a state that, but for modern critical care, human beings would not have survived to reach and have not evolved to endure. With this in mind, a final approach to hormonal therapies in critical illness is replacement of hypothalamic peptides by continuous (or pulsatile) infusion, which would be expected to reactivate normal pulsatile production of pituitary hormones. The primary advantage of this approach is that it maintains negative feedback on the pituitary and should theoretically prevent overproduction of end hormones, each of which has its own maladaptive effects at excessive concentrations. For example, although GH itself has been deemed unsafe for use in this population, infusion of GH secretagogues (e.g., GHRH, ghrelin) has been shown to normalize GH and IGF-I concentrations in a physiologic, pulsatile manner [132]. Co-administration of GH secretagogues, TRH, and pulsatile GnRH during the chronic state of critical illness has been shown to improve the function of all three axes simultaneously [133]. However, further research investigating clinical outcomes using infusions of hypothalamic peptides is required to definitively address this issue.

Case Study

An 8-year-old girl with a known history of immune dysregulation and frequent infections is admitted to the general pediatric ward with respiratory failure, initially necessitating bi-level positive airway pressure (BiPAP) while on antibiotics for community-acquired pneumonia. Overnight, she has increased work of breathing and requires increasing amounts of oxygen. Early in the morning of her second hospital day, she suddenly becomes unresponsive and hypotensive, and the pediatric critical care team is emergently called. While receiving vasoactive medications and intravenous fluid boluses, the critical care team emergently intubates her. As an induction medication for intubation, she receives etomidate. What aspects about her general past medical history, admitting history, and recent emergent treatment should the intensivist consider when thinking about her endocrinologic axes, their potential dysregulation, and his monitoring and management of these axes?

Review Questions

1. Which of the following is the constellation of laboratory abnormalities associated with hypothyroxinemia of non-thyroidal illness (or "sick euthyroid syndrome")?
   A. High TSH, low T3, low T4
   B. Normal TSH, low T3, low T4
   C. Normal TSH, high T3, low T4
   D. Low TSH, low T3, low T4
2. Cortisol concentrations rise acutely in critical illness leading to all of the following except:
   A. Increased blood pressure through enhanced inotropic and vasopressor response to catecholamines
   B. Increased blood pressure through upregulation of inducible nitric oxide synthetase
   C. Increased free water clearance
   D. Increased protein catabolism, lipolysis, and glycogenolysis via induction of insulin resistance
3. Which anabolic hormone therapy has been shown to safely and substantially counteract protein catabolism of critical illness leading to both improved short- and long-term outcomes in pediatric burn patients?
   A. Oxandrolone
   B. Growth hormone
   C. Insulin
   D. IGF-1

Answers

1. B
2. B
3. A

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Supplementary Information

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