Contemporary Endocrinology
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Sally Radovick
Margaret H. MacGillivray *Editors*

Pediatric Endocrinology

A Practical Clinical Guide

Second Edition

Humana Press

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A Practical Clinical Guide, Second Edition

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Preface

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

The scope of the text includes 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 for many articles 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 to consistently present the following: (1) an introductory discussion with background information, (2) a brief overview of recent progress on the mechanism involved, (3) a discussion of the clinical features that characterize each condition, (4) a delineation of the criteria used to establish a diagnosis, (5) a new section in this edition discussing the genetics of the disorder where relevant, (6) a therapy section which comprehensively reviews the options available, the risks and benefits of each approach correlated with clinical trial and outcome data, and also includes information on the long-term safety and efficacy of the treatment modality, (7) where relevant, psychosocial, and quality-of-life issues are discussed, and (8) finally, guidelines are cited when available.

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 on type 2 diabetes mellitus and obesity, dislipoproteinemias, and the treatment needs of children who have survived malignancies presenting with the endocrine sequelae due to the growing number of patients presenting with these disorders. Finally, the relatively brief discussions of skeletal dysplasias, non-thyroidal illness syndrome,

and autoimmune endocrinopathy of the previous text have been expanded to comprise additional chapters to provide more comprehensive information on these disorders.

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.

Baltimore, MD, USA — Sally Radovick
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Part I

Growth Disorders

Childhood Growth Hormone Deficiency and Hypopituitarism

1

Christopher J. Romero, Andrew N. Dauber, and Laurie E. Cohen

Abstract

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 the GH axis is important and follows below.

Keywords

Growth hormone • Growth hormone deficiency • Hypopituitarism • Pituitary

Introduction

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

The anterior pituitary is formed of three parts, namely the pars distalis (pars anterior or anterior lobe), the pars intermedia (intermediate lobe), and the pars tuberalis (pars infundibularis or pars proximalis), and forms 80% of the pituitary gland. In humans, the pars distalis is the largest part of the anterior pituitary and where most of the anterior pituitary hormones are produced [3]. The intermediate lobe is poorly developed in humans, and although it is only a rudimentary vestige in adults, it is relatively obvious in pregnant women and in the fetus [4]. The upward extension of the pars distalis onto the pituitary stalk forms the pars

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tuberalis, which may contain a small number of gonadotropin-producing cells [3].

Peptides 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 of several hormones that are synthesized there [5]. These 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 or 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 the GH axis is important and follows below.

GH Physiology

GH Gene

GH, which has a molecular weight of 22 kDa, is a single-chain α-helical non-glycosylated polypeptide with 191 amino acids and two intramolecular disulfide bonds. This mature hormone accounts for 75% of the GH produced in the pituitary gland [3]. There exists a 20-kDa variant form, which arises from alternative splicing during the processing of human GH (hGH) pre-mRNA and constitutes 5–10% of the total pituitary hGH [7, 8]. 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 (GH-R) [9].

The *GH1* gene encodes for GH and is part of a 50-kb cluster of five genes located on human chromosome 17q22-24: They include *GH1*, *chorionic somatomammotropin* (*CS*)*-like* (*L*), *CS-A*, *GH-2*, and *CS-B* [10]. The CS-L translated protein appears nonfunctional, while CS-A and CS-B encode human chorionic somatomammotropin (hCS) or human placental lactogen. 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 GH-R. Interestingly, hCS does not appear necessary for fetal or extrauterine growth nor does it appear essential for maintenance of pregnancy or lactation [11]. The *GH-2* gene product, which is 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 [12, 13].

GH Secretion (Fig. 1.1)

GH secretion follows a pulsatile pattern, secondary to the antagonistic influences 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 (GHRH-R), which is a G-protein-coupled receptor with seven-transmembrane-spanning domains with three extracellular and three cytoplasmic loops [14]. Activation of the GHRH-R results in an increase in cyclic adenosine monophosphate (cAMP) and intracellular calcium, leading to the activation of protein kinase A (PKA) [15, 16]. PKA phosphorylates and activates cAMP response element-binding protein (CREB), which binds to cyclic AMP (cAMP) response elements in the *GH* promoter to enhance *GH-1* gene transcription [17, 18]. 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) [19].

SRIF, a 14-amino-acid neuropeptide, negatively regulates GH release primarily via the

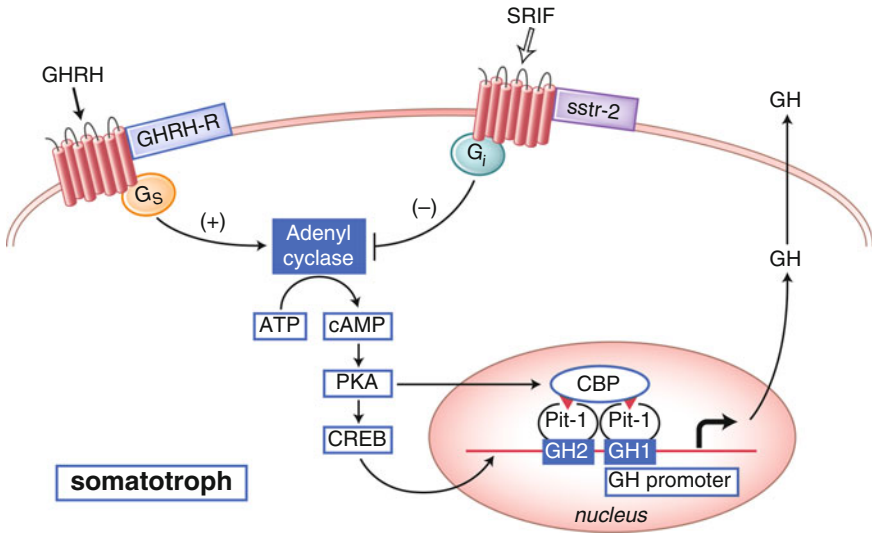

Fig. 1.1 Simplified model of *growth hormone* (*GH*) gene activation. GH synthesis and release from somatotrophs is regulated by growth hormone-releasing hormone (GHRH) stimulation and somatostatin (SRIF) inhibition. GHRH activation of its Gs-protein-coupled receptor leads to an increase in cyclic adenosine 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 Pit-1 and CREB-binding protein (CBP). SRIF activation of its Gi-coupled protein leads to a decrease in cAMP and a reduction in calcium influx

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, 25].

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 is probably secondary to the effect of gonadal steroids on GHRH [26–28]. Although hGH production continues throughout life, the levels decline in the elderly [29, 30].

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 [31, 32]. These peptides can initiate and amplify pulsatile GH release; however, this is accomplished via the GHS receptor (GHS-R), which is distinct from the GHRH-R [33]. 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 [34].

An endogenous ligand for the GHS-R named ghrelin has been identified and has been shown to stimulate GH release in a dose-related manner, as well as potentiate GHRH-dependent secretion of GH [35, 36]. 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 [37]. Several studies have demonstrated that ghrelin has a wide range of effects, including acting as a physiological mediator of feeding [38, 39]. Thus, it is difficult to separate the direct effects of ghrelin from those related to GH secretion.

GH Action (Fig. 1.2)

Approximately 50% of circulating GH is bound to GH-binding protein (GHBP). GHBP is produced in multiple tissues, with liver the

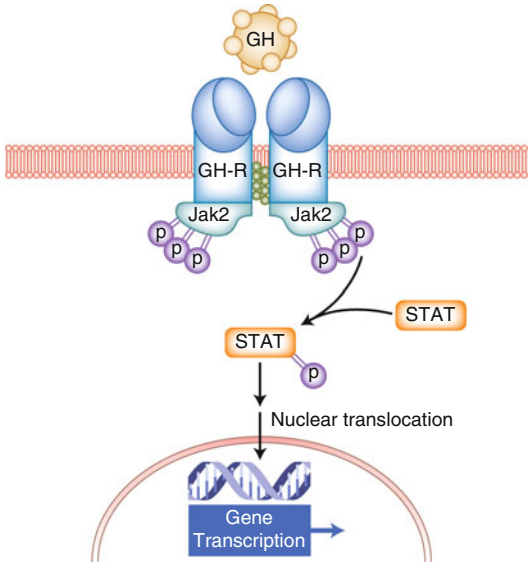

Fig. 1.2 Schematic model of growth hormone receptor (GH-R) binding and signaling. A single GH molecule binds asymmetrically to the extracellular domain of two receptor molecules, causing a conformational change. This leads to interaction of the GH-R with Janus kinase (Jak 2) and tyrosine phosphorylation of both Jak2 and GH-R, 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

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 GH-R levels and activity, yet its source or mechanism of generation is not entirely known. In rodents, it appears to be synthesized de novo from alternative splicing of *GH-R* mRNA. In humans, rabbits, and others, it may be shed from membrane-bound GH-R by proteolytic cleavage [9, 40].

The GH-R is a 620-amino-acid protein that belongs to the cytokine family of receptors [41]. It consists of a large extracellular domain, a single transmembrane helix, and an intracellular domain [42]. The highest level of *GHR* expression is in the liver, followed by muscle, fat, kidney, and heart. GH binds to a homodimer complex of the GHR in order to activate its intracellular signaling pathways. Although dimerization of the GHR was thought to occur after GH binding, recent data demonstrate that the subunits of the GH-R are constitutively dimerized in an unbound or inactive state [43, 44]. 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 that is transmitted via the transmembrane domain to the intracellular domain, allowing downstream kinase activation by phosphorylation of Janus kinase 2 (Jak2) [44]. Subsequently, the JAK2 molecule causes phosphorylation of critical tyrosines on the intracellular portion of the GH-R, which then provide docking sites for critical intermediary signal transducers and activators of transcription (STAT) proteins [45–47]. After phosphorylation, STATs dimerize and move to the nucleus, where they activate gene transcription [48, 49].

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 [50]. IGF-1, which is a basic 70-amino-acid peptide, is produced under the direction of GH predominantly in the liver [51]. It plays an important role in both embryonic and postnatal growth. Both systemic and local IGF-1 have been shown to stimulate longitudinal bone growth by increased osteoblast activity and increased synthesis of collagen [52–56].

Human fetal serum IGF-1 levels, which are approximately 30–50% of adult levels, have been positively correlated with gestational age [57, 58]. The levels of IGF-1 gradually increase during childhood and peak during pubertal development, achieving 2–3 times the normal adult values [59, 60]. IGF-1 production is also augmented by the rise in gonadal steroids, which contribute to the pubertal growth spurt. After adolescence, serum IGF-1 concentrations decline gradually with age [61, 62]. IGFs circulate within the plasma complexed to 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 [63, 64]. Six distinct human and rat

IGFBPs have been cloned and sequenced [65, 66]. IGFBP-3, which is GH dependent, is the major IGFBP in human serum and transports over 90% of the circulating IGF-1 [3].

The IGF-I receptor (IGF-1R), which is structurally related to the insulin receptor, is a heterotetramer comprised of two-membrane-spanning α (alpha) subunits and two intracellular β (beta) subunits [67, 68]. The subunits are linked by disulfide bonds and contain binding sites for IGF-I. The subunits 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, 69].

Growth Hormone Deficiency

Hypopituitarism can be caused by anything that damages the hypothalamus, pituitary stalk, or pituitary gland. The incidence of congenital GH deficiency has been reported as between 1:4,000 and 1:10,000 live births [70, 71]. 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 maternal hormones wane [72]. They generally have normal birth weights and lengths slightly below average [73]. The growth rate of a child with GH deficiency will progressively decline, and typically the bone age will be delayed. They develop increased periabdominal fat [74] and decreased muscle mass and may also have delayed dentition, thin hair, poor nail growth, and high-pitched voice [72]. 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 [72].

Congenital Forms of Hypopituitarism (Table 1.1)

Congenital cranial malformations, including holoprosencephaly, septo-optic dysplasia (SOD), and midline craniocerebral or midfacial abnormalities, can be associated with anomalies of the pituitary gland. These embryonic defects also include pituitary hypoplasia, pituitary aplasia, and congenital absence of the pituitary gland [6]. Clinically, they may be associated with pituitary hormone deficiencies or the risk for developing future hormone deficiencies. Although these conditions often have no identifiable etiology, ongoing advances in understanding 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.

Table 1.1 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)
GRHR receptor
Pituitary transcription factors
Hesx1 (Rpx)
Ptx2 (Pitx2, P-OTX2, Rieg)
Lhx3 (Lim-3, P-LIM)
Prop1
POU1F1 (Pit-1, GHF-1)
GH-1
Types Ia, Ib, II, and III
Multiple GH family gene deletions
Bioinactive GH
GH receptor
IGF-1
IGF-1 receptor
Stat 5b mutations
Idiopathic

GHRH receptor (*GHRH-R*) *mutations*: Mutations reported in the GHRH-R are often classified as a type of isolated GH deficiency. The little mouse (*lit*/*lit*), which demonstrates dwarfism and decreased number of somatotrophs, has a recessively inherited missense mutation in the extra-

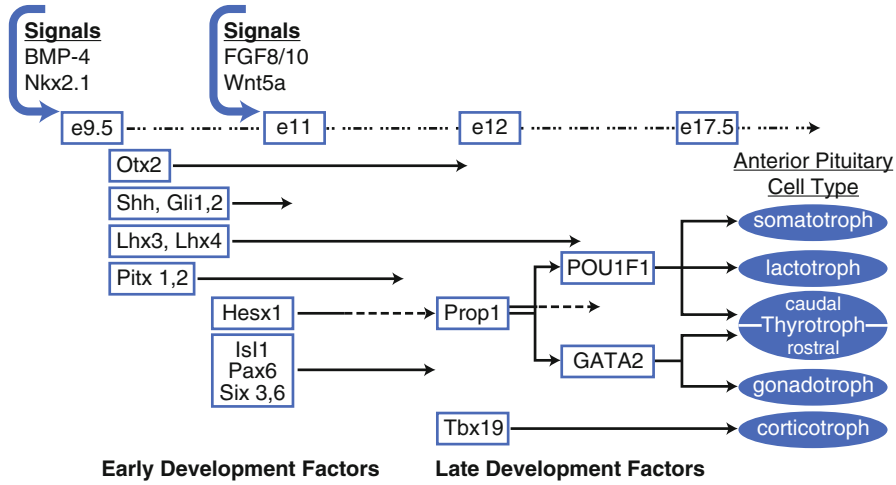

Fig. 1.3 Anterior pituitary development. The development of the mature pituitary gland initiates with the contact of the oral ectoderm with the neural ectoderm followed by a cascade of events consisting of both signaling molecules and transcription factors expressed in a specific temporal and spatial 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 morphogenic protein 4 (BMP-4) and 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 [121, 267, 268]

cellular domain of the gene for *Ghrhr* [75, 76, 77]. In addition to GH deficiency, these mice exhibit postnatal growth failure and delayed pubertal maturation [77]. In humans, two cousins presented clinically with the typical phenotype of severe GHD and were found to have a nonsense mutation in the human *GHRH-R* gene that introduced a stop codon at position 72 (E72X) [78]. A similar mutation is found in codon 50 in "Dwarfism of Sindh" [79].

Pituitary transcription factor mutations: 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. The genetic evaluation of patients diagnosed with idiopathic hypopituitarism has identified mutations in these factors accounting for pituitary dysfunction.

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 [80, 81], although no target genes for Rpx have yet been identified [82]. Hesx1 null mutant mice demonstrate abnormalities in the corpus callosum, anterior and hippocampal commissures, and septum pellucidum, presenting a similar phenotype to defects seen in humans with SOD [80]. Two siblings with agenesis of the corpus callosum, optic nerve hypoplasia, and panhypopituitarism 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 [80]. More recently, a novel I26T mutation in exon 1 was reported in a patient with early GHD, FSH/LH deficiency, and evolving TSH and cortisol deficiency, along with pituitary structural abnormalities but normal optic nerves [83].

Several investigators have organized screenings to assist in identifying mutations in *HESX1*. Thomas et al., for example, scanned 228 patients with a wide spectrum of congenital hypopituitarism phenotypes: 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 [84]. More recently, 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 [85].

Ptx2 (*Pitx2*): Ptx2 is a paired-like homeodomain transcription factor closely related to the mammalian *Otx* genes that 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* [86]. Ptx2 null mice showed embryonic lethality; however, a hypomorphic allele model of Ptx2 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 [87–89].

RIEG is the human homologue of Ptx2, 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 [90]. One group of investigators described mutations in six out of ten families with autosomal dominant Rieger syndrome [91, 92]. Five of the six mutations reported were in the homeobox region, and several show loss of DNA-binding capacity.

Lhx3 (*Lim-3*, *P-Lim*): 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 [93–95]. Lhx3 expression persists in the adult pituitary, suggesting a maintenance function in one or more of the anterior pituitary cell types [93]. 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 [93, 96].

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 [97–99]. Among 366 studied patients with IGHD or CPHD, only 7 patients from 4 families were found to have LHX3 mutations, suggesting *LHX3* mutations are a rare cause of CPHD [99].

Prop1: Prop1 is a paired-like homeodomain transcription factor with expression restricted to the anterior pituitary during development [2, 100]. During pituitary development, Prop1 acts as a repressor in downregulating Hesx1 and as an activator of POU1F1 [101]. A considerable variation in clinical phenotypes of patients with PROP1 mutations has been demonstrated, even in patients bearing identical genotypes [100, 102, 103]. Several reports have shown that hormone deficiency may be variable and dynamic; some patients may develop cortisol deficiency over time or hypogonadotrophic hypogonadism despite the progression into spontaneous puberty [100, 104–106].

Multiple nonconsanguineous patients from at least eight different countries have a documented recurring homozygous autosomal recessive mutation of PROP1, delA301,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 [107–109]. In one family, progressive ACTH deficiency was noted with age [110]. In another consanguineous Indian pedigree, a 112-124del mutation resulting in a premature stop codon at position 480 was identified, and in addition to GH, PRL, TSH, and gonadotropin deficiencies, affected individuals were also noted to have an impaired pituitary-adrenal axis [111]. Several other mutations in PROP1 have been described [100, 107, 112–114].

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 [115, 116]. 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 [116].

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 [117–120]. At least 28 different mutations have been described, with 23 demonstrating autosomal recessive inheritance and five demonstrating dominant inheritance [121]. 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 [118, 120, 122–129].

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 another mechanism responsible for CPHD [130]. 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 [121, 131].

Isolated GHD (*IGHD*): 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 [132–135]. These patients frequently develop antibodies to exogenous GH therapy, which is attributed to the lack of immune tolerance because of prenatal GHD [136, 137]. Some patients may eventually become insensitive to GH replacement therapy demonstrating a decreased clinical response; subsequently, recombinant IGF-1 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 [133, 138, 139]. 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 frame shift within exon 5. These changes ultimately affected the stability and biological activity of the mutant GH protein [140]. Several other deletions or frame shift mutations have been described by others [141–143].

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 [139, 140, 144–148].

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 [149, 150].

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-1 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 [151]. In another patient, an aspartic acid to lysine mutation at codon 112 was identified and suggested to prevent appropriate GH-R dimerization [152].

There are also patients with the phenotype of growth hormone insensitivity who do not demonstrate mutations of the GH-R gene, but have identified mutations in downstream GH-R signaling molecules. Homozygous mutations in the Stat5b gene, a major GH-dependent mediator of IGF-I gene transcription, have been identified as a cause of GH insensitivity [153, 154]. The first mutation characterized was a point mutation resulting in a marked decrease in phosphorylation of tyrosine [153], a critical step in the pathway to STAT activation of IGF-1 gene transcription; while the second characterized mutation was an insertion in exon 10, leading to early protein termination [154–156]. In addition to growth retardation, both patients had evidence of immune dysfunction presumably because Stat5b is involved in downstream signaling for multiple cytokines.

GH-R mutations: Laron dwarfism is an autosomal recessive disorder characterized by clinical features of severe GH deficiency along with low IGF-1 levels but with normal to high levels of GH after provocative testing [157]. Several deletions and point mutations of several GH-R exons have been described [158–167]. Many of these mutations affect the extracellular domain and, therefore, lead to absent or decreased levels of GHBP [168]. Recombinant IGF-1 therapy has been demonstrated to effectively treat these patients [169, 170]. 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 *GH-R* gene [162].

IGF-1 and IGF-1R mutations: A patient noted to have a homozygous partial IGF-1 gene deletion with undetectable levels of IGF-1 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 [171].

Studies with African pygmies demonstrate normal levels of hGH, but decreased IGF-1 levels and unresponsiveness to exogenous hGH. Although IGF-1 deficiency has been hypothesized, Bowcock et al. found no differences in restriction fragment length polymorphisms in the IGF-1 gene in Pygmy versus non-Pygmy black Africans [172]. Furthermore, Pygmy T cell lines show IGF-1 resistance at the receptor level with secondary GH resistance [173, 174]. In a recent study, it was demonstrated that adult Pygmies demonstrate a reduction in both GH gene expression (1.8-fold) and GH-R gene expression (8-fold). This decrease of the GH-R expression in Pygmies was associated with reduced serum levels of IGF-I and GHBP [175].

Abnormalities in the IGF-1R gene have also been reported and are often associated with intrauterine growth retardation (IUGR). Several heterozygous mutations of the IGF-1R gene, as well as an association with deletions in chromosome 15q, have been reported in patients with growth retardation [176–181]. 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-1 levels were found to be either normal or high, whether at baseline or after provocative testing.

Other patients are suspected to have IGF-1 resistance, as they have elevated GH levels and elevated IGF-1 levels [182–184]. In one patient, cultured fibroblasts had a 50% reduction

in IGF-1 binding capacity [183]. Another patient had a markedly diminished ability of IGF-1 to stimulate fibroblast α (alpha)-aminoisobutyric acid uptake compared to control subjects [184]. Their birth lengths, which were less than the fifth percentile, suggest the importance of IGF-1 in fetal growth.

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

Acquired Forms of Hypopituitarism (Table 1.2)

Head trauma can damage the pituitary stalk and infundibulum and lead to the development of transient and permanent diabetes insipidus, as well as other hormonal deficiencies [185, 186]. There are a number of reports suggesting an association between hypopituitarism and a complicated perinatal course, especially breech delivery [70, 187, 188]. 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].

Table 1.2 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
Hemachromatosis
Cerebral edema
Neoplasms
Craniopharyngioma
Germinoma
Hypothalamic astrocytoma/optic glioma
Cranial irradiation

Infiltrative conditions can also disrupt the pituitary stalk. Diabetes insipidus can be the first manifestation of Langerhans cell histiocytosis [189–191] or sarcoidosis [192]. Lymphocytic hypophysitis, usually in adult women in late pregnancy or the postpartum period, can result in hypopituitarism [193].

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 [194, 195].

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 [196]. Treatment of brain tumors or acute lymphoblastic leukemia (ALL) with cranial irradiation may also result in GHD. Lower radiation doses preserve pharmacologic response of GH to stimulation, but spontaneous GH secretion may be lost [197]. Discordancy between failure to provoke an adequate GH response to insulin-induced hypoglycemia but normal response to exogenous GHRH stimulation suggested that the hypothalamus is more vulnerable than the anterior pituitary [198]. More recent 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 postulate 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 time of increased GH demand [199].

The higher the radiation dose, the more likely and the earlier GHD will occur after treatment [200, 201]. 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

[200]. Higher doses also increase the likelihood of the development of other anterior pituitary hormone deficiencies as well [201]. Cranial radiation can also be associated with precocious puberty, leading to premature epiphyseal fusion [197], and spinal irradiation can lead to skeletal impaired spinal growth [202], both of which will further compromise adult height.

Diagnosis 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. In 2000, the Growth Hormone Research Society published its consensus guidelines on the diagnosis and treatment of GHD in childhood and adolescence [203]. In considering who should undergo evaluation for GHD, they stress the importance of first excluding other causes of growth failure and then assessing the patients for clinical features that can coexist with GHD. These features include hypoglycemia, prolonged jaundice, microphallus, and traumatic delivery in the neonate, as well as a history of cranial irradiation, head trauma, and central nervous system infection; family history of GHD and craniofacial midline abnormalities; and presence of other pituitary hormone deficiencies. 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-1 levels [204] and low peak GH levels after stimulation tests [205].

The majority of patients with GHD will present with short stature without any of these other features. Some suggested guidelines for further evaluation include height more than 3 SD below the population mean, height more than 2 SD below the population mean with a growth velocity more than 1 SD below the mean, or a very low growth velocity (less than minus 2 SD) irrespective of current height [203]. Conventionally, the gold standard for the diagnosis of GHD has been a peak serum GH <10 ng/mL after two different GH stimulation tests. This cutoff is completely arbitrary and has increased from <3 to <10 ng/ml as the supply of GH has increased with the production of recombinant hGH (rhGH). However, the sensitivity and specificity of these tests are limited due to their dependence on physiological parameters such as age, gender, and body weight; the implementation of different pharmacological stimuli; the arbitrary cutoff values; the poorly reproducible results; and the use of different laboratory techniques for the measurement of GH. Assessment of serum levels of IGF-I and its binding protein IGFBP-3 is a major advance in the diagnosis of GH deficiency. Ultimately, the diagnosis is based on the integration of auxological, biochemical, and radiographic criteria.

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. Radioimmunoassays (RIAs) and immunometric assays are the most commonly used laboratory techniques for determination of GH levels. Estimations performed by RIA use polyclonal antibodies, which render low specificity and higher GH levels when compared with the more specific immunoradiometric assays using two highly specific monoclonal antibodies. Discrepancies up to two- to fourfold have been reported among different assays [206].

A variety of pharmacological tests have been implemented to assess the GH secretory capacity of the pituitary gland [207]. They are expensive, not free of side effects, and require fasting conditions as high glucose levels inhibit GH secretion. GH provocative tests have been divided into two groups: screening tests including exercise, 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, sequentially or in combination, has been implemented [208, 209]. An inappropriate low secretory response in the second test supposedly is

confirmatory of GH deficiency. However, multiple studies have shown that children diagnosed with isolated GHD based on peak GH levels <10 ng/mL will have normal GH secretion on retesting both in childhood [210] and as adults [211, 212].

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 [213] or androgen [214] to Tanner stage I or II children has been recommended to distinguish between GHD and constitutional delay in growth and puberty [215], although there is no consensus on this recommendation. While children with GHD might have an attenuated response, those with constitutional growth delay will have a normal secretory pattern. In a study by Marin et al. [215], 61% of normal-stature prepubertal children who were not primed with sex steroids failed to raise their peak serum GH concentration above 7 ng/mL following a provocative test.

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. GH stimulation tests are reliable only in the diagnosis of severe or complete GH deficiency. In addition to their low reproducibility [216], a "normal" secretory response does not exclude the possibility of various forms of GH insensitivity or partial GH deficiency. Caution must be taken in obese children who undergo provocative testing for GH secretion, due to a negative impact of adipose tissue on GH secretion [217, 218].

Physiologic Assessment of Growth Hormone Secretion

In addition to pharmacological tests of growth hormone secretion, exercise testing has been implemented as a screening test for GHD, as exercise induces an increase in GH levels. Although it is simple, safe, and inexpensive, up to one-third of normal children have an absent GH response [219]. Additionally, 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 [220, 221]. 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 is still significant intraindividual variation and overlapping with the values found in normal short children [222]. Lanes et al. reported decreased overnight GH concentrations in 25% of normally growing children [223]. As sampling is required every 20 min for a minimum of 12–24 h, this test is not practical for routine clinical care.

GH induces the expression of IGF-I in liver and cartilage. The use of age and puberty-corrected IGF-1 levels has become a major tool in the diagnosis of GHD [224]. 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 [225, 226].

IGFBP-3 is the major carrier of IGF-1 [227]. It is GH dependent but has less age variation and is less affected by the nutritional status compared to IGF-I and, thus, may correlate more accurately with GH status [228]. 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 [229]. Similarly, normal values have been reported in children with partial GHD [225, 230].

Determinations of IGF-I and IGFBP-3 are reliable tests in the diagnosis of severe GH deficiency and have better reproducibility when compared with GH provocative tests. However, their sensitivity and specificity are still suboptimal [205]. The combination of a low growth velocity and IGF-I level is quite sensitive and specific for the diagnosis of GHD and may remove the need for provocative testing in patients [231] where other causes for growth failure, especially malnutrition and gastrointestinal illness, have been excluded.

Bone Age Evaluation

The evaluation of skeletal maturation is crucial in the assessment of growth disorders, as osseous growth and maturation is 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, 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 bone age (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 [232] and Tanner-Whitehouse 2 (TW2) [233] 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 [232]. 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. BA estimation has technical difficulties due to inter- and intraobserver variations as well as ethnic and gender differences among children.

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 S.D. (10 cm or 4 in) [234]. 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, chronological age, and BA obtained by the Greulich and Pyle method [235]. This method probably underpredicts growth potential [236]. (2) The TW2 method considers current height, chronologic age, TW2 assessment of BA, midparental stature, and pubertal status [233]. (3) The Roche-Wainer-Thissen method requires recumbent length, weight, chronological age, midparental stature, and Greulich and Pyle BA assessment [237]. (4) The Khamis-Roche algorithm (KR) 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 [238]. 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 [239]. In addition, predictions of adult height may be of limited value in patients with underlying pathology.

MR 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, pituitary stalk agenesis, and posterior pituitary ectopia [211]. Structural abnormalities are more common in patients with CPHD or panhypopituitarism (93%) and in those with severe GH deficiency compared to those with isolated GH deficiency (80%) [240]. 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.

GH Therapy

There is wide variability in the dose of rhGH used to treat GHD. Traditionally, rhGH dosage has been based on weight, and consensus guidelines recommend doses of 25–50 μg/kg/day given 6–7 days per week in children with the consideration of doses up to 100 μg/kg/day during puberty [203, 241]. High-dose therapy during puberty has been shown to increase near-adult height modestly without apparent adverse effects or increased rate of skeletal maturation [242]. There is great variability in response to rhGH therapy.

In order to decrease variability and improve adult height outcomes, two strategies have evolved to help refine rhGH dosing. The first strategy employs prediction models which calculate expected growth velocity based on baseline parameters [243]. 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 rhGH therapy, and height deficit from midparental target height have been found to be the most significant predictors of first-year growth velocity [244]. 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 rhGH doses based on a prediction model decreased variability in response without compromising efficacy [97].

The second strategy for refining rhGH 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 [245]. With this strategy, there is still a range of responses which depend on the individual patient's GH sensitivity.

Regardless of strategy used to select a dose for initiation of rhGH 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 [246, 247]. In patients with severe GHD defined as a peak GH level <5 ng/mL 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 [247]. A suboptimal response may be indicative of an incorrect diagnosis of GH deficiency, lack of compliance, improper preparation and/or administration, 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 rhGH. 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% [248]. Additionally, one can compare actual growth response to predicted growth response based on the aforementioned growth prediction models. As rhGH dose is included in the models, a poor actual versus predicted response indicates either decreased growth hormone sensitivity or noncompliance [247]. Finally, there is mounting evidence that underlying genetic variants in the GH-R influence response to therapy [249], but this area requires further research.

There have been attempts to further increase height gains in individuals with GHD who have a low predicted adult height through the use of gonadotropin-releasing hormone analogs to

suppress puberty. The data on this topic is conflicting, and recent consensus guidelines state that this practice cannot be suggested [250].

Monitoring of IGF-I and IGFBP-3 levels has gained wide acceptance to assess safety and compliance; however, their serum levels do not always correlate with the obtained increment in growth velocity. Although recommended by some [251], regular monitoring of the BA in children under GH therapy is questionable. Interobserver differences in bone age interpretation and erratic changes over time in osseous maturation make the estimation of adult height inaccurate. Similarly, predictions of adult height may be artifactually overestimated, as GH may accelerate the bone maturation in advance to any radiographic evidence [252].

Side Effects

Diabetes and insulin resistance: Despite the concerns of diabetes mellitus (DM) developing in patients under rhGH therapy due to its anti-insulin effect, a higher incidence of type I insulin-dependent diabetes mellitus (IDDM) in children and adults has not been reported [253]. Type II DM has been reported by some at a higher incidence in children receiving GH; however, others found no increased incidence of type 2 DM in rhGH-treated patients with a normal BMI [253]. Nevertheless, a high BMI is a risk factor for developing diabetes in GHD patients, and rhGH therapy may potentially accelerate the development of diabetes in predisposed patients [254].

Leukemia: Concerns regarding the development of de novo leukemia arose after a cluster of leukemia in patients under rhGH therapy was reported in Japan in 1988 [255]. A subsequent study, however, which looked at 32,000 rhGH recipients did not find significantly higher incidence compared to the general population [256]. Initially, three cases of leukemia in the United States were reported in 59,736 patient-years of follow-up, which was not significantly higher when matched by US age, race, and gender, yet three additional cases found in an extended follow-up suggested an increased minimum rate of leukemia (2.26 cases expected, $p = 0.028$). Five of these six subjects, however, had antecedent cranial tumors and four had received radiotherapy. More recently, the National Cooperative Growth Study (NCGS) published data to help address concerns about de novo leukemia in recipients without risk factors and report the safety issue has not been confirmed [257]. In patients with idiopathic GHD, there was no increase in leukemia [258].

Recurrence of central nervous system tumors: GH and IGF-1, which both have anabolic and mitogenic effects, have been suggested to cause proliferation of normal and malignant cells. Therefore, several possible mechanisms regarding rhGH's potential role in tumor growth have been investigated [259]. Initial data from the Kabi International Growth Study (KIGS) [260] and the NCGS [261] did not support an increased risk of brain tumor recurrence. Follow-up data by the NCGS in 2010, which essentially comprises 20 years of GH therapy and 192,345 patient-years, continued to report no increase in new malignancies or recurrences of CNS tumors in rhGH-treated patients without risk factors [257]. The development of second neoplasms (SN) in children treated with rhGH therapy, however, does appear to be increased especially in those with prior exposure to radiation [257]. Ergun-Longmire et al. reported that cancer survivors treated with rhGH 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 [262].

Skin cancer: The statistics of the NCGS have not shown a higher incidence of melanocyte nevi or skin cancer in individuals treated with rhGH [263].

Benign intracranial hypertension: This neurological complication has been described in patients receiving rhGH but with a low incidence. A prospective study collecting data on 3,332 children in Australia and New Zealand found a low incidence of 1.2 cases per 1,000 patients [264]. More recently, data from the KIGS further demonstrate the incidence is lower than previously

reported [265]. Nevertheless, an ophthalmologic evaluation is mandatory in rhGH recipients in the event of persistent headaches, nausea, visual symptoms, and dizziness.

Slipped capital femoral epiphysis (*SCFE*): The NCGS reported that children with GH deficiency were significantly more likely to develop SCFE while on rhGH (91.0/100,000 patient-years) than the general population. Typically, these children were older, heavier, and grew more slowly during the first year of GH treatment than those who did not [266]. Children with idiopathic short stature on GH treatment did not show an increased incidence (9.5/100,000 patient-years). More recently, data from the KIGS analysis demonstrated that the incidence of SCFE was comparable and even lower than previously reported, except for those in the congenital GH-deficient and Turner syndrome groups [265]. SCFE, however, can be associated with not only obesity but also untreated endocrine conditions (e.g., hypothyroidism) that affect growth, 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) [257, 265].

Conclusions

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 can also result in GHD. Diagnosis of GHD, however, remains problematic. Once it is diagnosed, rhGH therapy is an effective treatment.

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Growth Hormone Insensitivity

2

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Abstract

GH insensitivity (GHI) or resistance is defined as the absence of an appropriate growth and metabolic response to endogenous growth hormone (GH) or to GH administered at physiologic replacement dosage. The genetic disorders that interfere with the response to GH include mutations affecting the GH receptor (GHR), serum transducers, and activator of transcription 5b (STAT5b), acid-labile subunit (ALS), insulin-like growth factor I (IGF-I), and IGF-I receptor.

Keywords

Growth hormone (GH) receptor (R) • GHR gene mutation • Laron syndrome • GH-binding protein (BP) • Insulin-like growth factor I • IGFBP • Recombinant IGF-I • Serum transducers and activator of transcription 5b • Acid-labile subunit • IGF-I R • Noonan syndrome

The Growth Hormone–Insulin-like Growth Factor-I Axis

Growth hormone (GH) synthesis and secretion by the anterior pituitary somatotrophs is under the control of stimulatory GH-releasing hormone (GHRH) and inhibitory somatostatin (SS) from the hypothalamus (Fig. 2.1). Other GH secretagogues (e.g., ghrelin and various synthetic hexapeptides) may also play a role. 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 insulin-like growth factor (IGF)-I, with resultant inhibition of GH release [1].

GH bound to the soluble GH-binding protein (GHBP) in the circulation is 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 [2]. GHBP is the proteolytic cleavage product of the full-length membrane-bound receptor molecule [3].

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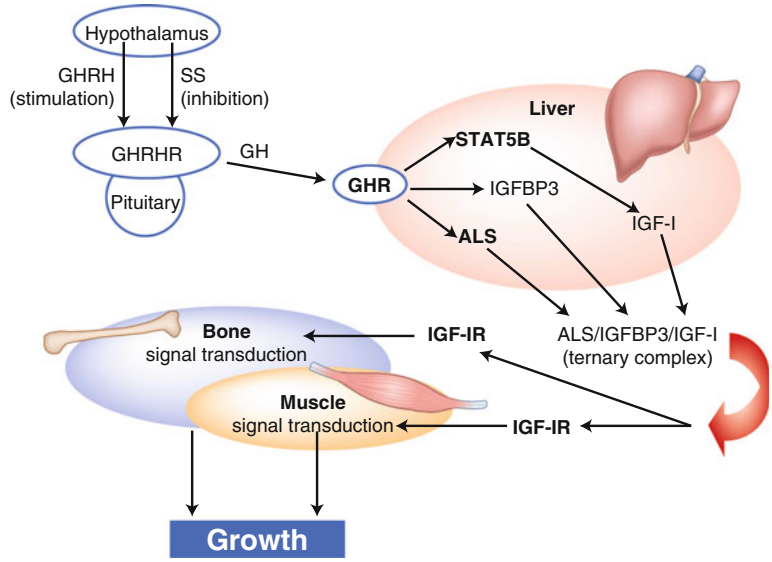

Fig. 2.1 Simplified diagram of the hypothalamic-pituitary-GH/IGF-I axis, showing mutational targets resulting in GH insensitivity indicated in **bold**

This characteristic permits assaying circulating GHBP as a measure of cellular-bound GHR, which usually correlates with GHR function [1].

The GH molecule binds to a molecule of cell surface GHR, which then dimerizes with another GHR molecule in the extracellular domain, so that a single GH molecule is enveloped by two GHR molecules [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 of cellular proteins. Included in this cascade are 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 growth hormone/prolactin/cytokine receptor family that includes receptors for erythropoietin, interleukins, and other growth factors [2].

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 six structurally related proteins with a high affinity for binding IGF. At least four other related proteins with lower affinity for IGF peptides have been identified and are referred to as IGFBP-related proteins [5, 6]. IGFBP3 is the most abundant IGFBP, binding 75–90% of circulating IGF-I in a large (150–200 kDa) complex which consists of IGFBP-3, an acid-labile subunit (ALS), and the IGF molecule. Both ALS and IGFBP3 are produced in the liver as a direct effect of GH. The ALS stabilizes the IGF–IGFBP3 complex, reduces the passage of IGF-I to the extravascular compartment, and extends its half-life. The remainder of bound IGF is in a 50-kDa complex with mostly IGFBP-1 and IGFBP-2.

IGFBP-1 production is highly variable, with the highest concentrations in the fasting, hypoinsulinemic state. The circulating concentration of IGFBP-2 is less fluctuant and is partly under the control of IGF-I; levels are increased in GHR-deficient states but increase further with IGF-I

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 to severe	Elevated	Decreased	Variable	Variable
Diabetes mellitus	None to mild	Elevated	Decreased	Decreased	Increased
Renal disease	Mild to severe	Normal	Decreased	Normal	Increased
Hepatic disease	Mild to 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 abnormal IGF-I

therapy of such patients [7]. The IGFBPs modulate IGF action by 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 actions [5].

Autocrine and paracrine production of IGF-I occurs in tissues other than the liver. In growing bone, GH stimulates differentiation of pre-chondrocytes into chondrocytes able to secrete IGF-I, which stimulates clonal expansion and maturation of the chondrocytes, with growth. It is estimated that at least 20% of GH-stimulated growth results from this autocrine/paracrine IGF-I mechanism [8].

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. Although the insulin receptor has a low affinity for IGF-I, IGF-I is present in the circulation at molar concentrations that are 1,000 times those of insulin. Thus, 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, especially in the IGFBP3-deficient state [9].

GH Insensitivity

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 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 (GHR), STAT5b, ALS, IGF-I, and IGF-I receptor (Fig. 2.1).

The conditions that have been associated with acquired GHI may not demonstrate low levels of IGF-I, or even consistent growth failure. Acquired GH resistance occurs in some patients with GH gene deletion for whom injections of recombinant human GH stimulate the production of GH-inhibiting antibodies [10]. 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 [11].

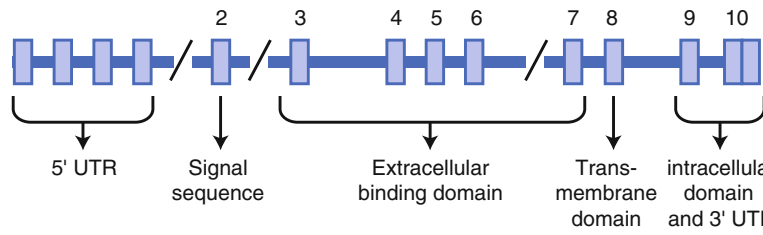

Fig. 2.2 Representation of the GHR gene. The *black horizontal line* represents intron sequence; *breaks in lines* indicate uncloned portions of the intron and the *boxes* represent exons, which are enlarged for clarity. UTR refers to untranslated regions of the transcripts. Translated regions are exons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

The Molecular Basis of GHI

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 9 coding exons. Exon 2 encodes the 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 3–7 encode the extracellular hormone-binding domain, except for the terminal 3 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 250 known patients with GHR deficiency (Laron syndrome), which result in a clinical picture identical to that of severe GH deficiency, 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 [3]; 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 mutations, missense mutations, frameshift mutations, splice mutations, and 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].

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 and whether associated with very low or unmeasurable GHBP, or with the more rare transmembrane defects that can be associated with elevated GHBP levels, result in a typical phenotype of severe GH deficiency (Table 2.2). In contrast, the intronic mutation present in the heterozygous state in a mother and daughter with

Table 2.2 Clinical features of severe IGF-I deficiency due to GH deficiency or GH receptor deficiency or STAT5b mutation

Growth • Birth weight—normal; birth length—usually normal • Growth failure, from birth, with velocity ½ 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 3rd 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

relatively mild growth failure (both with standard deviation score [SDS] for height −3.6), and resulting in a dominant negative effect on GHR formation, is not associated with other phenotypic features of GH deficiency [12]. 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 GH deficiency [13]. GHBP levels in the Caucasian patients 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 [14]. Naturally occurring truncated isoforms have also shown this dominant negative effect in vitro [15].

A novel intronic point mutation was discovered in a highly consanguineous family with two pairs of affected cousins with GHBP-positive GH insensitivity 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 pseudo-exon between exons 6 and 7, predicting an in-frame, 36-residue amino acid sequence, in a region critically involved in receptor dimerization [16].

Genetic Disorders Affecting GH-GHR Signal Transduction and Transcription

STAT5b

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 has emerged as the one that is crucial for growth [17]. The GH-activated GHR recruits the STAT5b which docks to specific phosphotyrosine 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, binding to DNA, interacts with other nuclear factors, and initiates transcription.

Ten patients have been described with seven autosomal recessively transmitted mutations of STAT5b [18]. Similar to children with severe GH deficiency and GHR deficiency, 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 similar to GH and GHR deficiency, postnatal growth shows rapid decline in SDS ranging from −9.9 to −5.6 at diagnosis, which was from 2.1 to 31 years of age. Serum concentrations of IGF-I, IGFBP-3, and ALS were markedly low; basal and stimulated GH concentrations were either normal or elevated. The features (growth patterns, facial disproportion, and biochemistry with the exception of GH-binding protein) have been identical to those of patients with severe GHR deficiency.

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 mutation. Reported abnormalities include T cell functional defects, low numbers of NK and γδT-cells, and IL-2 signaling defects.

PTPN11

Fifty percent of children with Noonan syndrome, which is characterized by short stature, cardiac defects, skeletal abnormalities, and facial dysmorphism, 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 Noonan syndrome who have this mutation have more severe statural deficit than those without the mutation. 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 [17].

Mutations of the IGF-I Gene

Failure of IGF-I synthesis due to a gene deletion was described in a patient with a homozygous partial deletion of the IGF-I gene [19]. His profound intrauterine growth failure (IUGR) persisted into adolescence and he had sensorineural deafness with severe mental retardation and micrognathia. Subsequently, a second patient was described with the same clinical phenotype but a different mutation also resulting in near absence of circulating IGF-I [20]. A third similarly affected patient had a defect in IGF-I synthesis resulting in production of a nonfunctioning IGF-I molecule circulating in high concentration [21].

The absence of the craniofacial phenotype of severe GH or GHR deficiency and the presence of normal IGFBP-3 in these patients, despite absent IGF-I function, indicate that the craniofacial features and low IGFBP-3 of GH and GHR deficiency are related to an absence of the direct effects of GH that do not act through the medium of IGF-I synthesis. It is also noteworthy that profound IUGR and mental retardation are not characteristic of GH or GHR deficiency, 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 been described, resulting in IUGR and postnatal growth failure, undetectable IGF-I by highly specific monoclonal assay but elevated levels with a polyclonal assay, microcephaly, and mild intellectual impairment, but with normal hearing [22].

Mutations of the Acid-Labile Subunit 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 are able to participate in protein–protein interactions; ~75% of the mature protein corresponds to the consensus motif for the LRR super-

family. 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, with 16 discrete homozygous or compound heterozygous mutations noted [23–29]. 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 patients, being better than target height in one patient, 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 GH insensitivity or deficiency emphasizes the compensatory capability of local IGF-I production [30].

Mutations of the IGF-I Receptor

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. 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 for study 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 IGFBP3 concentrations, a single patient was identified with compound heterozygosity for mutations of the IGF-I receptor resulting in amino acid substitutions. She had severe IUGR (birth weight 1,420 g at 38 weeks), poor postnatal growth, and elevated concentrations of IGF-I and integrated GH concentration when prepubertal, consistent with IGF-I resistance. The location of the mutations was within a putative ligand-binding domain and the heterozygous parents were subnormal in stature and 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, who had a heterozygous nonsense mutation reducing the number of IGF-I receptors on fibroblasts; two other affected first-degree relatives were identified [31]. In all, 17 cases in 7 families, each family with a unique mutation, had been described by 2010, with in vitro confirmation of failure of IGF-I binding and function [31–36].

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 and normal timing of puberty, and normal to markedly increased serum concentrations of IGF-I and IGFBP3. The effect of IGF-I gene mutations on intrauterine growth, however, is uniformly replicated in this less severe circumstance.

Epidemiology

Race/Nationality

Among the approximately 250 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 Arabs, Oriental, or Middle Eastern Jews, and the largest group, the genetically homogeneous 90+ 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. Recently, additional patients with the E180 splice mutation on the same genetic background have been identified in Chile 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) [9].

The individuals with STAT5b mutation include Kuwaiti siblings, two unrelated Argentinians, siblings from Brazil, one patient from Turkey, and one patient from the Caribbean [17].

ALS mutations were reported in three Kurdish brothers, three unrelated and two sibling Spanish patients, 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 [23–29].

IGF-I receptor mutations have been reported from the USA, Germany, Russia, Korea, Japan, and the Netherlands [31–36].

Gender

Among patients observed since the original description of the GHR deficiency syndrome by Laron, Pertzelan, and Mannheimer in 1966 [37] until 1990, a normal sex ratio was noted. The initial report of 20 cases from a single province in Ecuador included only one male [38], but subsequent observations from an adjacent province indicated a normal sex ratio, and a few more males were subsequently identified in the initial province [39]. 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.

Morbidity and Mortality

The only available report of the effect of GHR deficiency on mortality comes from the Ecuadorian population [39]. 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.

The complete lifespan included in the Ecuadorian cohort provided an opportunity to look at adult mortality risk factors. Twenty-three adults with GHD had elevated cholesterol levels, normal HDL-cholesterol levels, elevated LDL-cholesterol levels, and normal triglyceride concentrations compared to relatives and nonrelated community controls. It was postulated that the effect of IGF-I deficiency due to GHRD was to decrease hepatic clearance of LDL-C, because 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 [40]. Preliminary data suggest an increased cardiovascular mortality in the Ecuadorian GHR-deficient adult subjects. In contrast, there has been no death recorded from cancer, despite a high cancer mortality in relatives [41].

Clinical Findings (Table 2.2)

Growth

Individuals with GHI due to GHR deficiency usually have normal intrauterine growth [42]. 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. Thus, this intrauterine IGF-I synthesis does not appear to be GH dependent.

SDS for length declines rapidly after birth in GHR deficiency (Fig. 2.3) indicating the GH dependency of extrauterine growth. Growth velocity with severe GH deficiency or GHR deficiency is approximately half normal (Fig. 2.4). Occasional periods of normal growth velocity may be related to improved nutrition.

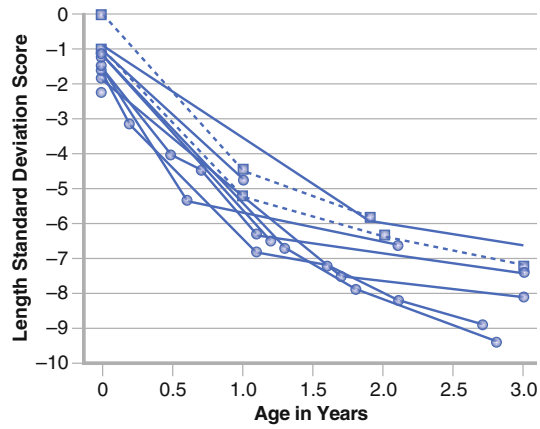

Fig. 2.3 Length standard deviation scores of nine girls from Ecuador (*open circles*, *solid lines*) and two brothers from southern Russia (*solid circles*, *dashed lines*) with known birth lengths, followed over the first 2–3 years of life [Adapted from *Trends Endocrinol Metab*, vol 5, #7, Rosenbloom AL, Guevara-Aguirre J, Rosenfeld RG, Pollock BH. Growth in growth hormone insensitivity, pp 296–303, Copyright 1994, with permission from Elsevier]

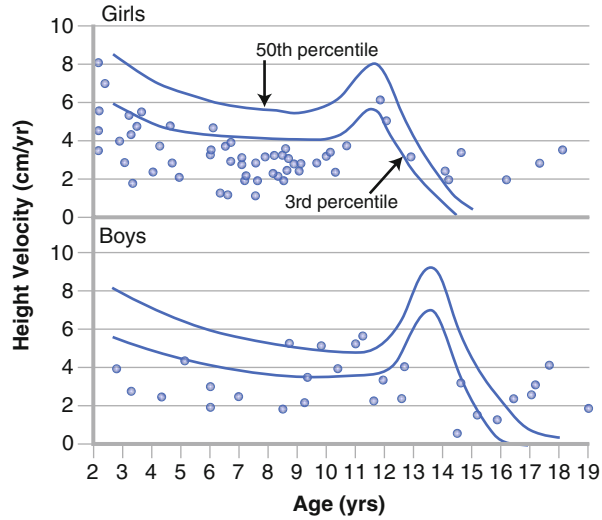

Fig. 2.4 Growth velocities of 30 Ecuadorian patients (10 males) with GH receptor deficiency; repeated measures were at least 6 months apart. Third and 50th percentiles are from Tanner JM, Davies PSW: Clinical longitudinal standards for height and height velocity for North American children. J Pediatr 1985; 107:317–329 [Adapted from *Trends Endocrinol Metab*, vol 5, #7, Rosenbloom AL, Guevara-Aguirre J, Rosenfeld RG, Pollock BH. Growth in growth hormone insensitivity, pp 296–303, Copyright 1994, with permission from Elsevier]

Despite normal sexual maturation, the pubertal growth spurt is minimal or absent in GHR deficiency, as documented in the most extensive available data, from Israel and Ecuador [42, 43]. The adolescent growth spurt is GH dependent, reflected in significantly elevated circulating levels of GH and IGF-I compared to preadolescence and adulthood [44].

Adult stature in GHR deficiency varies from −12 to −5.3 SDS in Ecuadorian patients and −9 to −3.8 SDS in others in the literature, using the US standards [42]. This is a height range of

95 cm to 124 cm for women and 106–141 cm for men in the Ecuadorian population. This wide variation in the effect of GHR deficiency on stature was not only seen within the population but also within affected families; such intrafamilial variability has also been described with severe GH deficiency due to GH gene deletion [10].

Some patients with GHR deficiency may have an appetite problem in addition to their IGF-I deficiency. Crosnier et al. [45] studied a child aged 3½ years with GHR deficiency 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 1,300 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 GH deficiency. The catch-up growth noted could not be explained by hyperinsulinism, which has provided the explanation for accelerated or normal growth in children with GH deficiency 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 patients with GHR deficiency without IGF-I replacement therapy, as noted in Fig. 2.4, might be explained by periods of improved nutrition alone.

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 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 [46]. 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 GHR deficiency as well as in severe GH deficiency [38, 47]. 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 [48].

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

Musculoskeletal and Body Composition

Hypomuscularity is apparent in radiographs of infants with GHR deficiency and is thought to be responsible for delayed walking, despite normal intelligence and timing of speech onset [48]. 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 GHR deficiency, 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 [49].

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 [38, 48]. That this feature is not peculiar to the Ecuadorian population or to IGF-I deficiency due to GHR deficiency has been confirmed by finding a Brazilian patient having a different GHR mutation with limited elbow extension [50] and observing this finding in all but the youngest patient in a family with eight individuals affected by multiple pituitary deficiencies [47]. The cause of this elbow contracture is unknown.

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 [48].

Reproduction

GHR deficiency 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 [39]. Females require C-section delivery.

Intellectual and Social Development

GHR Deficiency

Intellectual impairment was originally considered a feature of the Laron syndrome on the basis of uncontrolled observations [51]. Among 18 affected children and adolescents tested with the Wechsler Intelligence Scale for Children, only 3 had IQs within the average range [90–110]; of the remaining 15 subjects, 3 were in the low average range [80–89], 3 in the borderline range [70–79], 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 GHR deficiency, excluding 5 patients with mental disabilities who were in the original study [52]. 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 GHR deficiency 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 [53]. 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 [54].

The first controlled documentation of intellectual function in a population with GHR deficiency was in the Ecuadorian patients, 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 exceptional 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 [55].

STAT5b Gene Mutation

Consistent with other evidence that the IGF-I dependence 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.

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 GH and GHR deficiency, consistent with gene disruption studies in mice. The IGF-I-deleted mouse is neurologically impaired, while the GHR-deficient mouse is behaviorally normal [56, 57]. Thus, GH-dependent IGF-I production is not necessary for normal brain development and function.

IGF-I Receptor 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 1½ 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 [31–36].

IGFALS Mutation

Normal neurological development with *IGFALS* mutation would be expected, consistent with normal brain development in children with severe GH or GHR deficiency 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/autocrine) rather than endocrine.

Biochemical Features

Growth Hormone

Affected children with GHR deficiency 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 [58]. The GH levels show normal diurnal fluctuation [7]. Twenty-four-hour profiles demonstrate marked GH variability among adult patients with suppression by exogenous recombinant human IGF-I [7]. Thus, the normal sensitivity of the GH secretion is preserved, despite lifelong elevated GH levels and lack of feedback suppression from IGF-I.

Postpubertal patients with GHR deficiency may have normal or elevated basal levels of GH but invariably demonstrate hyperresponsiveness to stimulation, which is all the more impressive considering their obesity, which suppresses GH responses in normal individuals. In the Ecuadorian population, mean basal GH level in adults was significantly lower than that in children (11 ± 11 ng/ml versus 32 ± 22 ng/ml, $p < 0.0001$). This is thought to be related to the greater, though still markedly abnormal, IGF-I levels in the adults, resulting in some feedback inhibition of GH secretion [7, 48].

ALS mutations are associated with normal GH levels, despite very low circulating IGF-I concentrations. IGF-I receptor deficiency, which is an IGF-I-resistant state, rather than GH-resistant state, can also be associated with normal GH levels.

Growth Hormone-Binding Protein

Absence of GHBP in the circulation was initially considered a requirement for the diagnosis of GHR deficiency, 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 a number of patients. 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 patients 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. The presence or amount of GHBP measured did not relate to stature [48]. There were no age-dependent changes, indicating that the difference in IGF values between children and adults was not related to the GHBP levels and the GHBP levels did not correlate with stature or with serum IGF-I levels. 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 GHR deficiency.

Insulin-Like Growth Factors

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 GHR deficiency. IGF-II is not as severely suppressed, its reduction likely related to diminution of GHBP-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 388–792 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 suggests 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 [59]. The correlation of IGF-I levels with stature in adults with GHR deficiency indicates that, despite the markedly low levels, the influence of IGF-I on stature remains important in these subjects.

IGF-Binding Proteins

In IGF deficiency states that are the result of GH or GHR deficiency, IGFBP-3 is reduced, and as noted above, in children and adults with GHR deficiency, this reduction correlates with statural impairment [42]. In renal disease, elevated IGFBP-3 and IGFBP-1 and IGFBP-2 are thought to impair the delivery of normal levels of IGF-I [11].

Short-term and extended treatment of GHI with IGF-I has failed to result in increases in IGFBP-3 [7, 60–64], whereas treatment of GHD with recombinant human GH restores levels to normal. This indicates that IGFBP-3 production is under the direct influence of GH.

IGFBP-1 is elevated in GH and GHR deficiency; in GHR deficiency, 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 GHR deficiency and 175% of control in affected adults, a significant difference. The IGFBP-3 levels in adults with GHR deficiency are significantly greater than those in affected children [65].

Diagnostic Issues in GH Insensitivity

GHI due to deficiency is readily diagnosed in its typical and complete form because of severe growth failure, the somatic phenotype of severe GH deficiency, 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 GHR deficiency 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. [53] 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 GHR deficiency. Identification of heterozygous mutations, however, is not necessarily helpful because, as noted earlier, polymorphisms have been described which appear to have no phenotypic consequences.

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 GH or GHR deficiency, 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 cotransfection with wild-type GHR gene, to approximate the circumstance in vivo. Goddard et al. [66] 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 GHR deficiency in the homozygous state (R161C). Two other patients were heterozygous for this mutation. The other five patients included two who were heterozygous for the same novel mutation in exon 7 (R211H) and one each with novel mutations of exon 5 (C122X), exons 7 (E224D), and exon 10 (A478T). Expression in vitro of these four novel mutations involving the extracellular domain has shown functional effects, although cotransfection 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 GH deficiency. Five of the eight patients 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 recombinant GH treatment of idiopathic short stature (ISS).

The subjects studied by Goddard et al. [66] 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, that is, short children without a recognizable syndrome

or GH deficiency. Using a ligand-mediated immunofunction assay, Carlsson et al. [67] studied a large number of short children with known causes of growth failure such as GH deficiency 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 2 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. [68] 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 patients. 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 ~5,000 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 to have the novel diagnosis of "primary IGF-I deficiency," that is, normal GH responses with low IGF-I. The ISS group as a whole 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 [69]. 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 [70] 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. Even acutely, IGF-I levels decline substantially with fasting, which is considered a means of protecting against potential insulin-like effects on glycemia. 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 [71]. Nonetheless, promotional 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 [71]. These hypotheses were not data based 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 [72, 73].

The possibility of an effect of heterozygosity for a mutation which causes GHR deficiency 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 [74]. 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 [53].

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.

Treatment

Soon after the cloning of the human IGF-I cDNA, human IGF-I was synthesized by recombinant DNA techniques (rhIGF-I) and physiologic studies undertaken with intravenously administered rhIGF-I [75]. Subcutaneous preparations of rhIGF-I became available in 1990.

GHR Deficiency

During administration of rhIGF-I at a dose of 40 μg/kg sc every 12 h over 7 days to 6 Ecuadorian adults with GHR deficiency, hypoglycemia was avoided by having the subjects eat meals after the injections [7]. 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 2 children with GHR deficiency 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 [76].

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 IGF-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 13½ 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 recovery of denser and curly hair with filling of the frontotemporal baldness, the 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 [77]. Studies were done at doses of 40, 80, and 120 mcg/kg with pharmacokinetic profiles suggesting 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 IGF-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.

Table 2.3 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 Year 2	8.8 (1.9)[c] 7 (1.4)[c]	9.1 (2.2) 5.6 (2.1)	8.8 (1.1) 6.4 (1.1)	8.2 (0.8) 6.0 (1.3)[d]	8.0 (2.2) 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 Year 2	−5.8 (1.5)[c] −5.4 (1.8)[c]	−7.2 (1.8) −6.7 (1.4)	−7.5 (1.1) −7.0 (1.2)	−5.2 (1.7) −5.8 (1.2)[d]	−5.9 (1.8) −5.6 (1.8)[e]

[a]Includes 2 patients with GH-neutralizing antibodies
[b]Includes 8 patients with GH-neutralizing antibodies
[c]For 15 subjects treated for 4 years
[d]For 6 of the 9 subjects
[e]48 subjects

Seventeen prepubertal Ecuadorian patients were entered into a randomized double-blind, placebo-controlled trial of IGF-I at 120 mcg/kg sc bid for 6 months, following which all subjects received IGF-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 occur 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. 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 GHR-deficient patient with anorexia [45]. For those receiving IGF-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 IGF-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 IGF-I-treated groups. Initial hair loss occurred in 90% of subjects, similar to what is seen with treatment of hypothyroidism, reflecting more rapid turnover [62].

In the 2-year treatment study comparing 120 mg/kg bid dosage to 80 mg/kg bid treatment of GHR deficiency in Ecuadorian patients, no differences in growth velocity or changes in height SDS, height age, or bone age between the two dosage groups (Table 2.3). A group of six subjects receiving the higher dose followed for a third year continued to maintain second-year growth velocities. The annual changes in height 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 IGFBP3 levels to increase (Fig. 2.5). 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 [63, 77].

The Israeli report of 3 years' treatment of nine patients is the only one in which patients were given IGF-I as a single daily dosage (150–200 μg/kg) [78]. The European study group noted height

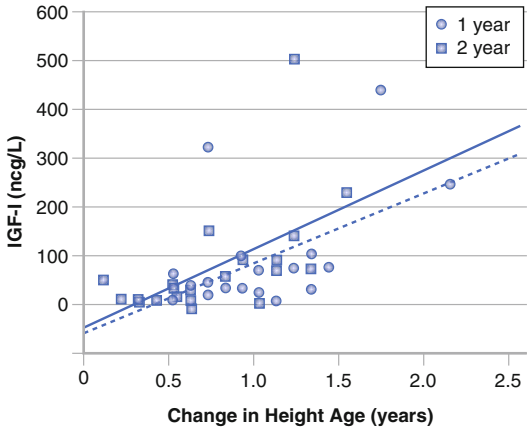

Fig. 2.5 Correlation of annual changes in height age and differences between baseline and trough levels of serum IGF-I with rhIGF-I treatment in 22 children with GHRD. The *dashed line* represents the 1-year correlation ($r=0.54$, $p=0.009$) and the *solid line* represents the 2-year correlation ($r=0.58$, $p=0.005$) [Adapted from The Journal of Endocrinology and Metabolism, Two year treatment of growth hormone receptor deficiency (GHRD) with recombinant insulin-like growth factor-I in 22 children: Comparison of two dosage levels and to GH treated GH deficiency. 82 (2), February 1997, pp 629–633. Copyright 1997, The Endocrine Society]

SDS improvement of 0.7 over 1 year and 1.2 over 2 years [64], 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 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 GHR deficiency, and fewer than 10% with GH inactivating antibodies (Table 2.3). The growth velocity increment in the first year was 4.3 cm in the European and mecasermin (Genentech/Tercica) study populations [79] 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 [79].

Comparison of the growth response of 22 rhIGF-I-treated GHR deficiency patients and 11 GH-treated GH-deficient patients in the same setting demonstrated mean growth velocity increment in those with GHR deficiency to be 63% of that achieved with GH treatment of GH deficiency in the first year and less than 50% in the second and third years (Table 2.3). The inadequate growth response compared to GH treatment of GH deficiency persisted over this extended 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 2.3. 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 [8]. Near-total deletion of the GHR in liver only in the mouse model had no effect on total body or bone linear growth [80].

Limitations of Endocrine IGF-I Replacement

The observation that growth failure due to GH insensitivity cannot be adequately corrected with endocrine IGF-I replacement is not explained by concomitant IGFBP3 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 GHR deficiency and increases further with rhIGF-I therapy [7].

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 GH deficiency? The dual-effector hypothesis remains the best explanation for inadequate growth response [8]. 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, IGF-I replacement therapy of GI may need to continue longer than GH treatment of GH deficiency to achieve more normal height. This goal will likely require suppressing adolescence in most children with GHI, using GnRH analogs [77].

In addition to statural attainment, goals of replacement therapy with IGF-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 lipoatrophic, increasing or sustaining the high-percentage body fat. Normalization of craniofacial features has also been apparent [81]. 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 GHR deficiency is to be expected with IGF-I therapy, but the reason for this increased risk is unknown. Leukocytes share in the general upregulation of IGF-I receptors in GHR deficiency and appear to function normally in this condition [82]. In a study of one affected infant (who died at 7 months with bronchitis) and five adults with GHR deficiency from Ecuador, Diamond et al. [83] demonstrated a variety of immune disturbances in the infant and three of the adults. The pathologic significance of these findings remains uncertain [84].

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, or 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 as a result of high susceptibility of IGF-I to post sampling proteolysis [71]. 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 [85]. 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 [86]. Wide fluctuations in IGF-I concentrations can be seen in normal prepubertal children, including levels <2 SDS [87]. Finally, IGF-I generation tests have poor reproducibility [88].

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 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 Lawson Wilkins 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 [89]. The manufacturer of IGF-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" [71]. Considering that fewer than 200 children with GH insensitivity due to GHRD, transduction defects, or GH-inhibiting antibodies had been identified worldwide in the previous 20 years, it is apparent that there was 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, Tercica 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 that was 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 μg/kg daily and the same dose of GH with once-daily injections of either 50, 100, or 150 μg/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 ≤11 years for boys and ≤9 years for girls. There are no growth velocity criteria. This study was likely to include a substantial number of children, especially males, with CDGM, the most common explanation for short stature in the growth clinic, and other normal short children. It is noteworthy that there was not an IGF-I monotherapy arm and that the IGF-I was 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 IGF-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 [7]. 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 subjects 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 [90]. Both of these are approximately 50% greater responses than in normals and indicative of better than normal GH sensitivity.

Safety Concerns with Recombinant IGF-I

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

hypoglycemia of GH deficiency which is corrected by GH replacement therapy, IGF-I treatment increases the risk of hypoglycemia in children with GHR deficiency. Hypoglycemia has been the most common early adverse event, reported in 49% of subjects in the largest series, including 5% with seizures [79]. 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 [62]. 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 [79]. 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 [91]. 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 IGF-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 [79].

Anti-IGF-I antibodies have developed in approximately half of the IGF-I-treated patients during the first year of treatment, but these have had no effect on response [78, 79]. Urticaria has been noted in subjects participating in the trial of IGF-I with GH. Transient elevation of liver enzymes has also been noted [78].

Coarsening of facial features with disproportionate growth of the jaw reminiscent of acromegaly has been common, particularly among those of pubertal age [77]. 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 [75]. 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 [92]. 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 [93]. 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 [94]. There have been two instances of anaphylaxis from rhIGF-I treatment [73].

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 [95].

GH Therapy

Five individuals with IGF-I receptor mutations have been treated with recombinant GH with four

having substantial improvements in growth velocity but without the impressive catch-up growth seen with GH treatment of GH deficiency; the exception was an individual who had no response over 6 months of trial. The patient with partial primary deficiency of IGF-I responded to supraphysiologic doses of recombinant GH with catch-up growth [22].

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.

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Skeletal Dysplasias

3

Robert C. Olney and Michael B. Bober

Abstract

The 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 but also for what these disorders teach us about the mechanisms and regulation of growth.

Keywords

Skeletal dysplasia • Osteochondrodysplasia • Dwarfism • Upper-to-lower segment ratio • Arm span • Achondroplasia • Hypochondroplasia • Léri-Weill osteodyschondrosteosis • Multiple epiphyseal dysplasia • Osteogenesis imperfecta • Acromesomelic dysplasia, type Maroteaux • Jansen-type metaphyseal chondrodysplasia • Blomstrand chondrodysplasia • Recombinant human growth hormone • Pamidronate • Bisphosphonates • C-type natriuretic peptide • Natriuretic peptide receptor B • Parathyroid hormone-related protein • Parathyroid hormone receptor • Fibroblast growth factor receptor-3 • Madelung deformity • Short-stature homeobox-containing gene • SHOX

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Introduction

The 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 but also for what these disorders teach us about the mechanisms and regulation of growth.

As of 2006, there were 372 described skeletal dysplasias, 215 of which were associated with one of 140 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 5,000 births [1, 3]. 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 requires 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 a certain elegance ("achondroplasia" just sounds better than "absence of cartilage growth"), it can make learning about the skeletal dysplasias somewhat intimidating. Table 3.1 defines many of the terms commonly used in the field, and Fig 3.1 shows their application to the anatomy of the long bone.

Table 3.1 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 the forearm is directed away from the midline distal to the elbow, 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
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

(continued)

Table 3.1 (continued)

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 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

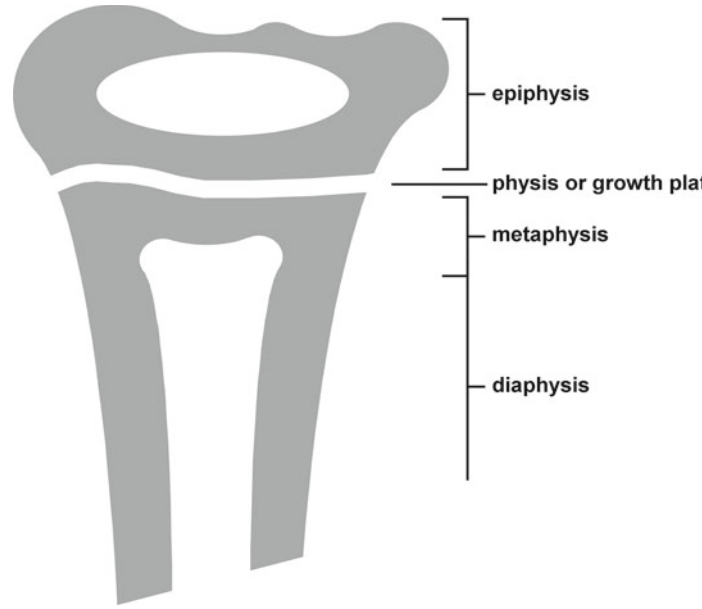

Fig. 3.1 Anatomy and nomenclature of the long bones

When to Suspect a Skeletal Dysplasia

When a child presents for evaluation of short 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 genetics 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. 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.

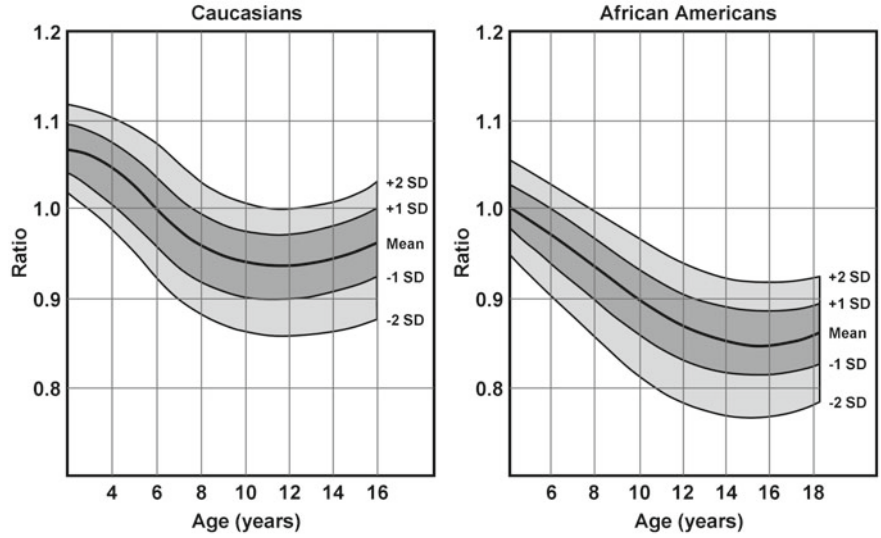

Fig. 3.2 Upper-to-lower segment ratio. The upper-to-lower segment ratio in childhood is shown for Caucasians (*left panel*, $n = 1{,}015$) and African Americans (*right panel*, $n = 1{,}015$). African Americans tend to have relatively longer limbs and a lower upper-to-lower segment ratio. Data is from McKusick [75]. Figure is reproduced from Hall et al. [4]. By permission of Oxford University Press

The skeletal dysplasias are of genetic origin and the majority are 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 newly available genetic tools, it is not uncommon 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 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 indicator 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. 3.2). A patient with a ratio that is greater than the 95th percentile has disproportionately short legs and suggests a short-limbed skeletal dysplasia. A patient with short stature and an upper-to-lower segment ratio less than the 5th percentile suggests the presence of a disproportionately short

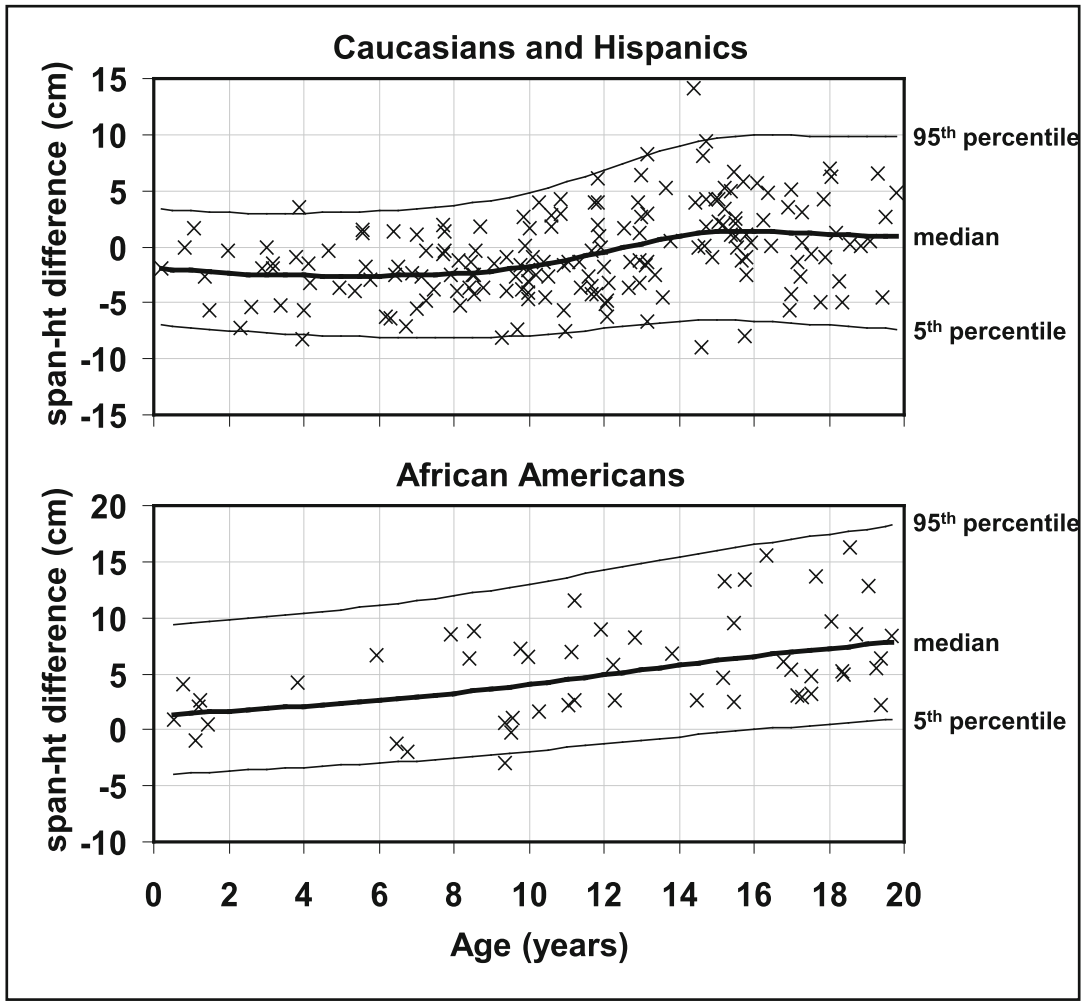

Fig. 3.3 Arm span/height difference. Arm span/height difference for healthy children in Florida was determined. Arm span was measured as described in the text. Height was determined by stadiometer. Curves were fitted using the LMS method [76], and the median, 5th and 95th percentile curves shown. Data 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*, $n = 53$)

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 as 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 [4].

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 outward. Marks are made at the tip of the middle finger of each hand, and the distance between them is 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. 3.3). An arm span/height difference that is less than the 5th percentile

Table 3.2 Reference range for arm span/height difference

	Arm span/height difference			
	Caucasion		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

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 [4]. However, the changes with pubertal status and racial differences make this simplistic guideline inappropriate. More appropriate guidelines are presented in Table 3.2.

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.

A thorough physical examination focused on the skeletal system is important. Observation of the child while standing will give 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 Hall et al. [4] 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-Weill 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/or campylodactyly are also important potential findings.

Initial 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

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, there are organized resources including the International Skeletal Dysplasia Registry at Cedars-Sinai Medical Center, Los Angeles, (www.csmc.edu/skeletaldysplasia) [2] and the European Skeletal Dysplasia Network (www.esdn.org) [5] which can review patient images and provide diagnostic possibilities. Local 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.

Common Syndromes

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 [6, 7]. The average adult height for men is 131 cm, with a range of 118–144 cm (3′10″ to 4′9″, SD score −8.3 to −4.6) and for women, 123 cm with a range of 113–137 cm (3′8″ to 4′6″, SD score −7.7 to −4.0) [8]. 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 [9]. 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, the torso is relatively long and narrow, and the head is large with frontal bossing. As a result of the defect in endochondral bone formation, the skull base and midface are affected resulting in midface 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 increased intervertebral space, dorsal scalloping, and lumbar interpediculate distance narrowing.

Achondroplasia is caused by a mutation in the fibroblast growth factor receptor-3 gene (*FGFR3*) [10]. Mutations which change the amino acid glycine to arginine at position 380 of the FGFR3 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 [11, 12]. 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 [13, 14]. A recent hypothesis is that sperm containing a *FGFR3* mutation has 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 [13]. 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 [15–17]. Studies generally show an improvement in height velocity during the first year of treatment and an average net gain in height SD score of 1.0–1.6 (8–14 cm). The body disproportion is reported to be worsened [16] or unchanged [15, 17] 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 [18]. In achondroplastic mice, overexpression of CNP [19], as well as exogenous administration of CNP [20], rescues the skeletal abnormalities, leading to the proposal of the use of analogs of CNP as a possible future treatment of achondroplasia in humans [12, 20].

Hypochondroplasia

Hypochondroplasia (MIM 146000) is a common skeletal dysplasia with incidence estimates ranging from 1 in 15,000 to 1 in 40,000 births [21]. The adult range height is 132–165 cm (4′4″ to 5′5″, SD score −6.3 to −1.7) in men and 127–150 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 [22]. 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 midface hypoplasia) are not necessarily 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 [23].

Like achondroplasia, hypochondroplasia is caused by mutations in *FGFR3* [21]. 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 (N540K) is the most common gain-of-function mutation to cause hypochondroplasia, but several others have been described. When there is an established clinical and radiographic diagnosis of hypochondroplasia, *FGFR3* mutations can be identified in about 70% of individuals. Thus, the possibility of genetic heterogeneity has been raised.

As with achondroplasia, rhGH seems to have some effect in hypochondroplasia, although studies are small and short term [24–26]. The studies show a height SD score improvement of as much

as 1.6 after 3 years of treatment [24]. The largest and longest-term study [27] showed that, while improving growth velocity and height SD score, rhGH also worsened the skeletal disproportion. Surgical limb-lengthening procedures (distraction osteogenesis) have been reported in adults with hypochondroplasia [28, 29] to improve height and skeletal disproportion but are by no means routine practice at this time.

Multiple Epiphyseal Dysplasia

Multiple epiphyseal dysplasia (MED) is a relatively common disorder with a prevalence of at least 1 in 20,000 [30]. 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. Generally 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.

MED 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). 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 rhGH in MED. Its effectiveness on height growth and body disproportion is unknown.

Léri-Weill Dyschondrosteosis

Léri-Weill dyschondrosteosis (LWD, MIM 127300) is a moderate 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 [31], a range that overlaps the healthy population. The short stature is of early childhood onset [32], with little change in height SD score during puberty [31]. The short stature is disproportionate and can be identified through a high upper-to-segment ratio (SD scores 2.9 ± 3.4) and low arm span/height difference (−5.1 ± 3.0 cm) [32]. As with height, there is an 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 [33]. This results in bowing of the radius and tilting of the articular surface of the radius toward the ulna and palm (Fig. 3.4). The resulting anterior displacement of the wrist gives the characteristic “bayonet” appearance of the wrist. Subluxation of the ulnar head makes it a prominent feature on the dorsal wrist.

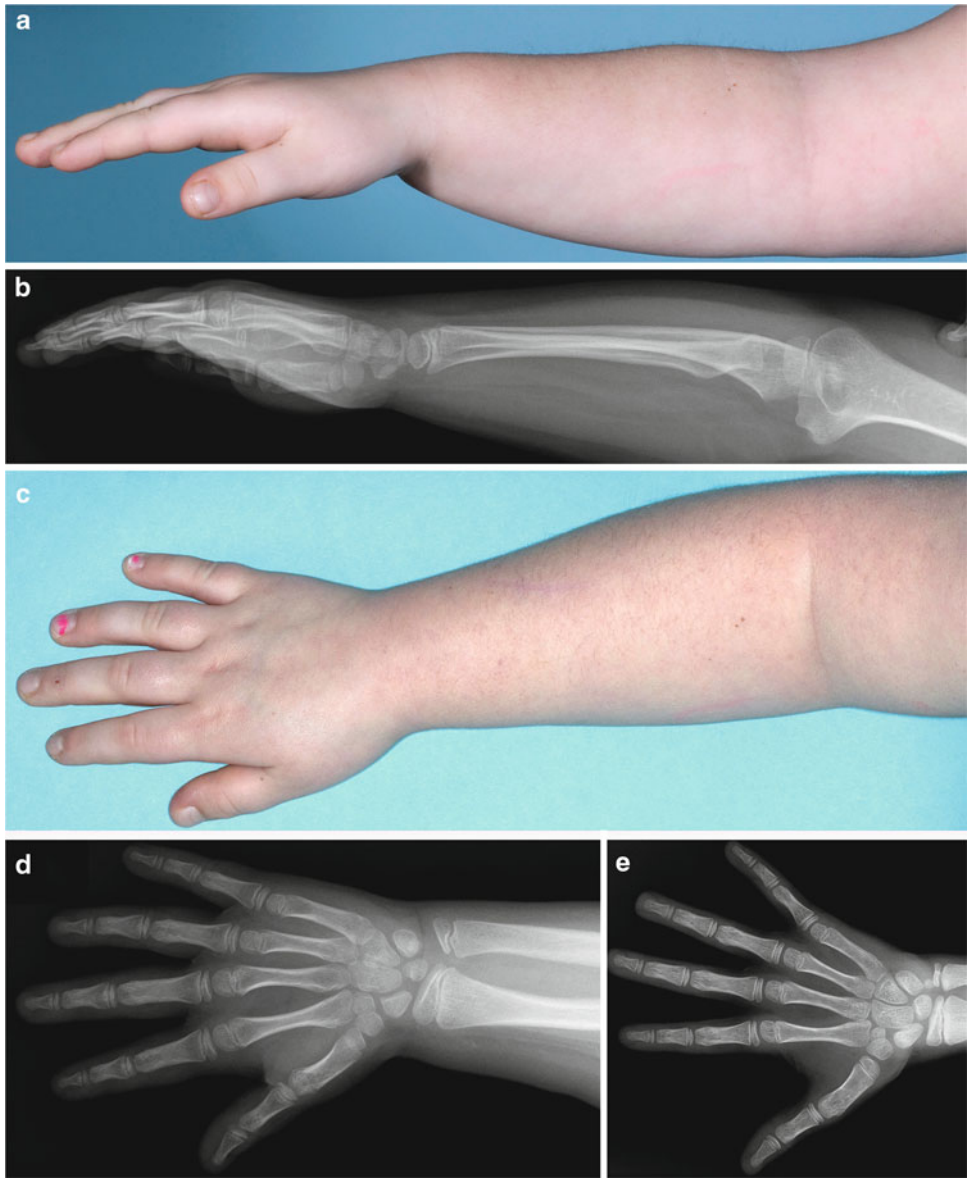

Fig. 3.4 Madelung deformity in a prepubertal child. An 8-year-old girl with Léri-Weill dyschondrosteosis (LWD) due to a partial deletion of Xp demonstrates Madelung deformity. The deformity is the result of the presence of physeal bar in the ulnar aspect of the growth plate of the distal radius. In prepubertal children, the findings can be subtle [33]. *Panel A* shows 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"). 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 fourth 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 [31, 77]

The deformity is detectable in young children but can be subtle [32]. 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 [32], and up to 88% in adults [31]. Women are more likely to be affected and are generally more severely affected. Other features of LWD include shortening of the fourth 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* [34, 35]. Microdeletions of *SHOX* enhancer regions have also recently been implicated [36]. The *SHOX* protein is a transcription factor that is expressed in epiphyseal growth plates [37], 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 [38]. By virtue of a missing X chromosome, girls with Turner syndrome have only a single *SHOX* gene. Although 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%) [32].

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 mutation, and 8 had deletions proximal to *SHOX* [36]. 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 routine care, and one study demonstrated a near final adult-height improvement over predicted height SD score of 1.3 ± 0.6 [39]. Trials in LWD are more limited but suggest an equally good response [40]. Both Turner syndrome and *SHOX* haploinsufficiency are FDA-approved indications for rhGH treatment [41].

Osteogenesis Imperfecta

Osteogenesis imperfecta (OI) is a heterogeneous disorder characterized by bone fragility and low bone mass predisposing to fracture. OI is estimated to occur in 1 in 10,000–15,000 live births. Extra-skeletal manifestations associated with OI can include blue sclera, dentinogenesis imperfecta (DnI), 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. [42] and distinguishes four clinical 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 five types (Table 3.3) [43]. Typically, patients with the moderate and severe forms of OI are readily

Table 3.3 Characteristics of osteogenesis imperfecta

Type (MIM #)	Inheritance	Prevalence (per 100,000)	Severity	Bone deformity	Fractures	Radiologic appearance	DnI	Short stature	Sclerae	Hearing loss	Gene(s) and mutations
I (166200)	AD	3–4	Mild	Uncommon	Few to 100s	Thin cortices, osteopenia	Rarely	None or mild	Blue	Present in about 50%	*COL1A1*, *COL1A2*; Large scale deletions, nonsense mutations, frame shift mutations
IIA (166210) IIB (610854)	AD AR	1–2	Perinatal lethal	Severe	Multiple	Severely deformed; broad, crumpled, bent femurs; small beaded ribs; minimal calvarial mineralization, platyspondyly	Yes	Severe	Dark blue	No	IIA: *COL1A1*, *COL1A2*; Missense mutations, splice site mutations IIB: *CRTAP*
III (259420)	AD, rarely AR	1–2	Severe	Moderate to severe	Multiple	Flared metaphyses ("popcorn"-like appearance in childhood), bowing, thin cortices, thin ribs, thin gracile bones, platyspondyly, severe osteoporosis	Yes	Moderate to severe	Blue	Frequent	*COL1A1*, *COL1A2*; Missense mutations, splice site mutations
IV (166220)	AD	~3–4	Moderate to mild	Mild to moderate	Multiple	Thin cortices, protrusio acetabuli	Sometimes	Variable	Normal to grey	Some	*COL1A1*, *COL1A2*; Missense mutations, splice site mutations
V (610967)	AD	0.4–0.6	Moderate	Moderate	Multiple	Hypertrophic fracture callus, usually of the femurs; mineralization of the interosseus membrane in the forearm	No	Variable	Normal	No	IFITM5
VI (610968)	Uncertain	0.4–0.6	Moderate	Moderate	Multiple	Similar to type IV	No	Mild	Normal	No	Unknown
VII (610682)	AR	Limited to a native Canadian population	Moderate	Rhizomelic shortening	Multiple	Similar to type IV, rhizomelic shortening	No	Mild	Normal	No	*CRTAP*
VIII (610915)	AR	Very rare	Severe	Severe, short-limbed	Multiple	Similar to type II/III, extreme skeletal undermineralization, bulbous metaphyses	No	Moderate to severe	Normal	No	*LEPRE1*
IX (259440)	AR	Very rare	Severe	Severe, short-limbed	Multiple	Similar to type II/III	Yes	Moderate to severe	Blue	No	*PPIB*

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. They commonly decrease 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, ligamentous laxity, and easy bruising. Dentinogenesis imperfecta may be present, but is not common in this group.

Approximately 90% of infants and children with clinical features of OI will have an identifiable mutation in either the type I collagen alpha 1 (*COL1A1*) or alpha 2 (*COL1A2*) genes. These mutations are inherited in an autosomal dominant manner. A small number (a subset of OI type II and types VII–IX) will have mutations in genes which are involved with collagen protein assembly. In general, these mutations are inherited in an autosomal recessive manner. 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 types II, III, and IV often have missense mutations that alter glycine residues in either *COL1A1* or *COL1A2*.

The front line of treatment of OI lies with the orthopedist who treats the acute fractures, as well as dentists for the treatment of the complications of dentinogenesis imperfecta, and the otolaryngologist for the treatment of hearing loss. The last decade has seen an increasing use of bisphosphonates in the treatment of OI. Bisphosphonates are analogs of pyrophosphate that bind to bone mineral and inhibit osteoclast function, reducing bone resorption. Glorieaux et al. [44] 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 [45] 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 [46] and delayed fracture healing [47]. At our center, we use the protocol reported by Glorieaux et al. [44], namely, pamidronate, 9 mg/kg/year, divided at dosing intervals based on age. The primary adverse effect is a febrile acute phase reaction that is common after the first dose. We pretreat with acetaminophen before each dose to decrease this effect. Some centers avoid infusions during active bone healing to avoid interference with callous remodeling. Other centers only delay infusions after osteotomies, but not after fractures. Transient hypocalcemia after an infusion is seen on occasion but is rarely of clinical significance. 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 [48]. Timing of the infusion cycles, dosing, and length of treatment vary considerably between centers with no clear consensus at this time [49, 50].

Due to the IV infusions, pamidronate treatment is costly and inconvenient. Recent studies of risedronate (an oral bisphosphonate), including a placebo-controlled randomized study in mild OI [51] and a randomized dose-ranging study of moderate to severe OI [52], have shown similar improvement in fracture frequency and bone mineral density with no reported adverse effects. However, a recent large, randomized, placebo-controlled study of alendronate (another oral bisphosphonate) showed improvements in bone mineral density, but no change in fracture rate [53]. There are larger studies in progress using zoledronic acid and risedronate acid.

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-I release in response to rhGH [54]. In short-term trials, rhGH improves both

linear growth and bone mineral density [55, 56] and a long-term randomized controlled study of rhGH is ongoing.

For the severe forms of OI, bone marrow transplant [57] and bone marrow-derived mesenchymal cell transplant [58] have shown promising early results.

Uncommon Syndromes: What They Teach Us About Growth

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 1–2 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 below normal or suppressed [59]. In 1995, it was reported that Jansen-type metaphyseal chondrodysplasia was caused by *activating* mutations of the parathyroid hormone receptor (*PTH1R*) [60].

In 1985, Blomstrand et al. [61] 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 [62]. 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 [63]. 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 [64].

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 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 [65]. 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)) [66] and bone morphogenetic proteins (BMP) [67] 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.

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. 3.5) [68]. Infants with AMDM usually appear normal at birth, although short birth length and subtle disproportion has been observed [69]. 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 [70]. It is a rare syndrome, with a prevalence of roughly 1:2,000,000 [71]. 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*) [70]. 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 [72]. 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 [18]. As such, CNP is a counter-regulatory mechanism to the Indian hedgehog/PTHrP pathway. This effect has lead to the exploration of CNP as a specific treatment for achondroplasia [12].

It was observed that the parents and heterozygous carrier siblings of patients with AMDM were shorter than the general population [70, 71, 73]. 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 [71]. In a study of 191 children with idiopathic short stature, we have identified a prevalence of 1.0% of heterozygous inactivating mutations in *NPR2* (publication pending), making this a not uncommon cause of idiopathic short stature.

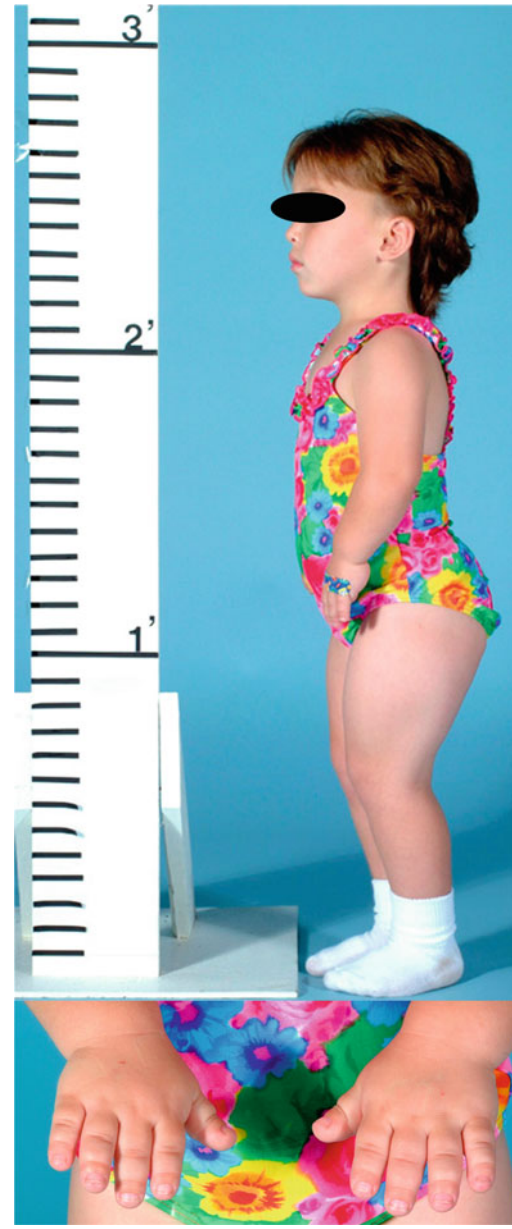

Fig. 3.5 A patient with acromesomelic dysplasia, type Maroteaux (AMDM). An 8-year-old girl with AMDM demonstrates severe short stature (height SD score of −8.5) with disproportionately short forearms/lower legs and severe shortening of the bones in the hands and feet. Her sitting height-to-standing height 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

Summary

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." [74] 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.

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Idiopathic Short Stature

4

Yung-Ping Chin and Pinchas Cohen

Abstract

ISS describes a category of children with severe short stature and poor growth velocity in the absence of an identifiable cause. After a thorough evaluation has been conducted, a number of therapeutic options may be considered. The approval of ISS as an indication for GH treatment poses further challenges in optimizing management of these patients.

Keywords

Idiopathic short stature • ISS • Short stature • Height • Growth • Growth disorders • Growth hormone treatment • IGF-1 • Insulin-like growth factor 1 • Aromatase inhibitors

Definition and Epidemiology

Idiopathic short stature (ISS) is defined by a height more than 2 SD score (SDS) below the corresponding mean height for a given age, sex, and population, without evidence of an underlying disorder. The majority of short children at or below −2 SDS fall into the category of ISS. Most studies have found the percentage of an organic etiology for short stature to be approximately 5%, but future genetics tests may increase this number [1]. The broad definition of ISS also includes short children previously designated with familial short stature (FSS) or constitutional delay of growth and puberty (CDGP), especially if their heights are below −2.25 SDS (1.2%). Children with ISS have normal birth weight and are growth hormone sufficient. ISS is not based on any positive findings in the diagnostic workup; rather, it is a diagnosis of exclusion. Thus, children born small for gestational age (SGA) and those with dysmorphic syndromes or clearly identified causes of short stature, such as endocrine deficiencies or chronic organic disease, should not be given this diagnosis.

Subcategorization

Three parameters are typically used in the subcategorization of ISS. The first criteria primarily distinguish between children with a family history

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of short stature and those without. The natural history of familial short stature (FSS) and nonfamilial short stature (non-FSS) is quite different with respect to height SDS at onset, predicted adult height, and target height. In FSS, the child is short compared to the population, but height is still within the expected range for parental target height. Future studies are likely to identify genetic causes for this. In non-FSS, the child is short for the population, and adult height achieved is below parental target height.

A second proposed subclassification for ISS divides ISS children into those with normal puberty vs. constitutional delay of growth and puberty (CDGP). Pubertal onset, not bone age, should be the main criterion for delay due to inherent problems with bone age determination [1]. In delayed maturation, predicted adult height is expected to be greater than current height SDS, but it is often substantially lower based on methods such as the Bayley-Pinneau [2, 3]. Height velocity is not as useful for subclassification due to variability of height velocity over time.

Another approach for possible subclassification involves the biochemical determination of IGF-1 levels and can help to differentiate between possible disorders of GH secretion, GH sensitivity, genetic factors affecting the growth plate, IGF resistance, and other causes.

Disorders of Exclusion

Certain disorders must be excluded in the diagnostic workup of ISS. Evaluation for dysmorphic syndromes is essential, given the increasing number of genetic tests available. Turner syndrome should be ruled out in all short girls. Testing for SHOX deletion or mutation should be done when clinical features suggest this, as SHOX defect is now an approved indication for GH therapy in the USA and Europe. Skeletal dysplasia should be considered when short stature is associated with abnormal body proportions.

Short stature in a child born small for gestational age (SGA), defined by weight and/or length <−2 SDS for gestational age, can be otherwise difficult to distinguish from ISS. Diagnostic evaluation should also include screening for systemic disorders such as celiac disease, hypothyroidism, anemia, and chronic inflammatory diseases. Regarding endocrine disorders, the investigation is primarily aimed at detecting hypothyroidism, Cushing's syndrome, growth hormone deficiency (GHD), and growth hormone resistance.

GHD is a difficult diagnosis to make in a definitive manner. Extreme forms of GHD usually present with severe growth retardation and are diagnosed early on. However, isolated partial GHD is much more difficult to separate from ISS. If GH therapy is initiated, GH resistance can be suspected if decreased responsiveness to GH is seen, and this could be due to a genetic defect in GH signaling.

Diagnostic Evaluation

There is no complete consensus on criteria that should be used when deciding to refer a child to a specialist. Referral should be made when the child's height is <−2 SDS compared with the population and when height is <−2 SDS compared with target height SDS based on parental height. Growth velocity <−2 SDS over a 1-year period also warrants further evaluation (see Fig. 4.1).

For children with a height of <−2 SDS, screening tests should be performed for disorders such as GHD, Turner syndrome, celiac disease, Crohn's disease, and renal acidosis, even with an unremarkable history and physical exam (see Table 4.1). A complete blood count, erythrocyte sedimentation rate, and C-reactive protein will help exclude anemia and infectious or inflammatory diseases. Electrolytes, bicarbonate, creatinine, calcium, phosphate, alkaline phosphatase, and albumin should all be evaluated. Screening tests for celiac disease include tissue transglutaminase antibodies and anti-endomysial antibodies, though intestinal biopsy remains the gold standard for diagnosis. TSH and free T4 should be tested to exclude primary, as well as central, hypothyroidism. IGF-1 level must be sent to evaluate for GHD. A karyotype should be performed in all girls with unexplained short stature

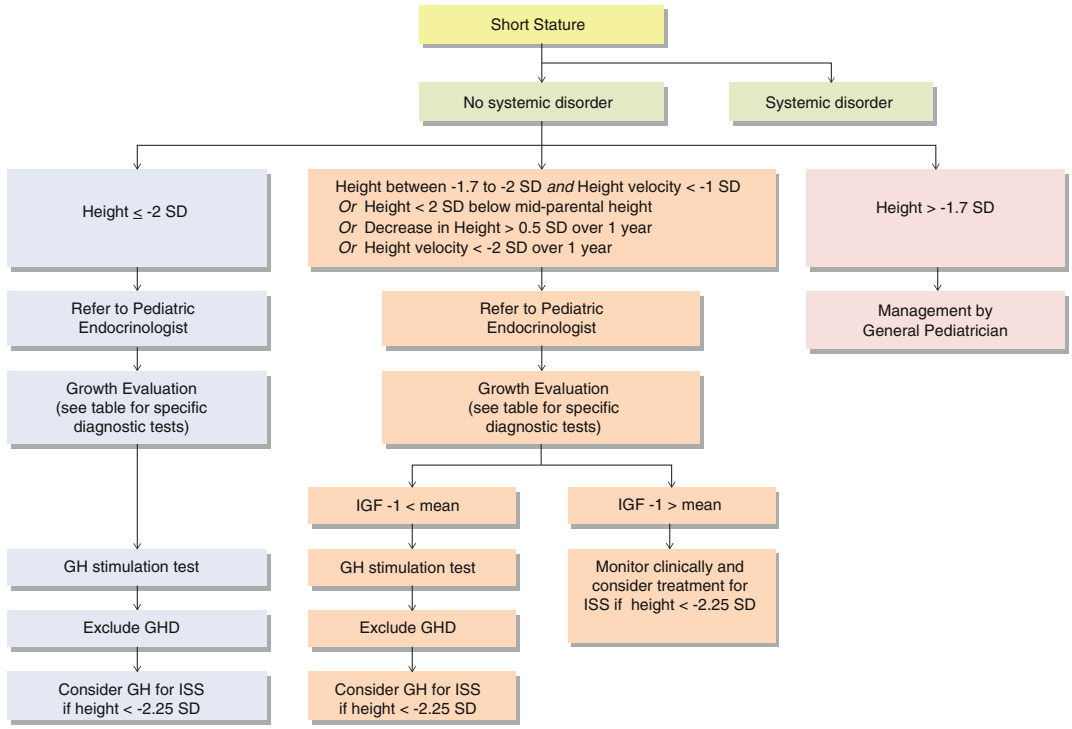

Fig. 4.1 Algorithm for evaluation of short stature. ISS = idiopathic short stature. GHD = growth hormone deficiency. SD = standard deviation

and in short boys with genital abnormalities. Bone age x-ray will give an indication for remaining growth potential. Specifically, bone age is usually delayed in secondary growth disorders and in many children with ISS. However, less bone age delay is seen in primary growth disorders. An absence of bone age delay is unlikely with GHD. If short metacarpals are noted, pseudohypoparathyroidism ought to be considered. A skeletal survey should be done when there is concern for skeletal dysplasia based on physical abnormalities.

Investigation of the GH-IGF axis should be performed in any patient with compatible history and physical exam, low height velocity, or IGF-1 levels below the mean for age (see Fig. 4.1). Based on a general consensus, GH testing is not required in a short patient with a height velocity at or above the mean, no bone age delay, and plasma IGF-1 level above the mean [4]. There is currently no absolute "gold standard" for diagnosing GHD. Low serum IGF-1 and/or IGFBP-3 are supportive but not diagnostic of GHD. The GH provocation test is not always a reliable tool for diagnosing GHD due to variation of testing methods, assays, and cutoffs, but it is still a required step in the evaluation of short children. In fact, very low values (5 ng/ml) are almost always associated with unequivocal severe GHD, although the category sometimes referred to as partial GHD (with stimulated GH levels of 5–10 ng/ml) clearly has some overlap with ISS. Similarly, stimulated GH secretion may not be decreased in a small subgroup of children who have atypical GHD (sometimes referred to as neurosecretory dysfunction) who may carry the diagnosis of ISS unjustifiably. In general, ISS patients will have normal 24-h GH production rates, but this test is not recommended due to a lack of adequate normative data.

Table 4.1 Diagnostic approach to short stature

• History and physical
• Auxological assessment of height and height velocity
• Tests for systemic and genetic disease
– Complete blood count (CBC)
– Erythrocyte sedimentation rate (ESR), C-reactive protein (CRP)
– Electrolytes, bicarbonate, creatinine, calcium, phosphate
– Alkaline phosphatase
– Albumin
– Celiac screen: tissue transglutaminase antibodies, anti-endomysial antibodies
– Karyotype
• Tests for endocrine disease
– Bone age
– TSH, free T4
• Static tests for the GH-IGF axis
– IGF-1, IGFBP-3
• Dynamic tests for GH secretion
– GH stimulation test
• Hypothalamic-pituitary radiological assessment
– MRI
• Dynamic tests for GH action
– IGF generation test (optional)
• Tests for specific genetic or molecular abnormalities

The choice of GH stimulation tests used varies considerably. All tests can have some adverse effects, though few problems are encountered in experienced centers. GHRH is not useful in children due to possible hypothalamic defects. Clonidine can cause hypotension and sleepiness, while arginine may result in nausea, vomiting, headache, and rarely hypoglycemia. Glucagon and propranolol can be associated with nausea, vomiting, hypoglycemia, and local reactions to injection. L-dopa in combination with propranolol can cause hypoglycemia. Furthermore, because false-positive tests occur due to lack of a "gold standard," testing with two different stimuli is required. Clearly, the reproducibility of GH tests is less than perfect. Use of the priming procedure, highly controversial and not universally accepted, involves the administration of high-dose sex steroid for a brief period in the days before a GH stimulation test in order to optimize GH secretion and decrease the chance of a false-positive result [1]. This mimics puberty, a time when GH secretion is greatest in a normal child.

Peak GH concentration <10 ng/ml supports the diagnosis of pediatric GHD, although new reference standards are currently being introduced. Measurement of spontaneous GH secretion is not indicated for routine assessment of GH status. IGF-1 and IGFBP-3 are dependent on GH secretion and action. IGF-1 is felt to be the most useful marker, but there is currently some variability among different IGF-1 immunoassays. IGFBP-3 levels are most helpful in the diagnosis of GHD in children younger than 3 years of age. MRI to evaluate hypothalamic-pituitary anatomy should be performed in children with confirmed GHD or if intracranial lesion is suspected; it is not necessary for patients with ISS.

Various mutations and polymorphisms in the GH receptor or downstream signaling cascade may be associated with a degree of functional GH insensitivity, but these are uncommon. A very small (currently less than 2–3%) but increasing number of patients with ISS have abnormalities of the GH receptor and in post-receptor GH signaling. There may be a spectrum of patients with GH insensitivity ranging from partial to complete. There may also be a subset of patients with IGF insensitivity. In a two-dimensional diagram illustrating abnormalities in either GH secretion or sensitivity, the more defined groups of GHD and GH insensitivity have clear defects in GH secretion or action, while the group classified as ISS shares some overlap with other categories and thus could fall almost anywhere within this diagram (*see Fig. 4.2*) [5]. GH insensitivity should be considered when IGF-1 is low and GH peak during stimulation test is high and when growth response to GH treatment is poor, despite an adequate treatment dose and good compliance with therapy. In patients whose height is <−3 SDS, GH-binding protein (GHBP) in serum, IGFBP-3, and acid-labile subunit (ALS) should be measured [1]. Low or undetectable GHBP and very low levels of IGFBP-3 and ALS are seen with homozygous mutations of GHR (classical Laron syndrome) [6]. Protocols for the IGF-1 generation test (IGF-GT) are available but not validated clinically and not recommended for routine practice. DNA screening for a GHR defect should be done when height is <−3 SDS, and for very low IGF-1 and IGFBP-3 levels (at

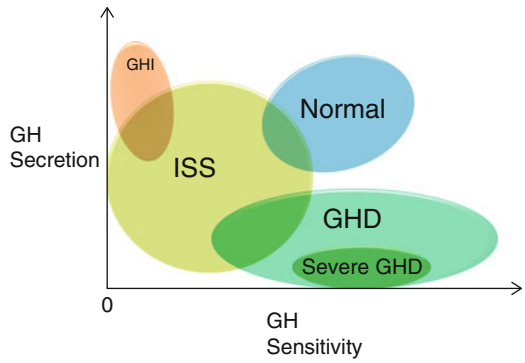

Fig. 4.2 Two-dimensional diagram of GH secretion and action disorders. ISS = idiopathic short stature, GHI = growth hormone insensitivity, GHD = growth hormone deficiency

least one <−3 SDS) and normal/high GH levels during stimulation test.

Genetic testing should be pursued when there is suspicion for a genetic diagnosis associated with short stature (i.e., Noonan syndrome or GH insensitivity syndrome). Mutations in the short stature homeobox (SHOX) gene are recognized as a possible etiology of growth failure in ISS patients; in some series, they are as frequent as 3–10% of ISS cases. SHOX gene analysis should be considered for a patient with findings compatible with SHOX haploinsufficiency.

Management

ISS should be managed by pediatric endocrinologists, and decisions should be evidence based. The child and parents should be counseled on GH treatment as a potential therapeutic option. Realistic expectations regarding height gain, clinical outcome, efficacy, and possible adverse effects must be discussed fully. Attainment of normal adult height is the primary goal, while achievement of normal adult height during childhood is the secondary objective.

Auxological criteria including age and height SDS are the most relevant factors when deciding to offer GH treatment. The shorter the child, the more consideration should be given to treatment with GH. In the USA and seven other countries, GH treatment is approved for children shorter than −2.25 SDS (1.2 percentile). ISS is defined by FDA approval as "height SD ≤−2.25 that is associated with a growth rate 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." Normal predicted adult height in a short child, as seen in constitutional delay, is considered a reason against GH therapy. Age should also be taken into account. Although no lower age limit has been defined, the optimal age for initiating treatment is from 5 years to early puberty. An age limit of 12–13 years has been used in clinical trials.

There are no biochemical criteria for initiation of GH treatment in ISS. Psychosocial consequences should also be considered in treatment decisions. Short stature may be a risk factor for psychosocial problems, such as social immaturity, bullying by peers, and low self-esteem. The ability to adapt to short stature varies greatly among individuals, and factors that may play key roles include parental attitudes and cultural views. Overall, studies have shown that short individuals function normally without evidence of significant psychosocial morbidity. It is important to evaluate a child's coping capacity and the likelihood that therapy will have a positive effect. One would be more likely to consider therapy if the child was affected psychologically by short stature than if the child was unconcerned about height. A child in psychological distress may warrant evaluation and counseling by a mental health professional. No data has been reported regarding psychological counseling, but interventions aimed at adaptive and coping strategies and social support should be considered.

Treatment with Growth Hormone

All relevant data (current and predicted height, pubertal status, bone age, psychosocial issues, etc.) should be considered when deciding on GH therapy for ISS. The expected result of GH treatment is an increase in height SDS and height velocity resulting in increased adult height. Hintz et al. initially showed that long-term administration

of GH to children with ISS increases adult height to a level above predicted adult height [7]. Features suggestive of a good initial response to therapy include change in height SDS >0.5/year, first-year increase in height velocity >3 cm/year, height velocity >+1 SDS in the first year, or being above the mean on the growth response curve constructed by Bakker et al. [4, 8]. Criteria for poor first-year responders include height velocity SDS <+0, change in height SDS <0.3, or <−1 SDS on the "Bakker" curve [4, 8]. If poor response is noted, compliance must be assessed first. Not only are serial IGF-1 measurements useful in assessing efficacy, safety, and compliance, but they are also a valuable tool for adjusting GH dose and modifying treatment protocols [9, 10]. The patient's age, pubertal status, and degree of growth retardation must also be taken into account when evaluating outcome. In most children with ISS, change in height SDS is the best indicator of response. If the growth rate is still inadequate after 1–2 years and higher doses of GH, treatment should be stopped or alternative therapies considered.

GH therapy in ISS results in a height velocity increase typically seen in the first year, which then tapers off gradually in subsequent years. The average height velocity in the first year of treatment is 8–10 cm/year (depending on dose), compared to 4–5 cm/year prior to treatment [11]. Growth velocity acceleration occurs at a dose close to replacement dosage (40 mcg/kg/day), with higher approved doses (67 mcg/kg/day) resulting in even more significant acceleration and better effect on final height [11]. Studies evaluating GH influence on rate of bone age progression and the onset of puberty have shown different results, but no effects were seen with dosages ranging from 30 to 67 mcg/kg/day. Regarding adult height, treatment with an initial dosage of 35 mcg/kg/day is associated with an average of 3–5 cm on final height, even if the dose is increased later on. Thus, it seems that the initial starting dose is critical with regard to taller adult height. All studies using a dose of at least 50 mcg/kg/day throughout the course of therapy gained an average of ≥7 cm [11]. The mean increase in adult height attributable to GH therapy (average duration 4–7 years) in ISS is >2–3 inches. compared with control groups [4]. Responses are highly variable and dose dependent. Those with the best growth response include children who are younger or heavier, receive higher GH doses, and are shortest relative to target height [4]. Interestingly, baseline IGF-1 levels which are inversely related to GH response in GHD have no relation to the GH response in ISS. Adult height outcome is influenced negatively by age at start and positively by midparental height, height at start, bone age delay, and first-year response to GH [4]. Regarding body composition, GH increases lean body mass, decreases truncal fat, and may increase bone mineral density. Data is still limited on the psychosocial effects of GH. Several studies found improvement in behavior and social functioning, while others have found little to no effect on psychological variables. Only a few studies have addressed the effect of GH on well-being and quality of life in adulthood, and the majority of those treated were satisfied with GH.

GH treatment dosage has traditionally been selected and adjusted by weight (standard dose per kg body weight or m^2 body surface area), with adjustments made using auxological parameters such as linear growth velocity. The usual maintenance GH dose varies from 25 to 50 mcg/kg/day for GHD and from 40 to 67 mcg/kg/day for ISS. A dose of 67 mcg/kg/day was recently approved for ISS based on long-term studies [12]. For other pediatric conditions, the upper limit of GH dosage is also around 70 mcg/kg/day (higher doses are approved for children with SGA, Turner syndrome, chronic renal failure, and during puberty). GH dose should be reduced if IGF-1 levels are elevated at >2 SDS. According to an IGF-based dosing study, ISS subjects required higher GH doses than GHD patients, suggestive of a degree of GH insensitivity in ISS patients [13]. Interestingly, ISS patients grow less well than GHD patients at the same IGF-1 levels that are achieved during therapy, demonstrating some degree of IGF insensitivity as well. Titration of dose based on higher IGF-1 resulted in greater growth responses. Thus, ISS patients may benefit from a higher IGF-1 SDS target during GH therapy (typically between the mean and +2 SDS).

Treatment with GH involves continuous evaluation of efficacy and safety with the option of changing or discontinuing therapy if response is poor, when acceptable height is attained, or if the patient wishes to stop treatment. Height, weight, pubertal development, and adverse effects should be assessed at 3- to 6-month intervals while on GH. Response to therapy should be evaluated by calculating height SDS, height velocity (cm and SDS), and change in height SDS. Bone age is usually performed at baseline, and annually after the beginning of puberty, to assess skeletal maturation. Monitoring for scoliosis, tonsillar hypertrophy, papilledema, and slipped capital femoral epiphysis should be performed. IGF-1 levels should be measured at least every 6–12 months to assess compliance and GH responsiveness. IGF-1 levels also help guide dose adjustment, but significance of abnormally elevated IGF-1 levels remains unknown. TSH and FT4 should be measured 3–6 months after treatment starts and then every 1–2 years, to ensure thyroid function remains normal during therapy. GH should be stopped when near-adult height is achieved (height velocity <2 cm/year and/or bone age >16 year in boys and >14 year in girls) or if height is well in the normal adult range.

Side effects of GH in ISS are similar to those reported in children on GH therapy for other indications, though less frequent. So far, GH seems to be a safe treatment; no long-term adverse effects have so far been documented. Data regarding posttreatment follow-up in ISS is lacking, since follow-up on adults who received GH treatment for ISS as children has not been reported. Epidemiological association between GH, IGF-1 levels, and neoplastic disease warrants continued monitoring of IGF-1 and IGFBP-3 levels vis-à-vis GH safety, even though this issue remains a theoretical concern as GH has not been proven to induce malignancy and true causality has not been established.

Given the high price of GH, a cost-benefit analysis should be presented to the family. The cost of GH is estimated at $10,000–20,000 per cm. The estimated cost per cm gain at a recommended dose of 50 mcg/kg/day was calculated at $27,200–54,400 [11]. It is currently not known how a gain in height relates to a change in quality of life. Although GH treatment for ISS is approved by the FDA, it is not universally reimbursed by insurance companies and HMOs [14]; in some regions and countries, it is not covered at all. Self-payment by families is common in some areas. There is continued controversy over the use of GH in ISS among pediatricians and pediatric endocrinologists. Those against the practice of "endo-cosmetology," a recently coined term describing the treatment of non-GHD short stature with growth hormone, consider ISS a normal variant of growth and contend that growth hormone will not substantially improve stature or quality of life [15]. Ultimately, clinicians should be advocates for their patients and serve their best interests.

GH Treatment Alternatives

Puberty modulators are sometimes given as an adjunct or alternative to GH therapy. The goal of such treatments is to prolong the available time for growth rather than increasing height velocity during therapy. Current data suggests that the long-term effect of GH may be slightly augmented by adding a GnRHa or another inhibitor of sex steroid effect on the growth plate, such as aromatase inhibitors. An adolescent with ISS in early puberty with predicted adult height <−2 SDS may be a candidate for combination treatment.

GnRH agonists (GnRHa) decrease growth rate and bone age progression, thus resulting in opposite effects on adult height. Monotherapy in both sexes has shown a small and variable effect on adult height gain and is not recommended. Combination therapy with GnRHa and GH has potential value in girls, but not in boys with ISS. Studies have shown that the duration of GnRHa treatment period is important with regard to final adult height (an additional height gain of 3–6 cm after 2–3 years of combination treatment). Thus, treatment should be given for at least 3 years, and GH therapy should be continued until growth is complete. Data is limited on the psychosocial consequences of combined GH and GnRHa treatment in ISS.

Aromatase inhibitors have emerged as a potential alternative means of slowing epiphyseal fusion and prolonging linear growth. They are being used increasingly for various endocrine disorders and have shown promising results in adolescent boys with short stature, both alone and in combination with GH. Estrogen is known to accelerate maturation of epiphyseal growth plates. Inhibition of aromatase facilitates growth in the presence of androgens while slowing bone age advancement by inhibition of estrogen production. Aromatase inhibitors have been shown to increase near-adult height in males with ISS. However, they are contraindicated for use in females. At the current time, these agents are not approved for use as height enhancers in any country, and their off-label use should be considered investigational. Combined therapy is sometimes considered if height prediction is below −2.0 SDS at the time of pubertal onset in males. There are few studies in the literature regarding use of aromatase inhibitors for treatment of boys with short stature, but short-term data in randomized controlled trials is reassuring; long-term safety and efficacy in males with ISS have not been demonstrated [16]. In a double-blind, placebo-controlled trial, letrozole slowed the progression of bone maturation in boys with short stature and delayed puberty who were also given testosterone [17]. In a randomized controlled trial by Hero et al., 2-year treatment with letrozole given as monotherapy improved predicted adult height as much as 5.9 cm in a group of prepubertal boys with ISS [18]. The same group of investigators also found that boys treated with testosterone and letrozole reached a higher near-final height comparable to their midparental height, whereas those who received testosterone and placebo did not [19]. Another study using anastrozole in boys with GHD showed a slowing of epiphyseal fusion and a net gain in predicted adult height, calculated based on bone age, of +4.5 cm after 2 years and +6.7 cm after 3 years of treatment, compared to +1 cm at both time points in the placebo group [20]. Short-term treatment showed no negative effects on bone mineral density or spermatogenesis [18, 21]. One recent study of aromatase inhibitors alone suggested that there are some late-onset vertebral bone density issues in children who started treatment in early puberty [22]. Therefore, if treatment with these agents is considered, it should begin after mid-puberty, and bone density should be assessed.

Testosterone is the most appropriate treatment for boys with severe CDGP with a predicted adult height well in the normal range. If predicted adult height is below normal, testosterone should not be used. The goals of treatment are to initiate or increase secondary sexual development while increasing height velocity and lean body mass. Low-dose therapy accelerates linear growth in the short term with little or no advancement of bone age or decrease in adult height potential. Doses ranging from 50 to 200 mg IM monthly have positive effects on growth velocity [11].

Oxandrolone, an orally administered synthetic androgen, is not a recommended treatment. It is a weak androgen with less virilizing effects than testosterone and carries a remote risk of hepatotoxicity. Several studies have shown that oxandrolone therapy for 3 months to 4 years increases height velocity in the short term with no adverse effects, but does not significantly decrease predicted or measured adult height [11]. This is likely secondary to corresponding bone age acceleration. Growth is due to androgenic and anabolic effects rather than upregulation of the GH-IGF axis.

Recombinant human IGF-1 therapy is approved for short stature with severe IGF-1 deficiency (<−3 SDS for height and IGF-1) associated with normal GH secretion in the USA, Japan, and Europe. For patients with Laron syndrome and other forms of GH resistance, this treatment is the only effective means of improving growth and final height [23]. It is a theoretical therapeutic option in ISS children who are extremely short and unresponsive to GH treatment. First-year results from a randomized study by Midyett et al. found that IGF-1 treatment of short children labeled as moderate IGF-deficient or IGFD (height <−2 SDS) with low IGF-1 levels (−2 SDS) was associated with age- and dose-dependent increases in height velocity [24]. However, there are currently no published data regarding the effect of IGF-1 therapy on final

height in children with moderate IGFD, and further studies are needed to evaluate efficacy and safety. The off-label use of IGF-1 in patients taller than −3 SDS should be considered investigational.

Conclusions

ISS describes a category of children with severe short stature and poor growth velocity in the absence of an identifiable cause. After a thorough evaluation has been conducted, a number of therapeutic options may be considered. The approval of ISS as an indication for GH treatment poses further challenges in optimizing management of these patients. Given the controversy that surrounds the use of GH and adjunctive treatments such as aromatase inhibitors in current practice, additional controlled studies are required to ensure the safety and efficacy of these interventions.

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

5

Steven D. Chernausek

Abstract

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. This is a significant problem because of the morbidity that accompanies IUGR. The effect of IUGR on subsequent growth and its amelioration by growth hormone (GH) is the focus of this chapter.

Keywords

Growth hormone • Small for gestational age • Intrauterine growth restriction • Insulin-like growth factor I • Short stature • Growth

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].

There are long-term sequelae of IUGR as well. Affected children may have poor school performance and attenuated intellectual development [4]. 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 [5]. Postnatal growth is also affected adversely. Somewhere between 10 and 40% of children who are born following IUGR remain growth-retarded in childhood [6, 7]. Many never reach normal adult size. The effect of IUGR on

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subsequent growth and its amelioration by growth hormone (GH) is 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. IUGR (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 usually not available, and therefore, IUGR is infrequently documented with precision and the phase of pregnancy during which the growth aberration occurred is rarely defined. 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 and hypoglycemia.

After birth, most SGA newborns increase their growth velocity substantially and eventually catch up [6, 7]. 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 [8]. 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 (Table 5.1).

Mechanisms of IUGR

A deficiency of insulin secretion (such as 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 [9]. More common fetal insults that produce IUGR are hypoxia and nutrient deprivation. Restriction of oxygen or nutrients results 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, 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 [10] and Randhawa [11]). 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. [12–15] The growth-promoting effects of the IGFs are principally mediated by the type I or IGF-1 receptor, a homologue of the insulin receptor. [12] The IGF-2 "receptor," in contrast, serves as a clearance mechanism of IGF-2 and thereby modulates tissue IGF-2 abundance. [16, 17] 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 [18]. Thereafter, the influence of IGF-2 declines and IGF-1, under the control of GH, becomes the dominant growth regulator of postnatal life.

Table 5.1 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	[21, 22, 81]
IGF2	Silver-Russell S	Severe pre- and postnatal growth failure	Epigenetic variation at imprinted locus	[82–84]
IGF1R	Short stature	Severe pre- and postnatal growth failure, variable CNS abnormalities	Variable increases in circulating levels of IGF-I	[21, 24, 26, 27]
INS	Congenital diabetes	Fetal growth retardation, diabetes mellitus	10% of permanent neonatal DM	[85]
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	[86–88]
Chromosome 6 ICR abnormality	Congenital diabetes	Fetal growth retardation, transient diabetes mellitus	70% of transient neonatal DM- paternal isodisomy or DNA methylation defect	[89]
INSR	Donohue (Leprechaun) S, Rabson-Mendenhall S	Fetal growth retardation, diabetes mellitus, moderate to severe insulin resistance	Treatment with rhIGF-I may be beneficial	[90, 91]
PTF1A, *IPF1*	Pancreatic agenesis	Fetal growth retardation, diabetes mellitus	Cerebellar involvement with *PTF1A*	[92]
FANC A-M	Fanconi S	Severe pre- and postnatal growth failure, absent thumb/radius	Associated with GH deficiency, hypothyroidism, hypogonadism, and malignancy	[93, 94]
BLM	Bloom S	Severe pre- and postnatal growth failure	Increased risk for neoplasms	[95]

Studies by Lupu et al. [18]. detail the extent to which the GH-IGF axis influences growth in the rodent. 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 seventeen percent of body size in the adult mouse relates to factors other than GH or IGF. This means that diseases or conditions that alter growth significantly likely will impact the GH-IGF system at some point, either limiting the production of the IGFs, reducing the abundance or function of the IGF receptor, or perturbing specific steps along the intracellular signal transduction pathway.

Though much of our insight into mechanisms comes from studies of rodents, many of the experimental findings have been confirmed in humans (Table 5.1). Children with GH insensitivity due to receptor deficiency are near-normal size at birth, indicating a modest role for GH in prenatal growth [19]. In contrast, children with significant disruptions in the IGF-1 gene [20–22] and IGF-1 receptor gene [23–29] show severe IUGR 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

[30–32], illustrate the pivotal role of the IGF axis in controlling prenatal and postnatal growth.

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 GH treatment to benefit patients with short stature 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.

Clinical Presentation

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 or evident.

A more common presentation is that of the short child between the ages of 3 and 8. 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 3–5 SDS 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.

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 failure. 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. Patients who are born SGA frequently show catch-up growth during the first year and 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 [6, 33, 34]. Patients who have shown no evidence for improved growth following birth need further evaluation.
2. *Is the cause of IUGR obvious*? Careful history and physical exam can be very helpful in explaining the IUGR. Maternal hypertension and poor weight gain during pregnancy all suggest a maternal factor. Dysmorphic features in the baby imply a syndrome associated with IUGR is present. Useful diagnostic tests for evaluating patients with IUGR are listed in

Table 5.2 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)

Table 5.2. 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 evaluation 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 would seem 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.

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-I [35]. Cutfield et al. [36] showed that circulating concentrations of IGF-I, while lower than normal in short SGA children, were higher than those with idiopathic short stature of the same age. Chatelain et al. [37]. 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 IUGR, studies by Boguszewski et al. [38] and de Waal et al. [39] 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 low overnight GH concentrations or low IGF-1 concentrations were associated with an improved response [40, 41], whereas later reports, examining greater numbers, found no predictive value in these measures [42–44]. 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

Table 5.3 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/d	35 μg/kg/d (1 mg/M^2/d)	35–70 μg/kg/d

Data are from somatropin package insert for United States, from report 3478/03 by the Committee for Proprietary Medicinal Products of the EMEA, and from Clayton et al. [45] for consensus statement. *SGA* small for gestational age, *SDS* standard deviation score

SGA, likely include a significant proportion that do 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-I in the face of relative IGF resistance.

Growth Hormone Therapy

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 5.3). There is now general agreement that one should consider administration of GH to significantly short patients who experienced IUGR. A consensus statement published in 2007 [45] 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 deal with the complex and controversial issues that surround the topic.

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 [46–48]. However, enthusiasm was diminished by the suggestion that the growth stimulation was not sustained [49] and that undesirable bone age advancement was negating the effect [50]. 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 (Fig. 5.1). Table 5.4 displays 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

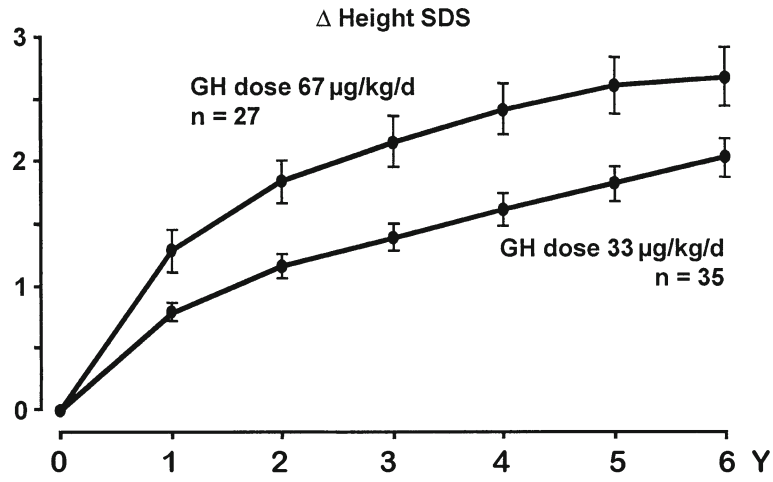

Fig. 5.1 Height SD score in patients with IUGR-associated short stature randomized to receive GH daily at 2 distinct doses. Patients were approximately 5 years of age on average at the start of treatment. Note the clear dose–response relationship most apparent in the first years of treatment. Figure is from De Zheger et al. (J Clin Endocrinol Metab 85: 2816, 2000, used with permission)

gains require a dose of at least 33 µg/kg/d 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 are 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 [51]. 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 suggests that the effect is not complete. Figure 5.2 shows 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.

Safety[1]

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 [52], 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 issue of insulin resistance is pertinent. Epidemiological and experimental data

[1]Note added in proof: Since submission of this work, there has been a preliminary report from the European Union SAGhE study suggesting an increase in overall mortality in children treated with growth hormone (Carel et al, J Clin Endocrinol Metab 97:416-25, 2012). Though this was not confirmed in a parallel study (Savendahl et al, J Clin Endocrinol Metab 97:E213-7, 2012), readers are encouraged to examine future reports on this topic.

Table 5.4 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/d)	SDS start	SDS end	SDS gain	Comments	Ref
Controlled clinical trial to final height	91 33	12.6 12.9	2.7 NA	66 0	−3.2 −3.2	−2.1 −2.7	1.1 0.5	Older subjects and short duration. RCT design provides proof of principal that GH therapy increases adult height	[96]
Clinical trial to final height	36 34	8.9 8.3	8.5 NA	33 0	−3.1 −2.2	−1.2 −2.0	1.9 0.2	Showed that duration of GH treatment prior to pubertal onset had positive impact on final height	[97]
Clinical trial to final height	28 26 15	7.9 8.2 7.8	7.9 7.5 NA	33 67 0	−2.9 −3.0 −2.6	−1.1 −0.9 −2.3	1.8 2.1 0.3	Shows what can be achieved with 7+ years of treatment. Modest or nonexistent dose effect in terms of final height	[98]
Clinical trial to final height	70 40	10.3 10.0	4.6±2.5 NA	20 0	−2.9 −2.8	−2.0 −2.2	0.9 0.6	Untreated "controls" had normal GH stimulation test; treated patients had GH peak <10 ng/ml	[99]
Registry analysis (NCGS)	270	6.9	4	40	−3.6	−1.8	1.8	Large population. Uncontrolled Survey with 46 patients in fourth year	[100]

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

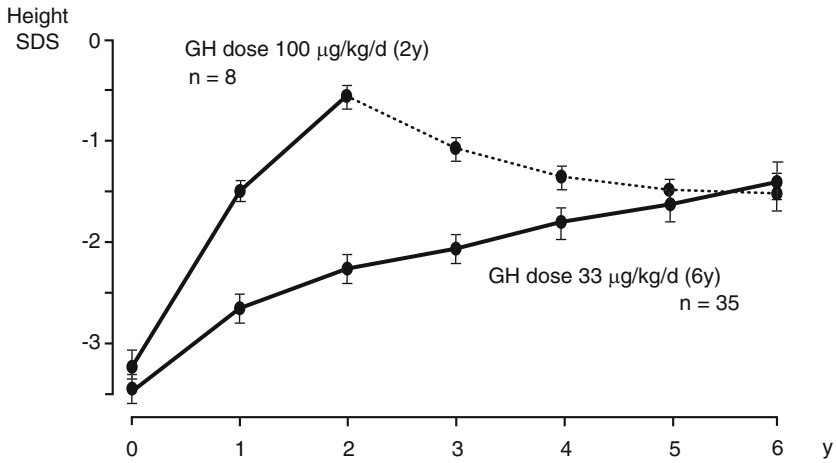

Fig. 5.2 Height SD score in patients treated with a 2-year course of high dose (100 μg/kg) daily GH and followed for four additional years untreated (*dotted line*). They are compared to patients treated with a lower dose of GH continuously for 6 years. Note that patients on high dose grew very well during GH therapy but that height SD score was not maintained when GH supplementation was withdrawn. Figure is from De Zheger et al. (J Clin Endocrinol Metab 85: 2816, 2000, used with permission)

indicate that humans born SGA have an increased incidence of obesity and type 2 diabetes as adults, with the implication that the period of fetal undernutrition results in a resetting of intrinsic insulin sensitivity [53, 54]. Patients with IUGR already show evidence of decreased insulin sensitivity as children [55]. 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 [56] and no clinically significant

impact on glucose or lipid metabolism [57]. 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 [58]. 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, [59, 60] 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 [61, 62]. However, large epidemiological studies find that risks for prostate cancer [63] and breast cancer [64] 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 [65]. This raises the question as to whether the high IGF-1 levels that generally accompany high-dose GH therapy might have adverse consequences over the long term.

Despite these justifiable concerns about GH safety, most data published to date are reassuring. Bell et al. [66] 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.

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 unlikely to benefit much unless they have concomitant GH deficiency. Though patients with specific genetic syndromes have rarely been evaluated in detail, there is really no reason to believe that for some the responses to GH would not equal 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 are 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 [27]. 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.

GH Dosing and Monitoring

Though relatively high doses may be ultimately required for the best growth response (the FDA-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. Each approach has its advantages and more experience is needed with various treatment regimens.

Patients should have reevaluation at a minimum of 6-month 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 [45]. 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 [58].

Periodic assessment of circulating IGF-1 during GH therapy has been recommended for patients receiving GH [67]. It is perhaps most valuable for determining dose replacement of GH for adults with GH deficiency [60] but has been advocated as a safety measure for other conditions where GH is used [68, 69]. 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 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-I concentrations above normal might be needed. 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. 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 resulting in catch-up when the patient is below the 5th percentile for height and to 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 should be within the normal range in most during the growth maintenance phase.

Future Directions

Use of GH has become established in the treatment of short stature that follows IUGR. Future studies need to define for physicians how the drug can be used with the greatest safety and efficacy. Should all patients receive the same, relatively high dose, or can dosing be tailored using markers of GH or IGF action that reflect individual sensitivity? The heterogeneous nature of this patient population continues to confound efforts to grasp all the variables influencing their

growth and GH response [70]. Prediction models that utilize existing baseline and/or initial response data have been developed and may prove useful in selecting optimal GH dose as well as the patients most likely to benefit [71–73].

The number of patients for whom a specific etiology cannot be established will lessen as methods to establish molecular-genetic diagnoses in IUGR patients improve and become more commonplace. Over 100 genes have been identified that determine adult height [74], and it is probable that many of these 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 design and optimize therapy.

The goal of GH therapy is a height in the normal range without complication. Although this is achieved in the majority [75], a small number still fall short of the goal (Fig. 5.3) because treatment was initiated too late, the short stature too severe, or the response to GH therapy was suboptimal. Therapies that might augment the response include adding recombinant human IGF-1 to the treatment and slowing epiphysial maturation with aromatase inhibition. These treatments have been evaluated in limited clinical trials and demonstrate efficacy [76, 77] but are considered experimental at this time.

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 [78, 79]. However, much more work needs to be done in this area [80], 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.

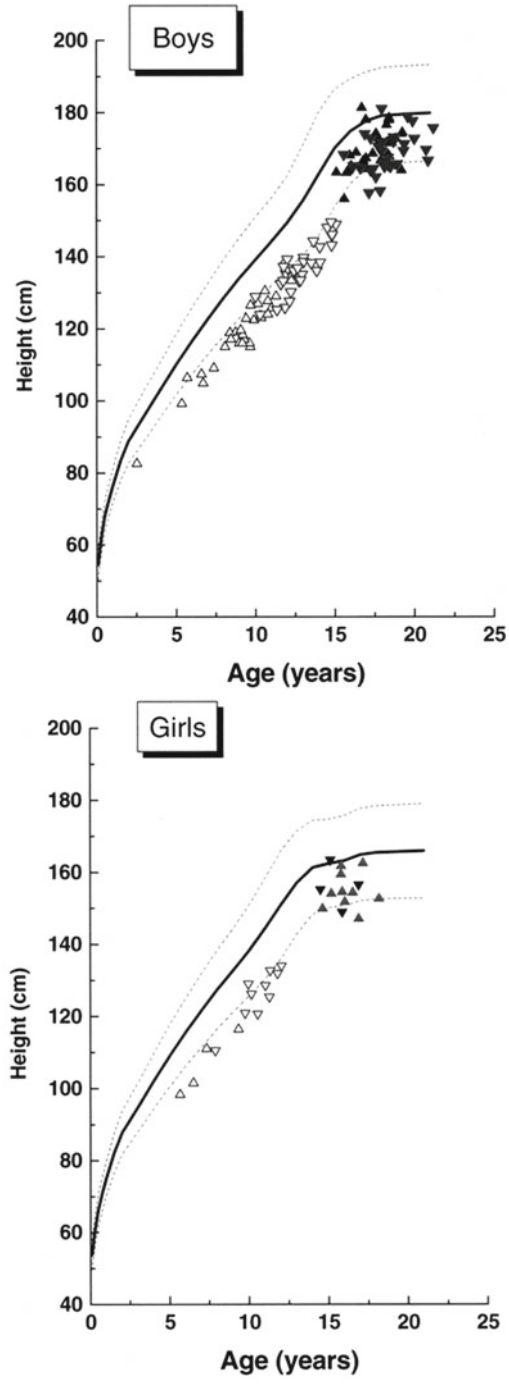

Fig. 5.3 Final height of children born SGA treated with GH. *Open triangles* are subject at treatment initiation. *Closed triangles* are the same individuals at final height. Figure is reprinted by permission from Macmillan Publishers Ltd: Pediatr Res 57: 216, copyright 2005

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Growth Hormone Therapy in Children with Prader-Willi Syndrome

6

Aaron Carrel and David B. Allen

Abstract

Prader-Willi syndrome (PWS), initially described by Prader, Willi, and Labhart in 1956, is characterized by obesity, hypotonia, hyperphagia, delayed motor skill acquisition, short stature, mental retardation, hypothalamic dysfunction, and hypogonadism. This article 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 growth hormone (GH) therapy. However, treatment of these children has raised awareness of other potential benefits of GH therapy, which in this particular group of patients may exceed linear growth promotion in importance. These include improvements in body composition, which leads to improved physical strength and function and increased energy expenditure.

Keywords

Prader-Willi syndrome • Growth hormone • Body composition • Strength

Introduction

Prader-Willi syndrome (PWS), initially described by Prader, Willi, and Labhart in 1956, is characterized by obesity, hypotonia, hyperphagia, delayed motor skill acquisition, short stature, mental retardation, hypothalamic dysfunction, and hypogonadism [1]. The genetic abnormality has been located on chromosome 15 (q11–13), a deletion of the paternal allele or presence of maternal disomy; a critical region of chromosome 15 is active only in the paternally inherited chromosome. Thus, PWS was the first human disorder associated with imprinting [2, 3]. It is

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now known that approximately 70–75% of cases of PWS are associated with absent expression of the paternal allele in the PWS region of chromosome 15q11–13. Approximately 25% of cases involve uniparental disomy, in which an individual inherits two copies of the maternal chromosome 15 and none of the paternal copy. Rare cases 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]. 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 the following: short stature, hyperphagia, sleep disorders, deficient growth hormone (GH) secretion, and hypogonadism [5]. Other central defining features in children with PWS include abnormal body composition with increased fat mass and decreased lean body mass [6]. 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 [7]. 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 PWS is well documented [8, 9]. This is distinguished from reduced GH secretion observed in nutritional obesity by low IGF-1 levels and abnormal body composition similar to that observed in patients with growth hormone deficiency (increased percent body fat and decreased FFM).

In hopes of alleviating abnormalities in linear growth and body composition, and because most children with PWS show evidence for subnormal growth hormone (GH) secretion, recombinant human GH therapy for children with PWS has been investigated. Several studies show that GH instituted during childhood for up to 4 years improves but does not normalize body composition, energy expenditure, and strength and agility in children with PWS [10–13], as well as in *infants and toddlers* with PWS [14, 15].

This article 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 GH therapy. However, treatment of these children has raised awareness of other potential benefits of GH therapy, which in this particular group of patients may exceed linear growth promotion in importance. These include improvements in body composition, which leads to improved physical strength and function and increased energy expenditure.

Growth and Growth Hormone in PWS

Growth of children with PWS is characterized by mild to moderate intrauterine (average −1 SDS) as well as postnatal growth delay. Often 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). The slow growth and delayed skeletal maturation observed in some, but not all PWS children contrasts with healthy obese subjects, in whom growth acceleration and bone age advancement are more commonly seen. 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 reported to be 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 by weight loss [16]. 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 [17]. Nevertheless, substantial evidence supports the existence of a true GH-deficient state in PWS. Children with PWS display borderline normal or diminished growth rates, in contrast to normal or accelerated growth typically seen in healthy non-PWS obese children. It is possible that overnutrition and hyperinsulinemia 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. Elevated levels of insulin, considered a possible cause of growth acceleration, are seen less in PWS compared to healthy obese children, but children with PWS do not display associated increased growth. Insulin levels are lower in children with PWS than in "healthy obese" children, suggesting relatively heightened insulin sensitivity compatible with reduced GH secretion [18].

Levels of IGF-1 are moderately 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. That nutrition-stimulated IGF-1 production is sustaining 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.

Finally, even children with PWS with normal weight/height ratios show low GH responses to provocation. 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 actually constitute the strongest 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.

Body Composition in PWS

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 body composition in the absence of obesity [19], and this has been confirmed by determination of reduced lean body mass and energy expenditure. [7] Between the second and fourth year of life, progressive obesity usually begins primarily as a consequence of excessive caloric intake but also due to decreased energy expenditure and reduced physical activity. The body composition of childhood PWS patients is characterized by a marked reduction in lean body mass associated with increased fat mass, even in those subjects who appear less obese. Thus, while caloric restriction may minimize weight gain, 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). 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 resembles that of severely growth hormone-deficient (GHD) individuals (i.e., reduced lean body mass and increased fat mass, bone mineral density, and energy expenditure) [20, 21]. This phenotype is clearly distinguishable from the parallel increase in lean body and fat mass observed in over-nourished 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 GH therapy [11, 22–24].

Effects of GH Treatment on Growth and Body Composition

Early studies of the effect of exogenous GH treatment of children with PWS focused on growth rate acceleration and improvement in stature as primary therapeutic goals. Multiple groups have 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 GH 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 GH administration [25].

Administration of GH 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 [26]. Similarly, children with PWS respond to GH therapy with improvements in body composition. In this population of children, these clinical effects are arguably more valuable than change in growth velocity. These findings emphasize the "non-growth" benefits of GH in clinical use. These GH effects have also been associated with improvements in body composition, height, and muscle endurance and power [27].

Consistent evidence shows that GH therapy for 12–48 months in children with PWS decreases fat mass, increases lean body mass, increases linear growth [12, 28, 29], and, in one study, increased fat utilization [10]. Specific changes in increased muscle mass and decreased body fat were seen with GH therapy compared to non-treated PWS children. Documented changes in physical function (strength and agility testing) in PWS children treated with GH, which translated to acquisition of new gross motor skills, appeared to be important "real-life" benefits for these children. While these findings suggested that GH 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.

Prolonged effects of GH 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 GH, 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 GH 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. Response of children with PWS to GH is greatest during the first 12 months with regard to growth rate, decreases in body fat, increases in REE, improvements in physical function, and laboratory alterations in carbohydrate and lipid metabolism. Thus, a diminution in response to GH during prolonged GH therapy, observed in virtually all growth studies of GH therapy, applies to other GH metabolic effects in children with PWS. Nevertheless, comparison of school-age children with PWS treated with GH since infancy showed significant gains in muscle mass, energy expenditure, and motor milestones [29].

Effect of GH 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 GH 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-GH-treated PWS controls, GH-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.

Effects of GH on Strength and Agility

Substantial documentation has accumulated to support beneficial effects of GH 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 GH 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 our subjects also claimed striking improvements in physical stamina, strength, and agility. Specifically, these included 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. [10–12, 28, 29]

The authors' research has included objective measures of changes in physical function during GH treatment, including a timed run, sit-ups, and weight lifting [30]. Improvements in running speed, broad jump, sit-ups, and arm curls after 12 months of GH treatment compared to controls were documented. Following 48 months of GH 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. 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. While these findings suggested that measured improvements in strength and agility were associated with "real-life" functional benefit to the children and their families, lack of a blinded, placebo-controlled study design admittedly weakened the scientific validity of these findings. Recently, however, we were able to compare findings from a study of early GH treatment of children with PWS who are now similar in age to those recruited for a previous randomized, controlled study of GH treatment for school-age children with PWS [29]. This afforded a unique opportunity to compare the effects of long-term GH treatment on body composition, physical function, linear growth, and lipid metabolism in two age-matched groups of children with PWS, one of which was GH-naïve and the other in whom treatment with GH was initiated prior to age 2 years. A comparison of these two groups provided the best illustration to date of the degree to which long-term GH therapy changed the natural history of growth, body composition, and physical function in children with PWS (Table 6.1). These data demonstrated that PWS children treated with GH 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. The following figures illustrate the differences between children with PWS who received GH from an early age to GH-naïve PWS children.

Body Composition (See Fig. 6.1)

Percent body fat and muscle mass (lean body mass) were assessed by DXA. Lower percent body fat was evident in early GH treatment

Table 6.1 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
BMI	23.7 ± 1.1	21.9 ± 1.2	0.33
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 F-test
[b]Least squares mean, adjusted for age and gender

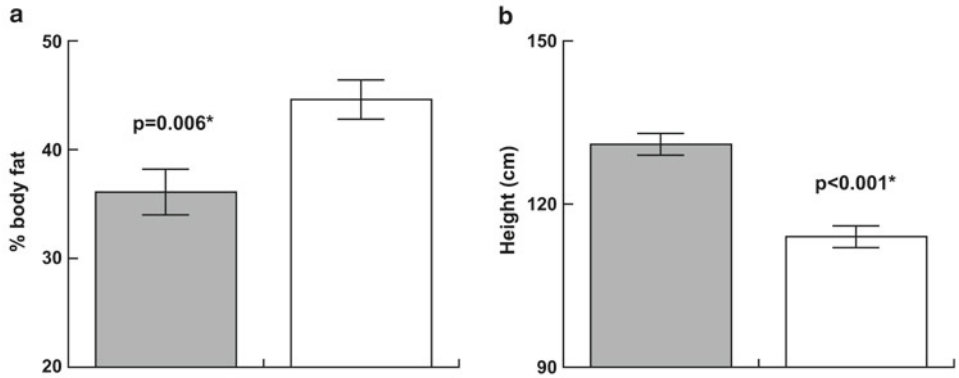

Fig. 6.1 Effect of growth hormone on anthropometrics in PWS (GH treated (shaded) vs. untreated). (a) Demonstrates % body fat and (b) demonstrates height. * Age and gender matched analysis using ANCOVA

children with PWS when compared to the GH-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 GH treatment-treatment group compared to the GH-naïve PWS subjects (24.1 ± 1.1 kg vs.16.7 ± 0.9 kg; $p=<0.001$).

Carbohydrate and Lipid Metabolism (See Fig. 6.2)

Carbohydrate and lipid metabolism were evaluated using fasting AM blood samples. Children with PWS who received early GH treatment were compared to the GH-naïve PWS children and demonstrated statistically significant lower total

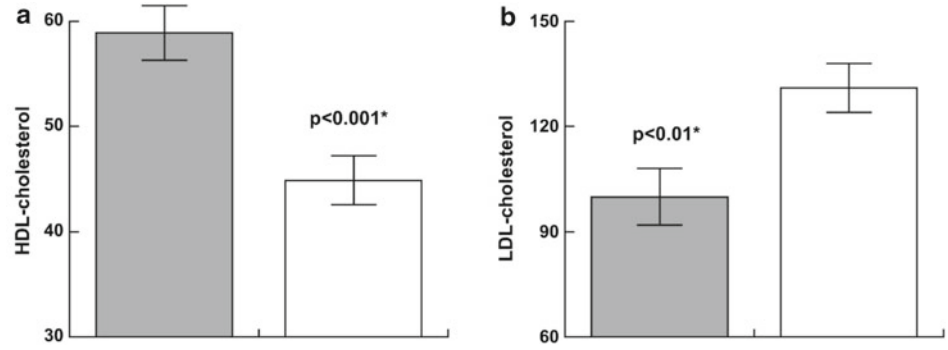

Fig. 6.2 Effect of growth hormone on lipids in PWS (GH treated (shaded) vs. untreated). (a) Demonstrates HDL and (b) demonstrates LDL. * Age and gender matched analysis using ANCOVA

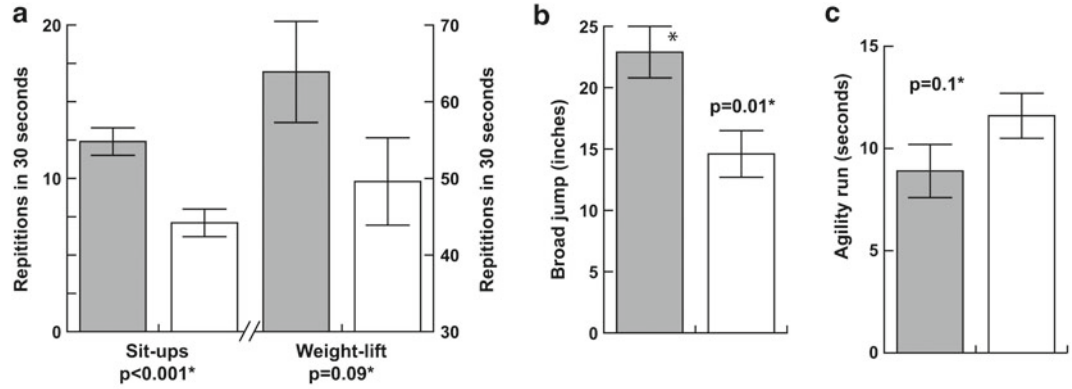

Fig. 6.3 Effect of growth hormone on strength in PWS (GH treated (shaded) vs. untreated). (a) Demonstrates repetitions in 30 s, (b) demonstrates broad jump, and (c) demonstrates agility. * Age and gender matched analysis using ANCOVA

cholesterol: least squares adjusted means of 177 ± 8.2 mg/dL vs. 190 ± 7.3 mg/dL, higher HDL 59 ± 2.6 mg/dL vs. 45 ± 2.2 mg/dL ($p = 0.0005$), lower LDL 100 ± 8 mg/dL vs. 131 ± 7 mg/dL ($p = 0.009$), and unchanged triglycerides 68 ± 11 mg/dL vs. 94 ± 12 mg/dL ($p = 0.1$). Glucose was not significantly different between the two groups. Fasting insulin was also was not significantly different between the two groups, 10 ± 1 mIU/mL vs. 7 ± 1 mIU/mL ($p = 0.1$).

Motor Strength (See Fig. 6.3)

A modified Bruininks-Oseretsky test was used to test strength and agility, with four sub-tests for different muscle groups of the body. Children with PWS who received early GH 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-lift repetitions (63.9 ± 6.6 vs. 49.6 ± 5.7; $p = 0.09$), although these did not reach statistical significance.

This analysis of similar-aged children with PWS, one group treated with GH for 6 years and the other naïve to GH therapy, offers a unique assessment of the degree to which early-in-life GH treatment alters the clinical course of this disorder. It also extends and tests the validity of

findings of previous studies of GH therapy in children under age 3 with PWS, none of which had a control group for longer than 12 months

Safety of GH Treatment in PWS

No adverse events have been reported in studies of children with PWS on GH with respect to glucose intolerance or scoliosis, two concerns that were raised in early GH studies. However, several cases of sudden unexpected death temporally associated with institution of GH treatment in children with PWS have been reported [31–34]. A causative relationship between exposure to GH and sudden death remains uncertain. Factors supporting a causative relationship include the occurrence of most deaths in the first 3–7 months of GH treatment and the known stimulatory effect of GH 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 GH therapy for 6–12 months improves respiratory function and carbon dioxide sensitivity in treated subjects. Taken together, these observations support the possibility of some additional early risk for GH-treatment associated sudden death (particularly in early childhood when lymphoid tissue growth is pronounced) followed by long-term benefit. It has also recently been suggested that partial adrenal insufficiency could be a contributing factor [35].

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 GH therapy in a way distinctly different from other GH treatment indications. In light of this potential rare by 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. The findings described previously, derived from comparison of long-term GH-treated and age-matched untreated children with PWS, suggest that the answer to the question is "yes." When compared to school-aged children with PWS who had not been treated with GH, the early-in-life GH treatment children showed, on average, the following:

1. 8.5% (absolute) reduction in body fat (19% relative reduction)
2. Nearly a doubling in broad jump and sit-up performance
3. 14 mg/dL higher HDL-C levels and 31 mg/dL lower LDL-C levels
4. Height increased by 16 cm

These differences compared to peers appear of sufficient magnitude to validate commonly heard (but difficult to test) parental reports of improved movement and agility, engagement in physical activities, and quality of life as a result of GH therapy.

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Turner Syndrome

7

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Abstract

Turner syndrome (TS) is one of the most common human chromosome anomalies. It occurs in approximately 1:2,000 female live births regardless of ethnic background. Girls with TS have an abnormal or missing X chromosome that causes short stature and may cause lymphedema, cardiac abnormalities, gonadal dysgenesis, dysmorphic features, nonverbal learning disabilities, and other problems.

Keywords

Gonadal dysgenesis • Growth failure • Sex chromosome abnormalities • X chromosome • Growth hormone • Ovarian failure

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Introduction

Turner syndrome (TS) is one of the most common human chromosome anomalies. It occurs in approximately 1:2,000 female live births [1] regardless of ethnic background. Girls with TS have an abnormal or missing X chromosome that causes short stature and may cause lymphedema, cardiac abnormalities, gonadal dysgenesis, dysmorphic features, nonverbal learning disabilities, and other problems [2, 3].

Pathogenesis

Approximately 50–60% of girls with TS are reported to have a 45,X karyotype. The loss of one X chromosome usually occurs as a result of a nondisjunction error during paternal meiosis since the single X chromosome in 60–80% of girls with TS is maternal in origin. Advanced maternal age may increase these errors [4]. These girls tend to have the most severe phenotype and are frequently diagnosed as newborns [5, 6]. 20–30% have structural abnormalities of the X chromosome such as rings, isochromosomes of the long arm, and partial deletions of the short arm. Thirty to 40% have mosaic patterns (karyotypes having two or more distinct cell types) involving the X chromosome such as 45,X/46,XX, 45,X/46,X,i(X), and 45,X/46,XY. This is thought to result from chromosome loss after fertilization [7]

In normal 46,XX females, either the maternal or paternal X chromosome is randomly inactivated in somatic cells during the late blastocyst stage and all descendants of that cell have the same inactive X. However, many genes escape inactivation [8]. In fact, about 15% of genes are expressed from both X chromosomes regularly and another 10% of the genes do so variably [9]. Many of the genes that remain active are located at the tip of the X chromosome and have homologous genes on the Y chromosome. These "pseudoautosomal" regions (PAR) are the only ones in which the male X and Y chromosomes recombine during meiosis.

Loss of all or part of an X chromosome may lead to clinical abnormalities through the following mechanisms: (1) haploinsufficiency of gene expression, (2) failure to express imprinted genes (genes expressed from a chromosome derived from one parent and not the other), and (3) unmasking of X-linked mutations.

Haploinsufficiency of Gene Expression

Most of the TS phenotype appears to be caused by haploinsufficiency of expression from genes that are normally expressed on both X chromosomes. To date, only one such gene has been identified with assurance—short-stature homeobox-containing (SHOX) gene [10]. SHOX belongs to a family of homeobox genes, transcriptional regulators that are key controllers of developmental processes [11]. Short stature, the most common physical finding in TS, is caused in large part by haploinsufficiency of the short-stature homeobox-containing (SHOX) gene on the X chromosome expression in chondrocytes. Localization of SHOX expression during embryogenesis also correlates with many of the phenotypic abnormalities found in TS. For example, SHOX expression in the developing limbs (particularly at the elbow, knee, and wrist) correlates with common skeletal abnormalities such as cubitus valgus, genu valgum, and Madelung wrist deformity [12]. Bone alterations including mesomelia (evidenced by reduced arm span) and tibial bowing suggest that SHOX haploinsufficiency particularly affects growth of the forearm and the lower leg. SHOX localization in the first and second pharyngeal arches which form the maxillary shelves, mandible, auricular ossicles, and the external auditory meatus [10] correlates with a narrow palate, retrognathism, prominent ears, recurrent otitis media, obstructive sleep apnea, and problems with sucking and articulation.

Brain natriuretic peptide (BNP) is the first transcriptional target of SHOX to be discovered [13].

BNP is primarily expressed by cardiac tissue and is best known for its natriuretic and vasodilatory properties. However, it is also expressed in other tissues, including the cartilage where it is necessary for normal chondrogenesis. Therefore, decreased expression of BNP secondary to SHOX haploinsufficiency may contribute to the short stature of TS [14, 15].

Haploinsufficiency of pseudoautosomal gene(s) has been postulated to cause maldevelopment of the lymphatics. Absence or hypoplasia of peripheral lymphatics causes generalized lymphedema, and a cystic hygroma, a collection of lymph in the mesenchyme of the posterior cervical region, may result. It is estimated that >99% of 45,X conceptuses do not survive beyond 28 weeks gestation [16] and that most die in the first trimester. It has been postulated that the prior cause of death is severe lymphatic obstruction that causes thoracic, pericardial and peritoneal effusions, and cardiac failure [16]. Others have hypothesized that myocardial hypoplasia is the primary defect causing lymphedema in utero [17]. In one study, a heart weight less than the third percentile was present in more than 90% of 117 mid-gestation fetuses with hydrops and phenotypic TS, but uncommon in fetuses with hydrops of other etiologies. Most recently, it has been postulated that early death may be due to defects in placental differentiation [18]. A webbed neck, low upward sweeping hairline, and low-set prominent ears often result during fetal development from a resolving cystic hygroma. Lymphedema and nail dysplasia may result from the more peripheral process. Structural abnormalities of the heart and vascular system such as coarctation of the aorta and bicuspid aortic valve are much more common in girls with coexistent cystic hygroma or lymphedema [19], suggesting that dilated lymphatics encroaching on cardiac outflow or disordered intramyocardial lymphatic development may be responsible for their development. Another possibility is that there is haploinsufficiency of a gene common to both the lymphatic system and the vascular system, from which it arises.

Zinn et al. have hypothesized the existence of a pseudoautosomal gene that causes the neurocognitive problems in TS. They identified a small 8.3 Mb interval of the distal Xp (Xp22.33) which is sufficient for expression of the TS neurocognitive phenotype [20]. Haploinsufficiency of two candidate genes, STS and NLGN4X, within the 8.3 Mb critical region are current candidates for this TS-associated neurocognitive phenotype. Nonetheless, the neurocognitive deficits observed in TS are certainly multifactorial and related to interactions between genetic abnormalities, gonadal dysgenesis and resulting estrogen and androgen deficiencies, and other unknown determinants.

Both X chromosomes normally remain active in oocytes. Haploinsufficiency of multiple genes on the X chromosome have been postulated to cause gonadal dysgenesis. Gonadal dysgenesis occurs in the vast majority of individuals with TS and is marked by massive apoptosis of germ cells in mid-gestation rather than a deficiency of germ cell formation [21]. Genes on both the X short arm (such as BMP15) and long arms (such as FMR1 and FMR2) are important for maintenance of ovarian function [22] and are likely to be involved. Although it was initially postulated that oocyte loss was caused by failure of normal meiotic pairing, loss of germ cells occurs at the very early stages of meiotic prophase even before chromosome pairing is established [23].

The genes involved in other problems associated with TS such as metabolic disturbances, renal abnormalities, and autoimmune diseases have not been identified.

Imprinted Genes

The phenomenon of imprinting is related to differential effects of the parent of origin of the single X chromosome in 45,X TS on the phenotype. The physical phenotype and response to growth hormone (GH) do not appear to be imprinted [24, 25]. However, Skuse and colleagues have suggested that an imprinted gene is involved in

social cognition [26]; although these human studies have not been confirmed [27], an imprinted gene, Xlr3b [28], has been demonstrated to influence cognition in an animal model of "Turner" 39,X mice [29].

X-Linked Recessive Disorders

Girls with TS are at increased risk for many of the disorders that are more prevalent in males than females such as mental retardation and autism. The mechanism is thought to be related to expression of X-linked mutations on the single X chromosome in 45,X TS. For example, girls with TS have a risk of red-green color blindness (an X-linked disorder) similar to that of boys. Case reports of other X-linked recessive disorders in TS include hemophilia B and Duchenne muscular dystrophy.

Functional Disomy

The mechanism for this genetic alteration relates to overexpression of X chromosome genes. For example, individuals with small ring X chromosomes often have a severe phenotype that is not typical of TS and includes mental retardation [30, 31]. In these cases, the loss of the XIST gene, which is involved in X-inactivation, may allow for normally inactivated genes to be expressed, thereby causing functional disomy.

Clinical Presentation

Introduction

Clinical presentations vary widely and are responsible in part for the broad range of ages at which the diagnosis of TS is made [32, 33]. For the majority of those diagnosed in prenatal life, the diagnosis is based upon an abnormal karyotype obtained for advanced maternal age, an abnormal serum screen, and/or ultrasound evidence of a cystic hygroma, hydrops fetalis, or cardiac defects. Girls diagnosed during infancy almost invariably have lymphedema with or without a webbed neck and other dysmorphic features [34]. In contrast, girls lacking these classic features are often not diagnosed until late childhood or adolescence when they are investigated for short stature and/or delayed puberty or as adults when they develop premature ovarian failure.

Lymphedema

Lymphedema is common in TS and begins in utero. Fetuses with 45X TS may present with increased nuchal thickness alone on ultrasound or more generalized lymphedema [35]. Mortality is high in this population. Virtually all girls diagnosed during infancy have lymphedema secondary to maldevelopment of the lymphatic system. Lymphedema differs from that seen in congestive heart failure, in that it is most prominent over the metatarsals and metacarpals, with a crease across the wrist and ankle joints. Lymphedema usually improves over the first few months of life but may progress 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 [36].

Dysmorphic Features

Most girls with TS have one or more dysmorphic features caused by lymphedema and/or skeletal abnormalities. However, the phenotype varies greatly and some girls with TS will have no or very subtle dysmorphic features. Of those diagnosed, more than half have a high-arched palate and/or retrognathia [34]. Ears are often low-set and posteriorly rotated with poor helix formation. The hairline tends to be low and upward sweeping, and in the minority, a webbed neck represents redundant skin that once stretched over a cystic hygroma. Ptosis, epicanthal folds, and downward-slanting palpebral fissures are common eye findings. Some degree of nail dysplasia is found in three-quarters. Nails tend to be small,

narrow, and inserted at an acute angle. Cubitus valgus and short 4th metacarpals are also common. About one-quarter of patients have pectus excavatum and a smaller number have hypoplastic breast tissue and inverted nipples [32].

Cardiovascular Abnormalities

Cardiovascular abnormalities are recognized as the most clinically significant anomalies of TS. 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. Aortic obstruction from a coarctation, which is usually periductal, may be minimal until the ductus arteriosus closes. Congestive heart failure can then develop rapidly. In older children, signs of coarctation generally include decreased pulse and blood pressure in the legs compared with the arms, and a systolic murmur radiating to the back.

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 [37]. 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%) [38]. The 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 [39]. For example, the right subclavian artery was aberrant in 8% and dilations of the innominate, left carotid, and left subclavian arteries were found in 22, 25, and 58% of the subjects, respectively. 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 [40]. Using current MRI techniques, that percentage appears to be much higher. 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 [39]. Patients with PAPVR had increased right heart mass ($p < 0.05$), increased ratio of main pulmonary artery to aortic valve blood flow ($p = 0.0014$), and increased right ventricular volume ($p < 0.05$). In one patient, surgery was required.

Aortic dissection is estimated to occur in 1.4% of the TS population [41]. 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% did not have any of these risk factors [42–44]. Nonstructural abnormalities such as hypertension, conduction defects, or mitral valve prolapse are also more common than in the general population, occurring in approximately 16% [37]. 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 [45].

Short Stature and Skeletal Abnormalities

Short stature is the single most common physical abnormality and affects virtually all individuals with TS. Untreated individuals achieve an average adult stature 20 cm shorter than that of their peers, which results in a height about 3 standard deviations (SDs) below the mean [46, 47]. This growth deficit is similar from country to country, and the individual scatter around the mean height is not significantly different from that of the normal population [48].

Growth failure is due to (a) mild to moderate growth retardation in utero, (b) slow growth

during infancy, (c) delayed onset of the childhood component of growth, (d) slow growth during childhood, and (e) failure to experience a pubertal growth spurt [49–51]. Girls with TS average −0.5 to −1.2 SDs below the mean for birth weight. In one longitudinal study, mean height SDS fell from −0.5 at birth to −1.5 at age 1 year and −1.8 at age 1.5 years [50]. Poor growth in the first year of life may be exacerbated in some by poor feeding due to oro-motor dysfunction and inadequate weight gain [52]. Problems that may contribute to poor feeding in TS are low orofacial tone, poor jaw strength, a low tongue position, an abnormally narrow palate, and broad lateral palatal ridges (the latter two being common in disorders in which there is a lack of tongue thrust into the palatal vault) [53]. Velopharyngeal and lower gastroesophageal tract dysfunction may also contribute to poor breast and bottle-feeding. This can be followed by reflux, gagging, and regurgitation of solids when they are introduced.

Growth during childhood is slow, there is a diminished pubertal growth spurt, and growth is often prolonged into the early twenties. Individuals with TS tend to appear stocky since they have a greater relative reduction in body height than in body width, and are often overweight. The increased relative width to height of the thorax accounts for the illusion of widely spaced nipples. Hands and feet are also relatively large [54]. Developmental abnormalities of individual bones are responsible for many common findings such as short neck, cubitus valgus, genu valgum, and short 4th metacarpals. The short neck is due in many cases to hypoplasia of one or more cervical vertebrae. Cubitus valgus occurs in almost 50% of patients and is caused by developmental abnormalities of the radial head. About 35–40% of patients have a short or borderline short 4th metacarpal and many have abnormally acute angulation of their proximal row of carpals. A short 4th metacarpal causes a depression instead of a knuckle when the fist is clenched.

Scoliosis and kyphosis are common. In a 2002 study of 25 patients between the ages of 5 years and 18 years, 20% were found to have scoliosis (lateral curvature >10°), and 40% were found to have excessive kyphosis (anterior-posterior curvature >40°) [55]. The youngest children with kyphosis and scoliosis were ages 5.9 and 9.8 years, respectively. The incidences of both skeletal abnormalities increased with age.

A delayed bone age is found in more than 85% of patients with TS, but the degree of delay is not uniform among bones. This finding likely reflects the estrogen deficiency that is present in many patients early in childhood. The delay is greatest in the phalangeal bones, intermediate in the carpals, and least in the metacarpals, radius, and ulna [56].

Osteoporosis and fractures are more frequent among women with TS [57]. Many girls have radiographic osteopenia and a coarse trabecular bone pattern, even in the prepubertal years. Although interpretation of most studies of bone mineral density (BMD) in TS is difficult due to reporting of areal rather than volumetric BMD, some conclusions can be made. Prepubertal girls with TS have decreased levels of markers of bone formation, consistent with a low bone turnover state and decreased bone deposition. There is a deficit in radial BMD, a largely cortical site [58]. Osteopenia at predominantly trabecular sites develops during adolescence, progresses in adulthood, and is associated with increased bone turnover. The pathogenesis of the demineralization is unclear, but is most likely an intrinsic bone defect that is exacerbated by suboptimal replacement of gonadal estrogen deficiency [59] Rubin, 1998 1427.

Orthodontic Problems

Individuals with TS have a posterior cranial base that is short and positioned at a shallow angle [60]. Because the mandible is pushed posteriorly and is relatively more hypoplastic than the rest of the face, retrognathia is common. There is an increased incidence of anterior open bite and lateral crossbite due to a narrow maxillary arch [61]. The palate is high-arched with unusual palatal bulges on the medial aspect of the posterior alveolar ridges. Girls with TS tend to have advanced dental age rather than the delayed

dental age expected for bone age-delayed individuals [62]. Tooth morphology is often abnormal: tooth crown size is reduced [63, 64] and roots tend to be short, placing these girls at an increased risk for root resorption [65]. Often, there is a need for orthodontic correction of these dental problems.

Hearing Loss

Ear and hearing disorders are very common problems among girls and women with TS. The majority of patients suffer from conductive hearing losses secondary to recurrent otitis media during infancy and childhood. The high incidence of otitis media in this population (60–80%) [66, 67] seems to result from an abnormal anatomical relationship between the middle ear and the Eustachian tube. A short, more horizontally oriented Eustachian tube in TS girls results in poor drainage and ventilation of the middle ear space and predisposes more nasopharyngeal microorganisms to reach the middle ear. Many girls require tympanostomy tube placement and a significant number 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 (air-bone gap >10 dB HL) was found in 43%. In addition, a mid-frequency sensorineural hearing loss (SNHL) between 500 and 2,000 Hz was present in 58% of the girls, four of whom required hearing aids [67]. The presence of such a mid-frequency dip appears to be a strong predictor for future rapid hearing decline with resulting social consequences [68]. SNHL has been reported as early as age 5 and appears to be progressive [69]. By their mid-forties, more than 90% of women with TS have a hearing loss >20 dB, with greater than 25% requiring hearing aids. Audiometry has revealed high-frequency (above that used for speech) SNHL in almost all individuals with TS studied (ages 6–38 years), suggesting "premature aging" of the cochlea [70].

Strabismus and Other Eye Problems

Ocular morbidity in TS is common. Strabismus is present in about one-third of the patients with TS, a frequency about ten times greater than that in the general population, and usually develops between 6 months and 7 years [71]. In a large report, 19% were reported to have amblyopia (loss of vision), most likely as a result of uncorrected strabismus. Esotropia (20%) was more common than exotropia (9%), and nearsightedness (40%) more common than farsightedness (13%) [72]. Anterior chamber abnormalities have also been reported to be more common in girls with TS and may present as congenital glaucoma [73]. Eight to 10% of the patients are also red-green color blind, an X-linked recessive trait, related to an X-linked mutation on the single X chromosome in 45,X TS [72].

Renal Abnormalities

Renal malformations occur in approximately 35–40% of individuals with TS [74, 75]. Of those with malformations, about half have abnormalities of the 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 [74]. Developmental abnormalities of the kidneys and collecting system predispose to urinary tract infections and possibly hypertension [75]. Vascular supply anomalies are observed with higher frequency [75].

Gastrointestinal Disorders

Elevated liver enzymes are often observed in patients with TS, who are usually asymptomatic. In a study of 218 adults with TS (mean age = 33 years), 36% had one or more liver enzyme levels higher than the reference level, the most prevalent being gamma-glutamate transferase (GGT) [76]. 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 [77]. An autoimmune pathogenesis may be also be operative in some cases since many have elevated antinuclear and/or anti-smooth muscle antibodies [78].

Celiac disease, an immune-mediated disease of the small intestines triggered by the ingestion of gluten-containing grains, occurs in about 6% of those with TS, a prevalence nearly ten times that in the general population. Although it can cause bloating, abdominal pain, and malabsorption, it may also present with growth failure alone [79]. Inflammatory bowel disease occurs in about 3% of those with TS [80], with Crohn's disease being at least as common as ulcerative colitis. Gastric and intestinal hemangiomas, telangiectasias, and phlebectasias are rare, but can produce massive gastrointestinal bleeding when present [81, 82].

Dermatological Problems

Individuals with TS often have nail dysplasia. Fingernails and toenails are small, hyperconcave, and deeply implanted secondary to the presence of lymphedema in utero. Hemangiomas are more common than in the general population and may be related to lymphatic abnormalities. They usually enlarge during the first year of life and then undergo slow regression. TS patients have an increased number of benign appearing melanocytic nevi (50%) that increase in size and number throughout childhood and particularly during adolescence [83]. Although there has been a theoretical concern for the effect of GH therapy on the growth of nevi, studies have failed to demonstrate a pathologic impact of GH therapy on the number or density of melanocytic nevi [84, 85]. Despite the increased numbers of nevi, the risk of melanoma in this population appears to be markedly reduced [86].

Pilomatrixomas, generally benign cutaneous tumors of the hair matrix cells, appear to be more common [87]. Other common skin problems include atopic dermatitis, seborrheic dermatitis, and keratosis pilaris [88]. Patients with TS have been thought to be at increased risk of keloid formation (hypertrophic scarring); however, this may simply reflect an increased frequency of surgeries involving the neck or upper chest, areas that are predisposed to hypertrophic scar and keloid formation. In one report, 5 of 92 patients with TS undergoing surgery developed keloids or hypertrophic scars [89].

Hypothyroidism and Other Autoimmune Disorders

Autoantibodies and autoimmune diseases are more common in individuals with TS than in the general population [90]. The most common autoimmune disorders in TS are Hashimoto's thyroiditis, celiac disease, and inflammatory bowel disease (IBD) [91]. In a study that evaluated 71 children with TS under 20 years of age (mean age of 11.4 years), 15.5% were hypothyroid, 17% were positive for thyroid peroxidase and/or thyroglobulin antibodies, and 33.8% had thyromegaly [92]. The frequency of thyroid abnormalities increased with age, with no abnormalities observed before 4 years of age. In one study, 18% of TS patients had celiac autoantibodies, and 26% of the antibody-positive patients had celiac disease (a prevalence of 4.5%) [93]. 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 [94]. TS patients may also be at increased risk for type I diabetes mellitus [57].

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 [57]. They were at increased risk for abnormalities constituting "the metabolic syndrome" including hypertension, dyslipidemia, type 2 diabetes, obesity, hyperinsulinemia, and hyperuricemia [57]. Body mass index (BMI) SDS begins to increase around the age of 9 years [95] and may exacerbate a tendency toward type 2 diabetes [96]. In one study of adult 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. VO2 max and physical activity were also significantly lower in TS. Interestingly, however, studies in adults have pointed toward β (beta)-cell failure rather than insulin resistance as the primary defect in glucose homeostasis [97, 98].

Gonadal Failure

Although the majority of girls with TS have gonadal failure and pubertal delay, some girls with TS enter puberty at a typical age. In an Italian retrospective multicenter study of 522 patients older than 12 years with TS, 32% of the girls with cell lines containing more than one X and 14% of nonmosaic, 45,X patients had spontaneous breast development [99]. Sixteen percent had spontaneous menarche that occurred at a mean age of 13.2 ± 1.5 years and a similar bone age. Although some developed secondary amenorrhea, others had regular menses for many years. Therefore, the diagnosis of TS should still be considered in girls with short stature, even if they are menstruating and should be considered in the differential diagnosis for women experiencing premature ovarian failure [100]. Spontaneous, unassisted pregnancy occurred in three patients (3.6%), of whom two had chromosomal abnormalities and malformations. Other studies have demonstrated a high risk of spontaneous abortion (25–40%), chromosomal abnormalities in the offspring (20%), and perinatal death (7%) [101]. When unassisted pregnancies occur, they are generally in patients with structural anomalies of the X chromosomes in which the Xq13-q26 region is spared or in patients with a mosaic karyotype containing a 46,XX cell line.

There is a broad range of gonadal dysfunction in TS, making it difficult to accurately predict who will enter puberty spontaneously and who will not. Traditionally, follicle-stimulating hormone (FSH) levels have been used. FSH follows a biphasic pattern in normal girls, with increased levels in infancy, decreased levels in childhood, and increased levels early in puberty. This pattern is exaggerated in girls with ovarian dysfunction. They have increased levels during the first 2 years of life which decline gradually to reach low levels (often indistinguishable from those in normal girls) between 5 and 10 years of age and rise again to castrate levels around the usual age for puberty [102, 103]. In healthy girls, serum inhibin B and estradiol levels follow a similar biphasic age pattern with high levels at 3 months of age and low levels during the prepubertal period [104, 105] with levels rising at puberty [106, 107]. In a longitudinal study of 70 TS girls with or without spontaneous puberty, Hagen et al. predicted ovarian failure in 20/20 patients with undetectable inhibin B on repeated measures, while 9/10 with detectable inhibin B entered puberty spontaneously [108].

Fertility is an area of great concern for women with TS [109]. Traditionally, assisted pregnancies using donor eggs have been the principal means by which women with TS achieved pregnancy. Recently, cryopreservation of ovarian tissue or oocytes from the TS individual has been offered in experimental protocols [110].

Girls with karyotypes containing Y material, such as 45,X/46,XY, are at increased risk for developing gonadoblastomas [111]. A gonadoblastoma is a gonadal tumor in which normal

components of the ovary, such as oogonia, granulosa-Sertoli-type cells, and thecal-Leydig-type cells, are present in varying amounts within circumscribed nests. The latter may produce sex steroids, which may cause virilization or occasionally feminization, depending on the predominant sex steroid produced. Although the pure gonadoblastoma is not a malignant tumor, the germ cell component may invade the ovarian stroma, producing a germinoma which is potentially malignant [112]. Occasionally a more malignant tumor, such as embryonal carcinoma or choriocarcinoma, may develop in a gonadoblastoma.

Using standard cytogenetic techniques, approximately 5% of patients with TS have Y chromosomal materials, and of those, gonadoblastoma has been thought to develop in 15–25%. Fluorescence in situ hybridization (FISH) studies using Y-specific DNA probes have demonstrated that the percentage of girls with TS having Y chromosome material is higher; however, the occurrence of gonadoblastoma in this population seems to be low [113]. Therefore, cytogenetic screening for Y chromosome material on peripheral karyotype is the current screen for increased risk for gonadoblastoma.

Learning Disabilities

Individuals with TS are at increased risk for specific neurocognitive deficits and problems in psychosocial functioning. These problems include deficits in visual-spatial/perceptual abilities, nonverbal memory function, motor function, executive function, attention, and social skills [114, 115]. Abnormalities in cognitive function in TS are accompanied by structural differences in older children and adult brains. For example, parietal lobes, parietal-occipital areas, and prefrontal areas, areas known to be associated with visuospatial processing, are small when compared with controls [116].

Although their distribution of verbal IQs is relatively normal, lower performance, and full-scale IQs are more prevalent in this population due to lower scores on performance than verbal tasks. For example, results of Wechsler IQ tests in 226 women with TS revealed a 12-point discrepancy between mean verbal and performance IQs (101 versus 89). This verbal-performance IQ discrepancy, consistent with a nonverbal subtype of learning disability, has not been well correlated to age or karyotype [117].

The neurocognitive deficits put them at a higher risk for educational problems. Rovet found that 48.2% of girls with TS versus only 20% of control subjects were recognized by their parents as having problems at school [118]. Mathematics is particularly problematic, and girls with TS score significantly lower than control subjects in overall arithmetic achievement. In Rovet's study, girls with TS obtained a mean global mathematics score 2.1 grades below their current placement level [118]. In a more recent study, a higher percentage of girls with TS made operation and alignment errors on a mathematics calculations test than did controls or another group with mathematic difficulties (fragile X syndrome) [119]. 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 some evidence to suggest that obsessive-compulsive tendencies [120] and autism are more prevalent [121].

Tumors and Miscellaneous

Besides gonadoblastoma, the risks for most cancers do not appear to be elevated in TS. Exceptions may include colon cancer [57], neuroblastoma [122], and pilomatrixomas [87].

Diagnostic Guidelines

The diagnosis of TS requires that the individual have both phenotypic features and genetic features of TS. That is, they must have one or more

clinical features such as short stature as well as deletion of the distal end of Xp (Xp11.2-p22) where the majority of genes associated with TS features appear to reside. Recent guidelines established by the American College of Medical Genetics (ACMG) [123] suggest criteria for diagnoses in the prenatal versus postnatal periods and investigation of Y chromosome mosaicism as outlined below.

Most prenatal diagnoses of TS are made when karyotypes on amniotic fluid (less commonly by chorionic villous biopsy) are obtained for advanced maternal age, an abnormal maternal serum screen or abnormal fetal ultrasound. Although serum screens in pregnancy have been designed to pick up diagnoses of trisomies 13, 18, and 21, elevated levels of human chorionic gonadotropin (hCG) and inhibin and slightly low levels of alpha fetoprotein (AFP) and unconjugated estriol are associated with an increased risk of TS. Other fetuses with TS have karyotypes obtained for abnormalities such as increased nuchal thickness, cystic hygroma, or hypoplastic left heart. For fetuses diagnosed "incidentally," most have a mosaic karyotype. Although those with 45,X/46,XX mosaicism can have a severe phenotype, most will be phenotypically 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. 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.

Table 7.1 Guidelines: Screening girls for Turner syndrome

Karyotype any girl with one or more of the following[a]
Unexplained growth failure
Webbed neck
Peripheral lymphedema
Coarctation of the aorta
Delayed puberty
or
Any girl with at least two or more of the following
Nail dysplasia
High arched palate
Short 4th metacarpal
Strabismus

Adapted from Savendahl L, Davenport ML. Delayed diagnoses of Turner's syndrome: proposed guidelines for change. J Pediatr. 2000;137(4):458, with permission from Elsevier

[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 glasses

Genomic copy number microarray studies can be used to characterize genetic abnormalities but should not be used as a frontline screen for TS since 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 peripheral blood karyotype testing and the TS is still being considered based on the clinical features, then 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 [34].

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 to a center with growth retardation (less than −2 SD) and/or decreased height velocity identified 18 cases of TS, an incidence of 4.8% [124]. To facilitate timely diagnoses, Savendahl and Davenport have

suggested guidelines for screening girls for TS [34]. Modified guidelines are presented in Table 7.1.

Therapy

Introduction

The patient should be referred to a physician expert in the care of individuals with TS if at all possible. Primary care physicians and involved subspecialists should be aware of published consensus guidelines for their health supervision, most recently outlined by Carolyn A. Bondy for the Turner Syndrome Consensus Study Group in 2007 [2]. Health-care checklists can serve as reminders for routine evaluations. An example of one such checklist recently published by one of the authors [125] is presented in Table 7.2.

Lymphedema

Lymphedema usually improves over the first few months of life. However, it may be severe or recur with puberty or hormone replacement therapy [126]. Combined decongestive therapy (CDT) which uses manual lymphatic drainage, bandaging, exercises, skin care, and low stretch support garments is an effective and noninvasive treatment [127, 128].

Dysmorphic Features

Plastic surgery may be recommended for some individuals with severe webbed neck and/or ear anomalies. With all surgeries, the risk of keloid formation must be considered [129].

Cardiovascular Abnormalities

At diagnosis, all patients should have an imaging study done: echocardiography for young children and echocardiography plus cardiac MRI for adolescents and adults. Girls who had imaging studies performed when in utero should be reimaged after birth. Patients who underwent echocardiography only during childhood should be reimaged with a cardiac MRI when they can do so without sedation. An electrocardiogram should be done along with the imaging studies to evaluate for conduction/repolarization defects/arrhythmia. A pediatric cardiologist should direct the care of any patient in whom a cardiovascular malformation is detected. If appropriate, prophylactic antibiotics should be prescribed to prevent subacute bacterial endocarditis. Even in those with a normal baseline cardiovascular structure, a cardiology evaluation and imaging procedure should be repeated during adolescence and every 3–5 years thereafter to rule out dilation of the aortic root, a process that can be advanced even in the absence of clinical findings or other cardiovascular pathology. Patients should be encouraged to carry a medical alert card and demand evaluation for aortic dissection if they experience the sudden onset of chest pain. Blood pressure should be closely monitored. Hypertension is common, often worsens with age and obesity, and is the most easily modifiable risk for circulatory disease in this high-risk population. The risk for aortic dissection or rupture during pregnancy may be 2% or higher [130]. Therefore, TS is a relative—and sometimes absolute—contraindication for pregnancy. For example, some have recommended that coarctation of the aorta and/or BAV be absolute contraindications for pregnancy. Any woman considering pregnancy should consult with a cardiologist and be monitored carefully throughout the pregnancy.

Short Stature and Skeletal Abnormalities

Introduction

Once the diagnosis of TS is made, growth should be assessed regularly using a TS-specific growth chart. Use of a TS-specific growth chart will facilitate detection of concurrent problems that affect growth, such as hypothyroidism, and aid in

Table 7.2 Health-care checklist for individuals with Turner syndromea

Problems	Screening test/referral	Timing of tests			
		At Dx	Q visit	Q year	Other
Hip dislocation	Physical exam (including height, weight, BP, and calculation of BMI)	X	In infancy		
Feeding problems		X	In infancy		
Strabismus		X	4 mo–5 yrs		
Otitis media		X	All childhood		
Growth failure		X	All childhood		
Pubertal delay		X	Adolescence		
Scoliosis/kyphosis		X	While growing		
Dysplastic nevi		X	School-age on		
Lymphedema		X	Lifelong		
Hypertension		X	Lifelong		
Needs information/support	Refer to TSS, other support groups	X			
Structural renal abnormalities	Renal ultrasound	X			
Cardiac abnormality[b]	Exam by cardiologist; EKG; MRI/echo	X			Q 5–10 yrs
Conductive and SNHL	Formal audiology exam	X			Q 1–3 yrs
Gonadal dysfunction	FSH, LH	X			At ages 0.5–3 and 10–12
Strabismus and hyperopia	Formal eye exam	X			At 1–1.5 years
Celiac disease	Serum IgA, TTG IgA Ab	X			Q 2–5 yrs [begin ~age 4]
Autoimmune thyroid disease	T4, TSH	X		Begin ~age 4	
Developmental, educational, and social problems	Developmental, educational, and/or psychosocial exam	X			Before school entry
Palatal/occlusive abnormalities	Orthodontic evaluation				At age 7
Sexuality; school and/or work plans	Counseling			Begin ~age 10	
Renal and liver dysfunction	Cr, BUN, LFTs, CBC	X		Begin ~age 15	
Metabolic dysfunction	Fasting BG and lipids			Begin ~age 15	
Low BMD	DEXA scan				At ~age 18
GH action	IGF-I/IGFBP-3			During GH tx	

Reproduced with permission, Davenport ML. Approach to the patient with Turner syndrome. J Clin Endocrinol Metab. 2010;95:1487–1495, *Copyright 2010, The Endocrine Society*
Abbreviations: *BMD* bone mineral density, *BUN* blood urea nitrogen, *CBC* complete blood count, *cr* creatinine, *Dx* diagnosis, *BP* blood pressure, *Echo* echocardiogram, *EKG* electrocardiogram, *exam* examination, *IgA* immunoglobulin A, *IGFBP-3* IGF binding protein-3, *LFTs* liver function tests, *mo* months, *MRI* magnetic resonance imaging, *PE* physical examination, *SNHL* sensorineural hearing loss, *TTG IgA Ab* tissue transglutaminase IgA antibodies, *Q* every, *TSS* Turner Syndrome Society, *tx* treatment, *yrs* years

[a]These guidelines were adapted from Davenport and Calikoglu [188] and Bondy (A Guideline of the Turner Syndrome Study Group) [2, 3] and reflect the author's clinical practice. They suggest *minimal* routine screening evaluations. If the patient has a problem in one or more areas, she will generally be followed up by a specialist in those areas and evaluated more frequently

[b]If diagnosed in infancy or early childhood, an echocardiogram may be performed. An MRI should be obtained once the child is able to undergo an MRI evaluation without sedation

the evaluation of growth-promoting therapies. Growth charts for American girls [131] and Northern European girls [132] ages 0–3 years are available as well as growth charts for girls ages 2–18 years [133]. These growth data are applicable to girls with TS from the United States. Untreated patients are expected to follow a percentile on the TS curve throughout childhood and adolescence. As for the normal population, there is a strong genetic component to each individual's growth pattern. In fact, the height of any individual with TS is expected to be about 20 cm less than that of their midparental height (MPH).

The goals of hormonal therapies for growth are to (a) attain a normal height for age early in childhood, (b) progress through puberty at a normal age, (c) attain a normal adult height at a normal age, and (d) avoid the adverse effects of therapy [134]. Clinical observation has suggested, but not proven, that 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 careers [135].

The timing and administration of hormonal therapies for girls with TS are still evolving as experience is gained in their use. GH is the agent of choice. Clinical trials of GH have demonstrated that GH improves final height in girls with TS [136, 137]. When given in conjunction with GH therapy, anabolic steroids appear to have a beneficial effect [138–142]. However, anabolic steroids, including oxandrolone, when used alone, increase short-term height velocity, but do not appear to improve final height. Recent studies also suggest synergistic effects on growth with the combination of very low doses of estrogen and GH [137].

Effects of GH on Linear Growth

GH therapy is considered standard of care for girls with TS who have linear growth failure [2]. Girls with TS are not GH deficient, but supraphysiological doses of GH drive growth. Demonstration that GH is effective in increasing adult stature was firmly established in 2005 with publication of the first randomized, controlled study of GH therapy in girls with TS to final height [136]. Girls who were in the treatment arm received GH supplementation (0.30 milligram per kilogram per week (mg/kg/week) divided into 6 doses/week). After a mean of 5.7 years, the girls in the GH arm averaged 7.2 cm taller than those in the control arm. This increase in adult height is roughly average for the many studies in which height gains achieved by GH therapy were compared with the growth of historical controls and varied from no significant change to as high as 17 cm [115]. Factors that determine the effect of GH on height include age at initiation of therapy (the earlier the better), GH dose (the higher the better), use of anabolic steroids (an additive effect), and age at initiation of feminizing doses of estrogen (the later the better) [143].

Of the factors determining GH efficacy, young age at GH initiation is the most important [136, 144]. A randomized, controlled 2-year "Toddler Turner" study of GH therapy in 89 girls with TS whose treatment was initiated between the ages of 9 months and 4 years (mean age, 2.0 ± 1.0 year) indicated that GH therapy is effective beginning in infancy [145]. After 2 years, 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). These girls are being followed to final height in an observational study.

Early normalization of height has a number of potential benefits for girls with TS: negating the physical limitations of short stature, prevention of stature-related juvenilization, improvement in peer group integration, and the opportunity to initiate estrogen replacement at a physiologically appropriate age [145–147]. It is now recommended that treatment with GH begin as soon as growth failure (decreasing height percentiles on the normal curve) is demonstrated [2].

GH therapy should be optimized for each individual, given its high costs and potential risks. In one study, untreated patients with TS were treated initially with a GH dose of 0.23 mg/kg/week that was doubled or tripled when growth velocity declined to less than twice that of its pretreatment level. The estimated final height benefit was 10.6 ± 3.8 cm compared to 5.2 ± 3.7 cm in a 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 29% in the fixed dose group [148].

Early work by Rosenfeld et al. demonstrated an additive effect of oxandrolone, an anabolic steroid that cannot be aromatized to estrogen, on final adult stature when compared with historic controls [149]. 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, 10.3 ± 4.7 cm taller than their mean projected adult height [149]. Recently, Menke et al. examined the effect of oxandrolone in a randomized, placebo-controlled, double-blind, dose–response study in the Netherlands [138]. 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 those in 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.

It has long been thought that adult stature is improved by delaying estrogen therapy as long as possible [150]. Chernausek et al. conducted a multicenter study in which 60 girls starting GH therapy were randomized to initiate estrogen therapy (conjugated estrogens begun 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 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.

However, more recent studies indicate that good, if not better, height results can be obtained by starting estrogen therapy (specifically estradiol) at 12 years of age than at ages 14 years and above [151, 152]. Most reassuring is a recently published randomized, placebo-controlled study to adult height of girls with TS ages 5–12.5 years of age 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 doses. GH treatment (0.3 mg/kg/day divided into 3 dosed 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). The modest growth benefit observed with the combination of ultralow-dose childhood estrogen replacement and GH suggests that the practice of delaying estrogen therapy should be reconsidered.

Effects 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. GH treatment appears to exacerbate the disproportionate growth of feet and to modestly improve the disproportion between height and sitting height. There is no significant effect on relative width of the shoulders and pelvis [153].

Effects of GH on Bone Mineral Density

For girls with TS, GH therapy is likely to help maintain prepubertal bone mineral density (BMD) [154]. Preliminary BMD data on patients after long-term GH therapy show an absence of osteopenia [155].

Effects of GH on Craniofacial Development

GH therapy in girls with TS has not been demonstrated to have a significant effect on craniofacial

growth [156], with the exception, perhaps, of an increase in the length of the mandible [157]. 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 complete by age 6, whereas the synchondrosis of the mandible does not close until late adolescence and can be reactivated in adulthood. 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 rate of dental development.

Effects 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 very important question, and controlled studies are unlikely 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 [158], 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 [159]. 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 [160]. 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, greater popularity, and experiencing less teasing than the untreated group [161]. 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 final height [162].

Safety of GH Therapy

Extensive postmarketing surveillance programs have documented that side effects of GH therapy in children are relatively rare. However, families should be fully apprised of the risks associated with GH therapy, many of which are more common in girls with TS than other patients receiving GH. In the most extensive report to date, a study of 5,220 girls with TS who received GH revealed higher incidences for disorders for whom 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 [163]. 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 degree of pigmentation of nevi. No adverse effects of GH on cardiac size, aortic diameter, or cardiovascular function have been found [164, 165].

Increased insulin resistance has been of considerable concern in this population at high risk for diabetes. Insulin resistance increases, but there is generally no effect on glucose levels [166, 167]. In a study in which girls with TS 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 levels decreased to values close to or equal to pretreatment values after discontinuation of GH treatment [168]. 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 [169].

Safety of Anabolic Steroids

Side effects of anabolic steroids may include (a) virilization with development of acne, deepening

of the voice, and growth of facial hair; (b) transient elevation of liver function tests; (c) insulin resistance; and (d) premature skeletal maturation. It has been known for more than two decades that mild virilization can occur when oxandrolone is used at a dose of 0.125 mg/kg/day [170]. Recently, virilizing effects were documented at a dose of 0.0625 mg/kg/day, a dose commonly used in clinical practice, and slower breast development was reported at that dose as well as a lower dose of 0.03 mg/kg/day po [138].

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, since 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 [171]. Because GH therapy for this population is a pharmacological one, it requires somewhat higher doses than those used for GH-deficient patients. Standard GH therapy in the USA for TS is 0.375 mg/kg/week divided into 6 or 7 doses. Division of GH dosing into two shots per day does not appear to be advantageous over one shot per day [172]. 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).

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).

Orthodontic Problems

Because many girls with TS have orthodontic problems (narrow maxilla with high-arched and narrow palate, micrognathic mandible), early evaluation by an orthodontist is suggested. The timing of any orthodontic treatment to address dental malocclusion should take into consideration long-term growth-promoting therapies that may alter tooth and jaw alignment. In addition, because the dental roots are short, unnecessary tooth movement should be minimized to avoid root resorption and loss of teeth. Because of this, TS girls should be evaluated by a pediatric dentist around 2 years of age, and by an orthodontist around 7 years of age [2, 189].

Hearing Loss

Screening for hearing problems should be done as soon as the diagnosis of TS is made. Children with TS should have pneumatic otoscopy done at every visit to the physician, and tympanometry should be considered if otoscopy is equivocal. Any child who has fluid in both middle ears for a period of 3 months should undergo an evaluation by a pediatric otolaryngology [173]. In general, tympanostomy tubes are recommended when bilateral conductive hearing deficiency of at least 20 dB HL is associated with bilateral effusions for a period of more than 3 months. Chronic or recurrent middle ear disease should be managed aggressively to minimize the likelihood of conductive middle ear damage and permanent hearing loss. In patients with TS, early tube placement may be justified since otitis media with effusion is less likely to resolve spontaneously than in the normal population.

Because infection of the tonsils and adenoids may contribute to sinus and middle ear infections, tonsillectomy and/or adenoidectomy may have to be considered as additional therapeutic options for some patients. Patients should be warned to protect their hearing by avoiding exposure to loud noises unless appropriate ear protection is in use. Audiology evaluation should be part of the hearing screening both at the time of diagnosis, and during monitoring of the hearing function. It should be obtained on an annual basis for patients with ongoing problems of otitis media, or who have already developed hearing loss. In those TS patients without hearing loss, audiological surveillance should still occur every 2–3 years.

Strabismus and Other Eye Problems

Children should be evaluated for strabismus at every clinic visit between the ages of 6 months and 5 years of age. Because early correction of visual alignment is critical for normal binocular vision to develop, any child with strabismus should be referred immediately to an ophthalmologist for further evaluation. In fact, it may be prudent for every child with TS to have an ophthalmology exam before or at 2 years of age.

Renal Abnormalities

All patients with TS should be routinely screened by ultrasound for renal abnormalities. If no urinary tract abnormalities are found, the kidney evaluation can stop here, as it has been shown that during an average of 6 years of follow-up these patients do not develop renal problems [74]. If renal abnormalities are found, additional testing will be directed in collaboration with a pediatric nephrologist, as these patients are at increased risk of hypertension and urinary tract infection.

Gastrointestinal Disorders

GI bleeding should be in the differential diagnosis of children with anemia. Celiac disease and IBD should be considered for those with unexplained weight loss. Celiac disease should be screened for (by measurement of tissue transglutaminase IgA antibody titer) after the patient turns 4 years of age and repeated every 2–5 years, or as indicated by the review of systems. In older TS patients, rescreening for celiac disease also depends on the clinical presentation. Screening for liver disease has also been recommended during the school-age years and thereafter. It may be more important to do so in those patients who are obese, or are taking oral estrogen or androgen therapies. Steatosis and steatohepatitis or more serious pathologies need to be considered if an upward trend in the liver transaminases is detected.

Dermatological Problems

Because an increased tendency for keloid and hypertrophic scarring has been reported in TS, until the exact risk for the development of these lesions after surgical procedures is better understood, patients should be forewarned before having their ears pierced or undergoing surgical procedures that keloids may arise. The risk of keloid formation should certainly be discussed when cosmetic surgery is being considered, as the neck and chest are known to be higher-risk areas for these lesions. The patient, family, and physician should examine nevi regularly to look for dysplastic features such as asymmetry, border irregularity, color variability, and diameter greater than 5 mm. Patients should also be advised to limit sun exposure, as TS patients tend to have more of these nevi, especially on the skin of the face, neck, and upper extremities.

Hypothyroidism and Other Autoimmune Disorders

Because autoimmune hypothyroidism may develop insidiously, thyroid function tests (thyroxine, thyroid stimulating hormone) should be monitored yearly from 4 years of age onward. For those growing poorer than expected, antibody studies for celiac disease should be obtained.

Those with positive tissue transglutaminase antibodies should be referred for evaluation by a gastroenterologist.

Obesity, Lipids, and Glucose Homeostasis

Patients should be encouraged to maintain their weight within an appropriate range for height and receive early dietary counseling if necessary. Girls with risk factors for type 2 diabetes mellitus should be screened with fasting blood glucoses or a modified OGTT.

Gonadal Failure

The goals of estrogen replacement therapy in girls with TS are to normalize developmental changes in secondary sex characteristics including breast size and shape, uterine size and shape for possible reproductive function, bone growth and mineral accrual, cardiovascular function, and other estrogen-dependent processes.

Estrogen deficiency can exacerbate several problems associated with TS. In adult women, estradiol deficiency is known to cause cancellous bone loss, endothelial dysfunction, decreased insulin production, an abnormal lipid pattern, increased central adiposity, and early atherosclerosis. Indeed, oophorectomy is an independent predictor of myocardial infarction and coronary death. Estrogens are likely to be important in childhood as well. Estrogens are produced from birth and can be measured in the serum throughout childhood in girls when sensitive assays are used. The normal mid-childhood ovary is not entirely quiescent; plasma estradiol concentrations in healthy prepubertal girls, albeit low, are up to eight times as high as those in boys [174].

There are many options for hormone replacement therapy. However, systemic administration of estradiol, usually by transdermal application in a patch or gel, is the only form of therapy to achieve natural levels of estradiol in blood [175]. Oral estradiol undergoes extensive hepatic first pass metabolism, with most of it being transformed to estrone sulfate. Ethinyl estradiol, a potent synthetic estrogen with little hepatic metabolism used in the Ross low-dose estrogen trial [137], is not available commercially in the United States. Conjugated equine estrogen (CEE) contains multiple sex steroids, some of which are not found in humans. Although it was historically the estrogen of choice in the United States, there is no longer justification for its use in children.

Both oral and transdermal estrogens prevent atherosclerosis. However, oral estrogens have been demonstrated to increase resistance to activated protein C, decrease antithrombin III, increase C-reactive protein, increase GH resistance, increase SHBG, and cause triglyceride enrichment of low-density lipoprotein and high-density lipoprotein particles. TDE has little effect on these parameters [176]. None of these studies has been carried out in large randomized trials and most have involved postmenopausal women; therefore, their applicability to adolescents and young adults with TS remains to be proven. In a recent short-term metabolic study of GH-treated girls with TS, neither oral nor transdermal estrogen adversely affected measured metabolic parameters [177]. However, in another study in which prepubertal GH-treated girls with TS were randomized to oral conjugated estrogen or TDE for 1 year, the transdermal group had significantly greater increases in spine bone mineral density (BMD) and uterine growth [178].

A suggested protocol for puberty induction using TDE patches is presented in Table 7.3. Beginning estrogen replacement in early adolescence (11–12 years of age) allows puberty to begin and to progress at a normal age [179].

If TDE cannot be used, oral estradiol or ethinyl estradiol should be considered. The following doses are considered equivalent although equivalency depends on which assays and clinical end points are used: 100 μg TDE, ~2 mg oral estradiol, ~20 μg ethinyl estradiol, and ~1.25 mg CEE. Oral contraceptives should be avoided if possible because they have unnecessarily high estrogen/progestin concentrations.

Androgen replacement therapy is not standard of care; however, a recent randomized, double-

Table 7.3 Pubertal induction and maintenance estrogen therapy using transdermal estradiol (TDE): a protocol using low "growth-promoting doses" for 18–24 monthsa

Treatment (months)	Target E2 (pg/ml)[b]	E2 dose	Notes
			Consider initiation of puberty at age 11–12 years if there is no breast development
0	3–4	0.1 μg/kg	Cut patch and apply piece overnight only. Replace every night. Check E2 levels in a.m. before patch is removed
6	3–4	0.1 μg/kg	Apply piece of matrix patch continuously[c]. Check E2 levels any time. Change patch as directed (once or twice weekly)
12	6–8	0.2 μg/kg	
18	~12	12.5 μg	E2 levels below this are believed to accelerate growth more than bone maturation
24	~25	25 μg	
30	~37	37.5 μg	
36	~50	50 μg	Start progestin (earlier, if breakthrough bleeding occurs): 200–300 mg micronized oral progesterone for ~12 days/month qhs (causes drowsiness); or 5 mg oral medroxyprogesterone for ~12 days/month
42	~75	75 μg	
48	50–150	100 μg	Typical adult dose; may not be high enough to protect liver, arteries, etc.

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Abbreviations: *CRP* C-reactive protein, *E2* 17β estradiol, *qhs* before bedtime, *SHBG* sex hormone-binding globulin

[a]This protocol is but one of many that can be used. This specific protocol is utilized in the author's clinic and individualized depending upon patient circumstances and desires. For example, older girls may wish to be started at 25 μg

[b]To convert pg/ml to pmol/L, multiply by 3.671. E2 levels should be monitored using liquid chromatography/tandem mass spectroscopy (LC/MS/MS) technology

[c]Vivelle Dot, matrix transdermal patch, is small and tends to adhere well

blind, placebo-controlled, crossover pilot study of androgen replacement therapy (1.5 mg methyl testosterone) in TS demonstrated significant beneficial effects on bone health, lipid profile, body composition, and QOL, whereas cognitive functions were variably affected [180].

Long-term treatment with estrogen and progestin that is initiated during mid- to late adolescence and is continued throughout adulthood appears necessary for a normal peak bone mass to be achieved. Additional measures to prevent osteoporosis should be used, such as ensuring adequate calcium intake (>1,000 mg of elemental calcium daily in the preteen years, and 1,200–1,500 mg daily after 11 years of age), encouraging weight-bearing activities, and avoiding overtreatment with thyroid hormones [155].

Because gonadoblastomas may occur as early as young childhood, prophylactic gonadectomies are recommended at the time of diagnosis for most girls with karyotypes containing Y-chromosome material or marker chromosomes identified as Y material by FISH [181]. Recommendations for those found by PCR technique alone are less clear.

The reproductive options for women with TS continue to expand. For the rare woman who is fertile, prenatal amniocentesis is recommended because of the high rate of chromosomal abnormalities. Those who are sterile may choose to adopt children or undergo artificial fertilization. The clinical pregnancy rate achieved by embryo transfer in women with TS appears to be similar to that of other oocyte recipients with primary ovarian failure; however, a greater percentage (40%) end in miscarriage [182]. The greater miscarriage rate may be the result of uterine factors and an increased incidence of genetic abnormalities. Therefore, preimplantation genetic diagnosis should be performed before embryos are transferred. In addition, chorionic villous sampling and amniocentesis should be offered to all TS patients

who become pregnant. Careful assessment before and during follow-up of pregnancy is important because of the increased risk of cardiovascular and other complications. Several women with TS have died during pregnancy from aortic dissection. Many physicians have recommended that only one embryo be transferred at a time to avoid the additional complications caused by twin pregnancy [130, 183]. No matter how pregnancy is achieved, careful screening of the cardiovascular system is requisite given the increased cardiovascular demands [184]. There is continuing controversy about which women with TS should be allowed to undergo assisted reproduction. Absolute contraindications to pregnancy are surgically repaired coarctations or uncontrolled hypertension, while bicuspid aortic valve is viewed by many as a relative contraindication [185].

Learning Disabilities

Children with TS should undergo a baseline developmental evaluation at the time of initial diagnosis or at latest, during the preschool years. Academic tutoring, occupational therapy, and training in problem-solving strategies can help girls and women with TS cope with their visual-spatial and cognitive challenges. Individual psychotherapy may be required to address the social and emotional difficulties commonly experienced by individuals with TS [186]. Timely institution of estrogen therapy may be important since there is evidence that some neurocognitive deficits such as memory, reaction time, and speeded motor function result from estrogen deficiency and are at least somewhat reversible with estrogen treatment [187]. Many patients and their families benefit tremendously from support group programs, such as those offered by the Turner Syndrome Society of the United States (TSSUS), which can be accessed at http://www.turnersyndrome.org.

Tumors

Tumors in the TS population should be approached the same as those in the general population.

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Management of Adult with Childhood-Onset Growth Hormone Deficiency

8

David Michael Cook

Abstract

The patient with childhood-onset growth hormone deficiency (COGHD) with growth as the major endpoint of therapy during childhood presents a number of diagnostic and therapeutic challenges 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 the transition period remain persistently growth hormone deficient (GHD). These are predominantly in the "idiopathic" COGHD category, while the patients with severe pituitary damage represent the patients who almost always remain GH deficient. Preparation for diagnosing and treating these patients is the subject of this report. The stage for informing the patient and his or her family 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 GH therapy.

Keywords

Childhood-onset GHD • Transition patients • Hypopituitarism • Diagnosing growth hormone deficiency and pituitary tumors

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Introduction

The patient with childhood-onset growth hormone deficiency (COGHD) with growth as the major endpoint of therapy during childhood presents a number of diagnostic and therapeutic challenges 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

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period. Not all children entering the transition period remain persistently growth hormone deficient (GHD). These are predominantly in the "idiopathic" COGHD category, while the patients with severe pituitary damage represent the patients who almost always remain GH deficient. Preparation for diagnosing and treating these patients is the subject of this report. The stage for informing the patient and his or her family 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 GH therapy.

What Are the Differences Between Adult-Onset GHD (AOGHD) and Childhood-Onset (COGHD)?

The cause of GHD in children is usually idiopathic, associated with isolated GHD and discovered because of poor growth. In adults GHD is usually associated with some form of pituitary damage including a pituitary tumor, trauma to the pituitary, or irradiation to the brain. In adults this happens later in life and many times loss of additional hormones not just GH; it is no wonder the two syndromes are different. Phenotypic differences are less when children have a cause of GHD associated with multiple hormone deficiencies such as craniopharyngioma. Compared with patients with AOGHD, patients with COGHD have lower BMI, lower waist-to-hip ratio, lower serum insulin-like growth factor (IGF-1) and IGF-binding protein (IGF-BP3), and better QOL scores [1]. In contrast, patients with COGHD have more severe consequences compared to AOGHD in reduced muscle mass [2], bone mass [3], and cardiac function [4].

Causes of GHD in Transition Patients

Approximately 6,000 new cases of adults with GHD are diagnosed each year in the United States [5] with 15–20% of those cases representing pediatric cases coming from patients transitioning to the adult indication. Many of the adult cases thought to be idiopathic have now been recognized as due to head trauma which may have been seemingly slight or inconsequential but enough to cause pituitary or hypothalamic injury or interruption of the blood supply from the hypothalamus to the pituitary [6–10]. Table 8.1 gives an approximation of diagnostic causes of COGHD in one series [11]. Note the predominance of idiopathic COGHD as would be expected. Table 8.2 [12–15] is a synopsis of different society opinions over the last decade as to the diagnostic and treatment criteria for persistent GHD in the transition-period patient. Table 8.3 indicates the reasons to treat included in the various society consensus guidelines; note that all agree that the COGHD patient should be continued on therapy if proven to be persistently GHD, but adults with isolated idiopathic GHD have not been included all agree that the COGHD patient should be continued on therapy if proven to be persistently GHD, but that in adults, with isolated idiopathic GHD, has not been included in most guidelines; the reasons for this have been an abuse of this category in adults to overdiagnose many patients as GHD who are not clearly deficient.

Table 8.1 Etiology of COGHD in one selected series [11]

Idiopathic	1,366	72.3%
Organic	424	22.4%
Infection	6	0.3%
Craniopharyngioma	149	7.9%
CNS tumor	144	7.6%
Trauma	34	1.8%
Irradiation	73	3.8%
CNS defects	13	0.7%
Histiocytosis	5	0.3%
Septo-optic dysplasia	100	5.3%

Note the preponderance of idiopathic etiology in this cohort
Adapted from Ref. [11]

Which Patients Remain Persistently GH Deficient After Childhood?

The various causes of GH deficiency (GHD) in children differ dramatically from etiologies of

Table 8.2 Published consensus statements of COGHD patients transitioning to adult care

Society	Year	Journal
Eur Soc for Peds Endo	2005	Eur J Endo 152 p 165 (ref #12)
Endo Soc	2006	JCEM 91 p 1621 (ref #13)
GRS and Eur Peds Endo	2007	Eur J Endo 157 (ref #14)
AACE	2009	End Prac 15 Oct 2009 (ref #15)

Table 8.3 Approved diagnoses causing GHD in AGHD and COGHD

Society	Patient categories
GRS 1998	Pit/hypothalamic disease, COGHD, not TBI, isolated idiopathic adult GHD is OK
Endo Society 2006	Pit/hypothalamic disease, COGHD, TBI, isolated idiopathic GHD is OK
GRS 2007	Pit/hypothalamic disease, COGHD, TBI, isolated idiopathic GHD not OK
AACE 2009	Pit/hypothalamic disease, COGHD, TBI, isolated idiopathic GHD not OK

The idiopathic category is acceptable in childhood onset but not adult onset

GH deficiency in adults. Most adults, for example, have readily recognizable causes of pituitary insufficiency due to obvious insults such as a tumor of the pituitary, surgery to remove the tumor, or irradiation as part of the therapy of that tumor. There are, of course, a variety of other causes, but they are less common. More recent publications have suggested that there is an increased awareness of idiopathic causes of GHD in adults. This is similar to the pediatric experience where idiopathic causes predominate over organic causes. Brabant, looking at a large database of causes of GHD in adults, has observed that the incidence of an idiopathic etiology in adults has increased over the last decade [16]. One such explanation for this may be the increased awareness of head trauma or traumatic brain injury (TBI) might masquerade as an idiopathic etiology. It has been appreciated that TBI-induced GHD might occur in seemingly insignificant head injury by history but still result in elements of hypopituitarism. Apparently just the right force can result in shearing of the blood supply to the pituitary to cause hypopituitarism. In adults, just the presence of a small tumor may affect normal pituitary function. It is estimated that patients with pituitary tumors who have all other hormones of the anterior pituitary intact will still have 25–35% chance of being GH deficient [8]. As increasing damage occurs to the pituitary from the tumor or therapy and other hormones are lost, there is an increased incidence of If 3 or 4 anterior pituitary hormones are lost, there is 95–100% chance of GH deficiency. If we couple the diagnosis of GH deficiency in adults with a low-serum IGF-1 and three or four anterior pituitary hormone deficiencies, the probability of proving GH deficiency improves to a virtual certainty. Hartman has suggested that if the IGF-1 serum concentration is <84 ng/ml with pituitary damage and 3–4 anterior pituitary hormones lost, there is a 98% chance the patient is GH deficient [17].

Childhood causes of GH deficiency differ from adult etiologies. A majority of childhood etiologies are idiopathic and not associated with known injury to the anterior pituitary (Table 8.2) [11]. Many of the children with isolated GH deficiency of childhood, who do not have other anterior pituitary hormone deficiencies and have an idiopathic cause, do not remain persistently GH deficient [18, 19]. For this reason, laboratory testing of this latter group is suggested to reconfirm the diagnosis.

The laboratory documentation necessary to reconfirm GH deficiency after childhood depends first upon the physician's clinical suspicion that the young adult might be persistently deficient. Not only does the clinician have to be suspicious, he or she must suggest to the patient that there is a need for continuous replacement therapy into adulthood. In the current medical climate, the patient or the family may challenge the physician

Table 8.4 Metabolic consequences of stopping GH after the childhood indication is completed (Reprinted with permission)

1. Weight gain
2. Body composition changes (more fat)
3. Decreased bone density
4. Decreased exercise performance
5. Decreased ability to concentrate or study

Table 8.5 Reasons given by patients for not continuing GH therapy after the completion of the pediatric indication (Reprinted with permission)

1. The patient does not want to return to "shots"
2. The patient "feels fine"
3. The patient was told that he/she could discontinue after growth potential is achieved
4. The patient lacks motivation
5. The patient is not currently insured
6. The patient is not going to any physician much less an endocrinologist

as to whether the child should be continued on GH therapy. Typical consequences of GH deficiency that occur after stopping GH therapy in the average 18- or 19-year-old patient are listed in Table 8.4. The accumulation of weight, especially in the truncal area, and decreased exercise performance are the most commonly reported complaints. Occasionally the young adult will notice a decrease in the ability to concentrate or study. The major impetus to return to therapy will often come from the patient. Unfortunately, the average child does not want to return to the daily routine of receiving GH injections. In the future, this pattern will certainly change as pediatricians begin to inform patients that GHD may persist after the pediatric indication is completed. A list of reasons why COGHD patients who would be proven to need GH into adulthood but do not continue on GH after the pediatric indication are given in Table 8.5. Once the patient agrees to resume therapy, the next step is to verify if the deficiency is persistent. For patients who are panhypopituitary from causes such as craniopharyngioma, the documentation necessary for insurance approval will usually only consist of a single IGF-1 subnormal serum concentration after being off with GH for 1 month or longer. For those with isolated idiopathic causes, the documentation must be more rigorous and include a low-serum IGF-1 concentration coupled with one GH-release stimulation test to confirm the diagnosis. As in children, there is no foolproof magic stimulation test. There have been a number of society guidelines for the diagnosis of GHD in the transition period. Table 8.2 lists these in chronologic order, and Table 8.6 lists the cut points for stimulation tests approved by the various societies for the transition patient. The unavailability of GHRH to use in the arginine plus GHRH test has forced clinicians to pick an approved provocative test that will convince not only the clinician but also the payors (insurers) that the patient is indeed GHD. The insulin tolerance test is still the gold standard for testing. The society guidelines suggest that the patient be off GH for 1 month or longer, and then a stimulation test and serum IGF-1 can be performed to confirm the diagnosis. The cutoff for insulin tolerance testing is 5 ng/ml or higher to be normal or below 5 ng/ml to confirm deficiency. The absence of ITT capability in the office setting for some makes an alternative test necessary. After insulin tolerance testing the next test suggested is the glucagon stimulation test. The dose of glucagon is 1 mg intramuscular or 1.5 mg if the patient is 90 kg in weight or heavier. Sampling of blood for glucose and GH during the ITT is every 30 min for 90 min. The glucose should go below 40 mg/dl. The sampling for growth hormone during the glucagon test is every 30 min for 3 h. It is not necessary to sample glucose during the glucagon stimulation test, but it is necessary during the ITT to confirm that a proper hypoglycemic stimulus has been achieved. The cutoff for the glucagon test is 3 ng/ml [20, 21]. There does not seem to be a problem with either ITT or glucagon to result in false-negative testing. Further refinement of this test may uncover some categories where a different cut point may be necessary as has been confirmed with the arginine/GHRH test. When it was available, the combined arginine/GHRH test was especially worrisome in the idiopathic cause of GHD in childhood which appears to be a deficiency of hypothalamic GHRH. Another theoretical problem associated with the combined

Table 8.6 GH stimulation tests and suggested cut points by various consensus groups for transitioning patients

Society/year	ITT	Arginine	Arg/GHRH	Glucagon
Eur Soc Peds/2005	<5	<5	<5	<5
Endo Soc/2006	<5.1	<1.4	<4.1	Not offered
GRS/2007	<6	Not in obese, no cut point	BMI dependant; 4, 8, 11	<3
AACE/2009	<5	<0.4	BMI dependant; 4, 8, 11	<3

All values in ng/ml

arginine/GHRH test is the false-negative rate observed in patients with neurosecretory deficiency of GH, which might be observed in patients who have received cranial irradiation for childhood leukemia. In these two diagnoses (idiopathic GHD of childhood and CNS irradiation) the pituitary fails much later than the hypothalamus causing a deficiency in hypothalamic GHRH with an intact or functioning pituitary. Supplying GHRH in the test can result in a false-negative response.

It is estimated that around 40% of patients with idiopathic GH deficiency in childhood will not be GH deficient as adults. This fact underscores the need for complete laboratory testing for this group of patients if they are to be considered for GH replacement therapy. The literature would suggest that approximately 35% of patients with isolated GHD would revert to normal upon retesting after they have stopped GH therapy [22]. For idiopathic deficiency associated with multiple hormone deficiency the number drops to 11%, and for X-ray-induced cranial irradiation the rate drops to 3%. In craniopharyngioma the number of patients that have normal stimulation tests after GH therapy in childhood is close to 0. For this reason, many insurance companies will accept a low IGF-1 as adequate proof that the patient is persistently GH deficient. Maghnie has looked at the predictive value of pituitary magnetic resonance imaging (MRI) findings with the thought that small pituitary volume might be more predictive of chronic GH deficiency [23]. He separated patients into four groups. The first two groups consisted of one with small pituitary gland size and the second with normal-size pituitary based upon MRI findings. The other two groups consisted of one with stalk agenesis and the last with craniopharyngioma. The pituitary size was not helpful for predicting persistent GH deficiency, and insulin or arginine testing results were quite variable. Combining arginine with insulin-induced hypoglycemia demonstrated almost complete responsiveness in the first two groups and no responsiveness in the latter two groups. The study underscored the difficulties in making the diagnosis of GHD in patients with isolated GH deficiency. In summary, more stringent testing of patients with idiopathic GHD of childhood onset is necessary. To this end, insulin-induced hypoglycemia or glucagon is suggested to provide convincing evidence of persistent GHD. Less stringent tests such as L-DOPA or clonidine alone are not suggested since they are less stringent and can result in many false-positive results. Arginine alone has been considered a test by AACE guidelines [15] after ITT or glucagon, but the cut point used is very low (<0.4 ng/ml to make the diagnosis). The various society guideline cut points for all the discussed tests are listed in Table 8.6. Note the various opinions on the use of arginine alone with the GRS not endorsing this test, but AACE suggesting that it can be used at a lower more stringent cut point.

Why Should We Replace Young Adults with GH?

The indications for return of GH therapy in children who have completed growth targets and are in transition to adulthood are the same for adults who have developed the deficiency later in life. GH deficiency in adults is associated with increased mortality, decreased quality of life, and an increase in bone fracture rates. Other reasons include abnormal risk factors for accelerated atherogenesis, including increased cholesterol

and decreased 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. We now believe that the full bone maturation process continues to around age 30. Stopping GH at age 17 or 18 could theoretically inhibit this process and leave these patients at risk for early-age osteoporosis [24, 25]. The evidence for this phenomena is indirect but convincing. Ter Maaten has looked at a group of 38 patients who were GH deficient as children and received subsequent GH therapy for a period of 3–5 years [26]. This group showed marked improvement in leg muscle area, decreases in skinfold and intra-abdominal fat, and improvement in total bone mineral content. Kaufman has looked at the bone mineral content in GH-deficient males with isolated and multiple deficiencies [27]. In both groups bone density is decreased. Since mortality figures have to do with an older population and are only theoretically important when talking to an 18-year-old young man or woman, it is really the bone density and the risk for fracture that are the most important indications for continuing GH in the transitioning patient.

Various studies have reported decreased bone density in young adults who have been GH deficient as children. Not only are the bones decreased in density, they also seem to be small bones with small bone volumes [28]. There is no current data to suggest that these bones are at risk for fracture, but the assumption seems valid. We do know that maximum development of bones continues until age 30. If the young adult stops GH at age 17 and GH is necessary for bone maturation and development, a strong case can be made for continuing GH not only until age 30 but also lifelong. In adults, quality of life is dramatically improved with replacement of GH in GH-deficient adults [29]. In children, there does not seem to be this dramatic difference in quality of life change after replacing GH. The reasons for this are not clear. One suggested explanation is that adults remember their previous functioning level of energy they had before developing GH deficiency, and children do not. We do know that children who remain GH deficient after childhood may not achieve successful employment [30]. Quality of life issues do not emerge as an important reason to continue GH therapy after childhood. It must be kept in mind, however, just how important these quality of life questions can be when addressed not only to the patient but also to the spouse or the parent. The individual patient may not recognize the subtle consequences of chronic GH deficiency as well as his or her mother, father, or cohabiting partner. It is only when the patient returns to GH therapy that they realize what was missing. Burman demonstrated this in a quality of life study in adults [31]. This author studied GH-deficient adults taking GH replacement therapy by questioning the spouse on what changes they noticed in the functioning level of the patient. The responses of the spouses were statistically significant compared to those from the individual patients. Whether this same situation exists for young adults is not clear. What does appear to be clear is that almost every reason that can exist is operative in keeping many young adults from returning to GH replacement. Some reasons are listed in Table 8.5. The most common reason appears to be that the young adult does not want to return to "shots." These young adults often fall into other categories of not returning. The second is that the pediatrician did not discuss the possibility of continuing therapy after growth potential has been reached. This is clearly not an indictment of the pediatric endocrinologist, but only a fact of historical note. This is a very new idea and there was no need to introduce this concept to children as early as 14 years ago when GH was first approved for GH-deficient adults, and only in the last 20 years has the syndrome of GH deficiency in GH-deficient adults been recognized.

Another reason for not returning to GH therapy after childhood is the lapse of insurance coverage when the child leaves home. New insurance plans often have an exclusion of GH for adults, and patients may not realize that they signed up for a group plan with this exclusion. Lastly, there is the impression from clinicians, such as myself, that these patients lose energy and motivation

after stopping GH and cannot coordinate whatever it takes to return to GH therapy.

Contraindications for Continuing GH Therapy

Before reinstating GH therapy it may be important to consider the contraindications for GH that might exist or have developed since stopping GH therapy. The first and most important would be the development of a 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 pituitary and CNS area. 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. If the cause is idiopathic and the patient is proven to be GH deficient, an MRI is suggested if an anatomic cause has not been ruled out. The young patient may, for example, had had a lesser quality MRI or CT scan of his or her pituitary area, and a newer technique may reveal stalk agenesis. This finding may help to support the diagnosis and need for lifelong therapy. Finding an anatomic abnormality of the pituitary will also boost insurance approval and help endorse continued need for GH replacement therapy if an anatomic lesion is identified [32, 33].

Diabetes mellitus is another contraindication to GH therapy if the diabetes is associated with proliferative retinopathy. Types I and II diabetes are not contraindications. If the patient is diabetic and GH therapy has begun, the diabetic control will worsen before it becomes better because of the positive effect on body composition. The increase in body fat, especially visceral fat, is associated with insulin resistance in the untreated adult GH-deficient patient.

Administration of GH will aggravate the insulin-resistant state and aggravate glucose control. After a period of time, GH therapy will change body composition, improve insulin sensitivity, and return glucose control. This will usually translate to an increase in insulin requirements by about 20% or double the oral hypoglycemic drug therapy required to control glucose. Individual patients will differ. However, the patient should be warned that diabetes control will get worse before it gets better. Pregnancy is not an absolute contraindication for GH therapy. The placenta does produce human somatomammotropin during the third trimester or even earlier, and therefore, during the last 3 months GH is not necessary. Our usual way of treating the pregnant 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 the IGF-1 value in the normal range.

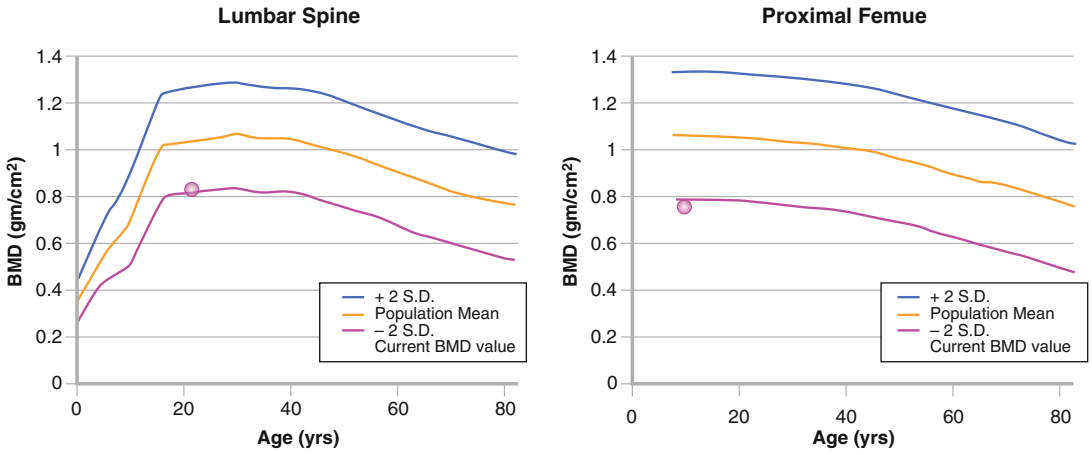
Table 8.7 GHRH plus arginine stimulation test for a 21-year-old COGHD patient with a BMI of 28

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

Peak value exceeds the cut point of 8 ng/ml for this BMI
Patient's BMI was 28

Clinical Examples of Young Adults Requiring GH Therapy

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 and gonadal function and a normal



	Bone mineral Density, gm/cm²	T-Score(Std Dev's from Young Adult Normal Mean)	%Change Since Last scan	First scan
Lumbar Spine	0.83	–2.0	N/A	N/A
Proximal Femur	0.66	–2.3	N/A	N/A

Fig. 8.1 Bone mineral density study of a 21-year-old woman with COGHD taken before restarting GH

Cortrosyn stimulation test. She was given an arginine plus GHRH stimulation test, and the results obtained showed a peak value of 9 ng/ml at 90 min (Table 8.7). She was told that she was not GH deficient because her BMI was 28 and therefore did not meet the normal cut point of <8 ng/ml for arginine/GHRH when BMI is between 25 and 30. She has gained 20 pounds 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 170 lbs and her BMI is 28. Her bone density reveals a *z*-score of −2.3 in her hip and −2.0 in her spine (Fig. 8.1) 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 test her with a test which activates the entire hypothalamic/pituitary unit and choose both insulin tolerance testing (Table 8.8) and glucagon testing (Table 8.9) (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 <5 ng/ml) and glucagon (cut point <3 ng/ml) (Tables 8.8 and 8.9). It is decided to put her on GH replacement. The dose selected is 50% of her

Table 8.8 Insulin hypoglycemia stimulation test for a 21-year-old COGHD patient who had a normal response to arginine plus GHRH and an abnormal response to glucagon

Time	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

Table 8.9 Glucagon stimulation test for a 21-year-old COGHD patient who had a normal response to arginine plus GHRH

Time (min)	Glucose (mg/dl)	Growth hormone (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

Table 8.10 Dose titration for a 19-year-old patient with autoimmune hypophysitis transitioning to the adult indication

Date (ng/ml)	mcg/d	IGF-1 (NL 180–780 mg/ml)
8/2008	800	60
10/2009	1,200	130
1/2010	1,600	204
3/2010	2,000	300
5/2010	2,400	480

The final plateau dose was 2.4 mg/d or 2,400 mcg/d

pediatric dose of 2 mg a day and you start her on 1.0 mg/day. Over the next 12 months her dose is titrated to her maintenance dose of 2 mg/day (see Table 8.10). She has a return of her energy and concentration power and does well in school. She also loses 20 lbs and her waist circumference drops 4 in. This case illustrates that many patients with idiopathic GHD of childhood will remain 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.

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. Because of the visual field abnormalities, he underwent pituitary surgery to remove the tumor. Subsequent to the surgery he was found to be growth hormone deficient. After a year of replacement therapy with testosterone, thyroid hormone, and cortisol and stable MRI image of his pituitary tumor, he returns for follow-up care. On physical exam he weighed 327 lbs and was 67 in. tall. His IGF-1 concentration was undetectable, and he had 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%. Because we were concerned about his glucose status we proceeded with GH therapy cautiously. He was started on 0.3 mg sc 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 II 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 sugar. 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 with higher doses as suggested by the work of Yuen [34, 35].

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 (nl182–780 ng/ml). 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 with normal responses greater than 5 ng/ml). Her 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 concentration.

Monitoring Therapy

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 GH and/or 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 the dose should be reduced to the next lower tolerated dose. The symptoms of GH should be covered by a discussion with the patient prior to initiating therapy. These can consist of muscle or joint pain, headache, edema, or carpal tunnel syndrome. The latter is managed by a 4-day holiday from drug therapy then resumption of the same dose.

The patient's weight should be followed, and the patient cautioned that weight loss is not usually observed with GH therapy but body composition changes are. From a low technology (and low cost) standpoint, waist and hip circumference should be obtained at 6-month intervals. Usually the waist circumference will change more quickly than the hips, or both may improve despite no significant change in weight. If insurance will allow, DEXA should be performed before GH therapy is begun. When ordering this procedure, the best parameters to follow are hip, spine, and body composition. The software for the latter determination is widely available and should be requested. Since bone density decreases after beginning GH therapy in the first 6 months, returns to baseline at 12 months, and increases from baseline at 18 months, we suggest the second DEXA study be performed no sooner than 18 months after beginning therapy, and then yearly thereafter [36, 37]. Serum IGF-1 concentrations should be followed at 4- to 6-week intervals until the plateau or maintenance dose is reached, and then every 6 months. The serum IGF-1 is more of a safety guide than an absolute concentration that defines the target dose. The IGF-1 should be used primarily as a guide to therapy. If the IGF-1 exceeds the normal 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. 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 sugar 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 patients if they have a family history of type II diabetes and are currently obese.

Summary

The treatment of young adults who have been growth hormone deficient as children is an emerging clinical science. The first and foremost important rule is to confirm the patient is persistently deficient, especially in patients who carry the diagnosis of idiopathic GH deficiency. 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. For both patients and physicians, the process of getting patients back on therapy will be very satisfying because of the return of energy and physical performance and restoration of body composition.

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Part II

Hypothalamic and Pituitary Disorders

Diabetes Insipidus

9

Abhinash Srivatsa and Frederick D. Grant

Abstract

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 analogues such as desmopressin. Treatment of nephrogenic diabetes insipidus typically depends upon reversal of the underlying cause, but pharmacological treatment may be partly successful.

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Diabetes insipidus (DI) • Polyuria • Vasopressin • Central DI • Nephrogenic DI • Desmopressin • Triple-phase response

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 analogues such as desmopressin. Treatment of nephrogenic diabetes insipidus typically depends upon reversal of the underlying cause, but pharmacological treatment may be partly successful.

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 1,000 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.

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 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, 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 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 excess 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. Nontraumatic central diabetes insipidus and most cases of nephrogenic diabetes insipidus present as chronic conditions. 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 anti-diuresis (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]. However, 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].

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 (Table 9.1). The underlying cause may not be apparent in nearly half of the cases of central diabetes inspidus [15].

Table 9.1 Causes of central diabetes insipidus (reprinted with permission)

Congenital:
Developmental defects: septo-optic dysplasia, other mid-line defects
Inherited genetic defects: familial diabetes insipidus, wolfram (DIDMOAD) syndrome
Pituitary injury
Head trauma
Supra-sellar tumors: craniopharyngioma, germinoma
Pituitary macroademona
Surgery
Vascular: cerebral aneurysm, intracranial hemorrhage, sickle cell disease
Infiltrative and inflammatory disorders:
Granulomatous diseases: histiocytosis, sarcoidosis, Wegener's granulomatosis, syphilis
Neoplasm: CNS lymphoma, leukemia, metastatic carcinoma (breast)
Infections: bacterial meningitis, tubercular meningitis, viral encephalitis
Autoimmune hypophysitis

Vasopressin deficiency may occur with a wide variety of congenital disorders, such as septo-optic dysplasia, that disrupt the normal development of the pituitary gland and other midline structures [16]. Diabetes insipidus is part of Wolfram's (DIDMOAD) syndrome that is characterized by central Diabetes Insipidus, Diabetes Mellitus, Optic Atrophy, and sensorineural Deafness resulting from mutation of the wolframin gene [17].

Familial diabetes insipidus is inherited as an autosomal dominant syndrome of vasopressin deficiency [18]. 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 [19], but this has

not been confirmed. Mutations have been identified at more than 30 sites within the vasopressin pre-prohormone [20, 21]. Most of these mutations are located in regions of the vasopressin precursor that do not encode the vasopressin peptide. Vasopressin deficiency resulting from one identified point mutation within the vasopressin peptide sequence is inherited as an autosomal recessive disorder. This mutation produces leucine-vasopressin that has a limited ability to activate the vasopressin receptor in the kidney [22].

Destruction of the pituitary gland, pituitary stalk, or hypothalamus can cause diabetes insipidus [13, 15, 23, 24]. Head trauma can cause transection of the pituitary stalk to produce diabetes insipidus. 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 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, 23–26]. Infiltration of the pituitary stalk can disrupt transport of vasopressin to the posterior pituitary. Germinomas, sarcoidosis, and histiocytosis X 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 stalk of posterior pituitary has been reported in children [27, 28]. 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 [29].

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 [30]. Most mutations associated with abnormal V2 receptor function are inherited as X-linked recessive disorders and impair the receptor response to vasopressin [31] by decreasing vasopressin binding or downstream signaling [32]. Recently, gain of function mutations at the same codon within the V2 receptor gene have been shown to cause chronic nephrogenic syndrome of inappropriate antidiuretic hormone action in two patients [33]. 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 [34, 35]. However, some of these mutations may impair the water channel function of the aquaporin [36] or prevent formation of aquaporin tetramers in the cell membrane [37].

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

Table 9.2 Reported causes of nephrogenic diabetes insipidus (reprinted with permission)

Congenital:
Inherited genetic disorders: mutations in V2 receptor or aquaporin 2
Renal malformations: congenital hydronephrosis, polycystic kidney
Acquired disorders:
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-infectious agents: foscarnet, amphotericin, methicillin, gentamicin
Anti-neoplastic agents: cyclophosphamide, isophosphamide, vinblastine, platinum
Other: phenytoin, acetohexamide, glyburide, tolazamide, colchicine, barbiturates

vasopressin resistance [38]. A wide variety of agents and processes have been associated with development of nephrogenic diabetes insipidus (Table 9.2). The precise mechanism by which most of these agents inhibit vasopressin action and exert their effect is not known. Some drugs, such as demeclocycline, appear to impair post-receptor signaling of the V2 receptor. Nearly half of all cases of drug-induced nephrogenic diabetes insipidus are related to the long-term use of lithium salts [39], 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 [40].

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 rule out 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 ruled out 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 maintain sufficient 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 bedwetting or frequent nocturia with high levels of urine output occurring during

periods of decreased water intake. Patients with well-compensated DI 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 DI 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 (Table 9.3). 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. In an individual with diabetes insipidus, impaired free water conservation permits 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.

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 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.

Table 9.3 Diagnostic testing for diabetes insipidus (summary) (reprinted with permission)

I. Basal testing:
A. Diabetes insipidus unlikely:
serum osmolality <270 mosm/kg, urine osmolality >600 mosm/kg, or urine output <1 l/m^2
B. Diabetes insipidus likely:
serum osmolality >300 (or serum sodium >150 meq/l) with urine osmolality <300 mosm/kg
II. Water deprivation study:
A. Water deprivation:
1) Precede by overnight fast (if tolerated and if indicated by clinical circumstances)
2) Continue complete water deprivation until:
loss of >5% of basal body weight or
plasma osmolality >300 mosm/kg or
urine osmolality >600 mosm/kg
3) Also discontinue if signs of hemodynamic compromise (blood pressure, heart rate)
B. Vasopressin administration:
1) Parenteral administration of vasopressin analogue
Vasopressin (Pitressin) 1 μ/m^2
Desmopressin (DDAVP) 0.1 μg/kg (maximum 4 μg)
2) Differential response to vasopressin
Central diabetes insipidus:
decrease in hourly urine output
urine osmolality increases by 50%
Nephrogenic diabetes insipidus:
no decrease in urine output
no increase in urine osmolality
III. Saline 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
3) Urine output decreases and urine osmolality increases when plasma osmolality reaches vasopressin secretory threshold
4) Analyze relationship between plasma osmolality, urine osmolality, and plasma/urine vasopressin levels using appropriate nomograms [4, 5, 41, 43]

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, 25, 41]. 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 illustrated in Table 9.3, 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 a vasopressin analogue such as desmopressin) demonstrates whether the diabetes insipidus is due to vasopressin deficiency or an impaired renal response to vasopressin [6, 13, 25, 41]. 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 [25].

In some cases, the results of the formal water deprivation test may be inconclusive [5, 41]. 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, 25, 41]. 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, and (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 shows no 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. If further testing is desired, the week of therapy should facilitate recovery of the concentrating gradient in the kidney, which may 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 but usually are not required for the diagnosis of diabetes insipidus. However, in selected clinical circumstances a vasopressin level may be helpful [5, 41, 42]. A plasma vasopressin level obtained after water deprivation will distinguish between central and nephrogenic diabetes insipidus [42], particularly in cases where there is only a partial defect in vasopressin secretion or action [5, 41]. To be most informative, plasma vasopressin must be evaluated as a function of plasma osmolality [41]. Vasopressin levels can be increased by hypotension, smoking, and nausea, and these stimuli should be avoided during testing for diabetes insipidus [15, 41]. 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.

The saline infusion test [5, 15, 43] may be useful in patients in whom water deprivation cannot be performed because of hemodynamic instability or in whom it would be difficult to obtain cooperation with water deprivation [43]. For example, infants cannot tolerate an extended fast. 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, 43]. When the 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 [43]. 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, 25, 43]. This test also is useful in identifying patients with normal vasopressin secretory ability, but an altered osmotic threshold for the release of vasopressin [25].

Interpretation of the saline infusion test may be complicated by a number of issues. Vasopressin 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 [44]. Vasopressin levels rarely are assayed in hospital labs and, thus, the samples must be sent to a reference laboratory, which will 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].

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 [45]. Anterior pituitary microadenomas do not cause diabetes insipidus. The normal posterior pituitary typically has a characteristic bright

spot on MRI, and absence of this characteristic may suggest loss of vasopressin-secreting neurons or deficient vasopressin production. However, a bright spot may not be seen in up to one-fifth of normal individuals [45]. 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 increased vasopressin release in nephrogenic disease [46–48].

Careful attention to the pituitary stalk may reveal a lesion disrupting vasopressin transport and secretion. Further evaluation of such a lesion will depend upon the clinical history of the patient. The 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 endocrinological and neurosurgical consultants, as the biopsy is likely to cause permanent damage to the pituitary stalk. If no lesion can be seen, 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 [24, 26]. 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, 25].

Patients with nephrogenic diabetes insipidus should be evaluated to exclude an electrolyte disorder, such as hypercalcemia or hypokalemia, that may contribute to renal insensitivity to vasopressin. Even in the absence of mechanical urinary outlet obstruction, diagnostic imaging 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 [49]. Treatment of the diabetes insipidus should help reverse the hydronephrosis.

With a family history of diabetes insipidus, genetic studies may be appropriate to confirm the cause of diabetes insipidus in an individual patient. 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.

Treatment of 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 analogues such as desmopressin are the specific therapy for central diabetes insipidus [50] (Table 9.4). 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 [51, 52]. Desmopressin has two molecular alterations compared to native vasopressin: de-amidation of the amino terminal cysteine and replacement of arginine-8 with D-arginine. These

Table 9.4 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
Desmopressin acetate				
(DDAVP)	IV/SQ	4 mcg/ml	1–4 mcg/day (divided doses)	6–12 h
			0.02–0.1 mcg/kg/dose in young children	
(Desmopressin)	Rhinal tube	100 mcg/ml	5–40 mcg/day	12–24 h
(DDAVP)	Nasal spray	100 mcg/ml	10–40 mcg/day (10 mcg/spray)	12–24 h
(DDAVP)	Oral	100 mcg/tab	100–800 mcg/day (50–300 mcg bid/tid)	8–12 h

two alterations result in a compound with a prolonged half-life of antidiuretic activity and elimination of the pressor activity found in 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 9.4).

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. Nasal administration of desmopressin can also 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 some situations. The fixed dose of the spray precludes small adjustments of dose, and children may require doses smaller than 10 mcg. Some authors suggest diluting the desmopressin 1:10 in saline to facilitate administration of small doses by rhinal tube [23]. 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- to 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 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 with oral administration of 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. 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 anti-diuresis with an accumulation of excess free water and progressive development of hyponatremia [50].

In patients treated with desmopressin, oral intake of fluids must be driven by and regulated by thirst. Management of diabetes insipidus in patients 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. However, management of diabetes insipidus in these individuals requires vigilance by the patient, family, and physician.

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, 50]. 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 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 trans-sphenoidal pituitary surgery, parenteral administration is used because of the difficulty of nasal administration of desmopressin. Parenterally administered desmopressin also is used 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 (0.08–0.10 mU/kg) can be used in the immediate perioperative period or during other procedures, such as administration of chemotherapy, which have potential disruption of free water balance [53].

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]. Patients are 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 should still 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 the periodic breakthrough prevents the development of hyponatremia that may result with accumulation of excess free water [50].

Infants are a special challenge in the management of diabetes insipidus as fluid intake is linked to caloric intake and usually is regulated by parents or other caregivers. Their 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 low-solute formula (e.g., Similac 60/40) can reduce the urine volume by 20–30% because of the lower renal solute load. Some infants may benefit from the addition of the diuretic chlorothiazide that can increase urine osmolality and decrease urine output. It is available as an oral

suspension and can be given to infants with central and nephrogenic diabetes insipidus at a dose of 5 mg/kg two or three times a day. These two therapeutic interventions significantly reduce the amount of free water supplementation needed in infants with both forms of diabetes insipidus to about 20–30 ml for every 120–160 ml of formula [54]. 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 μg/kg once daily, 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 [55]. However, in general, 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.

Treatment of nephrogenic diabetes insipidus can be a challenging endeavor. Discontinuation or a decreased dose of the precipitating drug may permit remission of the diabetes insipidus. However, this must be done in consultation with appropriate specialists that 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. 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 [56]. Diuretics, such as hydrochlorothiazide and amiloride (Midamor), probably ameliorate diabetes insipidus by producing a mild chronic volume depletion that leads to increased volume reabsorption in the proximal tubule of the kidney. With decreased distal delivery of filtrate, there is an overall decrease in urine volume. Combined therapy with hydrochlorothiazide and amiloride has been reported to be successful [57]. Amiloride may decrease entry of lithium into tubular cells and thereby decrease the effect of lithium on vasopressin action, and sometimes amiloride therapy will provide complete resolution of lithium-induced nephrogenic diabetes insipidus [40]. 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 the 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 consideration of substituting other therapies for lithium.

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]. With appropriate treatment, diabetes insipidus does not cause mental retardation [58]. 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 rule out hypernatremia due to exacerbation of the diabetes insipidus or hyponatremia secondary to water intoxication. 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.

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Management of Acute and Late Endocrine Effects Following Childhood Cancer Treatment

10

Jill L. Brodsky and Adda Grimberg

Abstract

Recent advances in the treatment of pediatric cancers have resulted in increasing numbers of children surviving their malignancy. Current survival rates are approaching 75%, and it had been estimated that by 2010, 1 in 715 young adults would be a long-term survivor of childhood cancer. 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 in acute and late effects of treatment. Endocrine disorders affect up to 50% of childhood cancer survivors following chemotherapy and radiotherapy. This chapter describes 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.

Keywords

Cancer • Craniospinal irradiation • Chemotherapy • Growth hormone deficiency • Hypogonadism • Precocious puberty • Hypothyroidism • Adrenal insufficiency • Diabetes insipidus • SIADH • Cerebral salt wasting • Bone • Obesity • Metabolic syndrome • Diabetes mellitus

Introduction

Recent advances in the treatment of pediatric cancers have resulted in increasing numbers of children surviving their malignancy. Current survival rates are approaching 75%, and it had been estimated that by 2010, 1 in 715 young adults would be a long-term survivor of childhood cancer [1]. Therapeutic options consist of a combination of multi-agent chemotherapy,

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surgery, radiotherapy, and bone marrow or stem cell transplantation. Unfortunately, decreasing mortality from malignancy comes at the cost of increased morbidity resulting in acute and late effects of treatment. Among 10,397 survivors in the Childhood Cancer Survivor Study, the cumulative incidence of a chronic health condition reached 73.4% 30 years after the cancer diagnosis, with a cumulative incidence of 42.4% for severe, disabling, or life-threatening conditions or death due to a chronic condition [2]. Endocrine disorders affect up to 50% of childhood cancer survivors following chemotherapy and radiotherapy [3, 4]. Late effects may occur soon after treatment; however, they may not develop for many years after cure. Therefore, lifelong follow-up of survivors in a multidisciplinary setting is recommended to ensure early diagnosis, timely institution of appropriate treatment, and counseling when needed. This chapter describes 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.

Acute Effects of Treatment

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 [5–7]. 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, unmasking the diagnosis of DI. Therefore, pre- and postoperative assessment of the CRH-ACTH-cortisol axis is imperative. If preoperative testing is unfeasible, empiric coverage with stress-dose glucocorticoids should be provided for safety. 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.

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 axon to the posterior pituitary for storage pending release into the circulation. Intraoperative damage to ADH neurons during hypothalamic-pituitary surgery is one of the most common causes of central DI. However,DI in the immediate postoperative period may be only the first phase of the "triple-phase response" [8]. 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. 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).

Inappropriate ADH secretion due to retrograde degeneration of the ADH neurons that had been surgically interrupted is the cause of the second phase. This unregulated release of ADH

results in inappropriately decreased urinary output with high urinary osmolality (>200 mOsm/kg), hypervolemia, and modestly elevated urinary sodium levels (greater than 20–30 meq/l). 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.

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 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 [9]. Given that a majority of their nutrition is in liquid form as breast milk or formula, the use of desmopressin may predispose them to water intoxication and hyponatremia.

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, bleeding, 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 [10]. 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. This leads to increased urinary sodium excretion causing a decrease in effective arterial blood volume. This state of dehydration leads to baroreceptor stimulation, resulting in appropriate ADH release and urinary concentration. 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.

Chemotherapy

During treatment for malignancy, malnutrition and cachexia are common side effects. Without proper nutritional support, weight loss, organ dysfunction, and wasting may occur. Gastrointestinal side effects are common with both irradiation and chemotherapy, including emesis, diarrhea, malabsorption, stomatitis, and esophagitis. Glucocorticoids exacerbate cachexia by their counter-regulatory effects on protein metabolism, leading to muscle wasting and further decrease in activity level. Tumor-induced cytokine production initiates a cascade of metabolic events including glycogenolysis, lipolysis, proteolysis, increased resting energy expenditure, and gluconeogenesis [11].

Because the literature is heterogeneous regarding tumor type, stage, and outcome variables, it is difficult to determine the optimal route and content of supplemental nutrition. Due to the increased complications associated with parenteral nutrition, an enteral route should be attempted first using elemental or partially digested formulas [11]. 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 [12].

The metabolic effects of glucocorticoids include decreased peripheral insulin sensitivity [13], increased hepatic glucose production [14], and islet cell toxicity and apoptosis [15, 16]. 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 pediatric acute lymphoblastic leukemia (ALL), has toxic effects on the beta cell and has been associated with the development of transient or persistent diabetes [17].

Considering the myriad of metabolic influences of glucocorticoids and that their predominant effect appears to be 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 [18], phenylalanine derivatives [19], alpha-glucosidase inhibitors [20], thiazolidinediones [21,22], and insulin [23,24]. 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.

High-dose glucocorticoid treatment is the cornerstone of therapy for childhood ALL. It is given intermittently throughout the duration of treatment over a 2- to 3-year period. Additionally, high-dose steroid therapy may be used postoperatively in the treatment of brain tumors. It has been established that when glucocorticoid therapy has been used for a brief period lasting less than 10 days, therapy can be discontinued without taper without adverse event [25]. However, during longer courses of glucocorticoids, the hypothalamic-pituitary-adrenal axis may be suppressed and unable to respond to acute stress for several months and 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.

Alkylating agents commonly used to treat pediatric cancers, such as cisplatin, thiotepa, cyclophosphamide, ifosfamide, and carboplatin, form covalent linkages to phosphates, 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 down-regulation of *AQP2* expression [26]. The treatment of drug-induced nephrogenic DI is first centered on the removal of the offending agent. However, when this is not possible, thiazide diuretics are commonly used [27]. Thiazide diuretics reduce the glomerular filtration rate and enhance urinary sodium excretion at the expense of water [9, 28, 29]. The end result is increased proximal tubular sodium and water resorption.

Late Effects of Treatment

Growth Failure/Growth Hormone Deficiency (GHD)

Impaired growth resulting in reduced adult height occurs frequently in childhood cancer survivors. Causes are multifactorial and include growth hormone deficiency (GHD), central precocious puberty (CPP), hypothyroidism, and spinal irradiation. For patients exposed to irradiation, truncal short stature may occur with decreased skeletal response to growth hormone (GH) therapy [30–33]. Even without radiation exposure, linear growth in survivors may be affected by growth plate exposure to chemotherapy, which is currently under investigation [34, 35].

GHD can occur in childhood cancer survivors as the result of direct tumor invasion, debulking surgery, or irradiation therapy in the hypothalamic-pituitary region. GH is the most vulnerable anterior pituitary hormone and is often the only anterior pituitary deficit to develop after cranial irradiation [36, 37]. 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) [38, 39], 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 [31]. Age at treatment has been shown to be inversely proportional to the development of multiple pituitary hormone deficiencies. 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 [31]. Young children, compared to adults, have shown increased vulnerability to developing isolated GHD after treatment with total body irradiation (TBI) doses as low as 10 Gy [38, 40–43].

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 [44]. The preservation of basal GH secretion is hypothesized to be due to decreased insulin-like growth factor-1 (IGF-1)-dependent negative feedback. Additionally, radiation-induced reduction of somatostatin secretion has been postulated to result in greater tonic GH release from the remaining somatotrophs [45]. 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 [45, 46]. 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 [44, 47, 48].

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 cranial-spinal 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 [49]. Radiation exposure has been associated with a specific form of GHD called growth hormone neurosecretory dysfunction (GHNSD) [50–54]. These patients have a preserved peak GH response on stimulation testing, despite decreased endogenous GH secretion [55]. During puberty, patients with GHNSD will fail to mount an appropriate acceleration in growth velocity due to a lack of increased GH secretion [56, 57].

No gold standard has been established for diagnostic testing for GHD in children following cranial irradiation. IGF-1 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 workup for short stature. However, IGF-1 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-1 and IGFBP-3 (above -2 SD) can be seen in the setting of abnormal growth velocity and GHD [58, 59]. 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 (2000) advocated needing only one failed stimulation test to make the diagnosis [60], while the Pediatric Endocrine Society (formerly the Lawson Wilkins Pediatric Endocrine Society; 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 [61].

GH replacement therapy has been shown to increase growth velocity and adult height in childhood cancer survivors with GHD [34, 62]. However, survivors treated with spinal radiation doses exceeding 20 Gy respond less well to GH therapy and are at risk for developing disproportionate growth of the limbs in comparison to the trunk [63, 64]. This becomes most apparent during the time of the pubertal growth spurt [65]. 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 preexisting kyphosis and scoliosis that may require orthopedic intervention [66].

Children with a prior history of malignancy constitute approximately 20% of all pediatric patients treated with GH [61]. The mitogenic properties of GH and IGF-1 have prompted concern regarding the safety of GH treatment in survivors of malignancy. Existing evidence

indicates that GH treatment does not increase tumor recurrence in persons successfully treated for a primary lesion [67]. 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 [61]. 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 [68–70]. Patients with craniopharyngiomas, a benign tumor, may be treated with GH once the tumor has been adequately controlled or stabilized [61].

All cancer survivors are at risk for developing a second neoplasm. In a report from the Childhood Cancer Survivor Study on 361 GH-treated individuals, including 122 survivors of acute leukemia and 43 survivors of soft tissue sarcomas [70], data suggested that GH treatment may slightly increase the risk of a secondary solid tumor, particularly in acute leukemia survivors [70]. 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 [71]. The Genentech National Cooperative Growth Study (NCGS), a registry containing 20 years of NCGS safety data covering approximately 55,000 patients and nearly 200,000 patient-years of GH, concurred that GH exposure does not increase the 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 [72]. Additional studies have suggested an increased risk of second neoplasms in children with a history of leukemia subsequently treated with GH [67]. However, there was no association between increased risk and GH dose, duration, and overall mortality [71]. Therefore, ongoing surveillance of such patients for second malignancies is important.

While radiation-induced GHD is usually permanent, the need to reevaluate patients after reaching adult height for continued treatment remains controversial [60, 73–75]. 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 of quality of life [76–79]. 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 [80]. 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- to 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 [80]. 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- to 12-month monitoring should also include testing of the other anterior pituitary hormone functions, fasting lipid panel, and overall clinical status.

Thyroid

Thyroid abnormalities are common in childhood cancer survivors. Given the impact of thyroid status on growth and development, it is important to recognize these disorders early and initiate appropriate treatment. 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 [81, 82]. 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 [83]. Exposure to radiolabeled agents such as 131I-metaiodobenzylguanidine

(MIBG) [84] and 131I-labeled monoclonal antibody for neuroblastoma [85] treatment has been shown to result in primary hypothyroidism. Central hypothyroidism is recognized to affect up to 85% of patients 5 years after treatment with brain and nasopharyngeal tumors [86] treated with radiotherapy [81].

Direct or scatter radiation exposure to the thyroid is a significant risk factor for the development of benign and malignant thyroid lesions [87]. While 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 20–29 Gy, individuals treated with 30 Gy continue to have elevated risk compared to the general population [88]. 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 [89]. The etiology of thyroid cancer in this population is thought to be secondary to radiation-induced rearrangements of oncogenes *RET–PTC* [90, 91].

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 this population experiences on a day-to-day basis. The most sensitive indicator of central hypothyroidism is a blunted or absent nocturnal surge of thyroid-stimulating hormone (TSH). However, given the challenge of obtaining nocturnal surge studies, the clinician must rely upon measurement of TSH and free T4 levels. The TSH may be inappropriately low or normal in the presence of a low 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.

Given the increased risk of developing benign and malignant thyroid nodules in this population, some recommend obtaining a baseline screening thyroid ultrasound 5 years after completion of treatment, and then every third year if negative. Although ultrasound screening for thyroid cancer in the general population is not cost effective and could lead to unnecessary surgery due to false positives, some believe it would be worthwhile in childhood cancer survivors who received radiotherapy involving the head, neck, or upper thorax [92].

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 [86]. Since TSH levels are unreliable in a patient with secondary hypothyroidism, it does not need to be followed on therapy.

Gonadal Axis

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 has been associated with the development of CPP at both lower doses for leukemia treatment (18–35 Gy) and higher doses for brain tumor treatment (>35 Gy) [93]. The mechanism for CPP following irradiation is hypothesized to involve dysregulation of cortical influences on the hypothalamus and a release of the inhibitory GABAergic tone [94, 95].

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 [46]. 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 [39, 96].

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 [97]. Bone age advancement greater than two 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 injections [98] or annual subdermal implants performed under local anesthesia [99]. In general, this class of drugs is effective in retarding progression of secondary sexual characteristics, preventing menses, slowing bone age advancement, and increasing final height [100]. The injectable form is supported by more long-term efficacy and safety data, while the implantable approach may facilitate compliance by eliminating the need for a monthly injection.

Aromatase inhibitors were developed as adjuvant therapy for estrogen-responsive breast cancer. The primary aim of therapy in pediatrics is attenuation of the effects of estrogen on growth, skeletal maturation, and secondary sexual development [101]. Use in children and adolescents is still limited and off-label.

Delayed Puberty

Delayed puberty can result from primary gonadal injury or deficiency of central activating signals (hypogonadotropic hypogonadism). Cancer survivors with a history of exposure to abdominal, pelvic, and spinal radiotherapy and/or alkylating agents are at increased risk of gonadal failure. High-dose cranial irradiation (>50 Gy) is associated with hypogonadotropic hypogonadism within the context of combined hormonal pituitary deficiencies [102–104]. Following radiation therapy, gonadotropin deficiency is second in frequency to GHD.

Survivors who lose ovarian function during cancer therapy or within 5 years of completion are classified as having acute ovarian failure (AOF). 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 menopause [105, 106]. Of survivors who developed AOF, 75% had been previously exposed to abdominal-pelvic irradiation. More than 70% of patients who received at least 20 Gy to the ovary developed AOF. During childhood and adolescence, doses in the range of 10–30 Gy have been noted to cause AOF in the majority of patients [107–110]. Additionally, 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 [110].

In males, radiation therapy to the testes of more than 7.5 Gy and alkylating agent exposure have been associated with gonadal failure. 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.

Diagnosis 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. 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 [111]. 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 to avoid unintentional dosing of household contacts. Testosterone therapy cannot overcome damage to the spermatogenic cells.

Fertility Preservation

Surgical, medical, and technological advances have enabled the medical community to provide fertility options for patients with cancer. Treating oncologists need to consider fertility options in patients undergoing therapy. The ability of having genetically related children is an important issue for patients surviving cancer [112]. Direct uterine effects after abdominal irradiation in young girls have included irreversible changes in uterine musculature and blood flow, leading to future spontaneous pregnancy loss and intrauterine growth retardation of fetuses [113, 114].

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. Oral contraceptive pills have also been investigated as a method to suppress the ovaries during chemotherapy as a means of protection from cytotoxic agents. Both of these regimens have been studied in small cohorts of patients. Larger studies are needed before these approaches can be recommended as part of routine patient care.

Cryopreservation is a strategy for preserving future fertility that is available to both females and males. 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 [115]. 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. 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 [112].

Adrenal Axis

Outside of the use of glucocorticoids during treatment, ACTH deficiency in childhood cancer survivors is relatively uncommon [87]. Acutely, ACTH deficiency can result from direct tumor extension or following surgery in that region. 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. In children with brain tumors receiving radiation treatment to the hypothalamic-pituitary area (median dose 44 Gy), the 4-year cumulative incidence of ACTH deficiency was 38% [37]. In a second retrospective study of 310 patients with CNS tumors referred for endocrine evaluation, the greatest risk for developing ACTH deficiency was seen in patients with a history of craniopharyngioma, medulloblastoma, or >24-Gy cranial irradiation [116].

Water Balance

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 treated with desmopressin who 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 [117, 118]. Vaptans are a class of drugs that act as specific V2 receptor antagonists and 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 hyponatremia [119]. By inducing free water clearance, aggressive hydration was able to be administered 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.

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 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 obtaining 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 in the setting of fever, diarrhea, and tachypnea. To prevent dehydration and resulting hypernatremia, the amount of free water per day will need to be increased to account for these losses.

Obesity and Metabolic Syndrome

In the general population, obesity is a well-described risk factor for the development of comor-

bid conditions such as diabetes mellitus [120, 121], hypertension [122], dyslipidemia [123], and cardiovascular disease [124, 125]. Several studies have suggested a role for the therapies used to treat childhood ALL, particularly glucocorticoids, in the increased risk of obesity observed among survivors [126–130]. The Childhood Cancer Survivor Study has shown that cranial irradiation in doses of 20 Gy or more 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 [130]. In this population, cranial radiation exposure is associated with a greater rate of increasing BMI, particularly among women treated with cranial irradiation during the first decade of life. Additionally, patients who received TBI to prepare for bone marrow transplantation may develop features of metabolic syndrome without associated obesity [131]. 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) [132–137]. Early diagnosis and intervention have been shown to reduce associated cardiovascular morbidity and mortality [136].

During routine annual follow-up care, all survivors should have height, weight, and blood pressure measured; BMI calculated; and percentiles noted. The Children's Oncology Group's most recent "Long-Term Follow-Up Guidelines for Survivors of Childhood, Adolescent, and Young Adult Cancers" were published in 2008. They recommend fasting blood glucose and lipid profiles to be obtained 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. Consider evaluation for other comorbid conditions including dyslipidemia, hypertension, glucose intolerance, diabetes mellitus, hyperinsulinism, and insulin resistance as needed. Patients should receive counseling at each visit regarding diet and exercise. Health care professionals should be aware of this risk and interventions to reduce or manage weight gain are essential in this high-risk population [137]. Lipid abnormalities should be managed according to the most current practice management guidelines for the given abnormality.

Currently, the only treatments that are FDA approved for the treatment of type 2 diabetes mellitus in children are metformin [138] 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 which occur in approximately one-third of patients. Slowly titrating the dose over a period of 6–8 weeks and taking the medication with food help to alleviate these symptoms. Insulin is an option for individuals that 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.

Effects on Bone Strength

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. Pediatric cancer survivors have numerous risk factors for osteoporosis, including malnutrition [139], reduced muscle strength [140], chemotherapy [141], radiation exposure [142], hypogonadism, and glucocorticoid therapy [143].

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 [144]. Numerous studies utilizing dual-emission X-ray absorptiometry (DXA) have reported bone deficits in childhood ALL [145–147] at diagnosis and during treatment and have shown a significantly increased risk of fracture during treatment of childhood ALL, particularly during the maintenance phase of chemotherapy [148].

Avascular necrosis (AVN) is a well-recognized complication of current therapy for childhood ALL, with a 3-year cumulative incidence of 9.3% in children treated for ALL [149]. Dexamethasone has been implicated as the main etiological factor. Additional risk is associated with adolescence and Caucasian race [150–152]. AVN is often multifocal, most commonly affecting weight-bearing joints and may cause a significant degree of pain resulting in immobility.

DXA is the preferred method for clinically assessing bone mineral content and areal bone mineral density. 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, GHD, and 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 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 [153].

The increasing availability of magnetic resonance imaging (MRI) has enabled earlier radiological diagnosis of AVN, prior to joint collapse, while mild to moderate pain may be the only presenting symptom. The volume and extent of AVN measured on MRI have been shown to predict fracture and joint collapse [154]. 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 [154].

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 [155].

Good nutrition, sufficient intake of calcium and vitamin D, and weight-bearing activity are critical components to optimize skeletal health in the cancer survivor population. Surveillance for other hormonal deficiencies (gonadal failure, 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, has been shown to provide symptomatic relief in AVN. However, there is minimal literature demonstrating the efficacy of core decompression in the treatment of AVN. Additionally, there is scant literature on 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 [156]. Experience using oral alendronate resulted in gains in bone mineral density, improvement in motor function, and modest gains in health-related quality of life [157, 158].

Conclusions

In the United States, there are approximately 270,000 survivors of pediatric cancer, or about 1 of every 640 adults between the ages of 20 and 39 years [159]. It is now clear that damage caused by chemotherapy and radiation therapy may not become clinically evident for many years after treatment. 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 patients' quality of life and decrease morbidity and mortality.

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Endocrinologic Sequelae of Anorexia Nervosa

11

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Abstract

Anorexia nervosa (AN) is a severe psychiatric and medical condition once described as the "relentless pursuit of thinness." 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. Recent trends demonstrate an earlier age of onset of AN, and it is recognized that onset at a younger age is associated with poorer growth and bone health outcomes. 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.

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Anorexia nervosa • Malnutrition • Bone health • Bone mineral density • Hypothalamic amenorrhea

Introduction

Anorexia nervosa (AN) is a severe psychiatric and medical condition once described as the "relentless pursuit of thinness" [1]. The disorder affects 0.5% of adolescent females in the USA [2] and represents the third most common chronic disease among American females. The disorder is most commonly seen among adolescent girls, with estimates that 5–10% of cases occur in males. However, 19–30% of younger patients with AN are male, and the overall prevalence of this disorder among adolescent boys appears to be increasing [3–5]. 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. Recent trends demonstrate an earlier age of onset of AN [6], and it is recognized that onset at a younger age is associated with poorer growth and bone health outcomes [7, 8].

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 primary clinical features of AN by Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV), criteria are shown in Table 11.1. Of note, adolescent girls who are pubertal may fail to make normal weight gains during growth and may gradually fall below the 85th percentile of expected weight for height or lose the equivalent of 15% of expected body weight for height. Linear growth failure may also result from inadequate caloric intake at a critical stage of puberty, while small amounts of gonadal steroids may continue to be secreted, advancing bone age and resulting in the loss of final adult stature. The DSM is currently under revision, with a Fifth edition (DSM-V) expected to be published in May 2013. As of July 2011, proposed revisions include that AN may lead to weight that is less than minimally expected for age in children and adolescents and may also include that AN involves either intense fear of gaining weight or behavior that interferes with weight gain [9]. Additionally, the proposed DSM-V criteria do not include amenorrhea in the diagnosis of AN [9].

Table 11.1 DSM-IV criteria for anorexia nervosa

1. An intense fear of gaining weight or becoming fat, even though underweight
2. A disturbance in body image such that the patient feels fat even when emaciated
3. Refusal to maintain body weight over a minimal normal weight (weight loss leading to maintenance of body weight 15% below that expected for height)
4. Amenorrhea for three or more cycles

Hypothalamic–Pituitary–Adrenal Axis in AN

Patients with AN exhibit hyperactivity of their hypothalamic–pituitary–adrenal (HPA) axis [10, 11]. These patients typically have elevated serum cortisol concentrations, accompanied by increased corticotropin-releasing hormone (CRH) secretion and normal circulating levels of adrenocorticotropic hormone (ACTH) [11].

The elevation in cortisol could be secondary to increased cortisol production, decreased clearance, or a combination of both factors [11]. Boyar and colleagues [12] were the first to report decreased cortisol metabolism in AN, subsequently confirmed by other groups. Walsh and colleagues [13] noted that when body size was taken into account (cortisol production/kg), cortisol secretion was significantly increased.

Overactivity of the HPA axis appears to be 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 [14]. Patients with AN may also exhibit inadequate suppression of cortisol after an overnight oral dexamethasone challenge [13, 15, 16]. Estour and colleagues [17] administered dexamethasone intravenously to 15 patients with AN and observed nonsuppression 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 [10] found increased cortisol response to CRH, while Hotta and colleagues [18] 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 [19, 20], increased [21], or unchanged [22, 23] 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 [24]. Recently, we demonstrated 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 [25].

Insulin, Leptin, and Adiponectin Abnormalities

Studies examining the question of insulin dynamics in AN have yielded contradictory results [26], demonstrating both insulin resistance and insulin deficiency in these patients. Low fasting glucose and insulin concentrations have been reported in AN [26], as have both normal [27] and increased insulin sensitivity [28]. 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 [29]. 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 involved with adaptations to starvation states [30]. The subnormal plasma leptin concentrations seen in AN [31] 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 [32–34]. 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 [33].

Adiponectin, an adipokine produced by adipocytes, varies inversely with fat mass [34]. Low levels of adiponectin are associated with insulin resistance and hyperinsulinemia [35]. Adiponectin has been shown to be both elevated [38] and low [39] in adult women with AN. Similarly, adiponectin levels have been reported as normal [40] and elevated [41] in adolescents with AN, as compared to healthy controls. Alterations in adiponectin levels likely reflect low energy availability in these 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 [42, 43].

Growth Hormone Abnormalities

Elevated serum growth hormone concentrations are found in at least one-half of emaciated anorexic patients [44, 45] and return to normal with weight gain [46]. 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 [47]. 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 [48]. 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 [47]. Patients with AN exhibit impaired GH responses to L-dopa and apomorphine administration, and two reports have demonstrated that these findings persist even after nutritional rehabilitation [49, 50]. The GH response to arginine has been reported as normal in one study [51]. A paradoxical increase in GH secretion following a glucose load has also been reported by some investigators [52, 53].

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 [22] 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, that occur commonly in these patients despite normal to slightly subnormal T4 levels. Levels of thyroid-stimulating hormone (TSH) are within normal limits [56, 57] and are not related to body weight [56]. However, peak TSH response to thyroid-releasing hormone (TRH) stimulation appears to be delayed (e.g., to 120 min) [58] and may be augmented [55], suggestive of a hypothalamic defect.

Hypothalamic–Pituitary–Ovarian Axis Abnormalities

Amenorrhea is 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 [44]. A positive relationship between resting luteinizing hormone (LH) levels and body weight has been shown, and LH levels normalize with weight gain [59]. 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 [60]. 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 [59].

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 [44] and may persist despite weight restoration [61]. Return of

menstruation in patients with AN correlates with regaining weight, although not all patients recover menses [62]. 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 [63], percentage of ideal body weight [64], and body mass index (BMI) [65]. However, it has been shown that return of menses does not show a simple relationship to weight or body fat [66], although the majority of patients resume menstruation when weight has returned to at least 90% of ideal [46]. These findings are in accord with the work of Frisch and colleagues [67] 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 [63] and implies that body composition may be an important determinant of reproductive fitness in the human female [67]. However, identifying clinical features that identify 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 [68]. Following weight restoration and resumption of menses, patients with AN appear to have normal fertility [62], although this has not been well-studied.

Prolactin

Fasting morning concentration of prolactin is normal in adolescents and women with AN [56, 69], 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 [70].

Vasopressin and Oxytocin

Partial diabetes insipidus has been reported in AN [69, 71], as have abnormally high cerebrospinal fluid (CSF) arginine vasopressin (AVP) levels [72]. Patients with AN have also been shown to have decreased CSF oxytocin concentrations, along with reduced oxytocin responses to stimulation [73]. These findings appear to reverse with weight gain, suggesting that they may be secondary to malnutrition, abnormal fluid balance, or both [74].

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 [75, 76]. Studies of peptide YY (PYY), an anorexigenic peptide released by the gut, have shown conflicting results, with both elevated [77] and normal levels [78] observed in women with AN.

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 rapid bone accretion occurs during puberty [79–81]. A serious complication of AN includes profound deficits of both trabecular and cortical bone compartments [82–86] with spinal bone density reported to be greater than 2 SD below normal in 50% of young women with this disease [83]. The bone loss is so severe that clinical fractures at multiple sites have been documented in women during late adolescence and young adulthood [84, 85, 87]. The multiple factors contributing to bone loss in anorexia nervosa, including increased bone resorption and decreased bone formation, are summarized in Fig. 11.1.

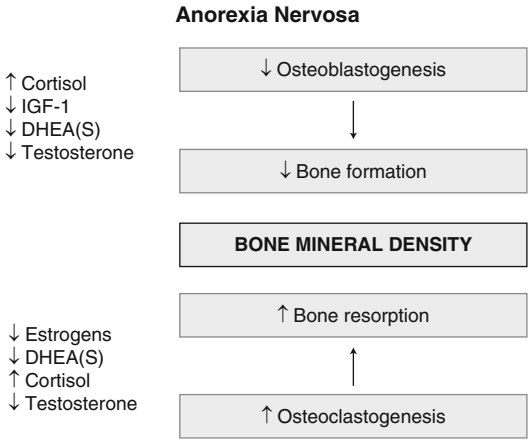

Fig. 11.1 Multifactorial etiology of bone loss in AN. Mechanisms behind the bone loss of anorexia nervosa are outlined

An uncoupling of bone formation and bone resorption is observed in women with AN [88], 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 [89]. 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 [89]. Oral contraceptives containing both estrogen and progestin are not adequate to halt the bone loss seen in AN [90, 91]. There also appear to be direct effects of undernutrition on bone, as IGF-I levels are subnormal and correlate with markers of bone formation [92, 93]. 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 [94]. 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 [95].

Deficiencies in androgens, notably DHEA, have also been demonstrated in some studies [20, 96] which may be clinically significant as DHEA appears to have both anabolic and antiosteolytic effects on bone [93, 97]. As noted earlier, our group recently demonstrated that therapy with combined therapy with DHEA and a combined oral contraceptive pill preserves BMD in young women with AN [26]. Lastly, while some studies have demonstrated that bisphosphonate treatment reduces bone turnover and increases bone mineral density in adolescents and adults with AN [98, 99], 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 [43].

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" which includes osteoporosis, amenorrhea, and eating disorders [100]. 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 [101–103].

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 [69, 71]. 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, or neurologic signs and symptoms. Other tests, including endocrinologic assessments, may be considered depending on the patient's presentation.

Management

There are limited evidenced-based guidelines for the treatment of anorexia nervosa, and treatment guidelines often rely upon expert recommendations [104]. 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 [65] found that BMI >16.4 ± 0.3 kg/m^2 was associated with an improvement in bone mineral density. Shomento and Kreipe [64] have found that a mean of >92 ± 7% of ideal body weight was associated with a return of menses. Golden and colleagues have 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. 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) [43]. Provision of adequate calcium and vitamin D intake are important during the critical period for bone accretion. These patients should receive 1,300 mg of elemental calcium and 600 international units of vitamin D daily [106], although appropriate supplementation doses are debated. Physical activity is associated with increased bone formation [107], and exercise regimens should be individually tailored to take into account hemodynamic stability, level of fitness, and extent of bone loss.

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.

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Part III

Adrenal Disorders

Adrenal Insufficiency

12

Kathleen E. Bethin and Louis J. Muglia

Abstract

Adrenal insufficiency is an important source of potentially life-threatening human disease. Defects at each level of the hypothalamic–pituitary–adrenal axis can yield impaired adrenal function that results in variable degrees of glucocorticoid or mineralocorticoid deficiency. In this chapter, we describe the physiology and regulation of adrenal steroid action, followed by presentation of mechanisms of primary, secondary, and tertiary adrenal insufficiency. The components of establishing a diagnosis of adrenal insufficiency along with its causation are summarized. Current pharmacological and psychosocial therapeutic interventions for disorders of impaired adrenal function conclude the overview.

Keywords

Adrenal • Steroids • Adrenal insufficiency • Congenital adrenal hyperplasia • Adrenocorticotropic hormone (ACTH)

Introduction

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

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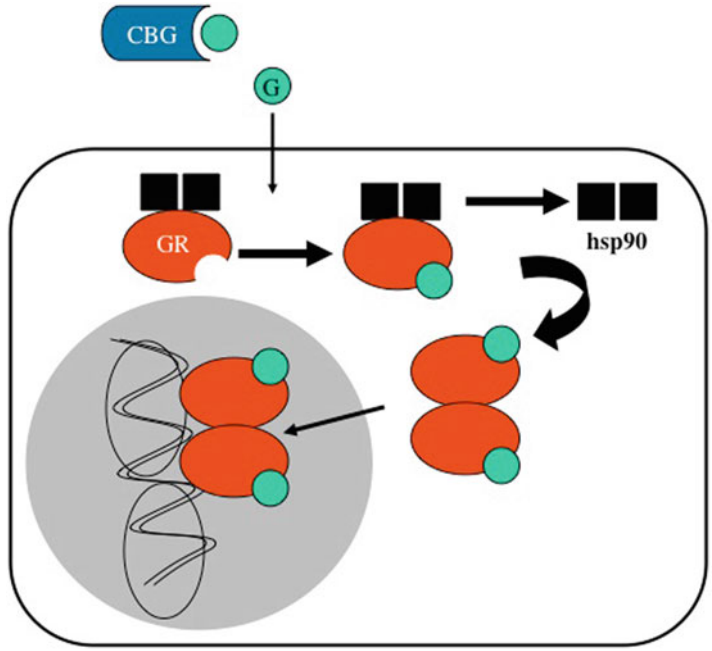

Fig. 12.1 Mechanism of glucocorticoid action. Glucocorticoids (G; small circles) circulate in the bloodstream primarily bound to corticosteroid-binding globulin (CBG). Glucocorticoid dissociates from CBG, diffuses across cellular membranes, and binds the cytosolic, heat-shock protein (hsp)-complexed, 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 of glucocorticoid response elements, recruitment of co-activators, or heterodimerization with other transcription factor partners (Reprinted with permission)

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 to 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. 12.1). Two distinct gene products exhibit high-affinity glucocorticoid (GC) binding, the mineralocorticoid (type I) receptor

and the glucocorticoid (type II) 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 beginning to be elucidated. One mechanism, for example, is that upon GR binding of GREs in nucleosome-packaged chromatin, co-activators (such as SRC-1/NcoA-1, TIF2/GRIP1, or p300/CBP) are recruited [18]. These GR–co-activator 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].

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. 12.2). 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 hypophysial 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. 12.3) [30, 31]. Cortisol then acts in a

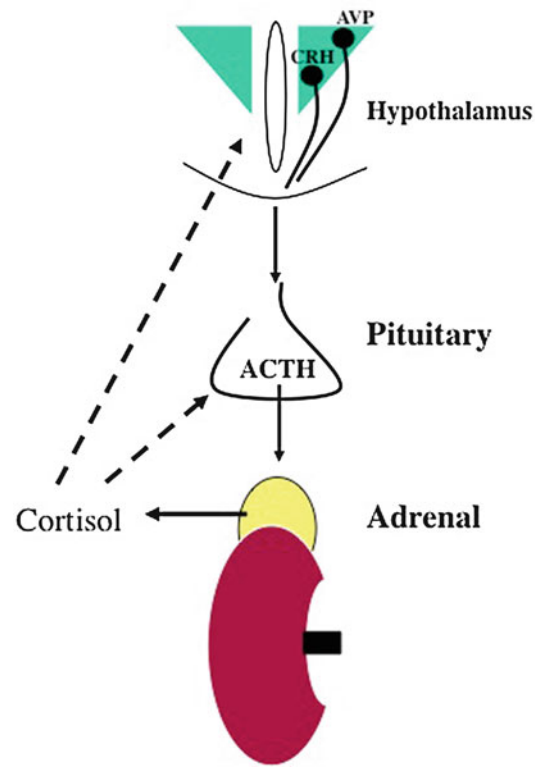

Fig. 12.2 Hypothalamic–pituitary–adrenal axis regulation. Stress, circadian stimuli, and glucocorticoid withdrawal stimulate cortical, hippocampal, and other higher neural centers to activate corticotropin-releasing hormone (CRH) and vasopressin (AVP) parvocellular neurons in the hypothalamic paraventricular 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 down-regulate excessive release of hypothalamic and pituitary mediators (Reprinted with permission)

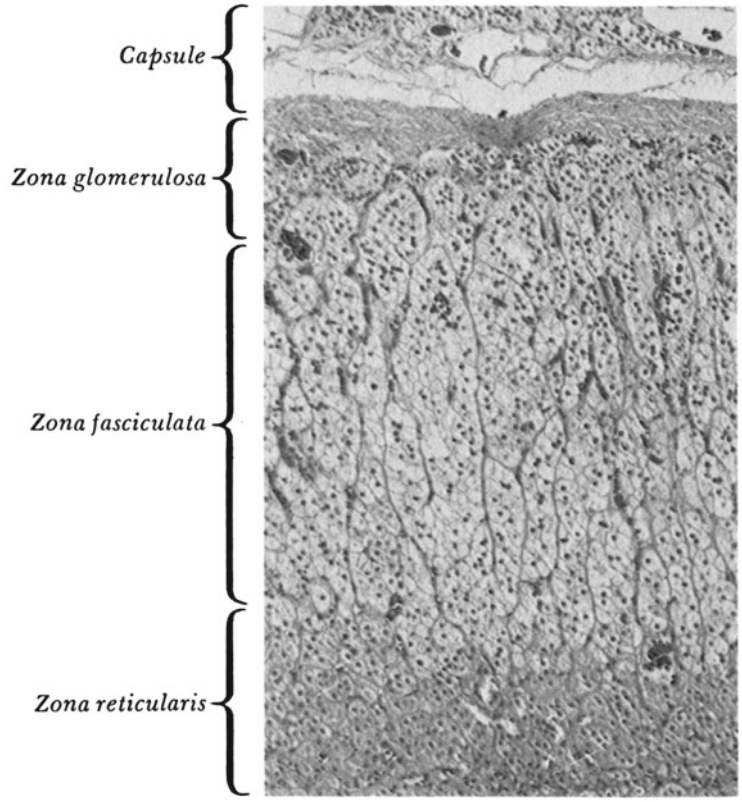

Fig. 12.3 Adrenal histology. Shown is a hematoxylin- and eosin-stained section of a normal human adrenal. Relative sizes and positions of the zona glomerulosa, fasciculata, and reticularis are indicated (X40 magnification). Reproduced with permission from Bethune (Copyright © Pfizer Inc. Reproduced with permission) [192]

negative feedback manner at central nervous system and pituitary sites to decrease excessive release of hypothalamic neuropeptides and ACTH. Conversely, when insufficient glucocorticoid is present in the circulation, 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, manifested as isolated glucocorticoid insufficiency.

Etiologies of Adrenal Insufficiency. *Primary Adrenal Insufficiency*. The most common cause of primary adrenal insufficiency, or Addison's disease, is autoimmune adrenalitis (Table 12.1). Antibodies that react to all 3 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

Table 12.1 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
Coccidiomycosis
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
de Morsier syndrome
Hydrancephaly/anencephaly
Pituitary aplasia/hypoplasia
Iatrogenic (Supraphysiologic glucocorticoids)

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sometimes completely disappear. Autoimmune adrenal insufficiency may occur as an isolated endocrinopathy or in association with other endocrinopathies [34, 36]. Addison's disease in association with other endocrinopathies can be subdivided into two groups: polyglandular autoimmune disease types I and II [15]. Polyglandular autoimmune disease III 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 I or autoimmune polyendocrinopathy candidiasis ectodermal dystrophy (APECED) 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's 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 I 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 II is much more common than type I and usually presents in adulthood or late childhood. Polyglandular autoimmune disease II is diagnosed when adrenal failure and autoimmune thyroid disease or type I

diabetes mellitus are present without hypoparathyroidism or candidiasis. Other diseases associated with this disorder include gonadal failure, vitiligo, diabetes insipidus, alopecia, pernicious anemia, myasthenia gravis, immune thrombocytopenia purpura, Sjogren's syndrome, rheumatoid arthritis, and celiac disease [34, 42, 43]. Much less is known about the etiology of polyglandular autoimmune type II other than an association with high-risk class II HLA alleles [45].

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. 12.4). 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. Males with 21-hydroxylase or 11β-hydroxylase deficiency do not manifest genital ambiguity, while those with 3β-hydroxysteroid dehydrogenase deficiency demonstrate under-virilization 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 [48]. 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 [49].

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 [50] which may result in adrenal insufficiency and 46, XY gonadal dysgenesis [51]. 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 [52].

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 [53, 54]. Defective ALDP function results in accumulation of very-long-chain fatty acid (VLCFA) demyelination in cerebral white matter and destruction of the adrenal cortex [55]. 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 [56–58]. 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 [59]. 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 [60].

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 [61–63]. Patients that also have achalasia and alacrima are classified as having Allgrove's

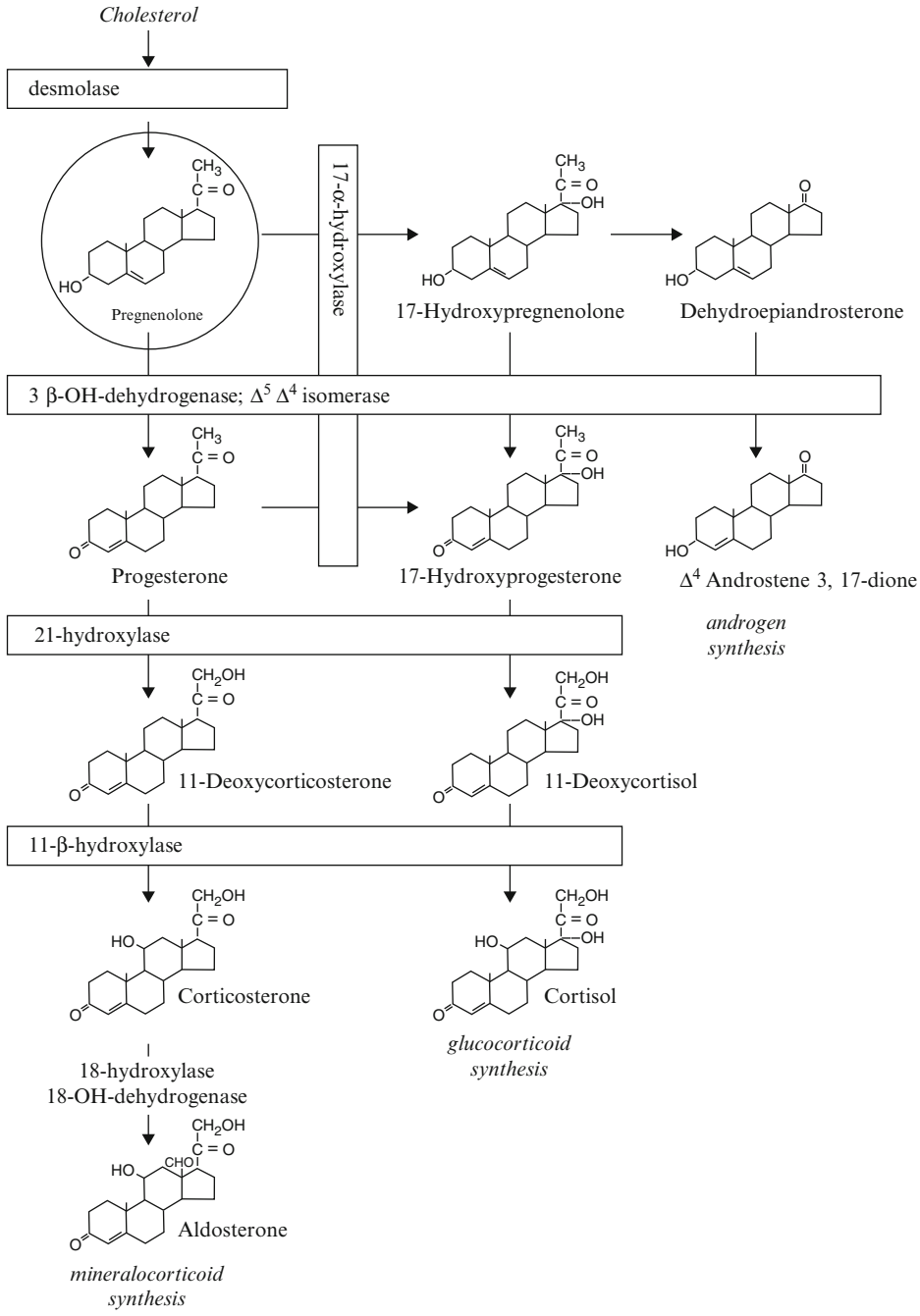

Fig. 12.4 Cortisol biosynthetic pathway. The enzymatic steps leading to mineralocorticoid, glucocorticoid, and adrenal androgen production are shown. Reproduced with permission from Bethune (Copyright © Pfizer Inc. Reproduced with permission) [192]

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*) [64] or steroidogenic acute regulatory protein (*STAR*) [65].

Wolman's disease, a rare autosomal recessive disease that results from complete deficiency of lysosomal esterase, is usually fatal in the first

year of life [66]. 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, cholesteryl ester storage disease [67].

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's sarcoma also is a significant cause of primary adrenal insufficiency in HIV-positive patients [68]. In addition, most patients with AIDS have decreased adrenal reserves as measured by prolonged ACTH stimulation [69].

Fungal disease has also been shown to cause primary adrenal insufficiency. Disseminated infection with histoplasmosis or blastomycosis may invade and destroy the adrenal glands [70, 71]. Cryptococcus and coccidiomycosis are rarer causes of adrenal insufficiency [72, 73]. Several drugs have been associated with the induction of adrenal insufficiency. Aminoglutethimide [74], etomidate [75], ketoconazole[76], metyrapone [77], and suramin [78] 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 [79, 80], barbiturates, and rifampicin [79] 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 [81].

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 [82]. 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 one year [83].

Several acquired and congenital lesions of the hypothalamus and pituitary are also causes of secondary/tertiary adrenal insufficiency (Table 12.1). 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 addi-

tional 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 development of the hypothalamus and pituitary associated with adrenal insufficiency include de Morsier syndrome (septo-optic dysplasia) [84, 85], 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 [86].

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 [87] reported small adrenal size in 3 children subject to postmortem analysis of the 10 individuals in the series, and a later study demonstrated evidence of central hypoadrenalism in 60% of PWS subjects undergoing a single-dose metyrapone test [88]. 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 [89, 90]. 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 [91, 92]. One kindred with suspected CRH deficiency and Arnold–Chiari type I malformation has been described [93]. While the mutation in this kindred is linked to the CRH locus, a specific mutation in CRH gene has not yet been defined.

Clinical Presentation

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 [94].

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 [95]. In children with polyglandular autoimmune syndrome type I, mucocutaneous candidiasis and hypoparathyroidism usually precede the appearance of adrenal insufficiency [41]. In X-ALD, neurological manifestations may either precede or follow the evolution of adrenal insufficiency [96, 97].

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's 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.

Diagnosis

Baseline Hormone Measurements. To verify suspected primary adrenal insufficiency in the patient presenting with classic signs and symptoms of Addison's disease, often little more is necessary than measurement of plasma ACTH, cortisol, renin activity, and aldosterone. Elevated ACTH and plasma renin activity together with low plasma cortisol and/or aldosterone confirms primary adrenal failure. In patients more than 6 months of age, the age at which the circadian pattern of glucocorticoid production has been established [98], not presenting in fulminant adrenal crisis, morning (approximately 8 am) ACTH and cortisol levels, along with electrolytes, plasma renin activity, and aldosterone, may establish the diagnosis. A morning cortisol of less than 3 mcg/dl is indicative of adrenal insufficiency, while concentrations exceeding 20 mcg/dl make adrenal failure quite unlikely [99]. 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 VLCFA measured to exclude X-ALD.

Screening for CAH caused by 21-hydroxylase deficiency on the newborn screen is universally required by law in all 50 of the United States [100]. 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 under-virilization 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/or 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 [101–103], random adrenal hormone measurements should be obtained on day of life 2 to 3. Additionally, the normal range of adrenal hormones differs for term and preterm infants and should be accounted for in interpretation of results [104–106]. 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.

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 [99, 107]. In the standard cosyntropin test, baseline ACTH and cortisol samples are obtained and then 250 mcg of cosyntropin is administered intravenously. If mineralocorticoid deficiency is also suspected, plasma renin activity, aldosterone, and electrolytes should be obtained with the baseline laboratories. Thirty and/or sixty minutes following cosyntropin administration, a second plasma sample is obtained for cortisol determination. Plasma cortisol concentration greater than or equal to 20 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 [108–111]. 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 to 60 minutes 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 [112].

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 [99, 113]. 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.

CRH Stimulation Test. To directly assess corticotroph function, ovine CRH 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 hours [114–116]. 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 definition of 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 hours. 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.

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 [117, 118]. 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) [119–121]. After an overnight fast, plasma is obtained at baseline, and then 30, 60, 90, 120, 150, and 180 min following injection. A normal response is achieved if peak cortisol exceeds 20 mcg/dl.

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 [122] and a multiple-dose form [123]. 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 [124]. 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.

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 [125] 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 [126–129]. Observation of calcifications in an older child is suggestive of tuberculosis or other granulomatous disease, while in an infant, the diagnosis of Wolman's disease should also be entertained. In a limited number of cases, CT-assisted needle biopsy for pathological diagnosis may be required.

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 [130]. 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 minutes after administering 1 mcg of cosyntropin intravenously is less than 9 mcg/dl [131–135]. Still others use a baseline cortisol of less than 7 mcg/dl [133, 136, 137] or a stimulated cortisol of <18 mcg/dl [133, 138] in response to low-dose cosyntropin as evidence of adrenal insufficiency [135]. 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 [139]. 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.

Diagnosing Adrenal Insufficiency in Neonates. Recently, it has been increasingly recognized that critically ill neonates may have relative adrenal insufficiency [140]. The fetal adrenal gland produces very little cortisol prior to 30 weeks gestation. This is partially due to lack of 3β-hydroxysteroid dehydrogenase activity prior to 23 weeks and partially due to suppression from maternal cortisol in preterm gestation. 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 cortisols [141–145]. 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.

Therapy

Primary Adrenal Failure. *Chronic Replacement*. The daily cortisol production rate in man is 5.7–7 mg/M^2/day [146–148]. Since the bioavailability of oral steroids is approximately 50% (but varies from individual to individual), the recommended dose for replacement hydrocortisone therapy is 10–15 mg/M^2/day divided 2–3 times per day [149, 150] (Table 12.2). It has been shown that twice-daily hydrocortisone produces a nonphysiological low cortisol level 2–4 hours prior to the next dose [151, 152]. Therefore, younger children, who are more prone to hypoglycemia when

Table 12.2 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 minutes to restore cardiovascular stability
Correct sodium at a maximal rate of 0.5 mEq/L to prevent central pontine myelinolysis
Stress dose steroid
Intravenous 100 mg/M^2 hydrocortisone, or if a new presentation, use 2.5 mg/M^2 of dexamethasone until ACTH stimulation test is done
Continue 100 mg/M^2/day hydrocortisone divided q 6–8 hours (or 2.5 mg/M^2/day dexamethasone) until stable for 24 hours
If a new presentation, perform ACTH stimulation test
Frequent assessment of electrolytes, blood glucose, and vital signs
Chronic replacement
Glucocorticoid replacement
10–15 mg/M^2/day of hydrocortisone divided BID–TID
Monitor clinical symptoms and morning plasma ACTH (Addison's 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 hours 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

(Reprinted with permission)

cortisol levels are low, or children with CAH, where efficient suppression of adrenal androgens is required, should receive hydrocortisone divided three 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 [100, 153–155]. Hydrocortisone tablets and liquid suspension are not equivalent. Hydrocortisone drug is unevenly distributed in the suspension and is not recommended for use in children [100, 156].

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 [157]. Because infant formulas are low in salt, infants are treated with 1–4 g per day of NaCl supplementation [158]. 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.

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 [159–164]. Estimates of the daily cortisol secretory rate in adults after surgery range from 60 to 167 mg/24 hours. Based on repeated cortisol measurements it has been estimated that adults undergoing minor surgery secrete 50 mg/day of cortisol [165, 166] and 75–150 mg/day after major surgery [163]. One comprehensive review of the literature [167] 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 30–100 mg/M^2/day of hydrocortisone with the onset of fever and gastrointestinal or other significant illness and continued for 24 hours after symptoms resolve [47, 168, 169] (Table 12.2). If the child has emesis within 1 hour of the dose, it should be repeated, and if emesis occurs again, an intramuscular injection of 30–100 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 30–100 mg/M^2 of hydrocortisone, and then continued on 30–100 mg/M^2/d divided every 6–8 hours for the next 24–48 hours postoperatively. There is no need to give extra fludrocortisone for stress; 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 should be infused over the first hour. Initially 100 mg/M^2 of hydrocortisone can be given intravenously and then 100 mg/M^2/day divided every 6–8 hours. In order to confirm a suspected diagnosis of adrenal insufficiency, equivalent doses of dexamethasone (2.5mg/M^2/day) should be given instead of hydrocortisone. 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/hr to minimize the risk for central pontine myelinolysis. Additionally, a glucose infusion should be continued during rehydration to avoid hypoglycemia.

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.

Special Considerations for Virilizing Forms of CAH. *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 3 times per day [47, 100, 170, 171]. 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 [100, 172–175]. 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 [176]. 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 [100, 177].

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 [178, 179]. 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, but it also stimulates ACTH secretion [180–182]. 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 [100]. Infants with CAH require approximately 1–4 grams per day of salt added to their formula or as supplementation to breast feeding [158].

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 [183, 184]. In any patient where normal levels of these precursors suppress growth, steroid doses should be reduced to maintain levels in the slightly elevated range [100]. 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.

Newer Therapies. *CAH*. A major shortcoming in the therapy of CAH is compromised final adult height [185–187]. 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. Currently, there is a trial underway of a modified-release hydrocortisone preparation to treat CAH [100, 188].

X-Linked Leukodystrophy. 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) have very little benefit [189]. 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 [55]. Bone marrow transplantation has shown some success when performed before significant cognitive changes have occurred [190, 191]. However, bone marrow transplantation does not reverse damage that has already occurred.

Psychosocial/Quality of Life

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.

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.

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.

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 [100]. 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 [100]. 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.

Adrenoleukodystrophy. The phenotype of X-ALD is variable with at least 7 clinical subtypes: childhood cerebral ALD, adolescent ALD, adult cerebral ALD, adrenomyeloneuropathy, Addison's disease only, asymptomatic, and heterozygote women. All of these subtypes can be present within the same family [97]. In 6–8% of cases, Addison's 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.

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Congenital Adrenal Hyperplasia

13

Christine M. Trapp, Lenore S. Levine, and Sharon E. Oberfield

Abstract

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. An abnormality in each of the enzymatic activities required for cortisol synthesis has 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. 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.

Keywords

Congenital adrenal hyperplasia • 21-Hydroxylase deficiency • Ambiguous genitalia • Nonclassic congenital adrenal hyperplasia • Newborn screening • Androgen excess

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. 13.1). An abnormality in each of the enzymatic activities required for cortisol synthesis has been described. As a result of the disordered enzymatic step, there is

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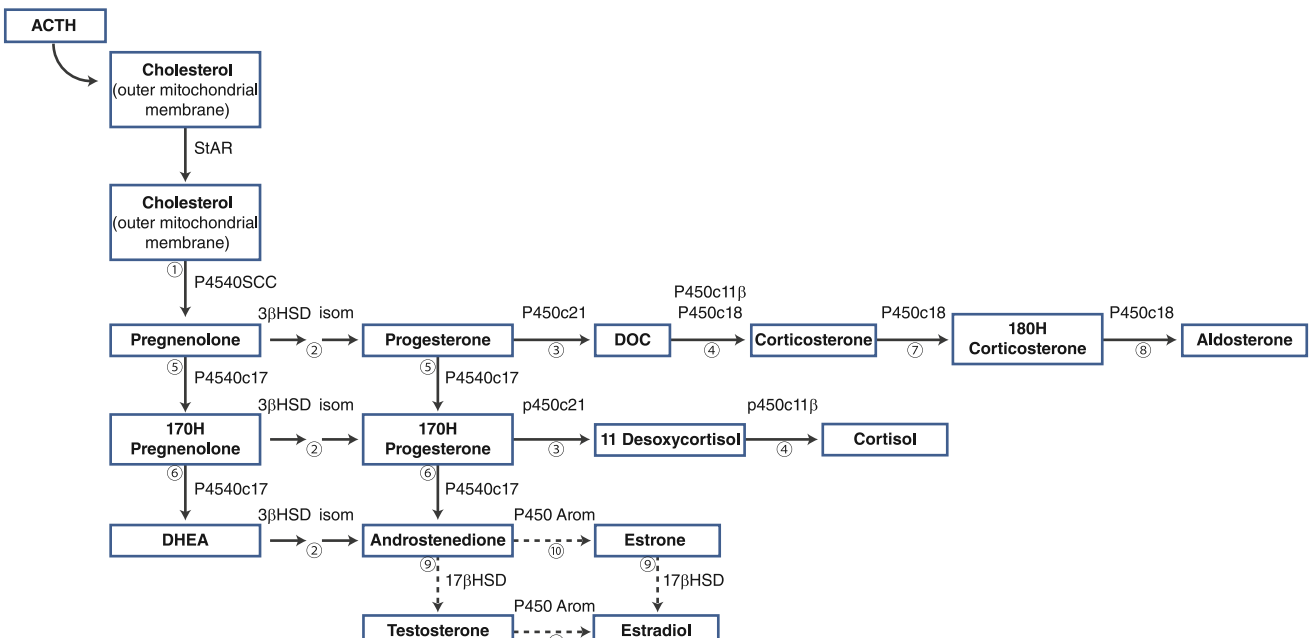

Fig. 13.1 Simplified scheme of adrenal steroidogenesis. Two reactions (*dotted arrows*) occur primarily in gonads, not in adrenal gland. Chemical names for enzymes shown above or to *right of arrows*; *circled* numbers refer to traditional names: (1) 20,22-desmolase; (2) 3β(beta)-hydroxysteroid dehydrogenase/isomerase; (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 13.1 Enzymes and genes in congenital adrenal hyperplasiaa

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

[a] Adapted with permission from Ref. [1] by the AAP

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 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 13.1. The clinical presentations of this family of disorders are presented in Table 13.2. 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.

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. 13.1). 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 ACTH 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; however, gonadal

Table 13.2 Clinical and hormonal data

Enzymatic deficiency	Signs and symptoms	Laboratory findings	Therapeutic measures
Lipoid CAH (cholesterol desmolase deficiency)	Salt-wasting crisis Male pseudohermaphoditism Incomplete female puberty	Low levels of all steroid hormones, with decreased/absent response to ACTH Decreased/absent response to HCG in males ↑↑ ACTH ↑↑ PRA	Glucocorticoid and mineralocorticoid administration Sodium chloride supplementation Gonadectomy of male pseudohermaphrodite Sex hormone replacement consonant with sex of rearing
3β(Beta)-HSD deficiency	Classic form: Salt-wasting crisis Male and female pseudohermaphroditism 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 pseudohermaphroditism 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 pseudohermaphroditism
21-Hydroxylase deficiency	Nonclassic form: Precocious pubarche Disordered puberty Menstrual irregularity Hirsutism Acne Infertility	↑ Baseline and ACTH-stimulated 17-hydroxy progesterone and pregnanetriol ↑ Serum androgens and urinary metabolites ↑ ACTH ↑ PRA Suppression of elevated adrenal steroids after glucocorticoid administration	Glucocorticoid administration

11β(Beta)-hydroxylase deficiency	Classic form: Female pseudohermaphroditism 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 pseudohermaphroditism
17α(Alpha)-hydroxylase/17,20 lyase deficiency	Male pseudohermaphroditism 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 pseudohermaphroditism ↓ PRA ↑ ACTH Hypokalemia Suppression of elevated adrenal steroids after glucocorticoid administration	Glucocorticoid administration Surgical correction of genitalia and sex hormone replacement in male pseudohermaphroditism 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

Adapted with permission from Ref. [136]

failure ultimately ensues (Table 13.2). Laboratory evaluation in patients with lipoid adrenal hyperplasia reveals low 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. 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 and most frequent in patients of Japanese and Palestinian descent [7, 8]. In this disorder, the gene coding for P450 SCC, a mitochondrial enzyme, has been normal in almost all cases studied (Table 13.1). 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. Common mutations in *StAR* have been identified in certain ethnic groups. Q258X accounts for the majority of mutations in Japanese and Korean patients, while R182L is found in Palestinians, R182H in eastern Saudi Arabians, and L260P in the Swiss [8, 9]. Recently, 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 [9]. Saenger et al. first described diagnosing congenital lipoid adrenal hyperplasia prenatally by measuring maternal estriol and amniotic fluid steroid levels [10]. It is now possible to diagnose congenital lipoid adrenal hyperplasia prenatally using targeted molecular genetic analysis for the c.201_202delCT mutation [8]. Other novel mutations in *StAR* are still being described [11].

While gonadectomy in affected 46,XY patients is currently recommended before puberty to prevent malignancy, of great importance 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 [9]. 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 [12]. Mutations in the P450scc gene have been described in two patients with lipoid adrenal hyperplasia: a 46,XY patient with a heterozygous mutation and a 46,XX patient with compound heterozygous mutations [13, 14]. Thus, 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 [7–17], the second one being P450-oxidoreductase deficiency [18].

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. 13.1, 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-hydrox-

ylase 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 ACTH.

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 3,000–12,000 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 13.2).

The 3β(beta)-HSD enzyme, located in the endoplasmic reticulum, mediates both 3β(beta)-HSD and isomerase activities (Table 13.1). In humans, there are two 3β(beta)-HSD isoenzymes, designated Type 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. A number of mutations in this gene have been described. 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 [19, 20].

In the past, signs of mild androgen excess in children and adults (precocious pubarche, acne, hirsutism, menstrual problems) have been attributed to a non-classic 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 non-classic 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 non-classic or mild form of the disease [19–26].

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 [27–29]. 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. 13.1). Similar to other forms of CAH, the deficiency in cortisol results in increased ACTH. Overproduction of DOC, a mineralocorticoid, ensues, producing 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 and are 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 13.2). Rarely, patients have been described with isolated 17,20-lyase deficiency.

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 13.1). At least 50 different genetic mutations of the *CYP17* gene have been documented with wide variation in the clinical and biochemical presentations, and novel mutations in the *CYP17* gene are still being described [30–32]. 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 [29]. The molecular basis for isolated 17,20-lyase deficiency has been elucidated [33–35].

21-Hydroxylase Deficiency

21-Hydroxylase deficiency is the most common form of CAH, affecting approximately 90% of individuals with CAH. It occurs with a worldwide frequency of approximately 1:15,000 newborns with increased frequency among certain ethnic groups (Yupik Eskimos; La Reunion, France). In this disorder, there is impaired ability to 21-hydroxylate progesterone and 17-hydroxyprogesterone to DOC and 11-deoxycortisol (S), respectively (Fig. 13.1). 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, resulting 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 ACTH secretion 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 13.2).

A milder non-classic 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 [36]. Salt-wasting is absent in the non-classic 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 13.2).

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 1,000 ng/dL. Testosterone levels are elevated to a variable degree and range from early male pubertal levels to levels in the adult male range (350–1,000 ng/dL). The 24-h urinary excretion of pregnanetriol and 17-ketosteroids, the metabolic products of 17-hydroxyprogesterone and androgens, respectively, are 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 non-classic 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 non-classic form [37]. Following ACTH administration, 17-hydroxyprogesterone may rise to levels of 2,000–10,000 ng/dL. Serum androgens are also less elevated compared to the classic form. Glucocorticoid administration results in a prompt decrease in the elevated hormones (Table 13.2).

21-Hydroxylation is mediated by P450c21, found in the endoplasmic reticulum (Table 13.1). 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 (*CYP21B*, *CYP21A2*) and one inactive (*CYP21P*, *CYP21A*). 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. Most patients are compound heterozygotes, having a different mutation on each allele. The severity of the disease is determined by the less severely affected allele. Approximately 75% of mutations are recombinations between the inactive *CYP21A* and the active *CYP21A2* gene, resulting in microconversions. Large gene conversions and gene deletions also occur.

The classic form of the disorder results from the combination of two severe deficiency genes, while the non-classic 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. Genotyping patients with CAH can be difficult given the complexity of gene duplications, deletions, and rearrangements within chromosome 6. More than 100 *CYP21A2* mutations are known; however, large deletions and a splicing mutation that abort enzyme activity encompass 50% of classic CAH alleles [38]. Point mutations, gene conversions, and gene duplications have been found in the mild *CYP21A2* deficiency genes. A valine-to-leucine substitution in codon 281 is a frequently found point mutation in non-classic 21-hydroxylase deficiency and is highly associated with HLA-B14DR1 [1–6].

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. 13.1).

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 (1,400–4,300 ng/dL) and DOC (183–2,050 ng/dL). Increased excretion of their metabolites, tetrahydro-11-deoxycortisol (THS) and tetrahydro-11-deoxycorticosterone (TH-DOC), 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. A milder form of 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) (Table 13.2).

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*) [39] (Table 13.1). 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 [40–44]. 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 [40, 45–48].

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 [49]. In 2004, the first reports describing mutations in the P450-oxidoreductase gene were published [50–53]. Since those initial reports, at least 50 additional patients with P450-oxidoreductase deficiency have been described. 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) [18]. *POR* knockout mice are embryonically lethal, most likely from extrahepatic *POR* deficiency; however, mutations in *POR* result in the variable clinical spectrum seen. Over 35 different *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 [18, 54, 55]. 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 [18, 54].

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 radio-ulnar synostosis, femoral bowing, fractures, and other skeletal deformities [56]. Not all patients with ABS exhibit genital abnormalities. 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 [57]. With P450-oxidoreductase deficiency, patients typically have increased ACTH, 17-hydroxyprogesterone, progesterone, and DOC with decreased cortisol and androgens postnatally, although steroid profiles can vary since *POR* deficiency affects multiple enzymes [18] (Table 13.2). 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 [18, 58, 59].

Because of the wide variability in phenotype and steroid hormone profile, the diagnosis and treatment of P450-oxidoreductase deficiency is not straightforward. 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 [18]. 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 [55, 59]. Because the majority of drugs are metabolized by hepatic P450 enzymes and *POR* mutants affect drug metabolism in vitro, drug metabolism may be abnormal in these patients and prescribing medications that are metabolized by P450 enzymes must be done with caution [18, 59]. Further study into the genetics and clinical outcomes of these patients is ongoing.

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.

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 10–20 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 [38]. Lower doses can often be used in the non-classical disorders. Whether the dose should be equally divided or a higher dose given in the morning or in the evening is controversial. 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 [38]. Some pediatric endocrinologists prefer cortisone acetate 15–20 mg intramuscularly every 3 days for the first 2 years of life. 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, 60]. 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- to 80-fold more potent [60, 61].

Mineralocorticoids

In the presence of aldosterone deficiency, fludrocortisone, a synthetic mineralocorticoid, is administered. The dose is usually between 0.1 and 0.3 mg daily. Current recommendations are that all infants with salt-wasting 21-hydroxylase deficiency require mineralocorticoid therapy, as well glucocorticoid treatment and sodium chloride supplementation [38]. 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.

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 testosterone patches 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.

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 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 [62]. 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 are 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 outcome in intersex patients. Many groups are exploring methods to improve outcome but there is at the present time no clear consensus on how this can best be achieved [63–66].

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, approximately 0.3 mg/kg IM every 28 days [67].

The use of a combination of an anti-androgen (to block androgen effect) and an aromatase inhibitor (to block conversion of androgen to estrogen) with reduced hydrocortisone and fludrocortisone doses has been reported in a 2-year study [68, 69].

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 suggested an improvement in predicted adult height, but long-term results and final heights were not available at the time [70, 71]. Although a 1- to 2-year nonrandomized study of children with CAH seemed to demonstrate an improved growth rate and height *z* score for bone age when growth hormone was administered, the current consensus statement recommends that further studies be undertaken to examine this issue [38, 72, 73]. Adrenalectomy has been reported in children with 21-hydroxylase deficiency and 11-hydroxlyase deficiency who could not be well controlled medically [74–77]. Bilateral adrenalectomy for CAH remains controversial. Studies of these new treatment regimens are required to determine if they result in better final outcomes.

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 the best measures of adequate glucocorticoid treatment in 21-hydroxylase deficiency [38].

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 13.2).

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 an additional measure of control [1–6, 38].

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. Cross-gender development and gender change from female to male have occurred [1–6, 64, 66, 78–90]. Decreased bone mineral

density in adult women with CAH has been reported [91]. However, in CAH children and adolescents on standard glucocorticoid therapy (10–20 mg/m^2/day), there is no evidence of decreased BMD assessed by DEXA when normalized for height [92–94].

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.

Prenatal Diagnosis and Treatment of CAH

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 [38]. 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. 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. Another newer technique for 1st-trimester sex determination involves isolating cell-free fetal DNA (ffDNA) from maternal plasma. This could allow for sex determination by the 7th week of gestation in women with high-risk pregnancies and potentially avoid treatment of non-affected fetuses [95]. De novo mutations, found in patients with CAH but not in parents, are found in 1% of disease-causing *CYP21A2* mutations [1–6, 96, 97]. Chorionic villus sampling, which can be performed at 10–12 weeks of gestation, is the preferred method over amniocentesis. If prenatal treatment is being considered, it must be instituted earlier in the 1st trimester before karyotyping and *CYP21A2* genotyping is determined [95].

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 [98, 99]. Lipoid adrenal hyperplasia has also been diagnosed prenatally using ultrasonography, amniotic fluid hormone levels, and maternal plasma and urinary hormone measurements [100, 101]. Theoretically, all forms of CAH can now be diagnosed prenatally by DNA analysis of chorionic villus cells.

Prenatal Treatment

As per the current consensus guidelines on CAH, prenatal treatment is regarded as experimental and no specific treatment protocols can be recommended [38]. 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 [102].

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 2 mg/day, has been administered in 1–4 divided doses. Dexamethasone is used since it is not inactivated by placental 11β(beta)-hydroxysteroid dehydrogenase type 2 [103].

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 had normal genitalia by report [104]. 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 United States 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. Long-term follow-up is limited and most infants have been followed only for a brief period of time. Detailed neuropsychologic evaluations have not been reported. 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 [96, 97, 105–108].

Cognitive and behavioral development of young children aged 6 months to 5 years treated prenatally with dexamethasone because of CAH risk was assessed by mother-completed standard questionnaires and compared with development of children from untreated CAH at-risk pregnancies. No significant differences in cognitive abilities or behavior problems were identified. Dexamethasone-exposed children were reported to demonstrate more shyness, emotionality, avoidance, and less sociability than unexposed children, although this has not borne out in all studies [109–111]. A recent small study found no differences in intelligence, handedness, or memory, but children who did not ultimately have CAH but were treated prenatally had poorer working memory, rated themselves lower in terms of scholastic achievement, and had increased anxiety [112].

Successful prenatal treatment has also been reported in 11β(beta)-hydroxylase deficiency [99]. 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 [38].

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 [106, 108, 113].

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 [105].

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 [114]. In one report, 30 of 44 dexamethasone-treated women indicated that they would decline prenatal treatment for a subsequent pregnancy [108].

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. 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 [115].

Treatment should be offered only to patients who have a clear understanding of the possible risks and benefits and who are able to comply with the need for very close monitoring throughout pregnancy and postnatally with continued follow-up of the prenatally treated child. 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 [105].

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 [116]. Shortly thereafter, a pilot newborn screening program was developed in Alaska [117]. Screening programs have been developed worldwide in various countries. As of 2009, all 50 states within the United States and 12 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 5,000 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 [38]. 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 [116–131]. 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 [132]. In the future, LC–MS/MS may become the method of choice for confirming positive results [133–135].

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. Prenatal diagnosis and treatment, as well as newborn screening, are now possible. 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.

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Cushing Syndrome in Childhood

14

Margaret F. Keil and Constantine A. Stratakis

Abstract

Cushing syndrome is a multisystem disorder resulting from the body's prolonged exposure to excess production of glucocorticoids. It is characterized by truncal obesity, growth deceleration, characteristic skin changes, muscle weakness, and hypertension. Most commonly, Cushing syndrome in childhood results from the exogenous administration of glucocorticoids. In this chapter, we present the causes and discuss the treatment of endogenous Cushing syndrome.

Keywords

Cushing syndrome • Adrenal • Pituitary • ACTH dependent • ACTH independent • Adenoma • Tumor

Introduction

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 nosology and therapy of Cushing syndrome have been made.

Cushing syndrome is a multisystem disorder resulting from the body's prolonged exposure to excess production of 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.

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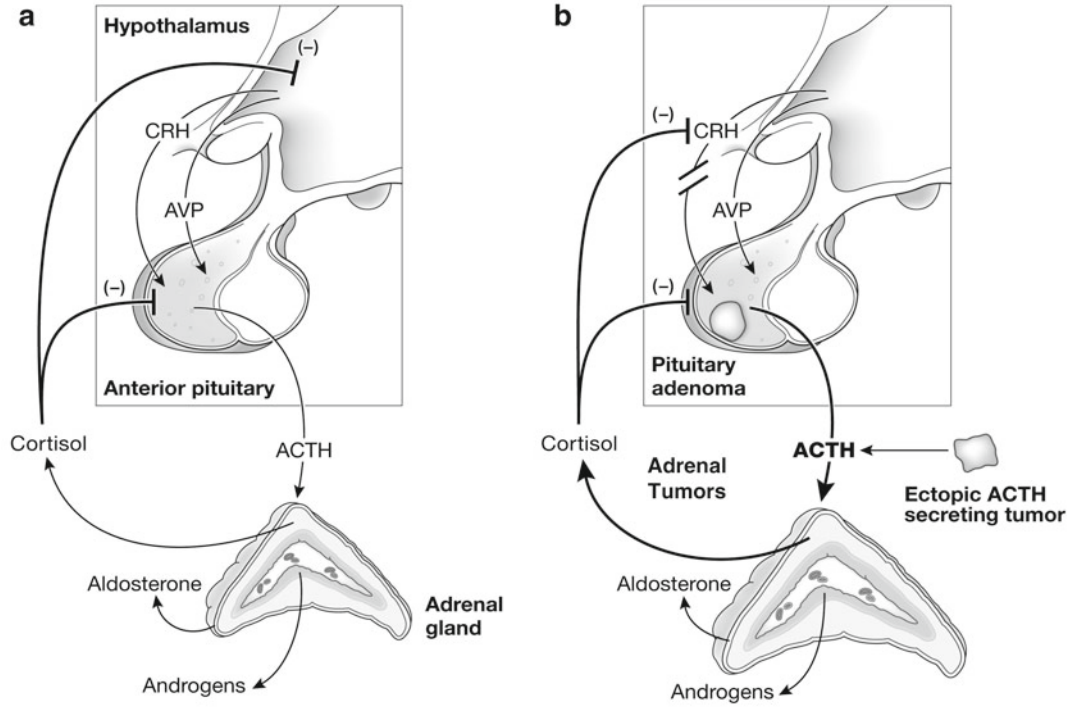

Fig. 14.1 (**a**) (*Left panel*) Physiologic regulation of cortisol secretion (*Abbreviations*: *CRH* corticotropin-releasing hormone, *AVP* arginine vasopressin, *ACTH* adrenocorticotropin). (**b**) (*Right panel*) Causes of Cushing syndrome; adrenal neoplasms include PPNAD, benign tumors, and adrenocortical carcinomas. *Straight arrows* represent stimulation

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. 14.1a). 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. 14.1b). 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 towards the primary cause of this disorder.

Epidemiology and 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. As in adult patients, in children with Cushing syndrome, too, there is a female to male predominance, which decreases with younger age; there might even be a male to female predominance in infants and young toddlers with Cushing syndrome [1, 3, 4].

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.

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. ACTH secretion in this disease occurs in a semiautonomous manner, maintaining 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 over-secretion by the hypothalamus or by an ectopic CRH source. However, this cause of Cushing syndrome has only been described in a small number of cases, and never in young children. 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. However, 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 [3–5]. 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.

More recently, bilateral nodular adrenal disease has been appreciated as a more frequent than previously thought cause of Cushing syndrome in childhood [5, 6]. 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 lentigines and myxomas. 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. The nodules are autonomously functioning, resulting in the surrounding atrophy of the cortex. Children and adolescents with PPNAD frequently have periodic, cyclical, or otherwise atypical Cushing syndrome.

Massive macronodular adrenal hyperplasia (MMAD) is another rare bilateral disease, which leads to Cushing syndrome [5]. The adrenal glands are massively enlarged with multiple, huge nodules that are typical, yellow-to-brown cortisol-producing adenomas. Most cases of MMAD are sporadic, although few familial cases have been described; in those, the disease appears in adolescents. In some patients with MMAD, cortisol levels appear to increase with food ingestion (food-dependent Cushing syndrome). These patients have an aberrant expression of the gastric inhibitory polypeptide receptor (GIPR) in the adrenal glands. In the majority of patients with MMAD, however, the disease does not appear to be GIPR-dependent; aberrant expression of other receptors may be responsible for the disease in these patients.

Adrenal adenomas or, more frequently, bilateral macronodular adrenal hyperplasia can also be seen in McCune–Albright syndrome (MAS) [7]. In this syndrome 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.

Molecular Genetics

Adrenocortical Hyperplasias

Aberrant cAMP signaling has been linked to genetic forms of cortisol excess. For example, MMAD may be associated with *GNAS1* mutations as seen in MAS or in some sporadic adrenal tumors [8]. In addition, micro-nodular bilateral adrenocortical hyperplasia (BAH), including PPNAD, is associated with germline inactivating mutations of the *PRKAR1A* gene [9]. Recently it has become apparent that several forms of micro-nodular BAH are not associated with inactivating mutations of the *PRKAR1A* gene but may occur as de novo or autosomal dominant inheritance of mutations, in the *PDE11A* or *PDE8B* genes [10, 11, 12].

Pituitary Corticotropinomas

Among functional pituitary tumors in early childhood, ACTH-producing adenomas are probably the most common although they are still considerably rare. To date, no genetic defects have been consistently associated with childhood corticotropinomas, which 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 [13].

Clinical Presentation

In most children, the onset of Cushing syndrome is rather insidious [1, 3, 4, 14]. The most common presenting symptom of the syndrome is weight gain (Table 14.1); lack of height gain concomitant with persistent weight gain is the most common presentation of Cushing syndrome in childhood. A typical growth chart for a child with Cushing syndrome is shown in Fig. 14.2.

Table 14.1 Clinical presentation of CS in pediatric patients (National Institutes of Health series—modified from Ref. [1])

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

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Other common problems reported in children include facial plethora, headaches, hypertension, hirsutism, amenorrhea, and delayed sexual development. Pubertal children may present with virilization. Skin manifestations, including acne, violaceous striae, bruising, and acanthosis nigricans are also common [2] (Fig. 14.3a–c). 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.

Diagnostic Guidelines

The appropriate therapeutic interventions in Cushing syndrome depend on accurate diagnosis and classification of the disease. The medical

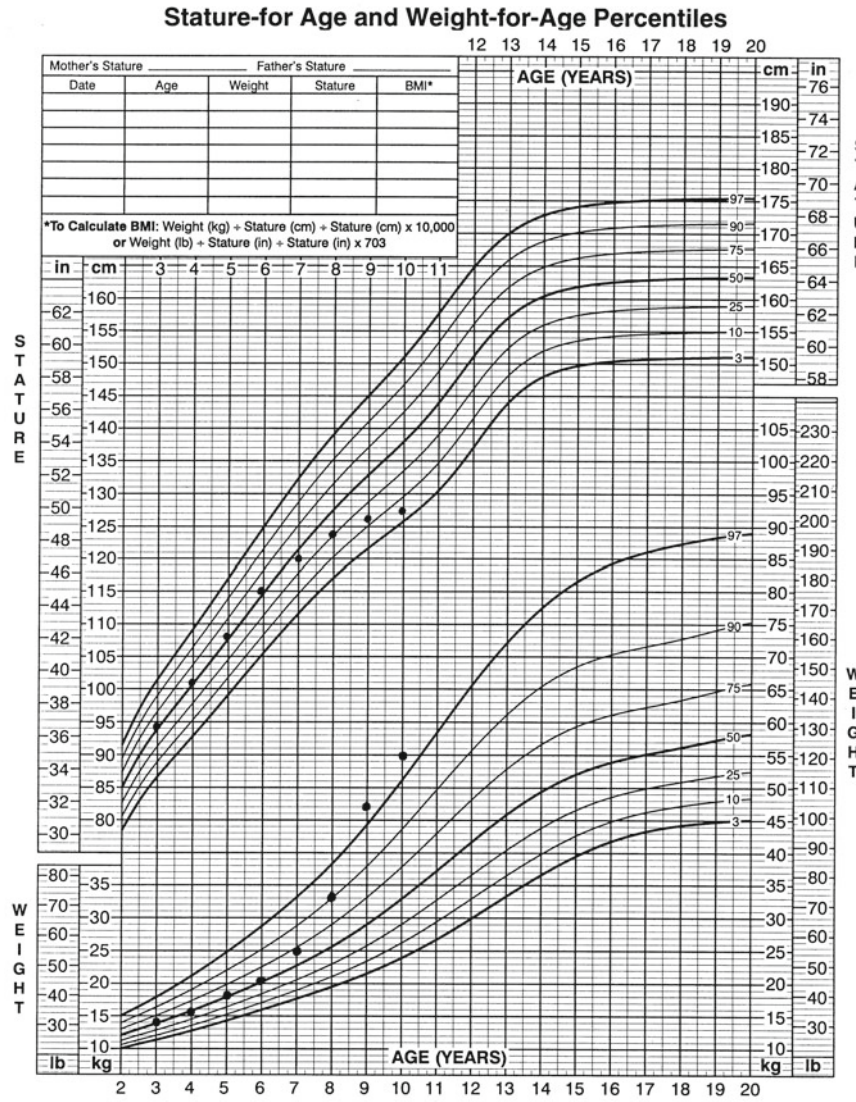

Fig. 14.2 Growth chart of a child with Cushing syndrome demonstrating growth rate deceleration with concomitant weight gain

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. 14.4.

The first step in the diagnosis of Cushing syndrome is to document hypercortisolism [15, 16], 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,

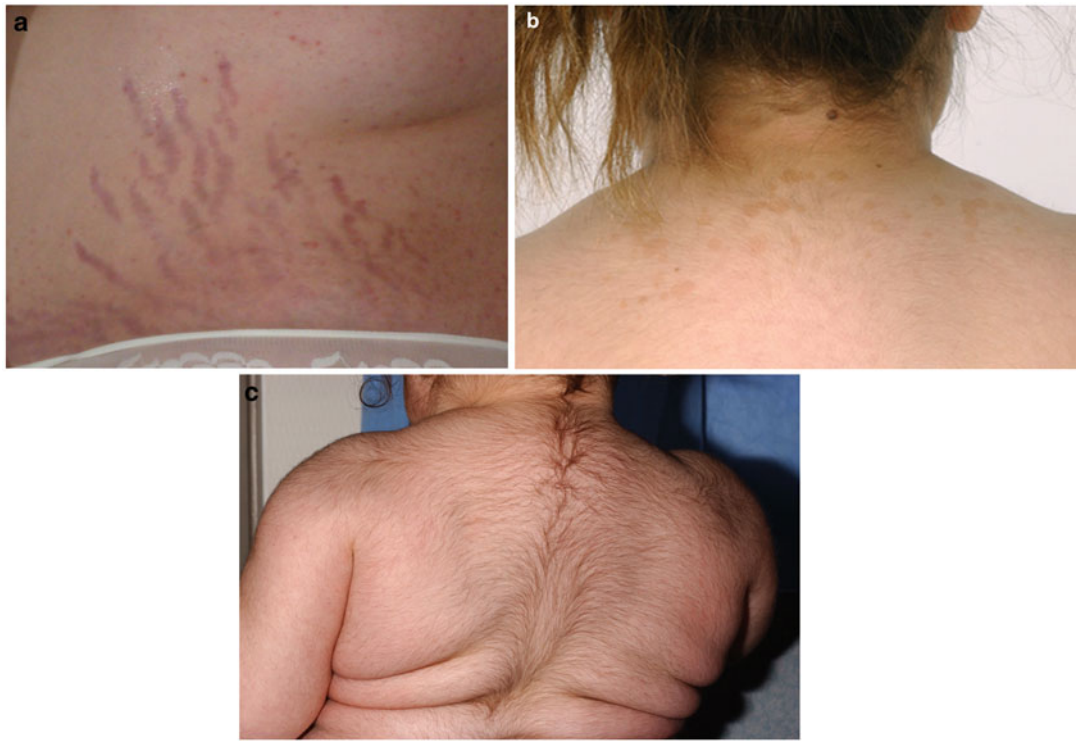

Fig. 14.3 Striae caused by endogenous Cushing syndrome in an 18-year-old girl (**a**), acanthosis nigricans and ringworm (*tinea corporis*) lesions in a 9-year-old (**b**), and hypertrichosis in a girl (**c**); both patients had long-standing Cushing disease

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 mostly 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) 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 [17]. This test has a low percentage of false normal suppression; however, the percentage of false positives is higher (approximately 15–20%). It should be remembered that the 1-mg overnight test, like the 24-h UFCs, does 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 is 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 [18]. 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

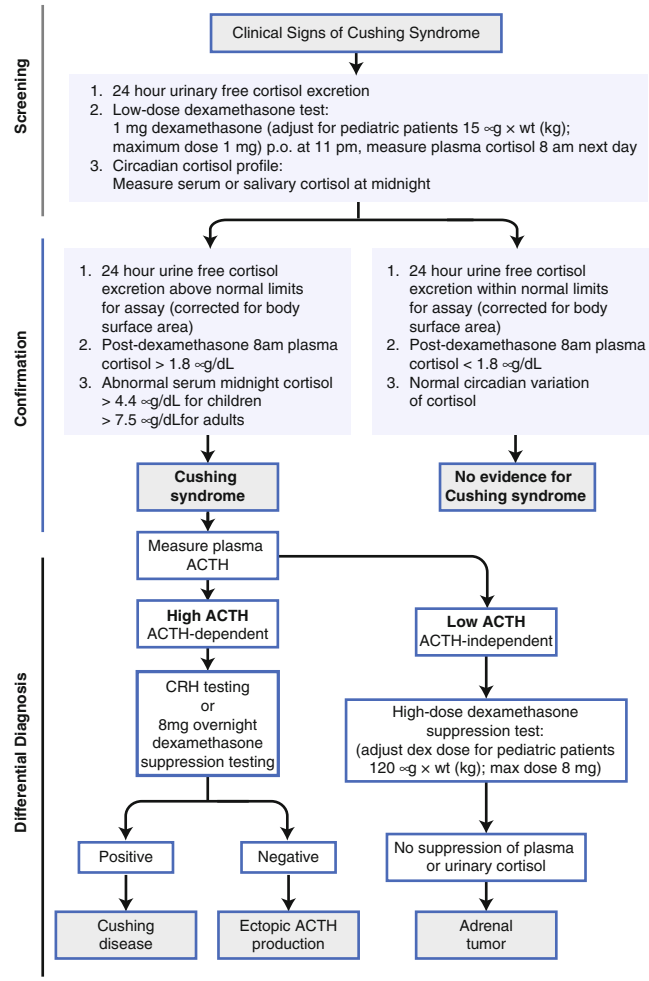

Fig. 14.4 Suggested diagnostic algorithm for the workup of suspected Cushing syndrome or hypercortisolemia. The details are discussed in the text; see also Ref. [6]

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, we recently reported that severe obesity (BMI >2 standard deviations) confounds the interpretation of the dexamethasone-CRH test. Confirmed height gain is a simple way to help distinguish children with pseudo-Cushing from those with Cushing syndrome [19].

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; we recently reported that 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 [15]. 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 or Liddle's test) is used to differentiate Cushing disease from ectopic ACTH secretion and adrenal causes of Cushing syndrome. The Liddle's 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. We recently reported in a pediatric population that 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 [15].

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 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 [11, 20].

We recently reported in a larger series of pediatric patients that 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) was the most rapid and accurate way for confirmation and diagnostic differentiation, respectively, of hypercortisolemia due to a pituitary or adrenal tumor [15]. However, for accuracy, diurnal testing requires an inpatient stay, and this may limit its use as a routine screening test [15].

An oCRH stimulation test may also be obtained for the differentiation of Cushing disease from ectopic ACTH secretion [21] and/or adrenal lesions. In this test, 85% of patients with Cushing disease respond to oCRH with increased plasma ACTH and cortisol production. 95% 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 and 45 min and an increase in the mean corticotropin concentrations of at least 35% over basal value at 15 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. Recently, we reported that post-contrast spoiled gradient-recalled (SPGR-MRI) was superior to spin echo (SE-MRI) in the detection of a microadenoma in children and adolescents with Cushing disease [22] (Fig. 14.5).

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

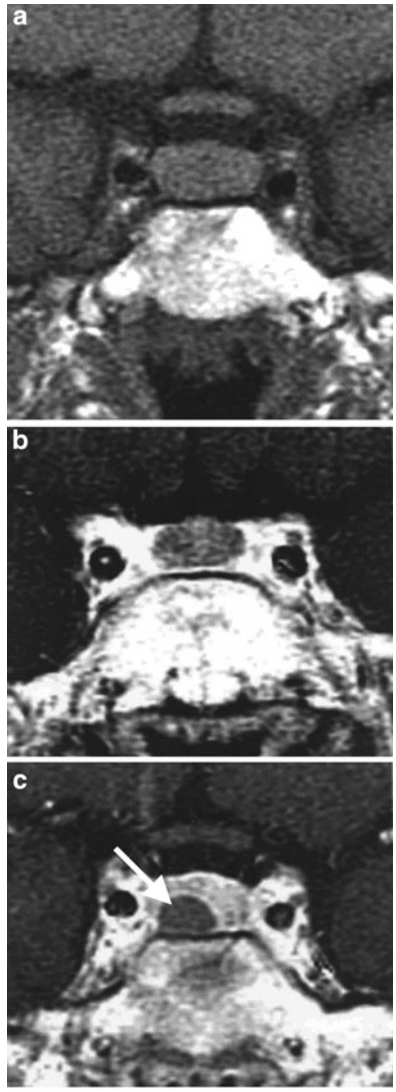

Fig. 14.5 MRI studies of a patient with a corticotropinoma detected by both SE- and SPGR-MRI in post-contrast studies. (**a**) Coronal pre-contrast SE images revealed no abnormality. (**b**) Coronal post-contrast SE images demonstrated a homogeneously hypoenhancing area in the right side of the anterior 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

presence of bilateral hyperplasia (micronodular or PPNAD) or the rare case of bilateral adrenal carcinoma. Most patients with Cushing disease 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.

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.

Since up to 50% of pituitary ACTH-secreting tumors and many of ectopic ACTH 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 [23]; however, we recently reported that it is a poor predictor of the site of a microadenoma in children [24]. 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, 5, 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.

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% [25]. 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%. 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 4,500/5,000 cGy total, usually given over a period of 5 to 6 weeks. Newer forms of stereotactic radiotherapy are now available as options for treatment of ACTH-secreting pituitary tumors. Photon knife (computer-assisted linear accelerator) or the gamma knife (cobalt-60) approaches are now available; however, experience with these techniques is limited especially in children. These modalities may be attractive because of the smaller amount of time required for these procedures and the possibility for fewer side effects.

The treatment of choice for benign adrenal tumors is surgical resection. This procedure can be done by either transperitoneal or retroperitoneal approaches. In addition, laparoscopic adrenalectomy is also available at many institutions. Adrenal carcinomas may also be surgically resected, unless diagnosed at later stages. Solitary metastases should also be removed, if possible [26]. Therapy with mitotane, which is an adrenocytolytic agent, can be used as an adjuvant therapy or in the case of an inoperable tumor. Other chemotherapeutic options include cisplatin, 5-flourouracil, suramin, doxorubicin, and etoposide. Occasionally glucocorticoid antagonists and steroid synthesis inhibitors are needed to correct the hypercortisolism. Radiotherapy can also be used in the case of metastases. The prognosis for adrenal carcinoma is poor, but usually children have a better prognosis than adults.

Bilateral total adrenalectomy is usually the treatment of choice in bilateral micro- or macronodular adrenal disease, such as PPNAD and MMAD. In addition, 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. Nelson syndrome, which includes increased pigmentation, elevated ACTH levels, and a growing pituitary ACTH-producing pituitary tumor, may develop in up to 15% of patients with Cushing disease who are treated with bilateral adrenalectomy. Children with long-standing untreated Cushing disease are especially vulnerable to Nelson syndrome after bilateral adrenalectomy.

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. Mitotane is an inhibitor of the biosynthesis of corticosteroids by blocking the action of 11-β-hydroxylase and cholesterol side-chain cleavage enzymes. It also acts by destroying adrenocortical cells that secrete cortisol. Other adrenal enzyme inhibitors, such as aminoglutethimide, metyrapone, trilostane, and ketoconazole, may also be used alone or in combinations to control hypercortisolism. Aminoglutethimide blocks the conversion of cholesterol to pregnenolone in the adrenal cortex inhibiting the

synthesis of cortisol, aldosterone, and androgens. Metyrapone acts by preventing the conversion of 11-deoxycortisol to cortisol. It can also cause hypertension secondary to the accumulation of 11-deoxycorticosterone. Trilostane inhibits the conversion of pregnenolone to progesterone. Ketoconazole is an agent, which inhibits several steroidogenic enzymes and is excellent in blocking adrenal steroidogenesis.

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.

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. During this period, glucocorticoids should be replaced at the suggested physiologic replacement dose (12–15 mg/m^2/day bid or tid). In the immediate postoperative period, stress doses of cortisol should be initiated. These should be weaned relatively rapidly to a physiologic replacement dose [27, 28]. 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 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.

Psychosocial Implications

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 psychopathology [29]. 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 [30].

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Part IV

Thyroid Disorders

Congenital Hypothyroidism

15

Cecilia A. Larson

Abstract

Congenital hypothyroidism remains the leading cause of preventable mental impairment worldwide. It is most often caused by iodine deficiency (endemic cretinism) or thyroid dysgenesis but can be caused by any defect in thyroid hormone production, regulation, or action. Because congenital hypothyroidism is common and newborns initially exhibit few specific signs or symptoms of the disorder, most developed countries offer universal newborn thyroid screening. With early identification and treatment of affected newborns, neurologic sequelae are minimized, and development in most adequately treated cases is normal.

Keywords

Congenital hypothyroidism • Iodine deficiency • Thyroid dysgenesis • Thyroid screening • Thyroxine-stimulating hormone

Introduction

Congenital hypothyroidism remains the leading cause of preventable mental impairment worldwide. It is most often caused by iodine deficiency (endemic cretinism) or thyroid dysgenesis but can be caused by any defect in thyroid hormone production, regulation, or action (see Table 15.1). Because congenital hypothyroidism is common and newborns initially exhibit few specific signs or symptoms of the disorder, most developed countries offer universal newborn thyroid screening. With early identification and treatment of affected newborns, neurologic sequelae are minimized, and development in most adequately treated cases is normal.

Recognition of iodine deficiency and attempts to eliminate the problem have been ongoing for decades, yet there remain significant areas of deficiency. Ongoing surveillance for iodine status is important as dietary deficiency tends to recur in certain populations and regions. While iodine deficiency can cause thyroid disorders in all ages, the fetus and newborn are at special risk for consequences of insufficient iodine due to the critical thyroxine-dependent intervals of neurodevelopment. For this reason, surveillance and treatments

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Table 15.1 Causes of congenital hypothyroidism (CH)

Primary hypothyroidism
Thyroid dysgenesis
Athyreotic
Ectopic
Hypothyreotic (ex hemithyroid)
Thyroid hormone dysgenesis—goitrous enzyme defect
Iodine deficiency
Iodine transporter defect
Peroxidase defect
Thyroglobulin synthetic defect
Peripheral thyroid hormone inactivation
Tumor deiodinase activity
Iodotyrosine deiodinase defect
TSH resistance (normal or hypoplastic gland)
Transient hypothyroidism
TSH receptor (TR-β)mutations
Gsα gene mutations
Maternal antithyroid medications
Maternal antibodies-Maternal thyrotropin receptor-blocking antibody (TRB-Ab)
Iodine
Idiopathic
Central hypothyroidism
Hypothalamic
Pituitary
Pituitary maldevelopment
Pit 1, TSH-β
Medications such as corticosteroids and dopamine

aimed at reproductive age women and newborns are of particular importance. Screening for iodine deficiency and its sequelae can be achieved by numerous means (thyroid ultrasonography, urinary iodine measurements, blood thyroid or iodine tests, and newborn screening). Of particular interest is the potential dual role of neonatal thyroid screening to detect both thyroid insufficiency in individual neonates and to detect populations at risk of iodine deficiency by monitoring the newborn population mean thyroxine-stimulating hormone (TSH) concentration [1].

Even in areas traditionally considered iodine sufficient, such as the United States, it is important to have surveillance for population iodine status, since recently, childbearing-aged women demonstrated a significant decline in dietary iodine intake [2, 3]. It will be important to monitor this trend and to track for potential consequences in terms of thyroid conditions in these at risk populations.

Aside from regional iodine deficiency as a cause of hypothyroidism in newborns, certain ethnic groups are at increased risk of developmental thyroid anomalies, suggesting there are heritable factors in thyroid development. Among the groups at increased risk are Hispanics, Chinese, Vietnamese, Asian Indians, Filipinos, Middle Easterners, and Hawaiians, whereas blacks are at reduced risk (1/3 the risk compared to whites) [4]. Thyroid dysgenesis is also twice as common in female newborns as males [5].

In iodine-sufficient areas, the leading cause of congenital hypothyroidism is thyroid dysgenesis, which accounts for about 80% of cases. Any defect in thyroid hormone production, regulation, and action can cause hypothyroidism, and Table 15.1 shows a categorization of types of congenital hypothyroidism. Thyroid hormone synthesis is dependent on sufficient iodine substrate, adequate iodine trapping, oxidation, and organification as well as sufficient production of thyroglobulin. Release of thyroid hormones from the thyroid gland is accomplished by proteolysis. Thyroid hormone is predominantly protein bound in the circulation, and peripheral conversion to active hormone is accomplished by the deiodinases. The active T3 binds to its nuclear receptors and transcriptionally activates genes with thyroid hormone response elements. Regulation of thyroxine production and metabolism is under control of the hypothalamic/pituitary axis (thyrotropin-releasing hormone and thyroid-stimulating hormone). Malfunction of any step of thyroid hormone production or regulation can result in hypothyroidism.

Thyroid Dysgenesis

The thyroid gland forms during the first trimester of development. It has a complex migratory development, arising from the median and lateral anlagen. The median anlage appears during the second week of gestation, expands into a bilobate structure adjacent to the heart, and is pulled caudally with the developing heart, completing its migration

by the 7th week of gestation. The lateral anlagen derive from the fourth branchial pouches and fuse creating the bilobate structure about the time the migration is complete [6]. It is notable that the most consistent developmental anomalies associated with congenital hypothyroidism have been cardiac [7–10]. The coincidence of timing and location of thyroid and cardiac embryonic development supports the theory of common cause for these anomalies. Since thyroid dysgenesis does not generally recur in families, it has been thought to be a developmental rather than a heritable condition, possibly associated with environmental teratogens. Seasonal variations in incidence of congenital hypothyroidism [11] have also been noted, giving further support to critical exposure (such as seasonal viruses) as a contributing factor to sporadic congenital hypothyroidism. However, as early as 1966, there was a report of dysgenesis in two pairs of monozygotic twins and in a mother and child [12]. The discovery of developmental genes, which play important roles in embryogenesis and developmental cell migrations, and the identification of mutations in these types of genes which have been associated with developmental anomalies including thyroid dysgenesis (e.g., PAX-8, TTF-2, and connexin) point to a role for genetic predisposition to thyroid dysgenesis [13–15] and may in part explain the observed higher incidence of congenital hypothyroidism among certain ethnic groups. It is likely that both genetic and environmental factors contribute to thyroid developmental anomalies.

Impaired thyroid hormone synthesis may be caused by insufficient intake of iodine, or by a number of mostly autosomal recessive defects which affect thyroid hormone synthesis. These include sodium-iodide symporter (NIS) which is responsible for actively transporting iodine into thyroid follicular cells, for which numerous mutations have been identified which can cause hypothyroidism [16–22].

Sufficient T3 must bind TR and activate thyroid-responsive genes for normal development to occur. Thyroid-responsive genes are present throughout the body, with specific time intervals of thyroid hormone responsiveness (see Fig. 15.1). Both human and experimental animal data indicate the critical role of thyroid hormone in development. Instances of maternal and fetal hypothyroidism (from iodine deficiency and untreated maternal thyroid-blocking antibodies) point to a critical role in neurodevelopment and hearing [23–26]. The primary effects in neurodevelopment are on the neural connections and arborization and myelination, which begins in utero and continues until age 3 years [27–29]. While bone age delay can begin in utero, overall growth is not compromised during gestation with growth retardation appearing only postnatally.

Clinical Presentation

Most neonates with congenital hypothyroidism appear clinically normal at birth. When signs and symptoms are initially present, they are nonspecific, making clinical detection difficult and often delayed. Early signs of possible congenital hypothyroidism include mottled and dry skin, lethargy, poor feeding, macroglossia, enlarged posterior fontanel (>1 cm), umbilical hernia, jaundice, constipation, hoarse cry, sleepiness, and hypothermia [30–32]. Of note, newborns with congenital hypothyroidism are not growth retarded at birth, although bone age may be delayed in cases of severe congenital hypothyroidism, most commonly with athyreosis. Hypothyroidism of longer duration is associated with decreased linear growth rates and epiphyseal changes. Pseudomuscular hypertrophy, delayed tooth development, and developmental delay can also occur.

Acquired hypothyroidism can occur at any age, and frequency increases with age. In the differential of "acquired" hypothyroidism in early childhood is congenital hypothyroidism not detected in the newborn period. This is particularly likely for partial thyroid dysgenesis or dyshormonogenesis which is compensated by gland hypertrophy for a variable period of time. Other causes of acquired hypothyroidism are autoimmune thyroiditis, thyroid dysfunction secondary to thyroid/hypothalamic/pituitary destruction due to chemotherapy, radiotherapy, iron, or other infiltrative processes. While some signs of hypothyroidism are consistent

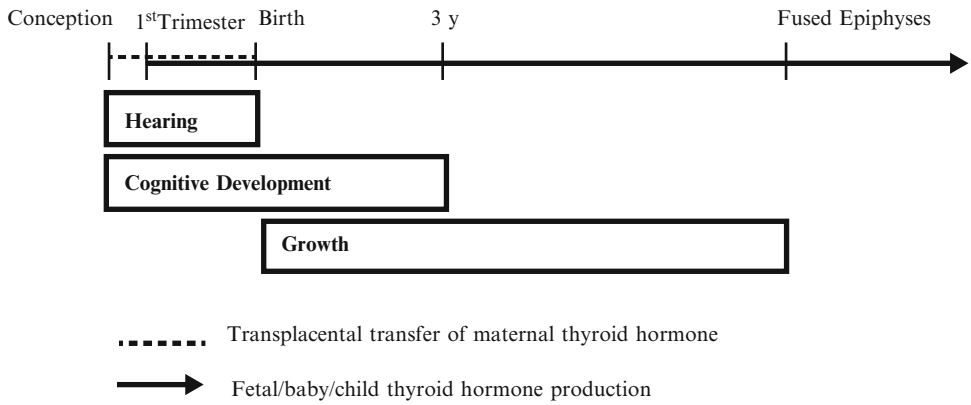

Fig. 15.1 Critical stages of irreversible thyroid hormone-dependent development

regardless of age at presentation, such as dry skin, constipation, and lethargy, other signs and sequelae are dependent on the developmental stage during which hypothyroidism occurs. Generally, all aspects of acquired hypothyroidism in adulthood are reversible with thyroxine treatment, and treatment delay does not cause any irreversible effects. In the developing fetus, newborn and child up to age 3, delay in treatment can cause irreversible developmental delays. The most sensitive clinical sign of hypothyroidism in the growing child is growth retardation. Decrease in linear and bone growth is characteristic of hypothyroidism, and examination of bone films and the growth curve can be extremely helpful in timing the onset of hypothyroidism.

Manifestations of hypothyroidism do not generally differ by sex of affected individual (other than menstrual irregularities which can occur in women with hypothyroidism); however, prevalence of thyroid disease (autoimmune and sporadic dysgenesis) is much greater in females (2:1 ratio for thyroid dysgenesis).

Cardiac malformations have been associated with thyroid dysgenesis but not with dyshormonogenesis, suggesting a unifying exposure or developmental gene which affects both thyroid gland formation and septation of the embryonic heart [33] rather than in utero hypothyroxinemia causing secondary cardiac malformations. TTF-2 has been associated with cleft palate and thyroid dysgenesis [14]. Some groups have reported hip dislocation, [9] though in other series, this has not been confirmed. In addition, the following are some of the syndromes associated with congenital hypothyroidism: Pendred syndrome, pseudohypoparathyroidism and hypoparathyroidism, Beckwith syndrome, Young-Simpson syndrome, and Sotos syndrome. Septo-optic dysplasia can be associated with varying degrees of hypopituitarism with growth hormone deficiency occurring with the greatest overall frequency and central hypothyroidism occurring in some cases [34].

Individuals with trisomy 21 are at increased risk for congenital hypothyroidism; in some studies, it has been found in 12.5% of Down's newborns [35, 36, 38–41]. Congenital hypothyroidism associated with Down's syndrome occurs with equal frequency in affected males and females (unpublished data from New England Newborn Screening Program) and is not associated with dysgenesis, suggesting that trisomy 21 does not affect development of the thyroid gland but rather has an effect on thyroid hormone synthesis and/or gland function. Recent reports suggest zinc deficiency in trisomy 21 patients may play a role in minor TSH elevations [37]. Individuals with Down's syndrome are also at increased risk of acquired hypothyroidism [38]. Since the signs of hypothyroidism can be mistakenly attributed to Down's (macroglossia, developmental delay, growth failure), routine interval TSH screening of all children with Down's is recommended.

Although the precise interval for screening has not been established, an approach which would focus on increased screening during the critical developmental phases would be newborn screening with T4 and TSH at 2 days, 2 weeks, and 2 months, then serum specimens, then q6–12 months up to 3 years of age, and annually thereafter (sooner if any signs or symptoms of hypothyroidism are noted).

Diagnosis

Newborn Screening

The role of newborn screening is to detect treatable, time-critical, newborn disorders which if undiagnosed would lead to significant morbidity and mortality. Newborn screening utilizing dried blood specimens collected on filter paper began in Massachusetts in 1962 with the introduction of the Guthrie bacterial inhibition assay (GBIA) to measure blood phenylalanine levels as a screen for phenylketonuria (PKU) [42]. In the 1970s, the ability to detect thyroxine and thyroid-stimulating hormone in dried blood specimens utilizing radioimmunoassay methodology was developed [43, 44] and subsequently incorporated into public health newborn screening programs. Currently, there is mandatory universal newborn thyroid screening throughout the United States, Canada, much of Europe, Australia, and some form of newborn thyroid screening (but not necessarily universally available) in parts of Asia, the Middle East, and Latin America. Newborn screening for thyroid disease has been one of the great public health success stories. Prior to screening, only one-third of hypothyroid infants were clinically detected before 3 months of age, and the majority of children had severe mental retardation, language, learning, and coordination difficulties [45]. With the introduction of screening, the age at detection has steadily declined. In the early phases of newborn thyroid screening, the target was to identify and initiate treatment by age 2 months. Currently with rapid specimen transport of newborn specimens collected on average day of life two and advances in technology which allow rapid T4/TSH analysis, many screening programs detect and initiate treatment within 1–2 weeks of age, allowing normal developmental outcome of individuals with congenital hypothyroidism [46]. Treatment with thyroxine is curative, making newborn thyroid screening cost effective [47]. The incidence of congenital hypothyroidism is approximately 1:4,000 in North America, though in Massachusetts the incidence has been rising and is currently 1:2,000 [48].

Types of Thyroid Newborn Screening Strategies

Primary T4, secondary TSH: all newborns screened for total thyroxine concentration, with triggered TSH for those with the lowest thyroxine values (below a cutoff value and for a certain percentile of the screened population, for instance, all T4 < 13 μg/dl and the lowest decile). This approach allows detection of central and peripheral hypothyroidism [49–51]. However, there is a broad range of normal thyroxine concentrations and low thyroxine is common in premature and sick infants and in individuals with thyroxine-binding globulin (TBG), or other binding protein, deficiencies. Binding protein deficiency does not require treatment, but is identified as a by-product of this screening strategy.

Primary TSH, with or without secondary T4: this strategy was initially adopted in European screening programs and provides a mechanism for monitoring for regions of iodine deficiency because it provides information about mean TSH for specific populations [1]. Another potential advantage of TSH screening is the theoretical enhanced detection of partially compensated hypothyroidism [52]; that is, T4 maintained in the normal range with elevated TSH. In a large study by Dussault in 1983 with simultaneous T4 and TSH (micromedic T4 assay and RIA TSH) in 93,000 consecutive filter paper specimens, the T4 assay had better precision, and there was similar sensitivity in case detections by either primary T4 or primary TSH screening, with false negatives ($N = 3$) by either approach including a case of central hypo which was detected only by the T4 approach [53].

Dual T4 and TSH: may be the most sensitive approach currently in use allowing detection of central hypothyroidism and euthyroxinemic hypothyroidism, but depending on cutoffs utilized, may result in less specificity and higher recall rates.

Free T4 is potentially the most sensitive and specific screening strategy. There is report by one program of a fT4 screening method utilizing filter paper specimens, but it has not been a widely reproducible method to date [54].

As with any laboratory test, the reference range for the normal population and the diseased population has to be established. Determining cutoffs for screening results is complex and should be periodically reassessed [55]. This is particularly true of endocrine newborn screening as timing and clinical status can affect the hormone reference range. At parturition and exposure to the cold, extrauterine environment, a neonatal TSH (and consequently T4) surge occurs within minutes of birth and subsides over the next 24–72 h. This surge also occurs, but in a stunted fashion, in preterm infants [56–58]. Newborn screening specimens collected at less than 24 h are enriched for mild to moderate TSH elevations which normalize on follow-up (increasing the recall rate); specimens collected at less than 24 h are also at jeopardy of masking hypothyroidism by showing a normal T4 which subsequently falls (T4 of maternal origin ± T4 surge). The ideal collection time for congenital hypothyroidism screening is probably 3–5 days of age to optimize both sensitivity and specificity of screening. In a review of the impact of early discharge on newborn screening, the higher recall rate and cases of missed diagnosis were noted [59]. Most screening programs require a follow-up specimen if the initial specimen is collected at <24 h to minimize the chance of a missed diagnosis.

Each laboratory should establish its reference range for its population and testing method.

In the New England Newborn Screening Program, when T4 measurement was changed from an RIA method to the AutoDELFIA method, a significant increase in mean T4 for newborns from 13 to 16 μg/dl was noted , and consequently the absolute T4 cutoff to trigger TSH testing was raised [60].

Work-Up of Screen-Positive Cases

Whenever notification of out of range newborn screening thyroid results is received, it is recommended that the newborn be promptly evaluated with a complete history and physical. History should include note of maternal/family history of thyroid disease, maternal medications (especially antithyroid medications or iodine), and baby medications (especially iodine, steroids, dopamine). Physical examination should include careful inspection for any signs of hypothyroidism, goiter, or sublingual masses.

Elevated TSH

Because the positive predictive value correlates with the degree of TSH elevation, a general guideline for management of newborn screening results is for TSH > 40 to collect confirmatory serum studies and initiate thyroxine while awaiting confirmatory test results. As a minimum, serum confirmatory studies should include T4 and TSH.

More modest TSH elevations have a lower positive predictive value. Thus, for TSH elevations in the 20–40 range, particularly if the T4 is in the normal range (>12), a follow-up filter paper or serum specimen is generally sufficient.

Generally accepted case definition for primary hypothyroidism is TSH>20 (or 25) on more than one specimen, collected after 24 h of age [61].

Low T4 and Non-elevated TSH

The differential diagnosis of low T4 and non-elevated TSH includes central hypothyroidism, acute illness, hypothyroxinemia of prematurity, and thyroid-binding globulin deficiencies. Central hypothyroidism is rare, occurring 1:50–100,000 births [49, 50]; it has been associated with hypoglycemia (due to adrenal insufficiency) and hypospadias in males and can be associated with certain syndromes such as SOD and midline craniofacial defects. A general approach to low T4 is to confirm the finding with another filter paper specimen and, if confirmed, to proceed to

serum fT4 testing in normal birthweight infants. Since hypothyroxinemia occurs in up to 50% of preterm infants (with 10% having T4 < 5 μg/dl), serial filter paper screening is generally sufficient. Preterm infants are at increased risk for delayed TSH elevations and this can be detected by performing the serial testing [62].

Role of Additional Confirmatory Testing

Bone age is useful for timing onset of hypothyroidism and for monitoring response to therapy; individuals with significant bone age delay at birth may be at risk for less than optimal outcome as it may be a marker for in utero hypothyroxinemia. In cases of bone age delay, prompt initiation of high thyroxine dose treatment is indicated. With prompt thyroxine, growth will normalize and bone age will also normalize.

Iodine (I-123) and technetium (TC99m) thyroid scans can be used to determine thyroid location and uptake. Iodine scans indicate not only iodine uptake but also organification of iodine. When the scan indicates athyreosis or ectopic thyroid (which combined account for about 80% of congenital hypothyroidism), it indicates need for lifelong thyroxine treatment, and any trials of thyroxine are not indicated. Thyroid scans require the administration of trace amounts of radioactive elements to the child.

Ultrasonography of the thyroid allows examination of the thyroid (without radioactivity) to determine if the thyroid is in the usual developmental location as a bilobed structure and, if in the usual location, whether it is hypertrophied suggesting dyshormonogenesis or iodine deficiency. While ultrasound is noninvasive and generally less expensive than other imaging methods, a potential drawback of ultrasonography is that it is often operator dependent.

Thyroglobulin (TG) absence can be associated with athyreosis and thyroglobulin synthetic defects, both of which require lifelong thyroxine replacement.

Thyroid-blocking antibodies (TBA), usually of maternal origin, can cause transient hypothyroidism of the newborn [63]. The risk for presence of maternal antibodies increases with maternal age, and transient hypothyroidism can recur with subsequent pregnancies. In cases of known maternal thyroid disease, maternal and/or baby antibodies should be considered as an early step in confirmatory testing. Neonatal hypothyroidism due to maternal antibodies is transient, usually lasting only a few months as maternal antibodies decline, and some have advocated for routine maternal thyroid screening for all children identified with out of range thyroid newborn screens.

TRH stimulation testing is indicated in cases of suspected central hypothyroidism, associated with low free thyroxine and non-elevated TSH. An exaggerated TSH response to TRH indicates hypothalamic dysfunction and should prompt a further investigation into the status of the hypothalamus and reason for insufficiency, and would generally include MRI of the hypothalamus. Failure to mount a TSH response to TRH indicates pituitary dysfunction which also warrants further investigation as to its cause and potential association of other pituitary insufficiencies.

While reduced thyroid-binding globulin (TBG) levels are indicative of TBG deficiency, in general, the specific measurement of TBG is not generally necessary, as confirmation of free thyroxine level within range is all that is necessary for follow-up of low T4 and non-elevated TSH. TBG deficiency in the absence of TSH elevation does not require treatment. Since TBG deficiency in most cases is X-linked, families should be counseled regarding the 1:2 risk of recurrence with subsequent male children.

Special subsets: premature and low birthweight infants. These infants represent a selected subpopulation at risk for suboptimal long-term outcome [64, 65]. Whether a portion of this impaired outcome is thyroid hormone dependent is not entirely known. Low T4 is known to correlate with risk of impaired neurodevelopmental outcome, including increased risk of intraventricular hemorrhage [66], and up to 50% of preterm infants are hypothyroxinemic [67]. In addition, premature infants are known to have higher iodine requirements, less mature hypothalamic/pituitary axis, and reduced activity of

deiodinases (especially in the central nervous system) which convert T4 to T3. Thus, thyroid hormone, iodine, and TRH treatments have been considered to improve the outcome of preterm babies, but none have demonstrated benefit to date, and additional studies in this area are needed [68, 69, 87–90]. Hypothyroxinemia of prematurity generally resolves by 6–10 weeks of age; however, some preterm infants go on to have delayed TSH elevations. For these reasons, serial screening is recommended for premature infants at 2, 6, and 10 weeks of age or until they reach 1,500 g or are discharged [62].

Acutely ill neonates also tend to have lower total thyroxine values. Because of the crucial role of thyroid hormone in the developing nervous system, some have advocated for empiric thyroxine treatment in acute illness, and trials on cardiac patients have been performed [70]. Treatment has not been harmful, but benefit has not been clearly established either. Recovery from acute illness can be associated with transient TSH elevations. Generally, sequential testing can help to distinguish these transient elevations from mild thyroid dysfunction. That is, over time, parallel increases in T4 and TSH suggest recovery from illness, and TSH should normalize within a week. Transient TSH elevations (without permanent congenital hypothyroidism) are frequently associated with congenital malformations and may represent recovery from acute illness. These elevations tend to be more modest elevations and are sometimes treated to protect the potentially vulnerable CNS. However, this group warrants a trial off thyroxine after the third birthday to determine whether thyroid dysfunction is persistent.

Blunted TSH response to hypothyroxinemia can occur in babies receiving transfusions, dopamine, and/or high-dose steroids. In these cases, serum fT4 and follow-up thyroid tests posttransfusion/treatment may be needed to determine if thyroxine treatment is necessary.

While newborn screening has benefited thousands of newborns in the USA (approximately 1,000 hypothyroid cases/year in the USA), screening has its limitations. As with any screening test, a normal result in the context of signs and symptoms of the disorder should not preclude further diagnostic testing and treatment if indicated. In any newborn or child with signs of potential hypothyroidism, serum T4 and TSH should be measured [45, 61].

Treatment

Levothyroxine is the treatment of choice for congenital hypothyroidism. Its long half-life allows daily dosing and no consequences of an occasional missed dose. Levothyroxine is converted to the active hormone T3, and in the brain, local T4 to T3 conversion is especially important adding to the rationale for treatment with levothyroxine in pediatric patients. Periodically, combination preparations of T4 and T3 have been advocated [71], but to date, there is no sufficient evidence to favor this approach which can be associated with risk of cardiac and other effects of T3 boluses. Furthermore, all the large-scale outcome studies for treated cases of congenital hypothyroidism have utilized levothyroxine.

Levothyroxine is available as a scored tablet of synthetic hormone in a variety of doses. Adverse consequences of treatment are minimal; in one case, there was report of reversible liver function abnormalities when levothyroxine was used in an individual with antibodies to the medication [72]. Prolonged hyperthyroxinemia can cause craniosynostosis (although this has been found in neonatal hyperthyroidism, it is not generally associated with treatment of congenital hypothyroidism) and osteoporosis. These adverse events can be avoided by regular monitoring of thyroid function tests and avoidance of overtreatment.

In treating congenital hypothyroidism, the aim is rapid normalization of total thyroxine (within 1–2 weeks of starting treatment); treatment should be initiated at 10–15 μg/kg/day, with the aim to keep the total and free T4 in the upper half of the normal range. TSH levels generally subside to the normal range within a month of starting treatment and thereafter should be maintained in the normal range. A rise in TSH while on treatment generally confirms the need for ongoing replacement therapy.

Levothyroxine tablets should be crushed and mixed with some milk and administered by syringe to infants. Soy formulas should be avoided as they interfere with absorption of the medication. Serum T4 (or fT4) and TSH should be monitored regularly starting at 2 and 4 weeks after medication has been started, every 1–2 months for the first year, and every 2–3 months to age 3 years and every 3 months until growth is complete. When dose adjustments are made, follow-up testing should be performed in 2–4 weeks [61, 73].

Resistance (poor absorption, enhanced clearance, pituitary/peripheral resistance) and noncompliance may present with persistent TSH elevation despite thyroxine therapy. In the case of poor absorption and enhanced clearance, the T4 is usually low. With resistance and noncompliance, the T4 is usually high or normal. In the noncompliant individual, there can be acute compliance with thyroxine causing the normal or high T4, but the TSH which has a longer half-life and time to equilibration will remain elevated, reflecting the prior state of hypothyroxinemia. Random and unannounced sampling of serum can help discover noncompliance. Resistance can be addressed by escalating the dose to determine a sufficient dose to normalize TSH; on occasions, other forms of thyroid hormone are needed for cases of resistance.

In cases of central hypothyroidism, assessment of the adrenal axis should be performed prior to starting levothyroxine to avoid precipitating adrenal crisis.

Outcome

There have been numerous studies of cognitive and developmental outcome of children identified with congenital hypothyroidism by newborn screening, and all have demonstrated excellent neurodevelopmental and growth when individuals were treated early and with sufficient thyroxine [23, 74–85]. For the most profoundly hypothyroid cases (athyreosis, maternal antibodies, very low T4 and high TSH, delayed bone age at diagnosis), there are certain cognitive defects which persist despite adequate treatment which are presumably attributable to maternal and fetal hypothyroxinemia. While IQ test scores are generally comparable compared to controls and siblings, there can be subtle defects in memory, attention, and visual-spatial processing [78]. These defects have not been found in cases of ectopic gland, presumably because there was sufficient thyroid hormone production by the partial gland.

More recent studies of outcome continue to support the notion that early and high thyroxine dosages will yield the maximal outcome, and if possible, treatment should be initiated before 2 weeks of age.

There are theoretical risks of overtreatment based on the clinical course of neonatal Graves' disease which can be associated with craniosynostosis, tachycardia and supraventricular tachyarrhythmias, poor weight gain and hyperirritability, and gut hypermotility. However, thyroxine treatment of congenital hypothyroidism does not increase the risk of craniosynostosis or supraventricular tachyarrhythmias [82], most likely because thyroxine is administered as T4 which is converted to T3 and even with high T4, the T3 is usually not elevated. Mild effects of excess thyroxine can occur with prolonged high T4 doses, but in general these affects are of little clinical consequence.

Thyroid Functional Outcome (Does Treatment Always Need to Be Lifelong?)

Since the critical period of thyroid hormone-dependent brain development is from fetal development to postnatal age 3 years, the recommendation is that thyroxine not be withdrawn until after the third birthday. For children confirmed to have ectopic or athyreotic hypothyroidism, no detectable thyroglobulin (prior to starting thyroxine), and for children in whom TSH elevation (>10) has occurred while on thyroxine after 1 year of age, there is no reason to attempt discontinuation of thyroxine. For the remainder of children treated with thyroxine, at the third birthday, thyroxine can be discontinued

or halved in dose for 30 days with serum thyroxine and TSH determination at that time [86]. An elevation of TSH confirms the need for continued thyroxine. If the dose was halved and there was no TSH elevation, at that point, the dose should be discontinued for 30 days with repeat thyroid studies. Once discontinuing thyroxine, it is important to advise of the signs and symptoms of hypothyroidism, and if they develop or there are any growth issues, repeat thyroid testing should be performed. Transient hypothyroidism occurs in 5–20% of cases and is more likely in cases of mild newborn screening TSH elevations, children with other malformations, children of mothers with Hashimoto's disease (and presumably transference of maternal antibodies to the baby), and former premature infants.

A more recently recognized area for concern and possible screening is maternal thyroid status early in pregnancy when the developing fetus is dependent on transplacental passage of thyroid hormone [24, 25].

Summary

Neurocognitive development, hearing, and growth are dependent on sufficient thyroid hormone in fetal and early child development. Iodine deficiency can affect maternal, fetal, and childhood thyroid function and remains the leading cause worldwide for treatable mental retardation. During early fetal development, transplacental supply of maternal thyroxine is crucial, and if necessary, if maternal thyroxine is available, it can continue to provide thyroxine to the developing fetus. At birth, there may be few signs or symptoms of hypothyroidism, making newborn screening for thyroid function a critical step in detecting congenital hypothyroidism, which if caught early is a treatable condition with normal or near normal developmental outcome. Individuals at risk for suboptimal outcome are those whose maternal thyroxine supply was insufficient in utero and those whose diagnosis of hypothyroidism was delayed or incompletely treated.

Some children, despite in range newborn screening thyroid tests, will develop TSH elevations later, and thus, any signs or symptoms compatible with hypothyroidism should be pursued with thyroid testing, regardless of the newborn screening thyroid results.

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Autoimmune Thyroid Disease

16

Stephen A. Huang

Abstract

Autoimmune thyroid disease affects approximately 2% of the female population and 0.2% of the male population. Its overall prevalence peaks in adulthood, but it is also the most common etiology of acquired thyroid dysfunction in pediatrics. This chapter presents a summary of autoimmune thyroid disease, discussing first chronic autoimmune thyroiditis and then Graves' disease, with an emphasis on their clinical management. Optimal quantities 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.

Keywords

Thyroid • Hypothyroidism • Hyperthyroidism • Autoimmune • Hashimoto's disease • Graves' disease • Thyroiditis

Autoimmune thyroid disease affects approximately 2% of the female population and 0.2% of the male population [1]. Its overall prevalence peaks in adulthood, but it is also the most common etiology of acquired thyroid dysfunction in pediatrics [2, 3]. This chapter presents a summary of autoimmune thyroid disease, discussing first chronic autoimmune thyroiditis and then Graves' disease, with an emphasis on their clinical management. Optimal quantities 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.

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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].

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Table 16.1 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 myxoedema, atrophic thyroiditis)
Status: Persistent hypothyroidism with increased TSH
2C Transient aggravation of thyroiditis (example postpartum thyroiditis)
Status: May start as transient, low 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 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)

Adapted from Davies, Thyroid 1993 [7]
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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 we now recognize as a form of 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 itself has been redefined in recent years (Table 16.1) [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 (deQuervain's) thyroiditis.

Pathophysiology

The activation of CD4 (helper) T lymphocytes specific for thyroid antigens is believed to be the first step in pathogenesis. Once activated, self-reactive CD4 T cells recruit cytotoxic CD8 T cells as well as autoreactive B cells into the thyroid. The three main targets of thyroid antibodies are thyroglobulin (Tg), thyroid peroxidase (TPO), and the thyrotropin receptor (TR). 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 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 (the Hurthle or Askanazy cell). In contrast, the gland of atrophic autoimmune thyroiditis is small with lymphocytic infiltration and fibrous replacement of the parenchyma.

Clinical Presentation

The presentation of chronic autoimmune thyroiditis includes either hypothyroidism, goiter, or both. A goiter or firm thyroid is the first physical sign of chronic autoimmune thyroiditis. Thyromegaly is typically diffuse with a "pebbly" or "seedy" surface

Table 16.2 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

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that evolves into a firm and nodular consistency [12]. As the disease progresses, subclinical and then clinical hypothyroidism appears. Symptoms of hypothyroidism may be subtle, even with marked biochemical derangement (Table 16.2). The initial history should include inquiries into energy level, sleep pattern, menses, cold intolerance, 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. 16.1) [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 differs clinically from true precocity by the absence of accelerated bone maturation and linear growth (Table 16.2).

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 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, 21].

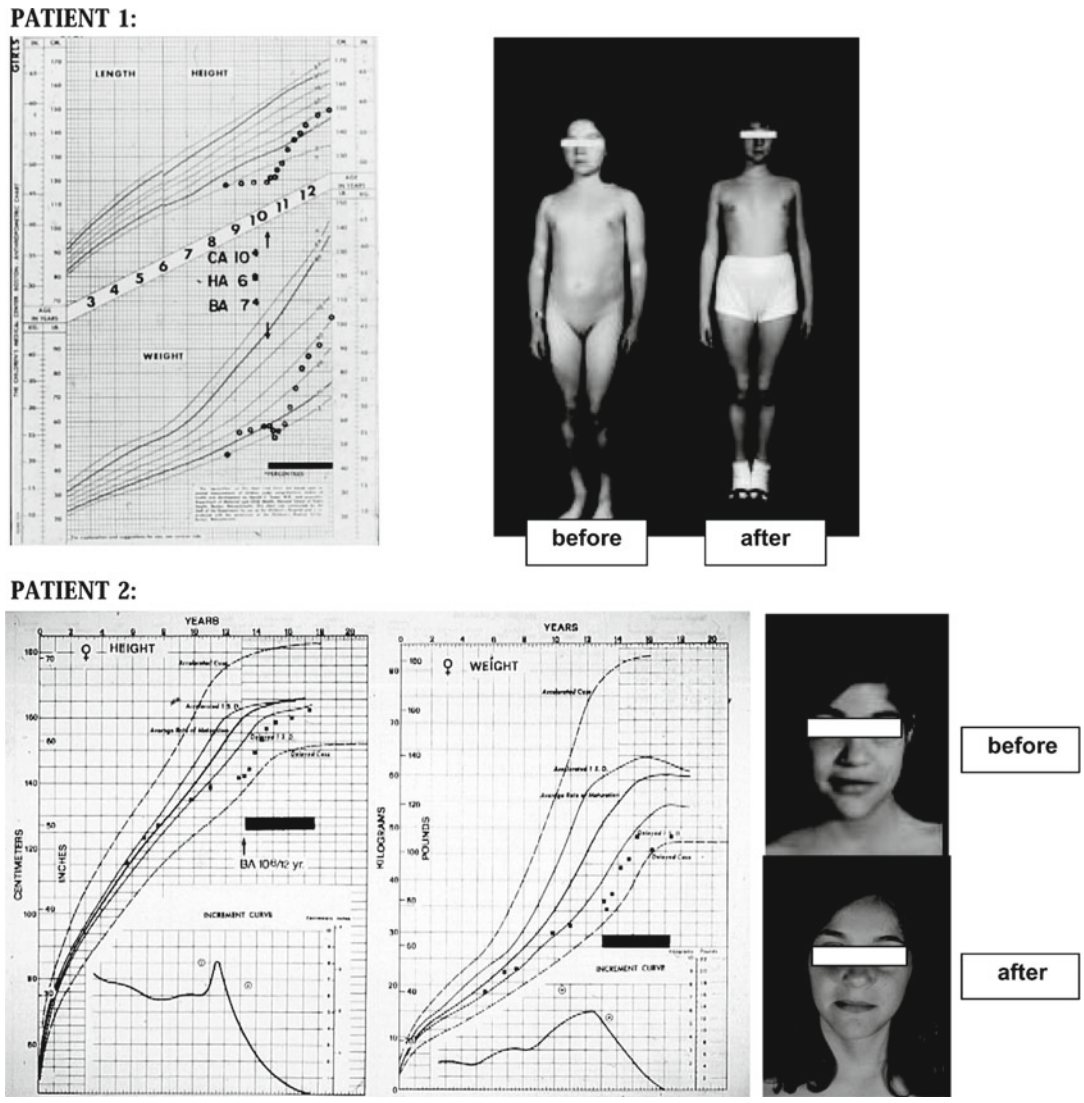

Fig. 16.1 Two patients with chronic autoimmune thyroiditis. Two patients with chronic autoimmune thyroiditis. The growth failure of hypothyroidism characteristically affects height to a greater degree than weight. The initiation of thyroid hormone replacement (*solid black bar*) is associated with an acute drop in weight due to the mobilization of myxedematous fluid, followed by an acceleration in linear progression or "catch-up growth." Breast development was noted in patient 1 which regressed after hypothyroidism was corrected. The interval between pretherapy and post-therapy photographs is 1 year for patient 1 and 6 months for patient 2. Charts and photographs are from the files of John F. Crigler, Chief Emeritus of Children's Hospital Endocrinology in Boston (Reprinted with permission)

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. For this reason, it is our practice to recommend thyroid

Table 16.3 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

Adapted from LaFranchi, Pediatric Annals 1992 [4]
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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 [22]. 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 [23].

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. 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 [24]. Some authors advocate a graded approach to the initiation of levothyroxine [25]. Alternatively, a starting dose can be estimated based upon the patient's age and ideal body weight (Table 16.3) [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 and we aim for a target range of 0.5–3 μU/ml. 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 16.4). 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 [26, 27]. 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 [28, 29].

Graves' Disease

Robert Graves reported the clinical syndrome of goiter, palpitations, and exophthalmos in 1835. In both adults and children, Graves' disease is the most common cause of hyperthyroidism [30, 31]. Hyperthyroidism is relatively rare in children (yearly incidence of 8–9 per 1,000,000 children less than 15 years old and 1 per 1,000,000 children less than 4 years old) [32, 33]. Girls are

Table 16.4 Conditions that alter levothyroxine requirements

Increased levothyroxine requirements	
Pregnancy	
Gastrointestinal disease	Mucosal diseases of the small bowel (e.g., sprue)
	Jejunoileal bypass and small bowel resection
	Diabetic diarrhea
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

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affected four to five times more frequently than boys, although no gender difference is noted under 4 years of age [32, 34].

Pathophysiology

Graves' disease shares many features associated with chronic autoimmune thyroiditis, including autoantibodies directed against thyroglobulin, thyroid peroxidase, and the sodium-iodine symporter. Hyperthyroidism is caused by thyroid-stimulating antibodies which bind and activate the thyrotropin 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 cause the extrathyroidal manifestations of Graves' ophthalmopathy and dermopathy, respectively.

Clinical Presentation

The 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 (Table 16.5). 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 rarer than in adults and tend to be less severe [34]. The pediatric literature cites a 25–60% frequency of ocular manifestations, but the majority are 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 [35]. As expected, these signs improve in most patients after restoration of the euthyroid state, and conservative management is generally recommended

Table 16.5 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

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so long as vision is not threatened [36]. Unique to pediatric Graves' disease is the acceleration of linear growth and bone maturation associated with prolonged hyperthyroidism [37].

Diagnosis

The term thyrotoxicosis refers to the manifestations of excessive quantities of 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' 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 without hyperthyroidism. 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 should be added 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 coupled with the chronicity of symptoms will be adequate to allow a diagnosis, but radionuclide studies using I-123 can provide confirmatory data (Table 16.6). 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. The RAIU will be low, distinguishing them from the more common Graves' disease. For thyrotoxicosis which has been present for more than 8 weeks, Graves' 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. If thyromegaly is subtle and eye changes are absent, an I-123 uptake, with or without a scan, should be performed. Autonomous nodules must be large to cause hyperthyroidism (typically 2–3 cm or more in diameter), so radioiodine scanning should be reserved for patients in whom a discrete nodule(s) is palpable. 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.

The sensitivity of serum thyrotropin-receptor antibody (TRAb) assays is cited to be 75–96%

Table 16.6 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 thyroiditis (painless thyroiditis, silent thyroiditis, postpartum thyroiditis)
Ectopic thyroid tissue (struma ovarii, functioning metastatic thyroid cancer)

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for TBII (a competitive binding assay with TSH) and 85–100% for TSAb measurements (a bioassay of TSH receptor activation) in untreated Graves' disease. A false-negative rate of 10–20% has been documented for serum thyrotropin-receptor antibodies in Graves' disease, presumably due to the inadequate sensitivity of the assays or the exclusive intrathyroidal production of autoantibodies [1, 30]. In practice, the measurement of thyrotropin-receptor antibodies is rarely necessary as the combination of thyrotoxicosis and high RAIU in the absence of a palpable nodule is virtually diagnostic of Graves' disease.

There is a subgroup of patients who have a subnormal but not severely depressed TSH (usually between 0.1 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 [38, 39]. 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 [40]. 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.

Antithyroid Medications

The treatment of Graves' hyperthyroidism may be divided into two categories, antithyroid medications and definitive therapy. The thionamide derivatives, Tapazole (MMI) and propylthiouracil (PTU), are the most commonly used antithyroid drugs [41]. 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.

Recent reports from the Food and Drug Administration's Adverse Event Report System and the United Network of Organ Sharing have heightened awareness of the risk of PTU-related liver failure [42, 43]. These studies support 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. Appropriate indications for PTU are still being debated, but in practice its use is now reserved for the specific situations of 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.

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 [44–46]. 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, we prefer monotherapy with MMI. 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 daily in most patients after the initial restoration of euthyroidism.

The first clinical response to medications is 2–4 weeks into therapy. Weight loss stops or weight gain occurs. 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 [47]. Families should be counseled that fever, sore throat, or 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% [48–50]. 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) [51, 52]. 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.

Definitive Therapy

The two options for the definitive treatment of Graves' disease are I-131 and thyroidectomy. Both are likely to result in lifelong hypothyroidism and there is disagreement in the literature as to their indications [53, 54]. Some centers consider these modalities as options for the initial treatment of pediatric hyperthyroidism [55–57].

However, as a remission of Graves' disease occurs in a significant percentage of children, we recommend that antithyroid medications be offered as initial therapy. If patient noncompliance 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 1,000 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 [58–60]. 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 [61– 63]. Some institutions administer an empiric dose of 3–15 mCi, or a dose based upon the estimated weight of the gland (50–200 μCi/gm of thyroid tissue) [58, 59, 64]. Efficacy is dependent upon both thyroid uptake and mass, and it is more logical to prescribe a dose which will provide approximately 200 μCi/gm 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.

$$\text{Dose I}-131 = \frac{\left(\begin{array}{l}200\,\mu\text{Ci/gm} \times \text{estimated} \\ \text{weight of thyroid in gm} \times 100\end{array}\right)}{\left(\%\text{uptake at 24hours}\right)}$$

The frequency of acute side effects is low although one recent paper describes vomiting in 4 out of 35 pediatric patients [57]. 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 [65]. 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 [48]. A short course of glucocorticoids is appropriate if there is rapid progression of ophthalmopathy or as prophylaxis in children with preexisting moderate to severe ophthalmopathy.

Thyroidectomy is rarely used electively for the definitive therapy of 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 [48]. In the authors' opinion, these average complication rates are unacceptably high given the benign nature of Graves' 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 [66]. 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 [67]. 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. 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 [68].

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', especially with the relatively high possibility of remission. We monitor thyroid function tests every 3 months in the growing child and 3 weeks after any medication adjustment with the goal of normalizing the TSH. 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 [69]. 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.

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 during at-risk pregnancies has been recommended as a predictor for the development of fetal/neonatal Graves' [70, 71]. 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 cotransfer of maternal thyrotropin-receptor-blocking immunoglobulins may delay the appearance of neonatal Graves' [72, 73]. 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 (0.5–1.0 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' was estimated to be as high as 25% in the past although it appears to be considerably lower today [64]. Potential long-term morbidity includes growth retardation, craniosynostosis, impaired intellectual function, and central hypothyroidism [64, 74, 75].

The half-life of maternal immunoglobulin is approximately 14 days, so most cases of neonatal Graves' 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 nonautoimmune etiologies are exceedingly rare but should be considered if thyrotoxicosis persists beyond 3 months of age.

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Non-thyroidal Illness Syndrome

17

Lisa D. Madison and Stephen H. LaFranchi

Abstract

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. 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. 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.

Keywords

Non-thyroidal illness syndrome—thyroid hormone changes • Thyroxine (T4) • Free T4 (FT4) • Triiodothyronine (T3) • Free T3 (FT3) • Reverse T3 (rT3) • Thyroid-stimulating hormone (TSH) • Thyrotropin-releasing hormone (TRH) • Thyroxine-binding globulin (TBG) • Transthyretin • Albumin • Hypothalamic–pituitary–thyroid (HPT) axis • Central hypothyroidism • Leptin • Paraventricular nucleus • Tanycyte • Cytokines • Cortisol • Deiodinase type 1 (D1) • Deiodinase type 2 (D2) • Deiodinase type 3 (D3) • Thyroid hormone receptor (THR) • Thyroid hormone

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transporters • Monocarboxylate transporter 8 (MCT8) • Heparin • Dopamine • Glucocorticoids • Furosemide • Salicylates • Preterm infants • Neurodevelopmental outcome • Cardiac-renal insufficiency • Psychiatric disorders • Depression • Bipolar disorder • Attention-deficit hyperactivity disorder (ADHD)

Introduction

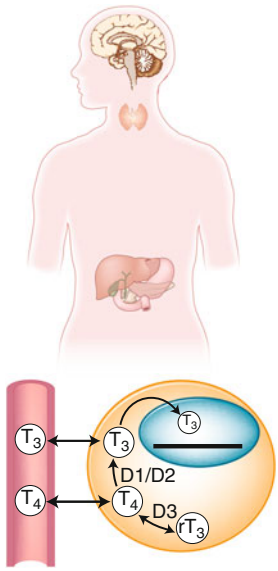
Non-thyroidal illness is the term used to describe the changes in thyroid hormone and thyroid-stimulating hormone (TSH) with acute illness not caused by an intrinsic abnormality of thyroid function [1]. In children, non-thyroidal illness is most commonly seen in acutely ill patients admitted to pediatric 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.

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 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



Hypothalamus	• Malnutrition →↑↓ TRH • Sepsis/Inflammation →↑ D2 (tanycyte →↑ T_3 →↓ TRH
Pituitary	• Cytokines →↓ TSH
Plasma	• Acute phase respones →↓ TBG →↓ TT_4 ↓ TT_3 • ↑Competitors for TH binding proteins →↓ TT_4 ↓ TT_3 • Free T_4 and $free_4$ may also fall due to a cental hypothyroidism
Tissue uptake	• ↓ T_4/T_3 uptake • ↑or unchanged thyroid hormone transporter
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. 17.1 Changes in thyroid tests during the course of NTI (from Ref. [1] © Society for Endocrinology [2011]. Reproduced with permission)

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 (see Fig. 17.1). 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.

Pathogenesis of NTIS–Thyroid Changes

Change 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).

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 nondeiodination pathways, manifested by increased levels of T3 sulfate and triiodothyroacetic acid (TRIAC) and tetraiodothyroacetic acid (TETRAC) in patients with NTIS [1].

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" proteins 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. Non-esterified 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].

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 co-activators and corepressors; there is some evidence that cytokines produced with acute illness compete with co-activators 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].

Summary of Pathogenesis of NTIS–Thyroid Changes (See Fig. 17.2)

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.

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%, 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, 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

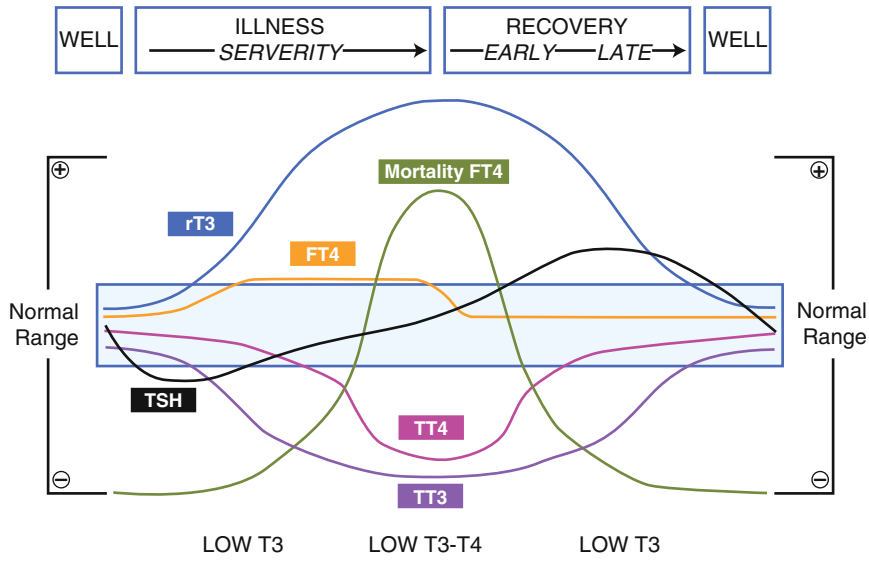


Fig. 17.2 Summary of the mechanisms that give rise to the serum thyroid hormone changes in the non-thyroidal illness syndrome (Reproduced with permission from Balogh et al. [20])

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.

Pediatric Clinical 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.

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 8–10-fold increase in thyroid gland volume, a 3–4-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 terms 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 7 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 comers 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]. A Cochrane database systematic review 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.

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 (PICU). 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.

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 [30]. 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 [31]. 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.

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 [32, 33]. TSH and total T4 are also suppressed after cardiac surgery, reaching a nadir within the first 24 h [33]. 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 [33]. Reverse T3 rises postoperatively, reaching a peak at about 24 h [33]. All of the changes in the HPT axis resolve gradually over the course of 5–7 days [33, 34].

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 circulatory 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 [32–34].

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 [32]. 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 [32, 34], 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 is 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 [34]. A recent meta-analysis, however, showed no evidence of alteration in postoperative mortality in the adult population with T3 supplementation [35]. 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 [36]. 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 [37]. 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 [38]. 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 [39]. 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 [31].

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 [40]. 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 [41]. 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 [42]. However, given that studies also report that TRH degradation is decreased in children with CRI [43], 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 [42].

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 [44]. 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" [45]. 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.

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 [46]. 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 [47]. Female offspring of parents with bipolar disorder appears to have a higher prevalence of autoimmune thyroid disease (16% vs. 4% in controls) [48].

Patients with depression tend to have normal to high T4 or free T4 levels and low TSH concentrations [49]. 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 [50].

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 [51]. However, when this question was examined from the perspective of children generally referred with ADHD, thyroid function tests were not suggestive of GRTH [52].

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 [53]. 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.

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.

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Resistance to Thyroid Hormone and TSH Receptor Mutations

18

Ronald N. Cohen

Abstract

Resistance to thyroid hormone (RTH) is a syndrome characterized by variable tissue hyporesponsiveness to thyroid hormone throughout the body. Classically, patients come to attention for a variety of reasons including goiter, abnormal thyroid function tests (TFTs), or neonatal screening programs. Biochemically, the syndrome is characterized by elevated thyroid hormone values in the setting of non-suppressed thyrotropin (TSH) levels. In most patients, hyporesponsiveness occurs in both the hypothalamic and pituitary as well as peripheral tissues. Resistance in the hypothalamus and pituitary leads to elevated thyrotropin levels, which stimulate the thyroid gland to increase production of thyroid hormone; however, reduced action elsewhere results in (to a greater or lesser degree) compensated thyroid hormone hyporesponsiveness. In contrast, TSH resistance is caused by mutations in the TSH receptor, and is characterized by a range of symptoms, from euthyroid hyperthyrotropinemia to frank hypothyroidism.

Keywords

Thyroid hormone receptor • Resistance to thyroid hormone • Thyrotropin (TSH) • TSH receptor • Thyroid function tests • Mutation • Development

Introduction

Resistance to thyroid hormone (RTH) is a syndrome characterized by variable tissue hyporesponsiveness to thyroid hormone throughout the body. Classically, patients come to attention for a variety of reasons including goiter, abnormal thyroid function tests (TFTs), or neonatal screening programs. Biochemically, the syndrome is characterized by elevated thyroid hormone values in the setting of non-suppressed thyrotropin (TSH) levels. In most patients, hyporesponsiveness occurs in both the hypothalamic and pituitary as well as peripheral tissues. Resistance in the hypothalamus and pituitary leads to elevated thyrotropin levels, which stimulate the thyroid gland to increase production of thyroid hormone; however, reduced action elsewhere results in (to a greater or lesser degree) compensated thyroid

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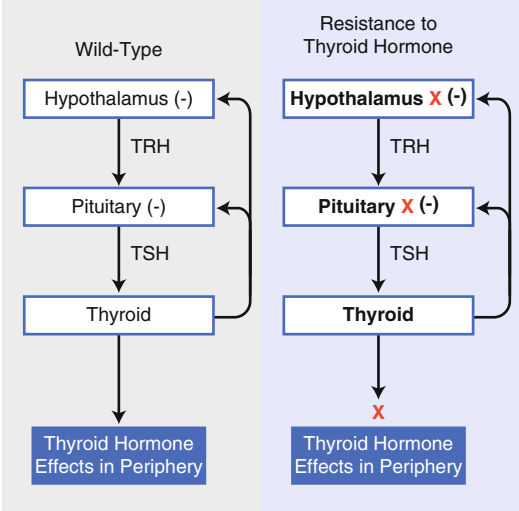

Fig. 18.1 The hypothalamic–pituitary–thyroid axis in RTH patients. TRβ mutations or other (as yet undefined) defects in patients with RTH lead to reduced thyroid hormone responsiveness in the hypothalamus and pituitary, resulting in increased production of thyroid hormone. Impaired thyroid action elsewhere in the body results in the clinical phenotype seen in patients with RTH. However, increased thyroid hormone action on TRα receptors leads to selective tissue hyperthyroidism (e.g., in the heart conduction system)

hormone hyporesponsiveness (Fig. 18.1). Thus, affected individuals do not show classic signs and symptoms of myxedema, but instead exhibit delayed growth, hearing defects, attention-deficit hyperactivity disorder (ADHD), and other symptoms [1]. The majority of patients with RTH have autosomal dominant mutations of the thyroid hormone receptor-β (TRβ) gene. Patients have been identified from a wide range of races and ethnic groups; the exact geographic distribution of the disorder is unknown but it has been estimated that RTH occurs in about 1 case per 50,000 live births [2]. Therapeutic strategies for RTH are not well-defined and treatment (if any) must be individualized. Most studies of patients with RTH 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 few years, additional syndromes of reduced sensitivity to thyroid hormone (but distinct from classic RTH) have been elucidated, and these will also be discussed below.

Mechanisms of Resistance to Thyroid Hormone

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 [3, 4]. NHRs contain a number of important domains: an N-terminal activation 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 also 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) [5, 6]. When TRs bind to "positive" TREs (pTREs) in the presence of thyroid hormone, 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 [7–9]. The molecular events leading to thyroid hormone-mediated transcriptional repression remain unclear. In contrast, TR action on pTREs has been better characterized. On these genes, TRs are recruited to pTREs whether or not thyroid hormone is present (Fig. 18.2). In the absence of the active ligand triiodothyronine (T3), the TR binds nuclear proteins termed corepressors, including the nuclear corepressor protein (NCoR) and the silencing mediator of retinoid and thyroid hormone receptors (SMRT) [10–15]. These cofactors, in turn, recruit a protein complex with histone deacetylase function [16–18], 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 [19]. Coactivators stimulate gene expression by increasing the degree of histone acetylation, modulating interacts with general transcription factors, and other mechanisms [20–28]. More recently, rapid non-genomic actions of thyroid hormone have been identified (independent of

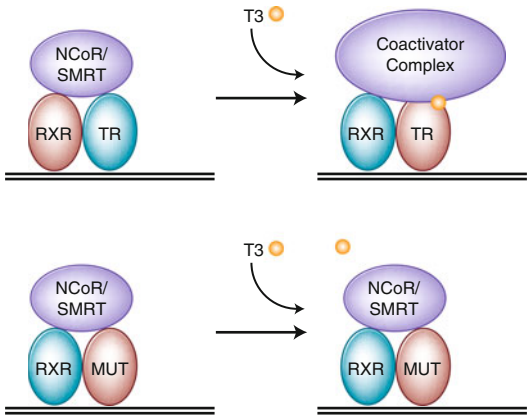

Fig. 18.2 Role of abnormal TR—corepressor interactions in the pathogenesis of RTH. The thyroid hormone receptor (TR) binds DNA as a TR-retinoid X receptor (RXR) heterodimer (or potentially as a TR-TR homodimer). The presence of ligand (T3) results in a conformational change in the TR, leading to dissociation 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

transcription) [3], though 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 on different chromosomes [29, 30]. The gene for TRα is located on chromosome 17; the gene for TRβ gene is located on chromosome 3. 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. In contrast, TRβ1, TRβ2, and the more recently described TRβ3 isoform [31] all bind thyroid hormone and differ only in their proximal region. Additional truncated isoforms for both TRα [32] and TRβ [31] have been identified, but their functions in vivo remain unknown.

Mutations in the TRβ gene have been identified in many patients with RTH, and in the vast majority of cases, the syndrome is inherited in an autosomal dominant fashion. A subset of RTH patients, however, does not exhibit TRβ mutations [33]. These patients may have defects in other proteins involved in thyroid hormone action [34], but such mutations have not been identified [35]. This is an ongoing area of research, and novel mutations will likely be identified in the future. A recent search for mutations in the retinoid X receptor gamma (RXRγ) gene, though, was not successful [36]. It has been hypothesized that some RTH patients may exhibit mosaicism for TRβ mutations [37].

Mutations of TRβ that cause RTH generally cluster in three "hot spot" regions of the gene [38, 39]. 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. 18.2). In a few patients, though, the defect is not with ligand binding per se, but with altered corepressor and/or coactivator recruitment [40, 41]. Interestingly, it has been shown that mutant TRs interfere with wild-type TR function, an effect has been termed "dominant-negative inhibition" [42, 43]. Recently, mouse models have clarified the role of the mutant TRs in the pathogenesis of RTH. Although complete knockout of TRβ produced mice with thyroid function tests consistent with RTH [44], "knock-in" of mutant TRs found in patients with RTH yielded mice with more severe resistance [45, 46]. Interestingly, knockout of TRα causes a syndrome of hypothyroidism with low TSH and growth arrest [47, 48]. Thus, a patient with a dominant-negative TRα mutation would be expected to have a different phenotype entirely.

RTH can be subdivided into generalized resistance to thyroid hormone (GRTH) and pituitary resistance to thyroid hormone (PRTH). In GRTH, the elevated thyroid hormone levels generated by resistance in 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 tachy-

cardia 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. Certain groups do not recognize the existence of PRTH as a distinct clinical entity [49], arguing that the same mutations have been reported to cause both GRTH and PRTH [50]. However, a careful evaluation of clinical and biochemical indices in a patient with RTH suggested that PRTH may very well exist [51]. 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 [52, 53]. 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 [54]. Another study identified differential recruitment of nuclear cofactors by a different TRβ mutation associated with PRTH [55].

Clinical Presentation

The clinical presentation of RTH is variable (Table 18.1). The initial family described by Refetoff et al. [56] 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, 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 TRβ allele [57]. 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 are inherited in an autosomal dominant fashion because mutant TRs exhibit dominant-negative inhibition over wild-type alleles.

Patients with RTH 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 [2]. Thyroid function tests are drawn, which reveal elevated thyroid hormone levels in the setting of a non-suppressed TSH (see "Diagnostic Guidelines" below). Infants with RTH may have congenital deafness, congenital nystagmus, neonatal jaundice, and hypotonia [1]. Patients with RTH may have an increased risk of developing autoimmune thyroid disease [58]. Individuals with RTH who have inappropriately underwent thyroidectomy or radioactive iodine treatment will exhibit signs and symptoms of hypothyroidism.

A National Institutes of Health study [59] evaluated a cohort of 42 RTH kindreds prospectively. There was autosomal dominant transmission in 22 kindreds, sporadic transmission in 14, and an unknown transmission in 6. 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 the patients with RTH compared to controls, and one-third of the patients had an IQ < 85. In contrast, only a few patients had actual mental retardation. Patients with RTH had a higher incidence of speech delay (24%), stuttering (18%), and hearing loss than controls. Although resting pulse was higher in patients with RTH, in this particular study, the correlation did not persist after adjustment for age (though it has been noted by other groups). Children with RTH exhibited delayed bone maturation. Bone age was delayed in 29% of patients, and 18% had short stature, though another study suggested that RTH is not associated with decreased final adult height [60].

As noted above, certain tissues demonstrate increased thyroid hormone-mediated effects in patients with RTH. 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 [61].

Table 18.1 Clinical characteristics of RTH in children

A. Physical exam
1. Goiter
2. Tachycardia
3. Short stature
4. Low body weight
B. Associated symptoms
1. Neurological
(a) Developmental delay
(b) Attention Deficit Hyperactivity Disorder (ADHD)
(c) Low IQ
2. ENT
(a) Deafness
(b) Speech impediment
(c) Recurrent ear, nose, and throat infections
C. Radiological findings
1. Delayed bone age
2. Increased thyroid ^{123}I uptake

Clinical findings found in patients with resistance to thyroid hormone. Derived from data in Refs. [1, 59]

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 [62]. 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, these results suggest an important role for thyroid hormone in these CNS regions. A study of children with ADHD with and without coexisting RTH examined the role of thyroid hormone therapy (in this case, L-T3) in ADHD [63]. The majority of patients with RTH 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 appears to be distinct from ADHD in patients without RTH [64].

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 noncommunicating hydrocephalus [1].

Diagnostic Considerations

Diagnosis in Children and Adults

The initial testing of a patient suspected to have RTH should include routine thyroid function tests. Patients with RTH 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 (Table 18.2), including methodological laboratory artifacts due to the presence of heterophile antibodies [65]. 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 can be of help. 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.

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 [66] and liver disease [67]), have elevated total T4 levels in the setting of a non-suppressed TSH. These patients, though, have normal free T4 levels, when measured directly or estimated based on THBR or 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 [68, 69]. These albumin variants have increased affinity for T4. Therefore, measurement of total serum T4 is elevated; correction of T4 based on T3RU or THBR 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.

Table 18.2 Causes of euthyroid hyperthyroxinemia

1. Methodological artifacts
(a) Antibodies to thyrotropin (TSH)
(b) Antibodies to thyroid hormones (T4, T3)
2. Binding protein abnormalities
(a) Acquired forms of increased TBG
– Estrogen use/pregnancy
– Liver disease
– Acute intermittent porphyria
– Other drugs (methadone, perphenazine, 5-FU)
(b) Inherited
– TBG excess
– Familial dysalbuminemic hyperthyroxinemia (FDH)
– Mutant transthyretin variants
3. T4 to T3 conversion defects
(a) Acquired
– Amiodarone
– Propranolol (high doses)
– Oral cholecystographic contrast agents
(b) Inherited (SBP2 mutations, possibly deidonase defects)
4. Miscellaneous causes
(a) Acute psychiatric illness
(b) High altitude
(c) Amphetamine use
(d) Thyroxine therapy
(e) 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

Certain medications such as amiodarone [70] 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 normal TSH and T3 levels, excluding the diagnosis of RTH. Finally, a few other conditions such as acute psychiatric illness [71] can also cause abnormal thyroid function tests that can occasionally be confused with RTH (Table 18.2).

Once these various conditions are excluded, the diagnosis is generally one of RTH vs. thyrotroph adenoma [72]. Differentiation between these two disorders can be difficult, but the following guidelines can be used to distinguish them:

1. Symptoms of hyperthyroidism
   Thyrotroph adenomas secrete abnormal levels of TSH leading to hyperthyroidism. In contrast, GRTH patients generally have a variable degree of compensated thyroid hormone hyporesponsiveness. However, patients with PRTH are thyrotoxic, so the presence of thyrotoxicosis does not fully exclude RTH. Tachycardia is a common finding in patients with RTH (even GRTH), and it cannot be used as a screen for hyperthyroidism.
2. Alpha subunit measurement
   TSH is composed of alpha and beta subunits. The alpha subunit is common to other glycoprotein hormones such as luteinizing hormone (LH), follicle-stimulating hormone (FSH), and chorionic gonadotropin (CG). Patients with thyrotroph adenomas generally have higher alpha subunit levels than patients with RTH [72], though there is significant overlap.
3. Family history
   Thyroid function tests from relatives should be tested because RTH is an inherited condition. In contrast, thyrotroph adenomas are generally sporadic in nature. However, patients with RTH can harbor de novo mutations. Therefore, a lack of family history does not exclude a diagnosis of RTH.
4. MRI abnormalities
   Most patients with thyrotroph adenomas have macroadenomas that can be visualized by MRI at the time of diagnosis. However, with improved diagnostic accuracy, it may be possible to diagnose thyrotroph adenomas earlier in their clinical course. Furthermore, patients with RTH may have incidental pituitary adenomas, mimicking a thyrotroph adenoma [73]. However, the presence of a pituitary adenoma does make the diagnosis of thyrotroph adenoma more likely. In addition, pituitary adenomas may co-secrete multiple hormones; thus, other anterior pituitary hyperfunction

points to a more likely diagnosis of thyrotroph adenoma.

5. Thyroid ultrasonography
   A recent study used Doppler ultrasonography to determine whether thyroid blood flow distinguishes between RTH and thyrotroph adenomas [74]. These investigators showed that parameters of thyroid blood flow normalized in T3-treated RTH patients, but not in those with TSH-secreting adenomas.
6. TRH stimulation testing
   Patients with thyrotroph adenomas usually have a flat response of TSH in response to exogenous TRH, because TSH secretion is autonomous. In contrast, TSH levels generally rise after a TRH infusion in patients with RTH. Currently, TRH testing may not be feasible outside of research protocols due to the lack of availability of commercial TRH.
7. Reduced responsiveness to exogenous T3
   An effective method to confirm a diagnosis of RTH (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. Unfortunately, patients with PRTH will also have reasonably intact peripheral responses to T3. Patients are generally admitted to a clinical research center for the duration of the protocol. A few protocols have been described, including one by Refetoff et al., where exogenous T3 is given in an escalating regimen [1]:
   (a) T3 25 μg po BID × 3 days.
   (b) T3 50 μg po BID × 3 days.
   (c) T3 100 μg po BID × 3 days.

   In pediatric patients, the middle 100-μg daily dose may be converted to 25 μg, for ages 1–3 (8–15 kg BW); 50 μg, for ages 4–9 (16–25 kg BW); and 75 μg, for ages 10–14 (26–45 kg BW), with the other doses altered accordingly. This type of approach has been successful, even in patients who have previously been inappropriately treated with radioiodine [75]. A variety of clinical parameters are measured, including weight, food intake, pulse, BMR, thyroid function tests, prolactin, thyroglobulin, cholesterol, triglycerides, creatine phosphokinase, ferritin, and SHBG. Alternatively, Safer et al. [51] used a mildly different protocol to evaluate a patient with PRTH. Adults were given T3 25 μg BID × 4 days, 50 μg BID × 4 days, and 100 μg BID × 4 days. Biochemical indices including TSH, ferritin, SHBG, ALT, AST, creatine phosphokinase, lactic dehydrogenase, total cholesterol, and fasting triglycerides were measured. Echocardiograms, sleeping heart rate measurements, ankle jerk relaxation time, and neuropsychiatric testing were also performed.
8. Thyroid hormone receptor mutations
   Most cases of RTH are associated with mutations in the TRβ gene. Ultimately, the most secure way to make a diagnosis of RTH is to demonstrate (a) elevated thyroid hormone levels in the setting of a non-suppressed TSH, (b) thyroid hormone hyporesponsiveness, and (c) a TRβ gene mutation.

Neonatal Considerations

If a child is born to a parent with RTH with a known TR 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 and DNA analysis [76], though the benefits of making the diagnosis at this stage of development have not been clearly established.

Therapy

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) [50]. The use of TRIAC (3,5,3′-triiodothyroacetic acid), a thyroid hormone analog with relative specificity toward the TRβ receptor [77, 78], has been advocated for use in patients with RTH, but its specific role has not been clearly defined. D-Thyroxine has also been used [79], though one study suggested it was less effective than TRIAC [80]. Novel TR analogs hopefully will be developed that activate mutant receptors [81]. In sum, 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 [82]. High miscarriage rates of wild-type fetuses of pregnant RTH mothers has been suggested to be due to high circulating levels of thyroid hormone [83]. A prenatal diagnosis of RTH was made [76] in a 29-year-old pregnant woman with at 17 weeks gestation. The fetus and mother were both found to harbor the identical TRβ 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 [50] 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 [50]. As noted above, patients with RTH and coexisting ADHD may improve when treated with thyroid hormone [63]. In any case, patients who require treatment should be followed closely, with careful evaluation of growth, bone age, and thyroid-responsive biochemical indices.

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 taken up into cells by a variety of transporter proteins [84]. 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 and hypotonia presenting in infancy or childhood [85, 86]. In these patients, serum T3 levels are elevated, 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 [87], 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 that did not require MCT8 for transport and may represent a novel modality for patients suffering from this syndrome [88].

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). SBP is involved in the incorporation of the unusual 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 [89, 90]. Recently, other kindreds with SBP2 mutations were found to have coexisting azoospermia, axial muscular dystrophy, photosensitivity, abnormal immune function, and insulin sensitivity [91], suggesting that SBP2 mutations produce a complex, systemic selenoprotein deficiency syndrome.

TSH Receptor Mutations

Introduction

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) [92]. 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 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 [93], but it was not until 1995 that the first patient with a TSH receptor mutation was firmly documented [94].

Mechanisms of Disease and Clinical Presentation of TSH Resistance

In 1995, Sunthornthepvarakul et al. documented the first case of a TSH receptor mutation leading to TSH resistance [94]. 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 TSH receptor corresponding to a region in the TSH extracellular domain [94]. In vitro data confirmed the mutant receptors exhibited decreased biological activity. Since that time, a number of other patients have been identified with TSH receptor mutations and TSH resistance, though additional patients have been identified without identifiable mutations. Most patients with TSH receptor mutations are either homozygotes or compound heterozygotes.

The TSH receptor is a transmembrane G-coupled receptor (Fig. 18.3). 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 [95]. The extracellular domain may inhibit constitutive activity of the receptor [96]. 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 [96]. The gene encoding the TSH receptor has been localized to chromosome 14q31 [97, 98].

There are two general modes of presentation for patients with loss-of-function germline TSH receptor mutations. The first is similar to the family identified by Sunthornthepvarakul et al. [94]. In these patients, high TSH levels are necessary to overcome partial TSH resistance, and patients remain euthyroid (compensated euthyroid

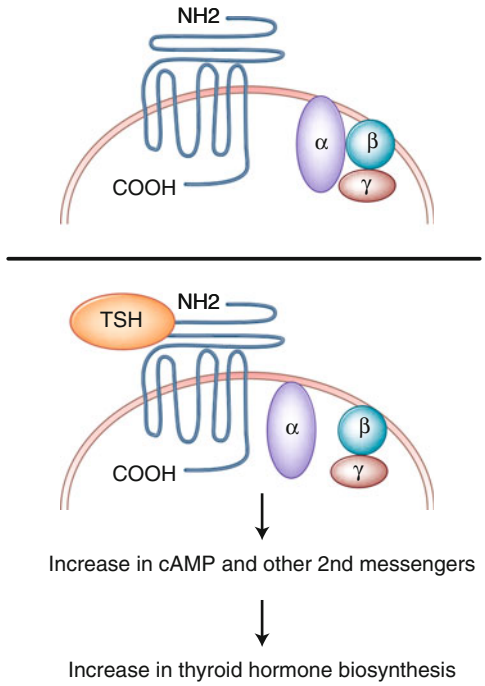

Fig. 18.3 Schematic diagram of the TSH receptor. The TSH receptor is composed of an N-terminal extracellular domain, a transmembrane region (which spans the plasma membrane seven times), and a cytoplasmic tail. Stimulation of the TSH receptor leads to G-protein dissociation and activation

hyperthyrotropinemia). Four additional families with these clinical characteristics were identified by de Roux et al. [99]. One patient had a homozygous mutation in codon 162 of the TSH receptor; 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 (F525L) domains [99]. Additional mutations have been identified from patients with euthyroid hyperthyrotropinemia [100, 101].

In contrast, other TSH receptor 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 [102]. Ultrasound evaluation revealed hypoplastic thyroid glands. A homozygous mutation of the TSH receptor 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 [102]. Severely affected patients have been identified by other groups as well [103–105].

Not all patients with resistance to TSH have mutations in the TSH receptor [106]. Patients with pseudohypoparathyroidism caused by mutations in GNAS may exhibit resistance to a variety of hormones including TSH [107]. Mutations in transcription factors involved in thyroid gland development such as Pax8 [108] and TITF1 (Nkx2.1) [109] 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 [110].

Diagnostic Considerations and Therapy

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 TSH receptor heterozygous carriers to be 1 in 172 in that population [111]. Thus, the prevalence of TSH resistance may be higher than previously appreciated.

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.

Conclusion

Hormone resistance leading to thyroid dysfunction occurs 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 rigorously. 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 TRβ 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 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.

Note Added in Proof

Recently, patients with mutations in TR alpha have also been described [112]. This new syndrome is characterized by cognitive deficits, constipation, and short stature. The index patient exhibited normal levels of TSH, mildly low free T4, and highnormal T3.

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Thyroid Neoplasia

19

Andrew J. Bauer, Steven G. Waguespack, Amelia Grover, and Gary L. Francis

Abstract

The World Health Organization divides thyroid neoplasms into thyroid carcinomas, thyroid adenomas, and other thyroid tumors. Other thyroid tumors and metastases from remote cancers to the thyroid are distinctly uncommon in children and are not reviewed here. This chapter is divided into 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.

Keywords

Thyroid • Cancer • Nodule • Child • Multiple endocrine neoplasia • Papillary • Follicular • PTC • FTC • MTC

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Background

The World Health Organization divides thyroid neoplasms into thyroid carcinomas, thyroid adenomas, and other thyroid tumors [1–3]. Other thyroid tumors and metastases from remote cancers to the thyroid are distinctly uncommon in children and are not reviewed here. This chapter is divided into 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 [4].

Thyroid Nodules

Estimates from ultrasound (US) and postmortem examination suggest that 1–1.5% of children and 13% of adolescents have thyroid nodules [5, 6]. It remains unclear how many of these nodules would ever reach a clinical threshold. Several risk factors are associated with thyroid nodules in children including iodine deficiency, prior radiation exposure, and previous thyroid disease. Childhood cancer survivors who were treated with radiation therapy are at high risk for nodules, which develop at a rate of about 2% annually and reach a peak incidence 15–25 years after exposure up to 20–30 Gy (2000–3000 rad) [7].

US is especially useful for interrogating nodules and for detecting additional non-palpable lesions in the contralateral lobe or abnormal cervical lymph nodes. Size alone does not predict malignant histology [8]. However, decreased echogenicity, heterogeneous echotexture, irregular nodule outline, subcapsular location, increased vascularity, and microcalcifications are more common in malignant nodules [9]. Unfortunately, none of these features can reliably distinguish benign from malignant nodules. For that reason, fine needle aspiration (FNA) is recommended (Fig. 19.1). When performed under US guidance, FNA offers good sensitivity (94%), specificity (81%), and accuracy (83.6%) in children [10]. Previously, results of FNA were reported as malignant, benign, indeterminate, or suspicious for neoplasm and nondiagnostic [11]. However, the 2009 consensus guidelines have now added two additional categories including suspicious for malignancy and follicular lesion of undetermined significance [12]. The risk of inadequate samples is reduced by having the cytopathologist attend and immediately review the slides.

Malignant or suspicious lesions warrant preoperative staging as outlined below for the initial surgery of differentiated thyroid cancers (Fig. 19.2). Follicular neoplasms should be removed. The majority of benign thyroid nodules appear to remain benign over time. However, DTC has eventually been detected in a small proportion (about 3%) of benign-appearing nodules that had initial benign cytology on FNA [13]. For that reason, if not removed, "benign" nodules in children should be followed with serial US. Lesions that grow or develop suspicious US features should undergo repeat FNA or be removed. Attempts to improve the diagnostic utility of FNA have focused on molecular markers that are upregulated in thyroid cancers [14]. However, these procedures remain experimental and are not yet recommended beyond a research context.

The vast majority of thyroid surgeries are performed through a transverse cervical incision, leaving a noticeable scar. Recently, novel surgical approaches using a robotic system most commonly through a trans-axillary approach have been studied for patients with benign-appearing thyroid nodules and multinodular goiter [15–17]. Initial data indicate the procedure is safe, but it is important to analyze the data as the technique is broadened to include adolescents and younger ages.

Differentiated Thyroid Cancers

Only 1.8% of thyroid cancers develop in children and adolescents, but the incidence may be increasing [18–20]. 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 [21]. Adolescents have a tenfold greater incidence of DTC and a female/male preponderance (5:1) that are not seen at

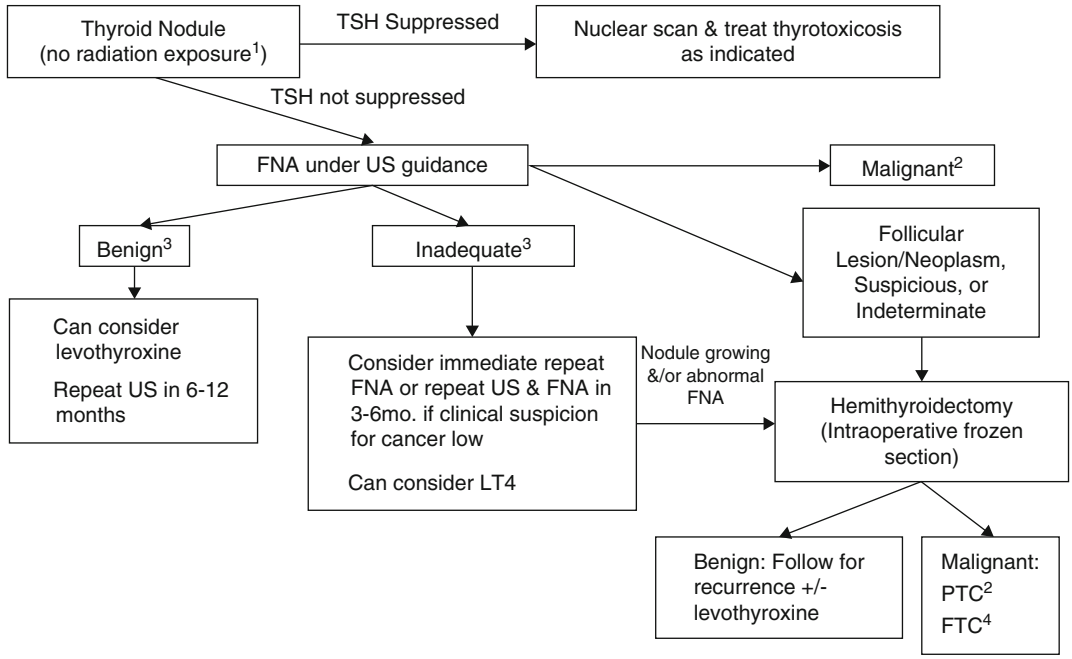

Fig. 19.1 Evaluation and treatment for thyroid nodules. Fine needle aspiration is the cornerstone to diagnosis and management. [1]Risk for cancer is higher in children with radiation exposure, this algorithm may not apply to irradiated patients. [2]See Fig. 19.2 (PTC algorithm). [3]Surgery can always be considered if concerning clinical features, size >4 cm, compressive symptoms, and/or patient preference. [4]Completion thyroidectomy and possible RAI

younger ages [19–24]. Estimates from the Surveillance, Epidemiology and End Results (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 [25]. Overall cure rates are high [24, 26–33], but children diagnosed prior to age 10 may have a higher risk of recurrence and death [34–37] although not all studies confirm this [38].

Evaluation, treatment and follow-up of children with DTC have generally followed adult guidelines [12, 39–41]. However, there are important differences between children and adults that impact this practice. First, thyroid nodules are fivefold more likely to be malignant in children (26.4%) than in adults (5%) [5, 42]. Second, children with PTC are more likely to have regional lymph node involvement, extrathyroidal extension, and distant pulmonary metastasis [26, 30, 32, 33, 37, 43–48]. Third, children are less likely to die from disease (2% cause-specific mortality), [33] and many children with pulmonary metastases (30–45%) develop stable yet persistent disease after RAI therapy [27, 33].

Furthermore, long-term (40 year) follow-up studies now indicate that children with DTC may have an increase in all-cause mortality attributed to second malignancies [33]. Whether this resulted from aggressive treatment or an underlying predisposition is unknown, but this concern has tempered the use of RAI, particularly for low-risk children. Adding to the lack of enthusiasm for 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 [49].

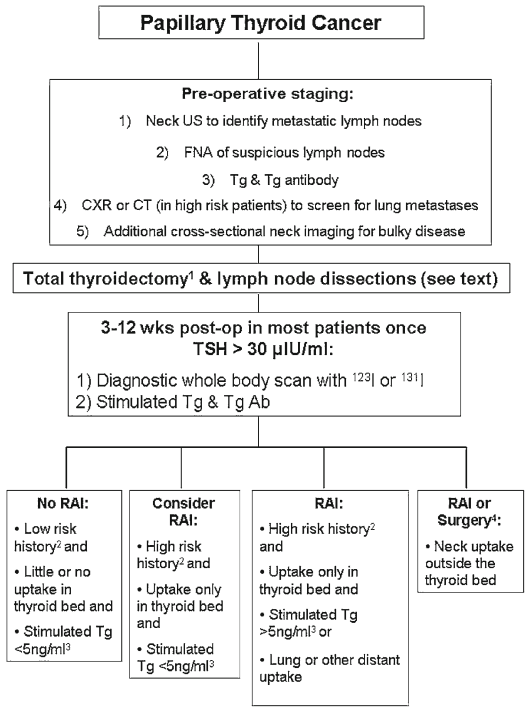

Fig. 19.2 Initial management for papillary thyroid cancer. Preoperative staging is vital to determine therapy. RAI ablation is indicated for high-risk patients but may be deferred for low-risk patients if close follow-up is assured (see text, for details). [1]Rare cases where lobectomy may suffice (see text). [2]Low risk: Primary tumor does not invade the trachea, recurrent laryngeal nerve, esophagus, or other vital structures; non-bulky lymph node presentation; no evidence of distant metastatic disease. High risk: Any of the previous features present. [3]Assumes negative Tg Ab. [4]RAI if no macroscopic disease on US; surgery if macroscopic disease on US

Papillary Thyroid Carcinoma (PTC)

PTC are generally well differentiated in children (Fig. 19.3) [50]. The major risk factor for developing PTC is radiation exposure to the thyroid [51, 52]. Survivors of childhood Hodgkin disease, for example, are 18.3-fold more likely to develop PTC [53]. The risk is greatest for those who received radiation at a young age and with doses up to 20–29 Gy [54]. RET/PTC rearrangements involving the "rearranged during transfection" (RET) oncogene are the most common molecular changes in PTC from children (10–80%) [55, 56]. In contrast to adults with PTC, mutations in the v-raf murine sarcoma viral oncogene homolog B1 (BRAF) gene are uncommon in childhood [55, 56]. Up to 5% of patients have a family history of PTC [57, 58] which may present earlier in life and may be more aggressive [59].

PTC most commonly presents as a palpable thyroid nodule, but PTC can also present as cervical adenopathy. PTC is frequently multifocal and bilateral, and it metastasizes to regional neck lymph nodes prior to lung. Distant metastases occur in 5–10% of children but typically only with extensive regional lymph node disease [32, 60].

Preoperative staging directs the initial management and generally includes a chest radiograph (CXR) or computerized tomography (CT) scan and comprehensive neck US to interrogate the contralateral thyroid lobe and the lymph nodes in the central and lateral compartments (Fig. 19.2) [61, 174]. Because most children with PTC have cervical node involvement, [26, 32, 43–47] preoperative US is necessary to identify children who require lymph node dissection. Neck CT or MRI should also be considered for locally extensive disease. However, if iodinated contrast agents are used, therapeutic RAI will need to be delayed 2–3 months. 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, positron emission tomography (PET) scanning is also not useful at this stage.

The TNM staging system can be used to estimate mortality risk [12, 62–64], but most young patients (<45 years of age) will be TNM stage I, and only the few with distant metastases will be stage II [65]. TNM stage I is diverse and includes incidental PTC, PTC with cervical node metastases, and grossly invasive PTC. Despite being TNM stage I, recurrence risk is much greater for patients with cervical node involvement or local tumor invasion [60, 66]. In fact, children with palpable cervical node metastases are more likely to recur (53% vs. 0%), persist (30% vs. 0%), and have multifocal disease (89% vs. 16%) and pulmonary metastasis (20% vs. 0%) than children

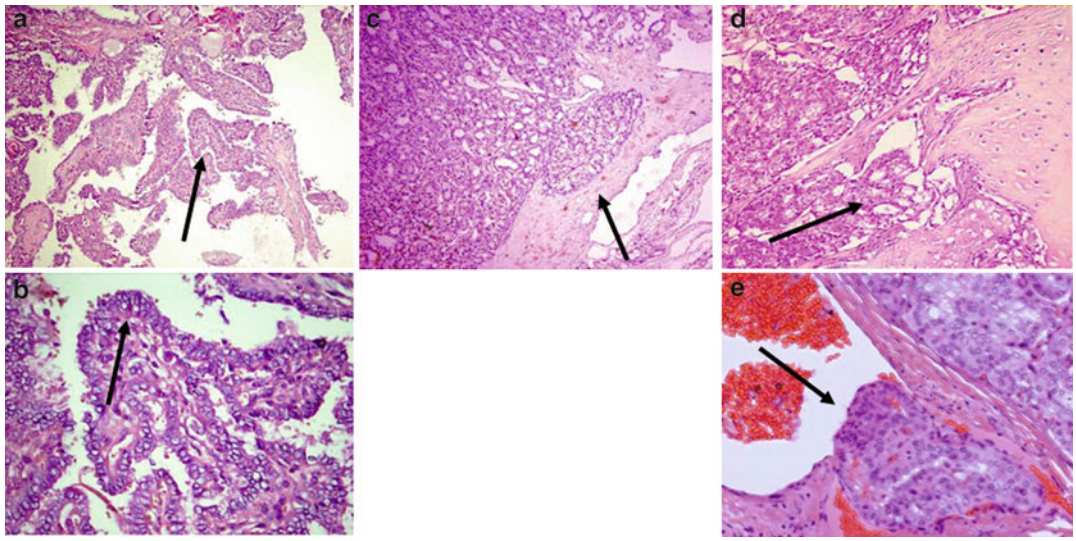

Fig. 19.3 Typical histology for DTC in Children. Hematoxylin and eosin staining of (**a**) typical PTC showing prominent papillae with fibrovascular cores (100×); (**b**) typical PTC at high power (400×) showing overlapping nuclei with intranuclear inclusions; (**c**) minimally invasive FTC showing invasion 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

without nodal disease [60]. Therefore, absence of cervical node disease is a strong indicator of low recurrence risk.

Initial Therapy for PTC in Children

Most surgeons perform a total thyroidectomy for children with more than incidental PTC. Recurrence risks are greater if lesser surgery is performed, [12, 24, 31–34, 46, 67–72] and serum thyroglobulin (Tg) is most sensitive for detecting disease after total thyroidectomy and RAI ablation [73–75]. Lobectomy and isthmusectomy may be adequate for low-risk patients with small (<1 cm) unifocal PTC but only if US shows absence of disease in the contralateral lobe and normal regional lymph nodes [12, 24, 39, 69, 71].

Total thyroidectomy should remove the entire thyroid, as residual normal thyroid tissue will take up the majority of RAI and prevent successful ablation of any residual microscopic disease. Thyroid surgery must be performed with attention to detail by an experienced thyroid surgeon [76]. A study evaluating over 5,800 patients showed a significant association between surgeon experience, complication rates, and length of stay [77]. 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 of the significant increase in complications during re-operative neck surgery [78]. Meticulous hemostasis and careful attention to the anatomy of the thyroid, parathyroids, and laryngeal nerves are essential to limit complications.

Voice change can result from injury to any of the external branches of the superior laryngeal nerves or the recurrent laryngeal nerves. If injury is bilateral, there is a risk for airway compromise. Careful visualization and use of electrodes to allow identification and nerve monitoring during surgery can be employed to reduce the risk of nerve injury. The risk of injury of the recurrent laryngeal nerve should be less than 3% after total thyroidectomy.

The parathyroid glands are also at risk. Their small size and delicate blood supply can lead to ischemia. The risk of permanent hypocalcemia is rare with lobectomy and 12% with total

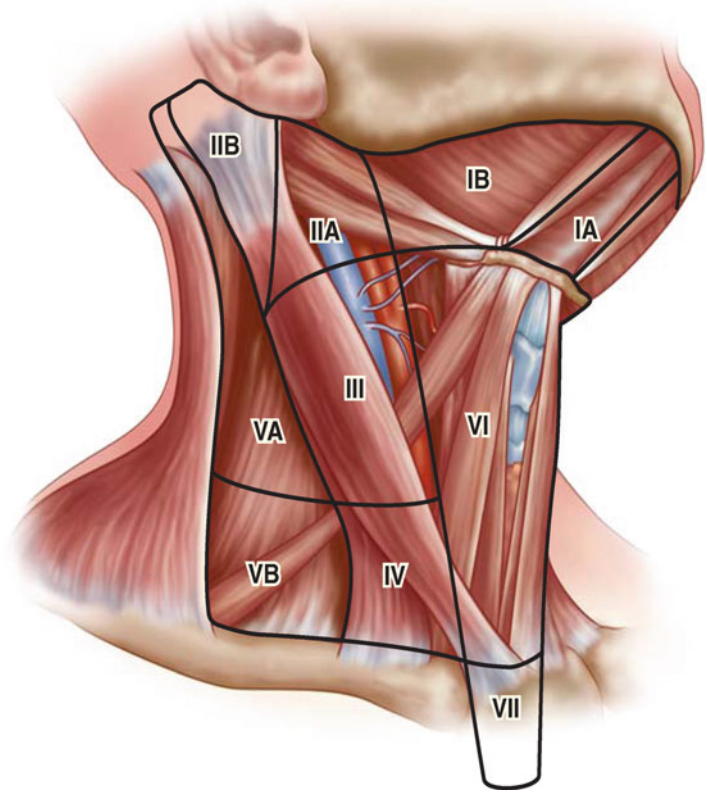

Fig. 19.4 Central and lateral cervical lymph nodes. Lymph nodes of the neck are divided into regions I through VII. Level I are submental and submandibular; level II are upper jugular; level III are midjugular; level IV are lower jugular; level V are posterior triangle and supraclavicular; level VI are delphian, prelaryngeal, pretracheal and paratracheal; and level VII are 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. Reproduced with permission [175]

thyroidectomy in children [76]. Temporary hypocalcemia (occurring in up to 50% of patients) is treated with oral calcium and vitamin D and resolves in 80% of cases [76, 79]. If the parathyroid glands appear threatened during surgery, autotransplantation should be performed at that time. Parathyroid hormone (PTH) levels may be helpful to identify those at greatest risk for postsurgical hypoparathyroidism [80, 81].

Lymph node dissection reduces the recurrence risk for children with PTC [76, 82]. All lymph node dissections should be comprehensive and compartment focused because recurrence rates are higher when "berry picking" is performed [83]. Although total thyroidectomy and central compartment dissections are associated with greater risks of hypoparathyroidism and recurrent laryngeal nerve injury [32, 67, 84], the risks are minimized when surgery is performed by a high-volume surgeon [71, 85].

Lymph node dissection can involve the central neck (levels VI and VII), lateral neck (levels II–V), or both (Fig. 19.4). Central dissection involves resection of lymphatic tissue from the hyoid bone to the left innominate vein and bilaterally to the carotid sheaths [86]. A lateral neck dissection usually removes the 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.

After surgery, patients are evaluated for persistent disease. Patients at low risk for recurrence (e.g., small unifocal tumors without lymph node disease) may be evaluated by US and a stimulated Tg, and they may be effectively followed in an expectant fashion. Conversely, patients at higher risk for residual or recurrent disease are

generally prepared for a diagnostic RAI scan along with a stimulated Tg and possible therapy with RAI (Fig. 19.2).

Children and adolescents with pulmonary or distant metastases are almost always treated with RAI, but RAI is more controversial for TNM stage I disease [29, 68, 87–89]. If RAI is prescribed, the TSH should be above 30 μIU/ml to facilitate uptake, [12, 24, 72, 90] and this can be induced by ≥14 days of thyroid hormone withdrawal [91]. Recombinant human TSH (rhTSH) using the typical adult dose can be used for remnant ablation in low-risk patients [92, 93] and may result in a lower absorbed radiation dose [94]. However, data on rhTSH in children are limited and retrospective [95, 96]. 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 normal 24-h urinary 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 [24, 70, 90]. Others suggest that ^{131}I doses should be based entirely on body weight (1.0–1.5 mCi/kg) [97, 98]. Dosimetry may be used to limit whole body retention to <80 mCi at 48 h and blood/bone marrow exposure to <200 cGy [12, 99, 100] and is useful in small children, children with diffuse lung uptake or significant distant metastases, and those undergoing multiple treatments [101]. Lesional dosimetry can be performed in children with substantial lung involvement or large tumor burden at other sites, such as bone [102–105]. A posttreatment scan to localize any metastatic disease should be obtained 5–8 days after RAI [12, 48].

In adults, one third of PTC are micro-PTC (<1 cm in diameter) detected by imaging or surgery for unrelated conditions [106]. The prevalence of incidental PTC in children is unknown, but detection is increasing. The natural history of micro-PTC is not well defined, but patients are commonly considered low risk [107, 108]. Unfortunately, lymph node metastases (43% of micro-PTC) and recurrence rates in adults are similar for micro-PTC (16.7%) and conventional PTC (21.3%) [106]. Very few data address micro-PTC in children. Based on the variable clinical course of micro-PTC, many clinicians perform US of the contralateral lobe and cervical lymph nodes. Those without involvement are followed expectantly after lobectomy with or without thyroid hormone suppression, while those with lymph node metastases are treated as if they had conventional PTC.

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 capsular invasion (minimally invasive FTC) and those with capsular and widespread vascular invasion (Fig. 19.3). Several genetic alterations have been reported in FTC [109, 110] including rearrangements of the peroxisome proliferator-activated receptor gamma (PPARγ) and the paired box gene (PAX-8) [111, 112]. FTC are typically unifocal and rarely metastasize to regional lymph nodes. However, FTC primarily develops hematogenous metastases, usually to lungs and bone, even without cervical node involvement. Therefore, most children with widely invasive FTC are treated with total thyroidectomy and RAI [113, 114]. Treatment of minimally invasive FTC is controversial for adults and children [115]. In a study of 37 young patients with minimally invasive FTC, disease-free survival was 92% at 10 years and none developed distant metastases [116], suggesting that minimally invasive FTC might be less aggressive in young patients. Lobectomy alone may be sufficient with close follow-up and possible TSH suppression.

Thyroid Hormone Suppression and Follow-Up

Postoperative TSH suppression is almost always prescribed for DTC in children, but optimal suppression is debated [117]. Some recommend

initial TSH suppression to <0.1 μIU/ml and relaxation to 0.5 μIU/ml following remission [97]. Recent American Thyroid Association guidelines are also utilized [12]. However, none of these recommendations has been validated in children. 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.

Follow-up for recurrence should be lifelong as all series have some recurrence after 20–30 years [30, 46, 107]. In children treated with RAI, initial surveillance usually includes neck US and measuring both suppressed Tg and TSH-stimulated Tg (± diagnostic RAI scan) [12]. Those with a negative stimulated Tg and negative US are defined as having "no evidence of disease," and TSH suppression and follow-up can be relaxed. In adults, an undetectable stimulated serum Tg is generally associated with remission [75, 118, 119], while Tg levels >10 ng/ml (off thyroid hormone) indicate likely residual disease [120]. 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 [121]. A significant increase in serial Tg levels indicates disease that might achieve clinical importance [122, 123].

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 RAI scans in children [124], and we do not know their serum Tg levels. Also unknown is how aggressive we should be in treating disease detected solely by abnormal Tg levels. Some opt to treat young patients until a negative RAI scan [29]. This "treat-to-negative-scan" approach is commonly used but does not take full advantage of serum Tg and thyroid US, which have detected disease in 23% of children when the scan is negative [125]. It is also possible for Tg levels to slowly decline in children 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. [23, 28, 126–128]

Thyroglobulin antibodies are detected in almost 25% of patients and interfere with serum Tg assays, rendering the Tg level uninterpretable [129]. In this setting, a decline in Tg antibody indicates declining disease burden, but it takes a median of 3 years to clear Tg antibody levels after cure of DTC [130]. A significant rise in Tg antibodies suggests possible disease progression.

Treatment of Residual/Recurrent Cervical Disease

Recurrent PTC develops in 30% of children, most commonly in the cervical lymph nodes [33]. In most cases, cervical disease can be treated with repeat surgery [129]. Surgical complications are more common with re-exploration of the neck but should be minimized when performed by a high-volume surgeon. Although many patients will be cured by repeat surgery, not everyone will develop an undetectable Tg level [129]. Nevertheless, if cervical recurrence can be removed, this is preferred over RAI, which is not very effective for macroscopic lymph node disease.

Treatment of Children with Pulmonary Metastases

RAI is indicated for patients with iodine-avid pulmonary metastases, but care must be given to select an effective dose and treatment frequency that will avoid adverse effects [26, 30, 32, 33, 37, 44–46, 48]. Empiric dosing may not be the best approach. Doses should be determined by the RAI uptake, patient age, and body size, complemented by dosimetry in some cases. The majority of children respond to ^{131}Iodine [131], and it has been suggested that patients with pulmonary metastases should be treated until disappearance of ^{131}Iodine uptake or until a cumulative dose of 22 GBq (600 mCi) has been given [131]. However, it is not known if less-aggressive therapy would also have been successful, and the cumulative RAI dose in children that is associated with increased risks of therapy

is also unknown. For patients with persistent RAI-avid disease, multiple high doses of RAI should only be given to those who are likely to benefit [97], and the decision to treat should be individualized and based on documented response to prior therapies. [131]

Newer Approaches for Children with Advanced DTC

Very rarely, children with DTC develop progressive life-threatening disease that is not amenable to surgery or RAI. In such cases, systemic therapy may be considered. Traditional chemotherapy has had limited success, [12, 132] but a variety of tyrosine kinase inhibitors show promise against refractory DTC [131–138].

Medullary Thyroid Carcinoma (MTC)

MTC is a rare disease in the pediatric population, with an annual incidence of <1 case/million/year [139]. It is a malignant tumor that arises from the neuroendocrine C cells of the thyroid, not from the thyroid follicular cells. Therefore, MTC does not produce Tg or concentrate iodine. With this in mind, Tg is not used as a biomarker, and radioiodine therapy is not indicated. However, the cells that comprise MTC produce calcitonin (Ct), as well as a variety of other hormones, such as carcinoembryonic antigen (CEA), that are used as biomarkers of persistent or recurrent disease.

MTC is a monogenic disorder associated with constitutive activating mutations in the *RET* proto-oncogene and the clinical syndromes of multiple endocrine neoplasia (MEN) 2A (including isolated familial MTC) or MEN 2B, depending on the mutation [4]. MTC is characterized by a predictable stepwise progression of histologic dedifferentiation from benign C-cell hyperplasia (CCH) to MTC [140–144]. In adults, MTC is most frequently a sporadic disease, diagnosed during routine evaluation of a thyroid nodule.

Mutations in the RET receptor are transmitted in an autosomal dominant mode of transmission with extremely high penetrance. Offspring of affected individuals have a 50% chance to carry the mutant allele. Once the *RET* mutation is identified, strong phenotype-genotype relationships allow for prediction of the rapidity with which the individual will develop MTC as well as the risk to develop other associated endocrinopathies (primary hyperparathyroidism and pheochromocytoma) [4, 145]. *RET* mutations are stratified by the American Thyroid Association into four risk categories that define the aggressiveness of the MTC and the age for initial screening, clinical evaluation, follow-up, and treatment (Table 19.1) [4].

The advent of genetic screening for MTC and MEN and the development of evaluation and treatment guidelines with age-based recommendations for prophylactic thyroidectomy have drastically reduced the incidence of metastatic MTC in children and adolescents and have improved long-term, disease-free survival [146, 147]. Unfortunately, adults are still often the proband case, and approximately 50% of them will have large tumors, regional metastasis (stage III and stage IV, TNM classification) at diagnosis, and poor prognosis [148].

Multiple Endocrine Neoplasia 2A

MEN 2A is the most common form of MEN and is associated with the triad of MTC, primary hyperparathyroidism (PHPT), and pheochromocytoma. Nearly 90% of patients with MEN 2A will develop MTC, up to 60% will develop unilateral or bilateral pheochromocytoma, and 15–30% will develop PHPT [145]. The most common mutations associated with MEN 2A are located at codons 609, 611, 618, and 620 on exon 10 and 634 on exon 11 [149]. The specific mutation, as well as the individual family history, can predict the aggressiveness of disease. Mutations in codon 609, 618, or 620 can also be associated with Hirschsprung disease, while mutations at codon 634 can be associated with cutaneous lichen amyloidosis [150–152], a dermatologic disorder that is typically located in the interscapular region of the back [153, 154].

Table 19.1 RET mutation classes, timing of thyroidectomy, and surveillance[a]

Risk level	Mutation at codon	Age for RET testing	Age for prophylactic thyroidectomy	Age at screening pheochromocytoma[b]	Age at screening PHPT[b]
D	918, 883	ASAP, before 1 year	ASAP, before 1 year	8 years, then annual	8 years, then annual
C	634	<5 years	Before 5 years	8 years, then annual (include 630)	8 years, then annual (include 630)
B	609, 611, 618, 620, 630[c]	<5 years	Before 5 years, consider later if stringent criteria met[d]	20 years, then annual	20 years, then annual
A	768, 790, 791, 804	<5 years	Before 5 years, consider later if stringent criteria met[d]	20 years, then annual	20 years, then annual

[a]Limited to common mutations—for complete information, please refer to the 2009 ATA guidelines (*modified from* [4])
[b]Pheochromocytoma screen (fractionated plasma or urine metanephrines); PHPT screen (albumin-adjusted calcium or ionized calcium)
[c]Although mutation 630 is considered level B for MTC, screening for pheochromocytoma and PHPT should occur by 8 years of age (follow level C)
[d]Criteria for later surgery include normal annual serum Ct, normal annual thyroid US, and a less-aggressive MTC family history

Adherence to the recommended age for thyroidectomy is critical in order to remove the thyroid before MTC develops or metastasizes. In rare cases, surgery may be delayed for low-risk mutations based on specific family history, preference or circumstances, but patients in whom surgery is deferred need to have meticulous follow-up to include normal annual basal serum Ct screening and normal annual neck US starting at 5 years of age [4]. Unfortunately, once clinically evident MTC develops, the likelihood for cure is low [146].

Multiple Endocrine Neoplasia 2B

MEN 2B is the least common of the MEN syndromes and in the majority of cases arise from de novo mutations in *RET* at codons 918 or 883 [149, 155, 156]. Unfortunately, MTC in MEN 2B is the most aggressive, with reports of metastatic MTC developing in patients as young as 9 weeks of age [157]. The most common sites of metastases include regional cervical lymph nodes and distant invasion to liver and bone [158]. PHPT is not a feature of MEN 2B, but patients with MEN 2B are at risk to develop pheochromocytomas, which have developed as early as 12 years of age [159].

MEN 2B is associated with specific physical features that may aid in diagnosis, including marfanoid habitus, pes cavus, pectus excavatum, proximal muscle weakness, neuromas of the lips, tongue and conjunctiva, and ganglioneuromas of the urinary and gastrointestinal tract [160]. In addition, infants may have decreased ability to produce tears and an increased incidence of constipation and feeding problems, subtle findings that may tip the clinician to look for other associated physical findings [150].

Familial Medullary Thyroid Carcinoma (FMTC)

FMTC is believed to be in the spectrum of MEN 2A, and there is similarity in the specific *RET* mutations that occur in FMTC and MEN 2A [149]. However, families affected by FMTC do not have a predilection to develop pheochromocytoma or PHPT. The diagnosis is established when at least four family members have MTC without other features of MEN [149]. However, before a diagnosis of FMTC is rendered, testing for the other endocrine tumors must be performed, and families should be counseled about potential signs and symptoms of the endocrine disorders associated with MEN 2A. There is also reduced penetrance for MTC in families with FMTC, and when it does develop, MTC typically develops later in life, sometime after the second and third decade [161].

Evaluation of Patients with Suspected MTC

Testing for a *RET* mutation should occur as soon after birth as possible if MEN 2B is suspected and between 3 and 5 years of age for other families with a known *RET* mutation [4]. With genetic testing, approximately 95% of patients with MEN 2A and MEN 2B and 88% of patients with FMTC will be found to harbor a *RET* mutation [145].

In contrast, a germ line *RET* mutation is found in only 2–9% of patients with apparently "sporadic" MTC, which occurs in adults with no family history of MEN or MTC [4, 161]. Although the chance of finding a mutation is low in sporadic MTC, *RET* testing should be performed because, if found, patients are at risk to develop other endocrine manifestations and may pass the gene mutation to their children. Genetic counseling must be a part of the evaluation process in order to provide information on risk of transmission to subsequent offspring and options for prenatal testing and to facilitate sharing of information with other first-degree relatives.

In rare families, *RET* mutations are not found, placing children at unknown risk for MTC. In that case, the method used for *RET* testing should be reviewed, as the lab may screen only for the most common abnormalities. In these cases, retesting should be considered in an alternate laboratory where complete gene sequencing is performed [4].

Infrequently, a child may be diagnosed with MTC while being evaluated for a thyroid nodule or enlarged cervical lymph nodes. In these cases there is a much greater risk for local invasion, metastases, and persistent or recurrent disease. In Europe, there are several groups that recommend routine screening of serum Ct during evaluation of all thyroid nodules [162, 163]. However, due to the lower incidence of sporadic MTC and/or MEN 2B in children, this practice has not been adopted in the United States [12].

In children who harbor a codon 630 or 634 mutation (MEN 2A) or a codon 918 or 883 mutation (MEN 2B) and are diagnosed and/or undergo surgery after 8 years of age, screening for PHPT (albumin-corrected calcium) and pheochromocytoma (plasma or urine fractionated metanephrines) should be completed prior to surgery [4].

In addition to Ct, MTC may produce carcinoembryonic antigen (CEA), chromogranin A, as well as a variety of other biologically active hormones to include corticotropic-releasing hormone (CRH), adrenocorticotropic hormone (ACTH), somatostatin, vasoactive intestinal peptide (VIP), insulin, and glucagon [4]. Secretion of biologically active hormones is typically associated with advanced disease and can be quite debilitating due to persistent, intractable flushing and diarrhea [4].

Total thyroidectomy with meticulous dissection of the central neck and dissection of clinically apparent disease in the lateral neck is the operation of choice for clinical MTC [164, 165]. However, prophylactic central neck dissection (level VI) is unnecessary in children who undergo prophylactic thyroidectomy as long as there is no evidence of lymph node metastasis [4]. In patients with MEN 2B who are >6 months of age, a serum Ct <40 pg/ml, primary tumor <5 mm (as determined by US), and no abnormal cervical lymph nodes suggest that disease is confined to the thyroid [4]. For those rare patients who undergo thyroidectomy beyond the ages recommended by the risk-stratified ATA guidelines, preoperative screening is essential (CT and US), and meticulous level VI lymph node dissection with microscopy may lead to improved disease-free survival if there is concern for lymph node metastases [166, 167]. If additional imaging is considered, the most sensitive methods to detect metastasis include US or contrast-enhanced CT of the neck, CT of the chest, MRI of the abdomen (liver), and Axial MRI or scintigraphy for bone [168].

Postoperative Ct (and CEA) levels should be followed on a regular basis beginning 2–3 months after surgery. It is best to wait a few months because Ct levels may fall slowly in some cases and raise false concern for residual disease [169, 170]. After 6 months, the Ct level is a reliable indicator of residual disease. Only one in 20 patients with undetectable Ct levels will recur over the next 5 years [171]. If Ct remains detectable, additional imaging should be performed (as outlined above and also guided by the Ct level) and tumor markers followed every 6 months to determine the doubling time, a sensitive marker of disease progression [172].

For children diagnosed with MEN, annual screening for pheochromocytoma should begin by age 8 years for those with mutations at codon 630, 634, 883, and 918 and by 20 years of age for patients with other *RET* mutations. Annual screening for hyperparathyroidism should begin by 8 years of age for patients with codon 630 and 634 and by 20 years of age for the remainder [4].

In contrast to children with DTC, patients with MTC need thyroid hormone replacement following thyroidectomy, not TSH suppression. MTC does not express the TSH receptor or the sodium-iodide symporter, so there is no benefit from TSH suppression or RAI therapy. Patients with persistent or recurrent disease may remain relatively asymptomatic for years, but once disease progresses, symptomatic care should be offered, as there is no known therapy leading to cure. Multiple studies are ongoing to examine the potential impact of chemotherapy targeting activated receptors (tyrosine kinase inhibitors) [173] and signaling pathways as well as vaccine-based therapies [4].

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Part V

Mineral and Bone Disorders

Abnormalities in Calcium Homeostasis

20

Ruben Diaz

Abstract

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 deposition and growth is 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 the urine. Extracellular calcium levels are set within a very narrow range by the concerted action of several regulatory "calciotropic" hormones on calcium handling by 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.

Keywords

Calcium • Hypercalcemia • Hypocalcemia • Vitamin D • Parathyroid hormone • Phosphate

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 deposition and growth is 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 the urine.

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Extracellular calcium levels are set within a very narrow range by the concerted action of several regulatory "calciotropic" hormones on calcium handling by 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.

Hormonal Regulation of Serum Ca^{2+}

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. In the distal tubule, 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 (CaR) [3] expressed in parathyroid cells. Elevations in serum Ca^{2+} activate the CaR which, in turn, mediates the inhibition of PTH secretion by a yet poorly defined mechanism. 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 CaR, 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 for 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 appears to serve important functions in cartilage formation 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].

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 a 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. 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 placenta, a significant source of calcitriol for the fetus, 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 [10].

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 [11].

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 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 osteoclasts 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.

Calcitonin

Calcitonin is a 32 amino acid peptide produced by thyroid parafollicular C cells and in lesser amounts by other neuroendocrine cells [12]. High Ca^{2+} elicits a rise in calcitonin secretion in parafollicular cells, a process mediated by the same calcium-sensing receptor expressed in parathyroid cells [3]. In almost all instances, calcitonin antagonizes the effect of PTH on 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 [13]. 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.

Calcium Homeostasis During Fetal and Early Neonatal Period

During fetal development, calcium homeostasis is affected by maternal Ca^{2+} levels [13]. 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 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 secretion is normally suppressed during fetal development since 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. The mother is the primary source of vitamin D during this period. 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 [14]. 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 [13]. The intestine progressively develops increased sensitivity and dependency on vitamin D for adequate calcium absorption. Infant's vitamin D levels correlate best with supplementation and sun exposure and not with breast milk intake, regardless of maternal vitamin D status.

Hypocalcemia

Hypocalcemia develops as a consequence of either reduced influx of calcium from the gastrointestinal tract or bone into the extracellular space or the 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 (Table 20.1).

Alterations in Calciotropic Hormones Causing Hypocalcemia

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 [15]. When familial, autosomal dominant, autosomal recessive, and X-linked recessive forms of hypoparathyroidism have been described. Mutations of GCM2, a protein linked to parathyroid differentiation, is a recently identified etiology of parathyroid agenesis [16]. In some instances, point mutations of the PTH gene in chromosome 11p15 lead to inappropriate expression of PTH and dyshormonogenesis. A form of autosomal dominant hypoparathyroidism is associated with sensorineural

Table 20.1 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
Dyshormonogenesis
Acquired hypoparathyroidism
Polyglandular autoimmune disease type I
Mitochondrial myopathies
Disorders of metal ion deposition
Radiation exposure
Idiopathic
Abnormal PTH secretion
Hypomagnesemia
Autosomal dominant hypocalcemia
Critical illness
Peripheral resistance to PTH
Pseudohypoparathyroidism types IA, IB, II
Pseudopseudohypoparathyroidism
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
Ca sequestration or clearance
Acute pancreatitis
Drugs: Furosemide, calcitonin
Decreased ionized calcium
Exchange blood transfusion
Alkalosis

deafness and renal dysplasia. 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 [17]. Hypoparathyroidism is also common in patients with Barakat syndrome and Kenny-Caffey syndrome, characterized by medullary stenosis of the long bones, short stature, hyperopia, and basal ganglia calcifications. 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 [18].

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 the 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 a polyglandular autoimmune disease type 1, an autosomal recessive disorder also associated with hypoadrenocorticism, hypogonadism, thyroid disease, type I diabetes mellitus, pernicious anemia, chronic active hepatitis, malabsorption, and manifestations of ectodermal dysplasia such as alopecia, vitiligo, mucocutaneous candidiasis, keratopathy, and enamel hypoplasia [19]. 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 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. The etiology of hypomagnesemia can be secondary to intestinal malabsorption or excessive renal wasting as seen in Bartter syndrome and renal tubular acidosis [20]. In autosomal dominant hypocalcemia, activating mutations of the CaR shift the curve of inhibition of PTH secretion to change the set point of serum Ca^{2+} to a concentration that can be sufficiently low to elicit adverse effects. Correction of hypocalcemia causes significant hypercalciuria as the ability of CaR to decrease tubular absorption of calcium also increases, augmenting the risk to develop urinary stones when compared to other forms of hypoparathyroidism. Finally, PTH secretion has been shown to be impaired in critical illness, perhaps mediated by an interleukin-mediated overexpression of CaR [21].

Tissue insensitivity to PTH has a clinical presentation very similar to hypoparathyroidism. Pseudohypoparathyroidism (PHP) describes various familial disorders, often inherited as autosomal dominant trait, that are characterized by the peripheral resistance to PTH [22]. 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 1a 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 has been characterized by the absence of urinary cAMP after 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 presumably preventing the activation of adenylyl cyclase by the PTH receptor. These patients can 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 action 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 and may represent the inheritance of the paternal defective gene, suggesting the presence of imprinting in the inheritance of this disorder. Type 1b PHP resembles type 1a 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 another variant where the phenotypic features are absent and infusion of PTH induces the normal elevation of urinary cAMP but without the expected phosphaturia, suggesting a defect distal to cAMP production.

Vitamin D Deficiency or Resistance

If vitamin D stores are depleted, intestinal calcium absorption can decrease sufficiently to cause hypocalcemia. In growing children, the negative calcium balance affects bone deposition and results 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 [23]. 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.

Other Causes of Hypocalcemia

When calcium handling by the gastrointestinal tract, bone, or kidney is abnormal or not responsive to calciotropic hormones, hypocalcemia 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.

Classification of Neonatal Hypocalcemia

Neonatal hypocalcemia has been traditionally described as "early" when it occurs in the first 72 hours of life or "late" when it occurs beyond that period of time [14] (Table 20.2). 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.

Table 20.2 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.

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 exam 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 also cause tetany. Hypocalcemia affects cardiac function by impairing myocardial contractility and prolonging the QTc interval, increasing the predisposition to cardiac arrythmias. 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 bone abnormalities commonly seen in rickets are a prominent feature of the physical presentation.

Other findings in the history and physical exam frequently prove useful in the determination of the etiology of hypocalcemia. If the phenotypic features of type 1 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 1.

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 often range 1.12–1.23 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 when done 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 Ca/urine creatinine, if >0.2 the predisposition to calcium deposition in the urinary tract and nephrocalcinosis increases. In healthy neonates and infants, this ratio can be more elevated. 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 since 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 or VDDR-II disorders.

Management of Hypocalcemia

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) 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 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 peripheral tissues and kidney.

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 that normocalcemia has been difficult to achieve without significant hypercalciuria, the addition of a thiazide diuretics has been shown to 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 longstanding 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 is, in most instances, 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, 1,200–1,600 U/day, or 50,000 U IM should be quite adequate since calcitriol production and action is 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 1,000 mcg/day) or chronic parenteral calcium to maintain normal serum Ca^{2+}.

In general, phosphate binders are not required to manage hyperphosphatemia in all forms of 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 remains 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.

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 has been 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 measurements are persistently low) or the hypocalcemia is resistant to oral calcium treatment, calcitriol could be administered to enhance calcium absorption.

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 also be divided into etiologies that involve abnormalities in calciotropic hormones or defects in calcium handling by organs targeted by these hormones (Table 20.3).

Table 20.3 Differential diagnosis of hypercalcemia

Alterations in hormonal response
Hyperparathyroidism
Excessive PTH production
Primary Hyperparathyroidism
MEN (type 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 syndrome
Vitamin D excess
Nutritional
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)
William's syndrome
Hypophosphatasia
Subcutaneous fat necrosis
Adrenal insufficiency
Pheochromocytoma
Vasoactive intestinal peptide-secreting tumor

Alterations in Calciotropic Hormones Causing Hypercalcemia

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) type 1 and 2A. 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 [24]. Familial forms of HPT, accounting for about 10% of all cases and comprising most cases of hyperplasia, are usually transmitted in autosomal dominant fashion. In type 1 MEN, the affected gene, menin, has been mapped to chromosome 11q13 [25]. 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 2A 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 2A. The affected gene is the RET proto-oncogene in chromosome 10q11.2 [26].

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 [27]. 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 CaR.

In Jansen syndrome, children present with hypercalcemia, a metaphyseal dysplasia and other skeletal findings consistent with HPT. Recently, the genetic defect has been identified as a mutation of the PTH receptor that renders it constitutively active [28]. These children have undetectable PTH levels as their parathyroids respond appropriately to hypercalcemia.

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, both caused by the presence of an inactivation mutations in one of the alleles coding for the CaR [29]. 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, are either homozygous for inactivation mutations of the CaR, 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.

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 also is 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 is 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 [30].

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 [31, 32]. 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 [33]. Increased prostaglandin E secretion by renal tubular cells in Bartter syndrome has been suggested to promote bone resorption [11]. Vitamin A excess has been shown to cause hypercalcemia likely mediated by the activation of osteoclast-mediated bone resorption [34].

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 a 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 [35, 36]. The hypercalcemia often resolves before the first year of life; however, hypercalciuria often persists.

Hypercalcemia, sometimes very severe and life-threatening, has been seen in infants 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 causes [37, 38].

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 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 2B, 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 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 (liquefied bone) 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, 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 [29]. 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 CaR 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.

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).

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 3,000 cm^3/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 is associated with significant gastrointestinal side effects and can cause adrenal insufficiency.

Pharmacological agents may become available in the near future that can activate the CaR and suppress PTH secretion in affected glands. Pharmacological agents, i.e., calcimimetics, that can activate de CaR and suppress the secretion of PTH are now available. 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. More recently, ^{99m}Tc-sestamibi scanning has shown some promise, especially in the visualization of adenomas [39]. Intraoperative measurements of PTH are now feasible, aiding the surgeon in his search for hyperplastic or adenomatous tissue since successful removal would be reflected by an adequate rapid drop of PTH levels [40]. 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 could easily be removed in cases of recurring hypercalcemia. Post surgical hypocalcemia is common and easily 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 takes place. The use of both calcium and phosphate supplements together with calcitriol is recommended. In some instances, permanent hypoparathyroidism ensues, requiring lifelong therapy.

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Rickets: The Skeletal Disorders of Impaired Calcium or Phosphate Availability

21

Erik A. Imel and Thomas O. Carpenter

Abstract

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 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 osteoid matrix) of trabecular and cortical bone. Children with untreated rickets may develop severe curvature deformities of the lower extremities, primarily due to the load of weight-bearing. This chapter will describe the pathophysiology and clinical diagnostic and treatment approach to rickets from a variety of calciopenic and phosphopenic causes.

Keywords

Rickets • Calcium • Phosphate • FGF23 • Vitamin D • Osteomalacia

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 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 osteoid matrix) of trabecular and cortical bone. Children with untreated rickets may develop severe curvature deformities of the lower extremities, primarily due to the load of

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weight-bearing. *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 is always present when children have rickets, but similar pathophysiologic mechanisms in adults are usually described as osteomalacia, rather than rickets, since epiphyses have fused, and the growth plate manifestations of rickets no longer can develop (although bowing may develop in mature long bones).

The terms rickets and osteomalacia should be distinguished from *osteopenia*, a general term referring to the appearance of overall 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 is to identify those with a bone density more than 2 SD below the mean for age, race, and sex as "low for chronological age." In children, osteoporosis further implies low bone density along with the presence of fragility fractures. In contrast to rickets or osteomalacia, a pure osteoporotic lesion is not manifest 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 result in radiographic evidence of osteopenia (however, the histologic features would 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 [1].

The major forms of rickets may be conveniently classified as *calciopenic*—those predominantly resulting from a reduced availability of calcium to the mineralizing skeleton—or *phosphopenic*—those resulting from a reduced availability of phosphate. These should be distinguished from several *rickets-like* disorders, such as hypophosphatasia and some skeletal dysplasias. Moreover, both the calciopenic and phosphopenic forms of rickets may be subclassified as those resulting from nutritional, inherited, or other causes (Table 21.1). However, studies of the murine growth plate suggest that local hypophosphatemia is the common factor central to most forms of rickets, including vitamin D deficiency [2].

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.

Clinical Presentation and Diagnostic Evaluation

History and Physical Examination

Despite the notion that nutritional rickets has been eradicated, the incidence of this disorder is still more common than most other etiologies encountered. Nutritional deficiency must be excluded as the etiology of any case 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, breast-fed premature infants commonly developed phosphate

Table 21.1 Classification scheme for calciopenic and phosphopenic rickets

	Calciopenic	Phosphopenic
Nutritional	Vitamin D deficiency	Phosphate deficiency
	Calcium deficiency	
Inherited	1-α-hydroxylase deficiency	FGF23 mediated hypophosphatemic rickets:
	(Vitamin D-dependent rickets Type I)	X-linked hypophosphatemia
	Hereditary resistance to $1,25(OH)_2D$	Autosomal dominant hypophosphatemic rickets
	(Vitamin-D dependent rickets Type II)	Autosomal recessive hypophosphatemic rickets
		Non-FGF23 mediated hypophosphatemic rickets:
		Hereditary hypophosphatemic rickets with hypercalciuria
		X-linked hypercalciuric nephrolithiasis
		X-linked recessive hypophosphatemic rickets
Other	Malabsorption of Vitamin D	
	Cystic fibrosis	
	Inflammatory bowel disease	
	Celiac disease	
	Short bowel syndrome	
	Impairment of hydroxylation of vitamin D	
	Severe hepatobiliary disease	
	Severe renal disease	
	Increased catabolism of vitamin D	
	Anticonvulsant therapy	

deficiency. 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, providing supplementary mineral content to ensure that the higher mineral needs of premature infants were met. Abuse or overuse of phosphate binders can impair intestinal phosphate absorption and result in phosphate deficiency, but is rarely encountered in children. 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 [3]. Fat malabsorption, with resultant fat-soluble vitamin malabsorption, and underlying renal or liver disease may be important factors in the development of rickets and should be identified in the history. It is particularly important to obtain a detailed family history with attention to bone diseases and fractures. A history of inborn errors such as those associated with renal Fanconi syndrome should be sought. Finally, in 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.

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-knee 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. In general, the calciopenic forms of rickets manifest both upper and lower extremity involvement, whereas phosphopenic forms involve the lower extremities to a greater extent.

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 [4]. 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. 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 [5]. 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.

Radiographic Abnormalities

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, when the epiphyses begin to fuse, 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, thinned compacta, and pseudofractures. As healing of rickets begins, radiodense lines are detectable adjacent to the metaphyses representing rapid calcification of the cartilage.

Biochemical Abnormalities

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, this measure 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 associated with lesser (1.5–3-fold) degrees of elevations. Alkaline phosphatase activity generally decreases with therapy for rickets, though normalization may not occur despite aggressive therapy in some forms of the disease. Adults with XLH often 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 distinguishes between the causes. The primary measured storage form of vitamin D is 25OHD. Low serum concentrations of 25OHD indicate vitamin D deficiency. This may result from insufficient dietary intake or malabsorption, among other causes. Children with nutritional rickets often have a mixture of calcium and vitamin D deficiency, and in some tropical regions, calcium may be the predominantly deficient nutrient. Partial treatment of vitamin D deficiency may increase circulating 25OHD as to render the value in the normal range if treatment is initiated prior to the time of assessment. 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 distinguish the patient's 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 a normal serum calcium level suggests the diagnosis of primary hypophosphatemic rickets, and should be followed by an accurate assessment of renal phosphate handling. For this assessment, timing of collection is important. The ideal method is to obtain a 2 h urine collection following an overnight fast (or for infants, fasting at least 4 h), with a blood collection 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 (and hence not residual urine produced overnight), with simultaneous blood collection can be used to screen; however, 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 [6] is 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; however, it does identify the *low* TMP/GFR values seen in phosphate-wasting disorders. An alternate calculation (termed TP/GFR for distinction) can be determined [TP/GFR = serum phosphate − (urine phosphate × serum creatinine/urine creatinine)] and has less deviation between fasting and phosphate loading conditions, but fasting conditions are still preferred [7–9]. The TMP/GFR is an indication of the serum phosphate level at 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 with phosphate levels within the adult normal range develop rickets, although the mechanism for this has not been established. As a rough 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 non-renal causes of hypophosphatemia.

Several phosphate-wasting disorders need to be considered, as treatment differs. To distinguish XLH from hypercalciuric variants of hypophosphatemia, e.g. 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/24 h; normal values for the fasting morning urinary calcium/creatinine in children greater than 4 years of age are <0.21 mg/mg, whereas in infants this value is higher and can vary considerably based on the nature of the diet. In addition, proteinuria or microglobulinuria is often present in patients with XLHN. TIO cannot be distinguished biochemically from primary hypophosphatemic rickets, 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 beyond childhood.

However, 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 [10]. 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 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.

Differential Diagnosis of Rickets

Nutritional Rickets

Surprisingly, the incidence of nutritional rickets from vitamin D deficiency in the United States remains greater than that of inherited forms of rickets. Consequently, although other forms of rickets are generally described as having normal 25OHD concentrations, it is possible to present with an inherited form of rickets plus be vitamin D deficient or insufficient. Despite vitamin D supplementation of milk, numerous reports describe nutritional rickets occurring with regularity in African-American children with a history of (often exclusive) breast-feeding [11]. 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. In our experience, many of the children are subsequently 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 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 USA [12]. One recent study of Gambian children suggests that the phosphate regulating hormone, FGF23, may be involved in the pathogenesis [13] of the disease as hypophosphatemia is described. However, in contrast with most FGF23-mediated rickets, circulating $1,25(OH)_2D$

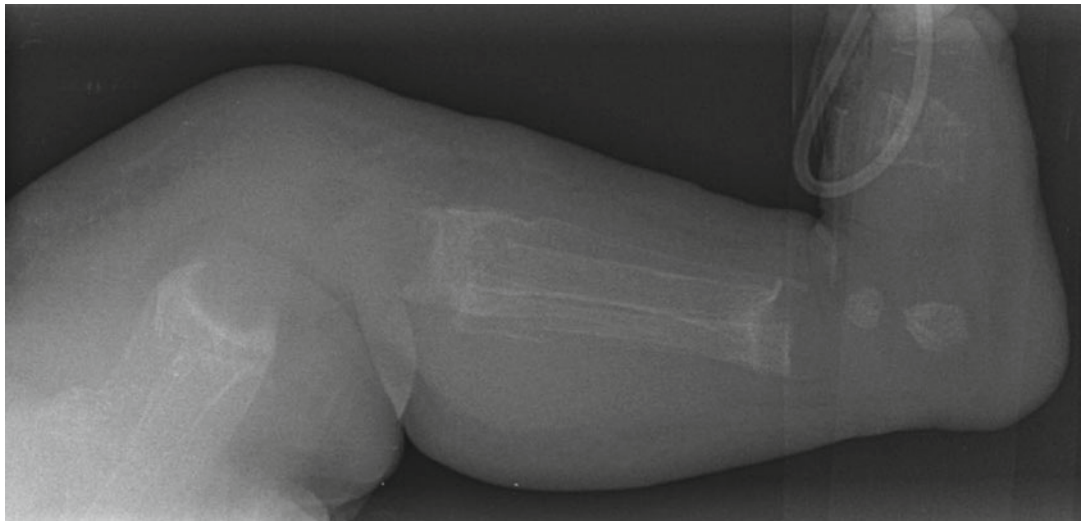

Fig. 21.1 A 25-week gestational age infant presented at 3 months of age with rickets of prematurity due to very low mineral and overall nutritional intake secondary to multiple comorbidities, including necrotizing enterocolitis and bowel resection, with intolerance of adequate enteral and parenteral nutrition volumes. This image demonstrates fraying and cupping of metaphyses as well as fractures. Bowing is not evident here due to lack of weight-bearing (X-ray from the files of Erik A. Imel)

levels were elevated [14], likely accounting for a secondary increase in FGF23 levels.

In contrast to this limited calcium supply, phosphate is nearly ubiquitous in human foodstuffs, 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 (see Fig. 21.1). While this condition is less common since the practice of adding breast milk fortifiers containing increased phosphate, among other nutrients, we have still seen rickets of prematurity when total nutritional intake was severely compromised due to comorbid conditions limiting ability to adequately provide phosphate and calcium.

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.

1α-Hydroxylase Deficiency (Vitamin D-Dependent Rickets Type I)

$1,25(OH)_2D$ is the most potent and active metabolite of vitamin D and circulates in concentrations 300–1,000-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 $1,25(OH)_2D$ [15]. Mutations in the gene encoding the cytochrome P450 moiety of the enzyme render a dysfunctional enzyme unable to donate electrons to 25OHD [16]. 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 inability to convert 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.

Hereditary Vitamin D 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 [17]. 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.

Onset of hereditary vitamin D resistance is typically 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 [18], and some patients may demonstrate oligodontia.

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 rickets (XLH). 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 [10]. 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 root canal 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 [19]. Affected patients have low serum phosphate levels, low indices of renal tubular phosphate threshold, and inappropriately low or normal $1,25(OH)_2D$ levels [4].

Patients with XLH have mutations in *PHEX* [20], 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*) [21]. FGF23 has critical roles in phosphate and vitamin D homeostasis. Bone-produced FGF23 circulates and interacts with FGF receptors in the renal tubule, in conjunction with the co-receptor, klotho. 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, 23]. Conversely, FGF23 is itself regulated through feedback mechanisms by both phosphate and $1,25(OH)_2D$ [24].

Other inherited renal phosphate-wasting disorders are less common. Autosomal recessive hypophosphatemic rickets (ARHR) has recently been identified due to gene mutations in *dentin matrix protein 1 (DMP1)* [25]. Such patients have similar features as those with XLH including the characteristic hypophosphatemia, phosphaturia, and low or normal $1,25(OH)_2D$, due to FGF23 excess. 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. Most recently another form of autosomal recessive hypophosphatemic rickets has been attributed to inactivating mutations in ENPP1 (ectonucleotide pyrophosphatase/phosphodiesterase 1), thought to regulate local concentrations of pyrophosphate at mineralization sites [26, 27]. Loss of function of this enzyme has been previously shown to be associated with generalized arterial calcification of infancy [28].

Activating mutations in *FGF23* are responsible for autosomal dominant hypophosphatemic rickets (ADHR) [29], another disorder with features similar to those of XLH, but with more variability in clinical presentation. 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 hypophosphatemia and osteomalacia developing as an adolescent or adult), resolution of the biochemical phenotype, or waxing and waning of the phenotype [30, 31]. The characteristic biochemical phenotype is identical to that of XLH, including increased FGF23 concentrations [31]. However, whereas the mutation causes resistance of FGF23 to proteolytic cleavage, the clinical waxing and waning features correspond to increases and decreases in FGF23 concentrations, suggesting that regulation of FGF23 is intermittently functioning appropriately. The mechanism for the varying FGF23 concentrations in ADHR is unclear.

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" [32]. 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 [33–35]. Tumors 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 in preliminary studies selective venous sampling has been reported to assist in localization of some tumors [36, 37]. However, in one study, among subjects without clear tumors on diagnostic imaging, no tumors were localized using selective venous sampling [36]. Thus the utility of this technique for routine use is not established. 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 [38].

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 [39]. Patients with linear sebaceous nevus syndrome also may develop FGF23 excess [40]. Recently FGF23 has been identified as the mediator of hypophosphatemic osteomalacia due to chronic iron infusions [41].

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 the renal sodium–phosphate cotransporter, NaPi2c, have been identified to cause HHRH [42–44]. In addition to rickets and osteomalacia, nephrolithiasis may occur in some patients and may be associated with specific mutations [45]. In contrast to XLH, the FGF23 concentration is reported as low normal in response to the hypophosphatemia in this disorder [42]. Most, but not all, cases have been described in North African and Middle Eastern populations.

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 Fanconi syndrome [46].

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) [47]. Lowe (oculocerebrorenal) syndrome, due to mutations in OCRL, which encodes an inositol polyphosphate 5-phosphatase [48], is usually manifest 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 [48]. In both conditions, as with other forms of the Fanconi syndrome, phosphate wasting occurs, often resulting in hypophosphatemia and occasionally overt rickets.

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 (TNALP). Over a hundred different TNALP mutations and both autosomal dominant and recessive inheritance are reported [49]. 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 TNALP 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 [49]. 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, though treatment with vitamin D may cause problems due to increased 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 blood), which may aid in diagnosis.

Radiographs may show "tongues" of lucency extending from the growth plate into metaphyses [49].

Other skeletal dysplasias include the *Schmid-type metaphyseal dysplasia*, an autosomal dominant disorder due to a mutation in the type X collagen (COL10A1) gene [50]. 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. However, for hypophosphatasia, a promising enzyme replacement therapy is currently in clinical trials.

Treatment

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-knee 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 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 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 stand to offer the most broad-reaching benefits. However, screening should be considered for high-risk groups, such as exclusively breast-feeding 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 breast-feeding 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 breast-feeding 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 [51]. Similar recommendations were recently made by the Institute of Medicine in their 2010 updated report, "Dietary Reference Intakes for Calcium and Vitamin D [52]." 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 1,000 IU daily for

infants to 4,000 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 [52]. Finally, although many published studies indicate a potential impact of vitamin D on a wide variety of nonskeletal conditions, there is insufficient evidence to justify basing dietary recommendations on nonskeletal targets of vitamin D at this time.

Vitamin D-Deficiency Rickets

Treatment of overt vitamin D-deficiency rickets requires higher doses 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 8,000 units/ml, but other formulations exist and the concentration should be confirmed. Vitamin D-deficiency rickets is typically treated with 1,000–2,000 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. If there is a concern about compliance, a single observed oral or IM dose of 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 several hours apart over a 1–3 day period.

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 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 [53]. We recommend either calcium glubionate (6.4% elemental calcium) or calcium carbonate (40% elemental calcium) in 2–4 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 bone, most children consume a phosphate sufficient diet. As vitamin D will also increase phosphate absorption, phosphate supplementation is not required in the setting 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/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.

Calcium-Deficiency Rickets

Although children with calcium-deficiency rickets significantly increase $1,25(OH)_2D$ in response to administered vitamin D, fractional absorption of calcium does not appear to change [54]. In fact, fractional calcium absorption was not positively related to baseline 25OHD concentrations [12]. Calcium repletion (1,000 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 [55]. Thus the primary treatment for this disorder is adequate calcium supplementation (30–50 mg/kg/day of elemental calcium).

1-α-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. Patients treated with calcitriol prior to the pubertal growth spurt have better height outcomes than those treated with nonactivated vitamin D metabolites through childhood [14]. Typically calcitriol is started at diagnosis in dosages ranging from 0.5 to 3.0 μg/day. Once normocalcemia and healing is attained, lower maintenance dosages of 0.25–2.0 μg/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 [14].

Hereditary Vitamin D 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 [56], 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 [56, 57]. Treatment is very challenging and should be performed by a pediatrician experienced in the management of metabolic bone disease.

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 5,000–10,000 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.

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 μg/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. In the presence of rickets, a calcium-containing phosphate binder (such as calcium carbonate) may be useful. Alternate phosphate binders such as sevelamer or aluminum hydroxide will not help rickets. CKD causes extreme elevations in FGF23 and in adults is associated with long-term mortality risk [58], though the precise role of FGF23 in CKD-related bone disease is not clear. Phosphate binders do decrease FGF23 concentrations, and sevelamer may have a greater effect in this regard [59].

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.

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 × phosphate product and precipitate soft tissue calcification. 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/dl, adjustments 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.

Phosphopenic Rickets

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.

X-Linked Hypophosphatemic Rickets (XLH)

Early treatment of XLH may be associated with improved long-term outcomes [60]; 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 trabecular bone [61, 62]. 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 a pharmacologic therapy rather than supplementation.

In XLH, phosphate is generally provided as 20–40 mg/kg/day of elemental phosphorus in 3 or 4 divided doses [63]. In early infancy, this amounts to a dose of 250–375 mg of elemental phosphorus, usually provided in 2 or 3 divided dosages, with the dosing interval limited by the ability to give smaller doses accurately. A useful preparation for infants is an oral Phospha-Soda solution containing 127 mg of elemental phosphorus per ml. Alternately a compounding pharmacy, usually at a children's hospital, may be able to make 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/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, it should be noted that parents need not 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 [64], 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 [61, 62]. 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/day [63]. Other active vitamin D analogs such as alfacalcidol may be used, but there is not 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 μg 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, but none have addressed final height in a controlled fashion. 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 [65, 66]. 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 [65, 67]. A review of seven trials of growth hormone therapy in XLH involving a total of 77 patients with this disorder concluded that the long-term impact of GH treatment on final adult height in patients with XLH remains unknown [68]. 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.

Short-term studies have indicated potential effects of calcimimetics on limiting the PTH increases triggered by an oral phosphate dose [69]. Furthermore, anecdotally, calcimimetics have been used to ameliorate tertiary hyperparathyroidism in XLH patients [70]. Calcimimetics may prove useful as adjunctive therapy in the future, though proper studies need to be performed.

As a result of studies implicating excess FGF23 activity as a central mediator of XLH, inhibiting the effects of FGF23 using a neutralizing antibody has shown correction of hypophosphatemia and skeletal improvement in the murine model of the disorder [71]. This approach is being further developed in initial human studies.

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 with 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.

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 [64]; 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 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 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 more severe hyperparathyroidism, especially with hypercalcemia. Calcimimetics may prove useful modifiers of this process, but data using this agent in long-term studies for XLH are not available.

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 [72, 73]. 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 [74]. 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 one to three years while on therapy.

Additional soft tissue calcifications occur. Severe hyperparathyroidism in XLH has been reported with myocardial and aortic valve calcifications [75]. Finally, calcifications of entheses and ocular calcifications are described. XLH patients typically develop enthesopathy, which becomes clinically evident by young adulthood in most patients [76, 77]. The mechanism of enthesopathy is not understood currently. Enthesopathy is not thought to be due to treatment; but there is also no evidence that standard therapy improves or prevents enthesopathy. 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 [77].

Hypervitaminosis D is manifest by hypercalciuria and/or hypercalcemia. This complication was frequently encountered with high-dose vitamin D therapy 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 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 is 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, it is not part of the standard clinical assessments. Indeed, several reports indicate that current therapy with calcitriol and phosphate may increase FGF23 concentrations [78–80], a finding recapitulated in the mouse model for XLH [81, 82]. 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.

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 (FD), 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 [31] 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, 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.

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; the authors have seen only minimal progression of findings in two subjects followed for 10–20 years from diagnosis.

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.

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. 1-α-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. A number of 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.

Conclusion

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.

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Part VI

Reproductive Disorders and Contraception

Turner Syndrome, Kallmann Syndrome and Noonan Syndrome

22

Diane E.J. Stafford

Abstract

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. Normal puberty is initiated by the onset of pulsatile secretion of gonadotropin-releasing hormone (GnRH) from the hypothalamus. These pulses cause release of luteinizing hormone (LH) and follicular-stimulating hormone (FSH) from the pituitary gland. These pituitary gonadotropins then circulate to the gonads and stimulate production of sex steroids. The differential diagnosis of pubertal delay is extensive but can most easily be divided into three categories: The first group represents temporary delays of puberty that are functional disorders, most commonly, constitutional delay of growth and puberty. The second is hypogonadotropic hypogonadism, in which hypothalamic or pituitary failure results in deficiency of circulating gonadotropins. Finally, hypergonadotropic hypogonadism 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.

Keywords

Delayed puberty • Gonadotropin-releasing hormone (GnRH) • Luteinizing hormone (LH) • Follicular-stimulating hormone (FSH) • Hypogonadotropic hypogonadism • Hypergonadotropic hypogonadism • Testosterone therapy • Estrogen therapy

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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

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defined as absence of an increase in 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 [1, 2]. 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 [3]. 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. 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.

Classification

Normal puberty is initiated by the onset of pulsatile secretion of gonadotropin-releasing hormone (GnRH) from the hypothalamus. These pulses cause release of luteinizing hormone (LH) and follicular-stimulating hormone (FSH) from the pituitary gland. These pituitary gonadotropins then circulate to the gonads and stimulate production of sex steroids. The differential diagnosis of pubertal delay is extensive but can most easily be divided into three categories: The first group represents temporary delays of puberty that are functional disorders, most commonly, constitutional delay of growth and puberty. The second is hypogonadotropic hypogonadism, in which hypothalamic or pituitary failure results in deficiency of circulating gonadotropins. Finally, hypergonadotropic hypogonadism 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 22.1 lists the various etiologies of hypogonadism resulting in delay or failure of pubertal development.

Delays of Pubertal Development

Delays of pubertal development are more common than failure of development and can most easily be divided into two groups: constitutional delay and delay caused by underlying chronic disease.

Constitutional Delay of Puberty

The most common cause of delay is constitutional delay of puberty and growth. These children represent the extreme of the normal physiologic variations in the timing of the onset of puberty. Children with constitutional delay are more likely to be short for age and with a history of relatively normal growth rate and to have delays in bone maturation. Frequently, there is a family history of late menarche in the mother or sisters or a delayed growth spurt in the father. Boys and girls with constitutional delay frequently have a positive family history of delay with both parents contributing to this genetic predisposition [4]. However, sporadic cases are also seen and a lack of family history does not exclude this diagnosis. The degree of delay in clinical manifestations of puberty is variable, but delay is usually not extreme, falling within the range of 2–4 years [3]. The diagnosis of constitutional delay is made more often in boys than in 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 [4, 5].

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 (Table 22.1). These disorders are usually associated with impaired availability or utilization of fuels, although clinical evidence of malnutrition may be absent. Similarly, patients with nutritional disorders, including anorexia nervosa, may present with

Table 22.1 Etiologies of delay or failure of pubertal development

Delays of puberty
Constitutional delay of growth and/or puberty
Sporadic
Familial
Chronic illness [gastrointestinal disease (inflammatory bowel disease, celiac disease), renal failure, hepatic disease, hematologic abnormalities (sickle cell disease, hemolytic anemia), malignancy, pulmonary disease (asthma, cystic fibrosis)]
Nutritional disorders
Malnutrition
Anorexia nervosa
Excessive energy expenditure, exercise
Endocrinopathies
Diabetes mellitus
Growth hormone deficiency
Hypothyroidism
Hyperprolactinemia
Glucocorticoid excess
Hypogonadotropic conditions
Congenital
Idiopathic hypogonadotropic hypogonadism
Kallmann's syndrome (Kal1, FGFR1, ProKR2, ProK2, CHD7, FGF8)
GnRH receptor defects
GnRH1 mutations
Kiss1 mutations
LHβ mutations
FSHβ mutations
PROP-1/LHX-3/HESX-1 mutations
Adrenal hypoplasia congenital (DAX1)
Panhypopituitarism
Septo-optic dysplasia
Prader-Willi syndrome
Laurence-Moon-Biedl syndrome
Acquired
Suprasellar tumors (craniopharyngiomas, etc.)
Histiocytosis X
Effects of radiotherapy
Effects of surgery
Cranial trauma
Effects of infection
Hypergonadotropic conditions
Congenital
Males
Klinefelter's syndrome (XYY)
Noonan's syndrome
Gonadal dysgenesis (XO/XY)
Defects in testosterone biosynthesis
5α-reductase deficiency
Partial androgen insensitivity
Anorchia (vanishing testis syndrome)
Leydig cell agenesis or hypoplasia
Females
Turner's syndrome (XO)
Gonadal dysgenesis (XO/XY, or XX)
Androgen insensitivity
Both sexes
Polymalformation syndromes
Acquired
Males
Bilateral orchitis
Surgical or traumatic castration
Chemotherapy, radiotherapy
Females
Surgical or traumatic castration
Premature idiopathic ovarian failure
Autoimmune ovarian failure
Chemotherapy, radiotherapy

delays in growth and/or pubertal development [6]. In the case of anorexia nervosa, the cause in most likely both lack of energy intake, as well as a central nervous system effect altering neuroendocrine control of gonadotropins [7, 8]. Excessive energy expenditure, such as that seen in young gymnasts and long-distance runners, causes pubertal delay by similar mechanisms [9, 10].

Endocrinopathies

Other endocrinopathies can also be associated with delays of puberty and growth. Isolated growth hormone deficiency is an important differential diagnosis with constitutional delay of puberty, since both present with decreased growth velocity for chronological 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 also may be caused by acquired hypothyroidism and hyperprolactinemia. Type 1 diabetes mellitus may also be associated with delays in pubertal development, even with optimal glycemic control [11]. Cushing's syndrome may cause delayed growth and puberty, though it may also result in premature development of sexual hair.

Hypogonadotropic Hypogonadism

Disorders in this category are characterized by low circulating levels of the pituitary gonadotropins, LH and FSH. This may be the result of genetic defects altering hypothalamic and/or pituitary development and defects in hormonal synthesis or action or may be acquired due to intracranial disease or trauma.

Idiopathic Hypogonadotropic Hypogonadism

Defects in the production or regulation of gonadotropin-releasing hormone (GnRH) in the hypothalamus, and subsequent lack of LH and FSH production by the pituitary, are the cause of idiopathic hypogonadotropic hypogonadism (IHH). Mutations in several genes have been found in patients with IHH. Identification of these genes has been difficult due to the rarity disease, small families due to reproductive abnormalities, incomplete penetrance and variable expressivity affecting phenotype, and overlapping phenotypes from numerous mutations [12, 13]. These single gene defects account for approximately 30% of all cases of IHH [14]. IHH is frequently divided into two categories: IHH with anosmia (Kallmann's syndrome) and normosmic hypogonadotropic hypogonadism (nIHH).

Kallmann Syndrome

Kallmann syndrome (KS) is a well-recognized form of hypogonadotropic hypogonadism consisting of gonadotropin deficiency accompanied by anosmia and represents approximately 10% of cases of IHH [14]. It is frequently transmitted as an X-linked recessive trait but may also have autosomal dominant inheritance with variable expressivity [15]. The X-linked form of Kallmann syndrome has been associated with defects in the *KAL1* gene, located on the pseudoautosomal region of Xp [15]. The protein product of the *KAL1* gene has neural cell adhesion molecule properties and provides scaffolding for GnRH neuron and olfactory nerve migration across the cribriform plate to their appropriate synapses [16]. In the absence of the KALIG protein, GnRH and olfactory fibers do not synapse properly, producing GnRH deficiency and anosmia. However, *KAL1* gene mutations account for only about 5–10% of individuals with KS. Autosomal dominant Kallmann syndrome has been associated with mutations in several other genes: *FGFR1* (KS2), *PROKR2* (KS3), *PROK2* (KS4), *CHD7* (KS5), and *FGF8* (KS6). These collectively account for 25–30% of KS [15]. The cause in the remainder of cases remains unclear. Individuals with Kallmann syndrome may also have a variety of other associated anomalies including visual abnormalities, renal agenesis and midline facial defects, congenital deafness, cryptorchidism, and microphallus.

Normosmic Idiopathic Hypogonadotropic Hypogonadism

Autosomal dominant forms of nIHH have also been associated with the genes implicated in Kallmann's syndrome, including *FGFR1*, *PROK2*, *PROK2R*, *CHD7*, and *FGF8*. The reasons for the variation in phenotype remain unclear. Autosomal recessive forms of nIHH have been associated with mutations in the GnRH receptor (*GNRHR*), *TAC3*, *TAC3R* and *KISS1R (GPR54)*, all of which cause variations in GnRH secretion or response [17]. Until very recently, no mutations in the GnRH gene itself had been identified. However, in 2009, Chan et al. described *GNRH1* mutations in patients with nIHH [18]. Identified mutations in these genes account for approximately 50% of cases of nIHH [12]. Digenic cases of IHH have been reported with individuals having mutations in two or more genes known to cause GnRH deficiency.

Other Inherited Forms of Hypogonadotropic Hypogonadism

Several other gene mutations have been shown to produce hypogonadotropic hypogonadism. DAX-1 (dosage-sensitive sex reversal, adrenal hypoplasia congenital, X chromosome) 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 congenita, an X-linked form of adrenal insufficiency due to lack of proper adrenal development [19]. 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 possess an AHC gene mutation [20].

Defects in the genes coding for the subunits of the pituitary gonadotropins have also 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 [16]. However, β-subunit mutations have been identified in both LHβ and FSHβ. Weiss et al. [21] 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 the LHβ gene that produced LH that was immunoactive and therefore measurable by current assays but had decreased bioactivity, resulting in delayed puberty. Similarly, mutations have been identified in the FSHβ gene, causing delayed puberty and hypogonadism. Matthews et al. [22] and Layman et al. [23] 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 exaggerated rise in LH to menopausal levels. 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 [16].

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 [24, 25]. *PROP1* is a transcription factor felt to be necessary for differentiation of multiple pituitary cell lineages, and its absence results in lack of proper pituitary cellular development. Mutations in *LHX3* and *HESX1*, other genes important in pituitary development and function, are also associated with IHH in the context of combined pituitary hormone deficiencies [26].

Leptin deficiency and leptin receptor defects have also been associated with hypogonadism. Patients with defects in leptin production or action typically have extreme obesity and hyperinsulinemia, in addition to hypogonadism [16].

Associated Syndromes

Hypogonadotropic hypogonadism is also associated with other more complex syndromes. Gross abnormalities of the pituitary gland, such as those seen in panhypopituitarism and septo-optic dysplasia, cause deficiencies in all pituitary hormones including gonadotropins. Hypogonadism is also a frequent feature of other genetic syndromes. A recent report found a mutation in the *CHD7* gene in an individual with delayed puberty associated with CHARGE syndrome [27]. Hypogonadotropic hypogonadism is also seen in Prader-Willi syndrome and Laurence-Moon-Beidl syndrome, among others. 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 regulation of this complex system.

Acquired Causes of Hypogonadotropic Hypogonadism

Intracranial disease processes, or therapy designed to treat such diseases, are well-known causes of hypogonadotropic hypogonadism. Histiocytosis X may be associated with HH, depending on the extent of pituitary involvement. 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 sensi-

tive to radiation effects than somatotrophs, making gonadotropin deficiency unusual in the absence of growth hormone deficiency [28]. Cranial trauma causing hypothalamic or pituitary damage, as well as infection involving these areas of the brain are also associated with gonadotropin deficiency.

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 inappropriate elevation of LH and FSH.

Klinefelter's Syndrome

Klinefelter's syndrome is the most frequent form of hypogonadism in males with an incidence of approximately 1:500–1,000 males [29]. The pubertal delay in this syndrome is caused by seminiferous tubule dysgenesis. These children have a karyotype of 47 XXY or its variants, including mosaicism, 48 XXXY, 49 XXXY, and male 46 XX. 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; micropenis; small, firm testes; poor muscular development; borderline IQ; and poor social adaptation. Physical examination also typically reveals so-called "eunuchoid" proportions with an upper to lower segment ratio of less than one, demonstrating increased long bone growth.

Turner Syndrome

Turner syndrome, characterized by a karyotype of XO or its mosaics, is the most common cause of primary hypogonadism in females. The syndrome is characterized by abnormal 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, giving variable progression through puberty, but patients seldom reach menarche. In girls presenting with short stature and pubertal delay, possible phenotypic features of Turner syndrome should be evaluated and a karyotype considered.

Vanishing Testes Syndrome (Congenital Anorchia)

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 either fail to develop secondary sexual characteristics or have incomplete progression through puberty, depending on the presence of Leydig cell tissue. Unlike males with abdominal cryptorchidism, these patients have no increase in testosterone levels following human chorionic gonadotropin administration.

Congenital Leydig Cell Aplasia

Male patients with this disorder have poor Leydig cell development, either aplasia or hypoplasia. Several patients with Leydig cell hypoplasia or aplasia have been shown to have mutations in the luteinizing hormone receptor gene [30–33]. 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 ele-

vated, implying resistance. Women identified with LH receptor defects appear to present with amenorrhea [32].

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 XX or XY karyotype. Females with Noonan syndrome undergo normal pubertal development and have normal ovarian function. 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 [34, 35].

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 45XO/46XY mosaic. In cases of 46 XY, genetic studies have shown microdeletions on either the Y [36] or the X chromosome [37]. Girls with pure gonadal dysgenesis may have complete lack of pubertal development, or some degree of breast development, but remain amenorrheic. Pelvic ultrasonography reveals a normal, prepubertal uterus, without measurable ovaries. Pure gonadal dysgenesis may also be associated with other malformations or trisomy 13 and 18.

Ovarian dysgenesis may also be associated with defects in the FSH receptor gene. 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 present on ultrasound examination, possibly due to residual receptor activity [38].

Gonadal dysgenesis causing male pseudohermaphroditism and hypogonadism is also associated with Denys-Drash syndrome (nephropathy, Wilms tumor, and genital abnormalities) and WAGR complex (Wilms tumor, aniridia, genital abnormalities, and mental retardation).

Defects in Testosterone Biosynthesis

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 both with the location of the enzyme defect in the pathway of steroid production.

Cholesterol side-chain cleavage deficiency (20-22 desmolase) results in total lack of masculinization in males and a severe neonatal salt-losing syndrome. 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 [39, 40].

17α-hydroxylase is essential for biosynthesis of androgens, glucocorticoids, and estrogens. Deficiency causes pseudohermaphroditism 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 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. These patients have elevated plasma androstenedione and estrone levels with low or normal testosterone [39, 40].

5α-Reductase Deficiency

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 this enzyme 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 [39].

Androgen Insensitivity

Androgen insensitivity syndrome (AIS) is a heterogeneous disorder caused by mutations in the androgen receptor gene [41, 42]. Phenotype in affected patients varies greatly from normal female 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 [41, 43]. Karyotype in these patients is 46 XY, and under the influence of Mullerian inhibitory substance, 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. However, they frequently present with primary amenorrhea. Phenotypic males will have variable progression through puberty depending on the activity of the androgen receptor.

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 [44]. 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 [45].

Autoimmune ovarian failure is seen in girls, either as an isolated autoimmune phenomenon or, more frequently, in association with polyglandular autoimmune failure. Idiopathic premature ovarian failure is also an infrequent cause. As with boys, ovarian failure may also be associated with chemotherapy, or radiation therapy, as well as surgical or traumatic injury to the ovary.

Evaluation of Pubertal Delay

History

A thorough medical history and family history are essential in the evaluation of pubertal delay. In cases of constitutional delay of puberty, a family history of late development may be present in up to 90% of cases. A family history of significant pubertal delay, treatment for such delay, or a history of infertility may point to an underlying genetic abnormality. The review of systems must probe for historical details that are consistent with systemic disease or chronic disorders associated with pubertal delay. Review of previous growth data, including weight for height, is essential. Plotting growth data on a longitudinal growth curve (e.g., Bayer and Bayley [46]) is useful to demonstrate a pattern of growth consistent with constitutional delay. These patients frequently have relatively slow growth in childhood. Growth data may also point to an underlying systemic disorder. Low weight for height may indicate nutritional disorders or underlying gastrointestinal disease. Patients with hypothyroidism, glucocorticoid excess, or Prader-Willi syndrome may have slight or significantly increased weight for height.

Physical Examination

Physical examination should include careful evaluation of pubertal staging, as subtle changes may indicate the spontaneous onset of puberty and alleviate the necessity for further evaluation.

Boys: 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. 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 [47]. Gynecomastia is a common finding in early puberty but may also be associated with Klinefelter's syndrome or partial androgen insensitivity.

Girls: Breast development in girls 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.

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. In patients with Klinefelter's 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. Physical abnormalities suggestive of genetic syndromes such as Turner, Noonan, Prader-Willi, Klinefelter's, and Kallmann syndrome as described above should be specifically evaluated, as well as finding which could suggest underlying chronic illness or endocrinopathy.

Initial 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 erythrocyte sedimentation rate for evaluation of inflammatory disease, complete blood cell count, electrolytes, renal or liver panel, or gastrointestinal studies.

Bone age evaluation is frequently helpful in the assessment of delayed puberty. Skeletal age more closely correlates with sexual development than does chronologic age. A skeletal age of 10 in girls and 12.5 in boys usually correlates with the onset of pubertal development [48, 49]. 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 (2 SD), 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 or urine gonadotropin levels (LH and FSH). If elevated, demonstrating hypergonadotropic hypogonadism, the etiology for gonadal failure should be further investigated based on the differential diagnosis in Table 22.1. However, 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.

LH and FSH pulsatility, as measured by 24-h sampling, has been used in several studies to distinguish between constitutional delay of puberty and hypogonadotropic hypogonadism. While such detailed study is not recommended in the routine evaluation of pubertal delay, these studies may provide useful insight. Odink et al. [50]

concluded that low FSH levels in children of adolescent age (random FSH of less than or equal to 1.11 IU/L in boys or 2.86 IU/L in girls) discriminated patients without LH pulse from those with normal pulses. A similar study also revealed that basal FSH levels of less than 1.2 IU/L were highly correlated with hypogonadotropic hypogonadism [51]. Thus, random FSH may be a useful tool in distinguishing constitutional delay from hypogonadotropic hypogonadism.

Assessment of estradiol levels is infrequently helpful in early puberty. Elevations are reassuring for onset of early puberty, but levels below the limit of many standard assays may 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 [52].

Karyotype determination, while not routinely indicated, should be carried out if physical examination suggests the presence of a genetic syndrome such as Klinefelter's, Turner, Noonan, or Prader-Willi syndrome. It should also be performed in cases of hypergonadotropic hypogonadism to evaluate for gonadal dysgenesis.

Cranial magnetic resonance imaging should be performed in patient suspected of having intracranial lesions or defects on the basis of initial 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 deferred in most cases.

Determination of the etiology of hypogonadotropic hypogonadism and distinguishing this permanent deficit from constitutional delay may be extremely difficult. As a result, conservative management with observation of over 6 months to 1 year may be warranted. However, in cases of clearly permanent hypogonadism, therapy should be initiated at a normal pubertal age to avoid the delay of growth and psychological effect of pubertal delay. Cases of probably constitutional delay must be evaluated on an individual basis for psychological distress and subsequent need for intervention.

Treatment of Delayed Puberty

Boys: Testosterone therapy is utilized for induction of puberty in boys with constitutional delay of puberty, hypogonadotropic hypogonadism, and hypergonadotropic hypogonadism. While several preparations of testosterone are available internationally, testosterone esters are the most commonly used. These compounds must be used intramuscularly to avoid hepatic metabolism. Testosterone enanthate and cypionate have longer duration of action than testosterone propionate. Three transdermal systems for delivering testosterone are currently available: a scrotal patch, a nongenital patch, and a gel formulation. When applied daily, these transdermal systems result in similar testosterone concentrations to those seen in normal young men in magnitude and diurnal variation.

While most physicians advocate a period of "watchful waiting," including periodic evaluation, reassurance, and psychological counseling, in boys with probable constitutional delay of growth and puberty, a short course of testosterone therapy may be initiated in order to stimulate pubertal development in some cases. A low dose of testosterone enanthate (50–100 mg given intramuscularly every 4 weeks) for 4–6 months will stimulate linear growth and secondary sexual characteristics without inappropriately accelerating bone age [53]. When distinction between constitutional delay and true hypogonadotropic hypogonadism is difficult, a short course of therapy (4–6 months) followed by discontinuation and monitoring for 4–6 months for progression of puberty 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 [54, 55]. 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.

Testosterone esters are also appropriate therapy for permanent hypogonadal states. Testosterone enanthate, administered by intramuscular injection, is the most common method of pubertal induction and maintenance for 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 gradually increased to a full adult dose of 200 mg every 2 weeks. When hypogonadism is diagnosed at a prepubertal age due to known abnormality, testosterone therapy may be started as early as a bone age of 11–12 years in order to decrease the psychological disturbance associated with delays in pubertal development [55–57].

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. 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 [58, 59]. More experience is needed with these preparations though they may provide a means for slower increases in testosterone levels than is available with testosterone esters.

The side effects of androgen therapy are associated with their physiologic effects. Frequent side effects include acne, oily skin, and some gynecomastia (due to aromatization to estrogen). Beneficial effects include decline in total plasma cholesterol and LDL concentrations, increased lean body mass, and decreased risk of osteoporosis based on improvement in bone mineral density [56].

Girls: Estrogen therapy induces breast development in girls with hypogonadism, with either long-term low doses or gradual increases in dose providing adequate time for pubertal growth and gradual breast development. There are many methods of pubertal induction and no optimal method has been identified. 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.

Estrogen alone is used in the early phase of pubertal induction. In cases where further growth is desired due to short stature, low doses of estrogen should be used for 6–12 months. Estrogen replacement can be given as conjugated estrogen (Premarin), micronized estradiol (Estrace), or transdermal 17-beta estradiol (Vivelle or Estrace). Premarin (conjugated estrogen) may be used at a dose of 0.3 mg every other day for 6 months followed by an increase to every day for 6–18 months. As breast development and pubertal growth increase, this dose can be increased to 0.625 mg. The full replacement dose of conjugated estrogen is 0.625–1.25 mg daily. A starting dose of 0.5 mg of Estrace (micronized estradiol) given orally on a daily basis can also be used. This dose may be continued for 12–18 months and then increased to 1 mg. Alternatively, transdermal matrix patches such as Vivelle Dot can be cut without decreasing their effectiveness and can therefore provide the lowest possible doses to the patient. A typical dose for initiation of puberty would be 6.25 μg (1/4 of a 25 μg patch) changed twice per week. As with other methods, the dose can be gradually increased depending on the extent of growth and breast development to a full replacement dose of 75–100 μg twice weekly [60].

With each of these therapies, a progestagen should be added after 12–24 months of therapy, preferably before spontaneous menstrual bleeding occurs. This can be given as Provera (medroxyprogesterone) at a dose of 5–10 mg or Prometrium (micronized progesterone) at a dose of 200 mg daily for 5 days. The progestagen can be increased to 10 days per month when breast development is complete [60]. Dose and duration should be tailored to the individual patient based on occurrence of side effects, such as nausea. The simplest regimen for the adolescent patient in continuous estrogen therapy with medroxyprogesterone added in the first 10–14 days of the calendar month. After adult doses of estradiol and progesterone 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 progestagen.

Hormone replacement therapy is associated with some behavioral changes in hypogonadal adolescents. Specifically, boys have an increase in nocturnal emissions and touching behaviors at higher doses [61]. However, in at least one study, estrogen and testosterone therapy were found to have minimal effects on behavior problems or mood in adolescents. In this study, low-dose estrogen therapy was associated with an increase in withdrawn behavior [62], but no other association was found when assessed using a variety of behavioral testing tools.

GnRH and Gonadotropin Therapy

In some cases of hypogonadotropic hypogonadism, pulsatile administration of gonadotropin-releasing hormone has resulted in induction of puberty [63]. However, as a practical matter, such therapy is most frequently used for stimulation of spermatogenesis or induction of ovulation in infertile adult patients. Similarly, gonadotropin therapy has not been commonly used in adolescents but reserved for therapy for adult infertility.

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Precocious Puberty: Clinical Management

23

Henry Rodriguez and Grace C. Dougan

Abstract

Precocious puberty has been a focus of interest for both the pediatric endocrinologist and the primary pediatrician for many years. Thirty years ago (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 the etiologies of previously uncharacterized disorders of precocious puberty.

Keywords

Precocious puberty • Gonadotropin-releasing hormone (GnRH) • GnRH agonist (GnRHa) • Follicle-stimulating hormone (FSH) • Luteinizing hormone (LH) • Androgen • Estrogen • Central precocious puberty (CPP) • Peripheral precocious puberty • Gonadotropin-independent precocious puberty

Introduction and Normal Development

Precocious puberty has been a focus of interest for both the pediatric endocrinologist and the primary pediatrician for many years. Thirty years ago (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

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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 the etiologies of previously uncharacterized disorders of precocious puberty.

The Hypothalamic–Pituitary–Gonadal Axis

The onset of puberty requires activation of the hypothalamic–pituitary–gonadal axis. Maturation of the hypothalamic–pituitary–gonadal axis initially occurs by mid-gestation [1]. 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 in such a way 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 interestingly are one of the few cell types that originate outside of 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. 23.1). The initial secretion of GnRH appears unrestrained and occurs between 100 and 150 days of gestation. The pulsatile secretion is essential to the activation of the pituitary gonadotropes, and the pulses must be of sufficient amplitude and frequency to regulate the release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH). The frequency of the pulses has been shown to alter the pattern of LH and FSH secretion such that faster frequencies increase gonadotrope release of both LH and FSH, slower frequency pulses increase the secretion of FSH relative to LH, and a constant infusion inhibits the release of both LH and FSH [3]. Maturation of negative feedback to the effects of sex steroids occurs after 150 days gestation with a progressive decrease in GnRH secretion resulting in low level 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. These gonadotropin levels lead to transient increases in sex steroids in infants that can approximate those 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 such as those with Turner syndrome [1]. Beyond 3–4 years of age, until puberty, the mechanism by which GnRH secretion is inhibited is less well understood since 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 [8, 9].

At the time of the 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 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 exhibit an estrogen-induced LH surge that leads to ovulation.

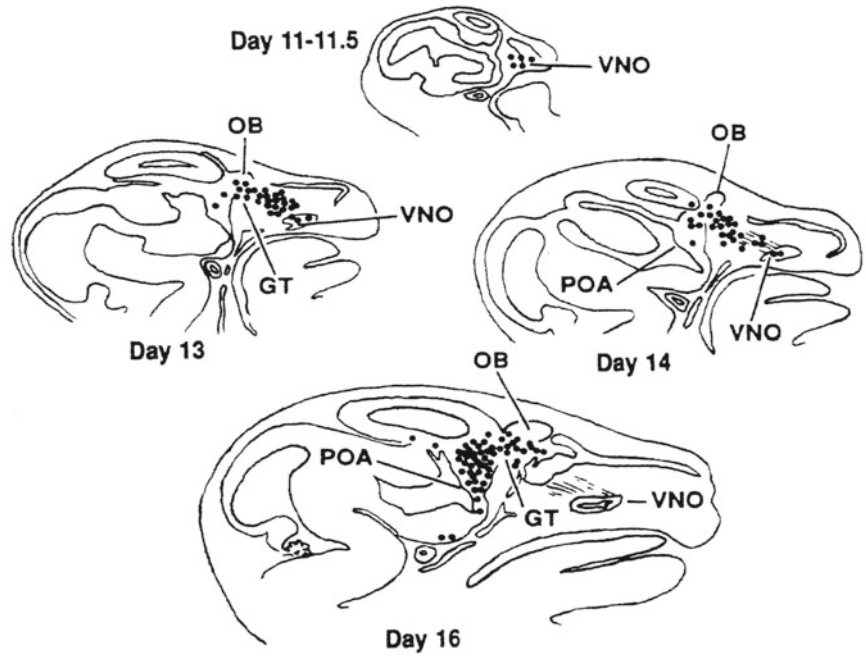


Fig. 23.1 Ontogeny of the GnRH neurosecretory neurons in the mouse. The migratory route of the GnRH neurons is indicated by the *black dots*. Their progression is illustrated according to embryonic day of development. At days 11–11.5, the neurons are in the area of the vomeronasal organ (VNO) and the medial wall of the olfactory placode. By day 13, the 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 (Adapted from Schwanzel-Fukuda M, Pfaff DW. Origin of luteinizing hormone-releasing hormone neurons. Nature. 1989;338:161–4; with permission)

Normal Pubertal Development

Normal puberty involves activation of both the hypothalamic–pituitary–gonadal axis (gonadarche) as well as 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 prior to 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 a consequent increase in adrenal androgen production [13, 14].

The 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 3 cm^3 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 appears within a few months. Tanner stages of genital and pubic

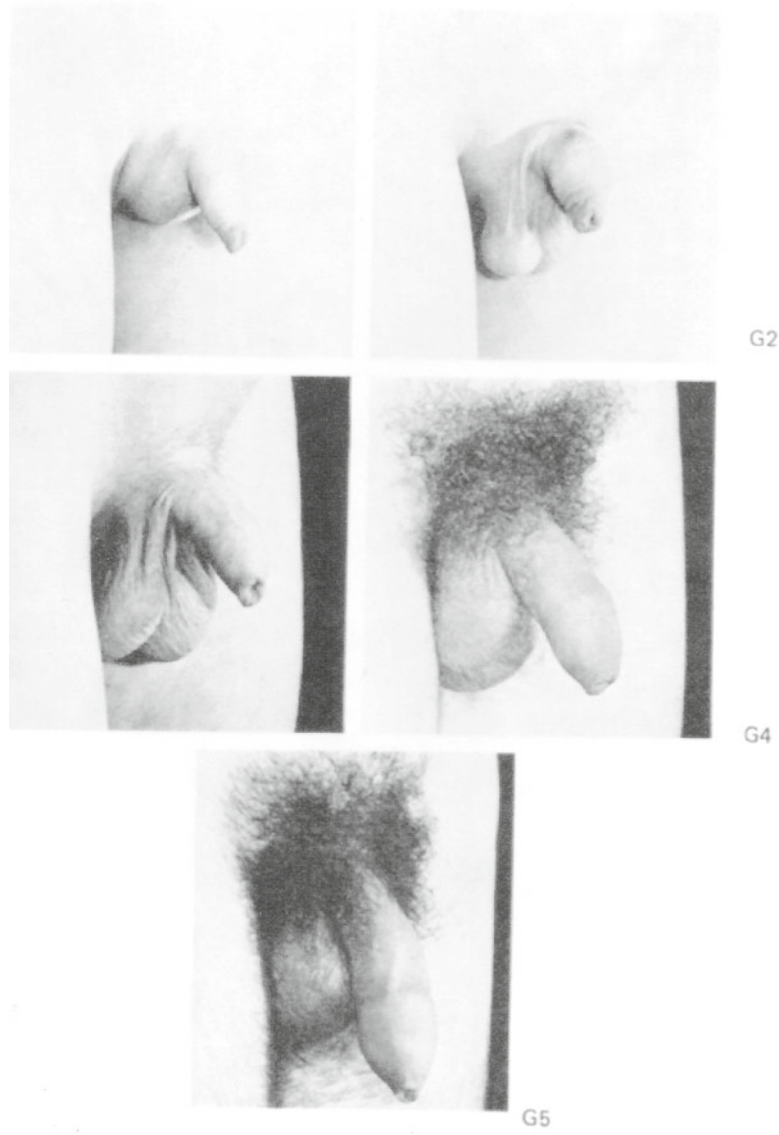


Fig. 23.2 Tanner stages of male genital and pubic hair development according to Marshall and Tanner and Reynolds and Wines (Photos from Van Wieringen JD, Wafelbakker F, Verbrugge HP et al. Growth diagrams 1965 Netherlands: Second National Survey on 0–24 Year Olds. Netherlands Institute for Preventative Medicine TNO. Groningen: Wolters-Noordhoff; 1971; with permission)

hair development are illustrated in Fig. 23.2. 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 can lead to transient breast budding or gynecomastia in a majority of pubertal boys [15].

Puberty in females is heralded by breast development, or thelarche, between the ages of 8 and13 years. Breast development may be unilateral for 6 months prior to the development of the contralateral breast. Pubic hair appears within a few months during Tanner 2 breast development with menarche typically occurring approximately 2 years after the onset of puberty, during Tanner

Fig. 23.3 Tanner Stages of female breast and pubic hair development according to Marshall and Tanner and Reynolds and Wines (Photos from Van Wieringen JD, Wafelbakker F, Verbrugge HP et al. Growth diagrams 1965 Netherlands: Second National Survey on 0–24 Year Olds. Netherlands Institute for Preventative Medicine TNO. Groningen: Wolters-Noordhoff; 1971; with permission)

4 breast development. This age range was called into question in 1997 by a study coordinated by the American Academy of Pediatrics [16]. Analysis of data collected by primary physicians on 17,077 girls of mixed ethnic background suggested that pubarche may occur as early as 5 years in African-American females and 7 years of age in Caucasian females. Menarche is not a presenting feature of puberty 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 are summarized in Fig. 23.3. As indicated in Fig. 23.4, in contrast to

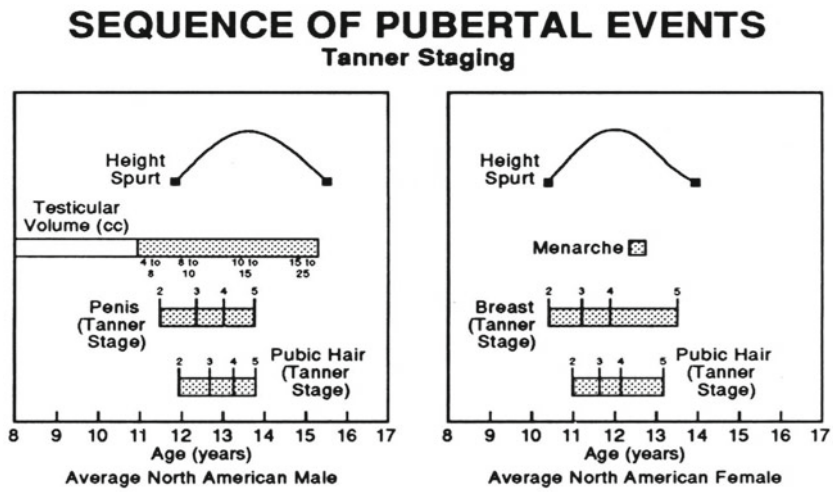


Fig. 23.4 Sequence of pubertal development in males and females. Relative growth velocity, testicular volume or menarche, and Tanner staging are indicated for age (From Tanner JM. Growth at Adolescence. Oxford, England: Blackwell Scientific Publications; 1962. p. 30–36; with permission)

males, the pubertal growth spurt follows shortly after the onset of puberty. 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 (acidifies), and leukorrhea appears. The uterus, as evaluated by pelvic ultrasound, is judged as pubertal in configuration when the body of the uterus is larger than the cervix, with a length >3.5 cm or a volume greater than 18 mL.

Precocious Puberty: Definitions

Recent discussions among pediatricians and pediatric endocrinologists have focused on the determination of the current definition of precocious puberty in females. In 1997, the Pediatric Research in Office settings 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. More recent data by Biro and colleagues on almost 2,400 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 early on but become the shortest adults [17]. This study also highlighted the finding that puberty starts earlier in females with greater body mass index (BMI), specifically with increased adiposity. This is not an insignificant position, as it implies that pubertal development is not precocious in Caucasian females older than 7 years and African-American girls older than 6 years. This prompted a review of the literature and the issuance of standards of practice by the Pediatric Endocrine Society (formerly the Lawson Wilkins Pediatric Endocrine Society) [18]. The Drugs and Therapeutics and Executive Committees of this body concluded that "recent data demonstrate that in the United States, the onset of puberty in girls is occurring earlier than previous studies have documented, with breast

and pubic hair development appearing on average 1 year earlier in white girls and 2 year earlier in African-American girls." They 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. It must be noted that many in the field of pediatric endocrinology think that the adoption of these standards was premature and carries the risk of overlooking pathology. In our opinion, until additional studies are performed, premature sexual development in Caucasian girls younger than 8 years and African-American girls younger than 7 years deserves a medical evaluation [19].

Precocious puberty is secondary to either central or peripheral mechanisms. Several forms of premature puberty, including both premature thelarche and premature pubarche, are generally 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 in Table 23.1. In contrast, gonadotropin-independent precocious puberty occurs in the absence of HPG axis activation and may be secondary to numerous etiologies, including those listed in Table 23.2. Because of the significant morbidity associated with many of these lesions, 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 final adult height.

Table 23.1 Differential diagnosis of gonadotropin-dependent precocious puberty

Idiopathic
Sporadic
Familial
Adoption from developing country
Following chronic exposure to sex steroids
Central nervous system disorders
Hypothalamic hamartoma
Congenital anomalies
Hydrocephalus
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
Russel–Silver syndrome
Williams syndrome
Klinefelter syndrome
Cohen syndrome
Pallister–Hall syndrome
Solitary maxillary incisor

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

Table 23.2 Differential diagnosis of gonadotropin-independent precocious puberty

Autonomous gonadal function
McCune–Albright syndrome
Peutz–Jeghers syndrome
Familial male 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
Testicular
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

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 increasing attention to studies that question the benign nature of premature adrenarche [20–25].

Premature Thelarche

Premature thelarche is the isolated premature appearance of breast development in girls that occurs during the first 3–4 years of life [26], with a peak prevalence in the first 2 years [27, 28]. 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 [29]. 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 the onset of development is prior to 2 years of age [27, 28],

Furthermore, girls with more than Tanner 2 breast development are also less likely to have breast tissue regression [30].

The precise etiology of premature thelarche remains unknown. It has been postulated that in some girls, the glandular breast tissue is particularly sensitive to low levels of circulating estrogen [31, 32]. It is important to exclude 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 [33]. Increased levels of sex-hormone-binding globulin (SHBG) levels 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 [34]. Serial and stimulated gonadotropin measurements in these girls have revealed elevated FSH levels consistent with those seen in early puberty [35–38]. It has therefore been suggested that premature thelarche 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 an 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 [39]. 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 usually a helpful diagnostic test. On occasion, girls with premature thelarche may 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 the bone age might be slightly advanced in obese children. 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 [26, 28, 37, 40].

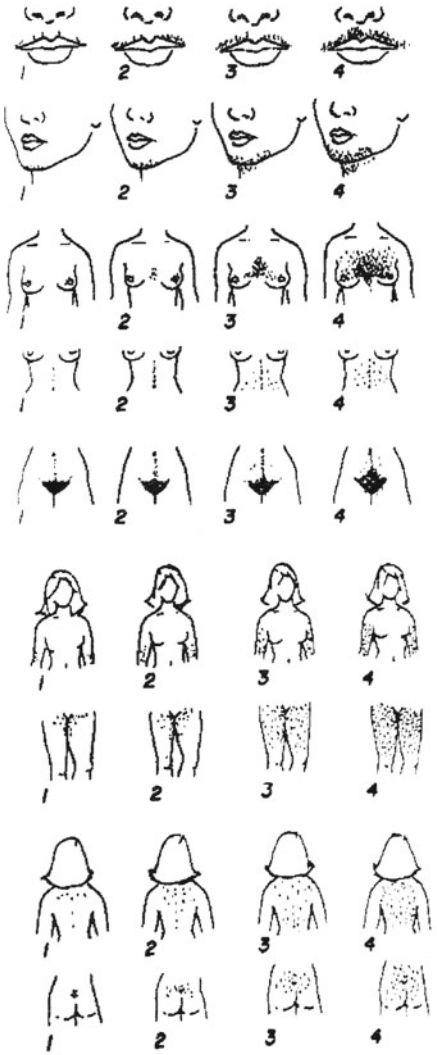
Premature Pubarche

Premature pubarche is defined as the appearance of pubic and/or axillary hair prior to 8 years of age. The child with premature pubarche typically presents with the early appearance of pubic and possibly axillary hair. In addition, there may be children that also manifest features of mild hyperandrogenism, including apocrine or adult body odor and comedones. The pubic hair is generally limited to Tanner stage 2 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. The presence of clitoromegaly is highly suggestive of serious pathology and is not seen in benign premature pubarche. Similarly, hirsutism is not a feature of benign premature pubarche. If considerable hirsutism exists, it can be quantified utilizing the Ferriman–Gallwey index (Fig. 23.5).

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 puberty and adrenarche may be occurring in younger females, particularly those of African-American descent [16]. However, we continue to recommend that caution should be exercised in assigning a diagnosis of "normal variant" pubertal development to females younger than 7–8 years of age. As many as 20% of girls with premature adrenarche have been reported to progress into central precocious puberty [41]. Benign premature adrenarche does not alter normal pubertal progression and is generally thought to be self-limited. However, recent studies suggest that premature pubarche may not be completely benign. These studies indicated higher association with later hyperandrogenism, menstrual irregularities, and infertility in females. These studies suggest that premature adrenarche may be the presenting feature of polycystic ovary syndrome (PCOS) [20]. 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) [42–44]. IUGR, in turn, has been associated with an increased risk of metabolic syndrome (insulin resistance, hypertension, central adiposity, and dyslipidemia) in adult life [45]. Since additional prognostic indicators for PCOS and metabolic syndrome do not exist, long-term follow-up of these individuals may be warranted [46].

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



Site	Grade	Definition
1. Upper Lip	1	A few scattered hairs at outer margin.
	2	A small moustache at outer margin
	3	A moustache extending halfway form outer margin.
	4	A moustache extending to mid-line
2. Chin	1	A few scattered hairs.
	2	Scattered hairs with small concentrations.
	3 & 4	Complete cover, light and heavy.
3. Chest	1	Circumareolar hairs.
	2	With mid-line hair in addition.
	3	Fusion of these areas, with three-quarter cover.
	4	Complete cover.
4. Upper Abdomen	1	A few mid-line hairs.
	2	Rather more, still mid-line.
	3 & 4	Half and full-cover.
5. Lower Abdomen	1	A few mid-line hairs.
	2	A mid-line streak of hairs.
	3	A mid-line band of hair.
	4	An inverted V-shaped growth.
6. Arm	1	Sparse growth affecting not more than a quarter of the limb surface.
	2	More than this; cover still incomplete.
	3 & 4	Complete cover, light and heavy.
7. Forearm	1, 2, 3, 4	Complete cover of dorsal surface; 2 grades of light and 2 of heavy growth.
8. Thigh	1, 2, 3, 4	As for arm.
9. Leg	1, 2, 3, 4	As for arm.
10. Upper Back	1	A few scattered hairs.
	2	Rather more, still scattered.
	3 & 4	Complete cover, light and heavy.
11. Lower Back	1	A sacral tuft of hair.
	2	With some lateral extension.
	3	Three-quarter cover.
	4	Complete cover.

Fig. 23.5 The Ferriman and Gallwey system for scoring hirsutism. A score of 8 or more indicates hirsutism (From Hatch R, Rosenfield RL, Kim MH et al. Hirsutism: implications, etiology, and management. Am J Obstet Gynecol. 1981;140:815, the chart is adapted from Ferriman D, Gallwey JD. Clinical assessment of body hair growth in women. J Clin Endocrinol Metab. 1961;21:1440, by permission)

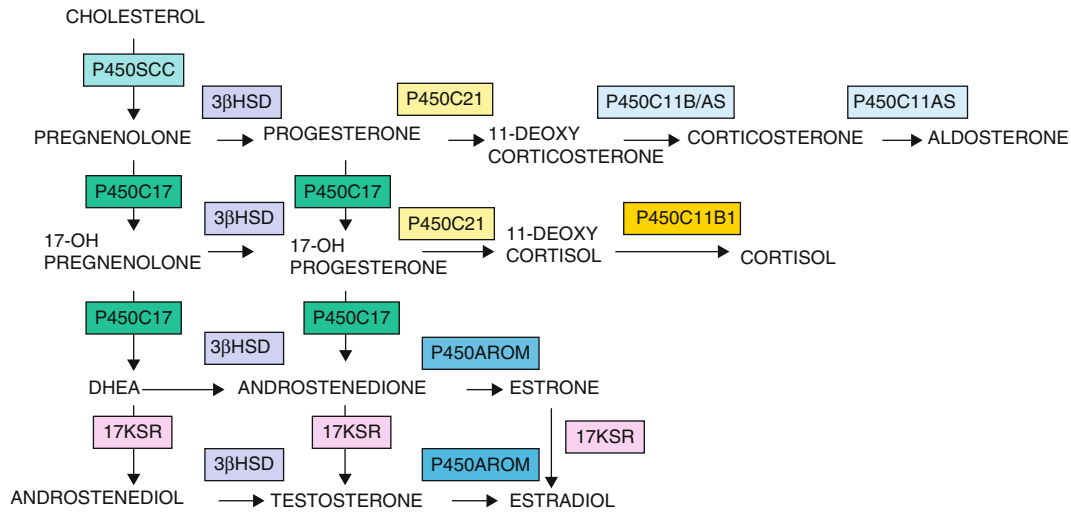


Fig. 23.6 Biosynthetic pathway of adrenal steroidogenesis

raise the 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. 23.6). 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). 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 excess quantities of these precursors lead to a 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 leading to 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 (nonclassical congenital adrenal hyperplasia—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 [47]. Studies of screening for NCAH in females with premature adrenarche report incidences ranging from 6 to 40% [46, 48, 49].

Virilizing tumors of the adrenal 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.

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 2–3 pubic hair development [50]. 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 100 μg 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 computed tomography (CT) scan or ultrasound should be used to identify mass lesions if significant virilization occurs 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.

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 [51–53].

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 development follows the sequence of normal puberty, however beginning at an earlier age. The etiology is most commonly idiopathic in girls, but is identified in the majority of boys [54, 55]. The reason for this sex difference is unclear. However, it has been proposed that the female axis is more readily activated by various factors. As listed in Table 23.1, 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.

The most common identifiable cause of CPP is a hypothalamic hamartoma. It has been identified in 10–44% of CPP cases [55]. These "tumors" are usually benign congenital malformations arising from disorganized central nervous tissue including GnRH neurosecretory neurons. They are best visualized by magnetic resonance imaging (MRI) and typically appear as a pedunculated mass attached to the hypothalamus between the tuber cinereum and the mamillary bodies, just posterior to the optic chiasm [55, 56]. Rarely, these tumors may be associated with gelastic (laughing) seizures, secondary generalized epilepsy, behavioral difficulties, and variable cognitive deficiencies [56]. Most hamartomas rarely enlarge, 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 [57]. Like other children with CPP, children with hypothalamic hamartomas respond well to GnRH analog therapy [56, 57].

A variety of other CNS lesions can result in central precocious puberty (Table 23.1). It is believed that they also cause disruption of 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 [58], 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 [59]. CNS irradiation with doses as low as the 18–24 Gy used in CNS prophylaxis therapy for childhood leukemia can also predispose children to central precocious puberty. In these cases, the age of onset of precocious puberty is often correlated with age at the time of radiation therapy.

Central precocious puberty 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 as a phenomenon secondary to the primary peripheral cause of precocious puberty. Patients with uncontrolled congenital adrenal hyperplasia, virilizing adrenal tumors, McCune–Albright syndrome, familial male precocious puberty, ovarian tumors, and exogenous sex-steroid exposure have all been reported to develop central precocious puberty, usually after successful treatment of the primary disorder has lowered the sex-steroid levels [60–63]. The mechanism responsible for activation of the GnRH neurosecretory neurons in these cases is unknown. It has been hypothesized that "maturation" of the axis leads to disinhibition of the GnRH pulse generator. The degree of bone age advancement appears to be correlated with the 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. Prior growth data are invaluable in determining if growth acceleration has occurred. A thorough physical examination to accurately document growth parameters as well to assess for the presence of acne, dark marks, adult body odor, breast development, axillary and pubic hair, estrogenization of the vaginal mucosa, and physiologic leukorrhea is critical. 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, 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 [64] and the use of GnRH agonists, such as leuprolide and nafarelin to induce gonadotropin release [65–67]. 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 [68–71]. Peripubertal increases in LH are first seen at night followed by greater increases in daytime levels [72]. 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.

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 will indicate the degree of sex-steroid exposure and consequence to skeletal maturation with ascertainment of growth potential. Pelvic ultrasonography can reveal adrenal and ovarian lesions and can 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 [73]. Therapy for gonadotropin-dependent precocious puberty must first address the etiology of the disorder. Consultation with experienced pediatric oncologist and/or pediatric neurosurgeons should be sought for potentially invasive CNS lesions. Early exposure of the epiphyses to elevated estrogen in the female and 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 reducing 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®). They act by interfering with steroid genesis 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 final height. "Super-agonist" therapy with long-acting GnRH analogs is currently the most widely used and effective therapy for CPP. These agents were initially utilized in the treatment of prostate cancer because they induce a "medical gonadectomy" and limit the further growth of testosterone-responsive tumors. 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 consequent decrease in LH and FSH, 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, the most widely used agent in the USA is Lupron® (depot leuprolide acetate) given as 0.2–0.3 mg/kg i.m. every 28 days. Longer acting preparations and an implantable GRNH agonist implant, histrelin, are gaining favor. 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 [74]. 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 improvements in adult height when compared with predicted adult height at the start of therapy and with untreated historic controls [75–77]. Studies to document final 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 [78].

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 [79, 80]. In a number of studies, addition of growth hormone therapy has been shown to improve growth velocity and predicted adult height [81]. The effect on final height has been favorable with an average gain to final adult height of 7.9 cm [157]. 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 [82] and at the attainment of final height [83]. 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 [84].

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 the child's 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 [85].

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 (Table 23.2). They range from exposure to exogenous sex steroids 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.

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 be capable of causing gonadotropin-independent precocious puberty [86–88]. More recent investigations have also suggested that specific environmental pollutants may exhibit estrogenic effects and might be a cause of premature sexual development [33].

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 [89], 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.

McCune–Albright Syndrome

McCune–Albright syndrome (MAS) is characterized by the triad of gonadotropin-independent precocious puberty, polyostotic fibrous dysplasia of bone, and irregular café au lait lesions ("coast of Maine") [90]. However, the syndrome is occasionally difficult to diagnose due to its variable phenotypic expression. 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 alpha subunit of the stimulatory guanine nucleotide binding protein (G protein), $G_s\alpha$ [91, 92]. 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 $G_s\alpha$ gene are found in both endocrine and non-endocrine tissues of patients with MAS [93]. The clinical presentation is extremely variable ranging from patients with multiple endocrine and non-endocrine abnormalities to hyperfunction of the ovary alone [94]. Affected endocrine organs may include the gonads, thyroid, adrenals, pituitary, and parathyroids [95–97]. Non-endocrine disorders may include hepatobiliary dysfunction, hyperplasia of the thymus, spleen, pancreas, gastrointestinal polyps, and abnormal cardiac muscle cells [98]. MAS also can present with only fibrous dysplasia lesions of bone and precocious puberty in the absence of cutaneous lesions [95].

The inheritance of MAS is sporadic and has been reported in all ethnic groups [99]. It is most frequently diagnosed in females, although it occurs in both sexes [100]. The most common presentation is a girl with precocious puberty secondary to estrogen production of autonomously functioning ovarian tissue [101]. 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 [99].

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 (central precocious puberty).

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 [62, 102]. 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 [103]. It has been reported to modestly control breast development and menses; however, growth velocity and skeletal maturation were unaffected. Testolactone, a weak aromatase inhibitor, has been reported to effectively decrease estrogen levels, prevent menses, and also improve predicted height [104]. Unfortunately, compliance has been 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 [105]. Ongoing studies are investigating the use of potent aromatase inhibitors. Our own experience has suggested that 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 [106]. Despite these difficulties, 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 the bone disease cause significant morbidity. Anecdotally, there appears to be an increased prevalence of breast cancer in young women who had a prior history of precocious puberty (author's observations).

Familial Male Precocious Puberty

Familial male 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 [107–111]. The Leydig cell produces testosterone

constitutively despite suppressed gonadotropins. Mutations are typically autosomal dominant; however, sporadic mutations have also been identified [112]. 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 [95]. It is interesting to note that no other signs of LH hypersecretion in females, such as polycystic ovarian 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, modest testicular enlargement, penile growth, increased musculature, bone age advancement), spermatogenesis, and growth acceleration. The testes are small for the degree of virilization and demonstrate Leydig cell hyperplasia [113]. Serum testosterone levels are in the adult male range and baseline, and GnRH-stimulated gonadotropin levels are suppressed [114]. 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 [115–117].

Therapy targeting androgen synthesis and action has been fairly effective. Early therapy utilized medroxyprogesterone acetate (MPA) [118] to inhibit steroidogenesis. It was modestly effective in decreasing testosterone levels and decreasing growth velocity. However, its effects on glucocorticoid synthesis and testicular morphology limit its application. More effective therapies include ketoconazole [119] (an antifungal and inhibitor of the 17,20-lyase activity of P450c17—Fig. 23.6) and a combination of spironolactone (an androgen receptor blocker) and testolactone (an aromatase inhibitor) [120]. Ketoconazole has been associated with toxicity (rash, nausea, headache, hepatotoxicity, pneumonitis, and renal failure) and requires careful monitoring [121]. 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. As in MAS, GnRH agonist therapy is indicated if CPP occurs following long-term sex-steroid level elevations [122].

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. 23.6). 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).

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.

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 [123]. 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 [124]. The majority of ovarian tumors produce estrogen, causing feminization, but they may also produce androgens, causing virilization [123, 125]. 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 one series of 17 granulosa/theca cell tumors [126]. The diagnosis is usually based upon 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 [127]. 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.

Testicular Tumors

Leydig cell tumors account for only 3% of all testicular neoplasms [128], 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 [129]. Surgical resection of these tumors will halt pubertal development [130]. 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.

Adrenal Tumors

Adrenal tumors typically present with virilization; however, feminization may occur [131]. 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, 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.

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 [132], lungs [133], mediastinum [134], pineal gland [135], basal ganglia, thalamus, and hypothalamus [136]. In boys, hCG simulates Leydig cell production of testosterone. 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 [137]. 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 [138]. Tumor markers that have been quite useful in the diagnosis and follow-up of these tumors include alpha-fetoprotein, human chorionic gonadotropin (hCG), and pregnancy-specific beta 1-glycoprotein [139]. 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 [140]. This increased tumor risk has been identified even in those males with low level mosaicism for Klinefelter syndrome [138].

Severe Hypothyroidism

Severe hypothyroidism may rarely present with precocious puberty (Van Wyk–Grumbach syndrome). The cardinal sign of this disorder is the

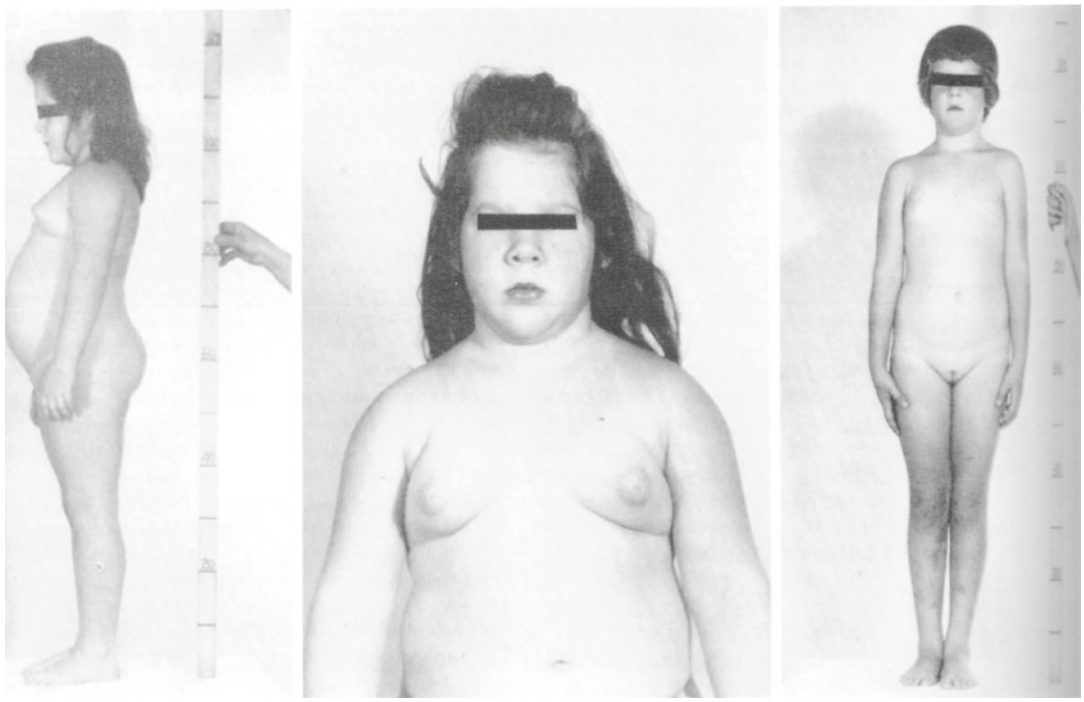


Fig. 23.7 *Left* and *center*, Precocious puberty in a 7½-year-old female with severe, chronic hypothyroidism secondary to autoimmune thyroiditis presenting with breast development, vaginal bleeding, and galactorrhea (height, −1 SD; bone age 5 3/12 years). *Right*, identical female after 8 months of thyroid hormone replacement therapy. Her height increased 7 cm; she had a decrease in breast size and cessation of galactorrhea (From Williams Textbook of Endocrinology, 9th Edition. 1998, WB Saunders; with permission)

child presenting with sexual precocity, poor growth, and skeletal delay. Girls may present with breast development, galactorrhea, ovarian cysts, and vaginal bleeding [141, 142] (Fig. 23.7). 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 [143]. 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 [144] and stimulation of gonadotrope FSH secretion may occur by elevated TRH levels [143]. We have seen several patients with primary hypothyroidism and precocious puberty that had a pubertal gonadotropin profile classical for central precocious puberty.

Gynecomastia

The reported prevalence of pubertal gynecomastia in boys has ranged from 30 to >90% [145]. Prepubertal gynecomastia, on the contrary, is quite rare and almost always abnormal. The published data 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 [146], exogenous estrogen exposure [88, 147, 148], endocrine disruptors, and "idiopathic" [149]. It is likely that some familial cases of gynecomastia are secondary to an aromatase excess syndrome [150]. 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 are currently being investigated for this indication.

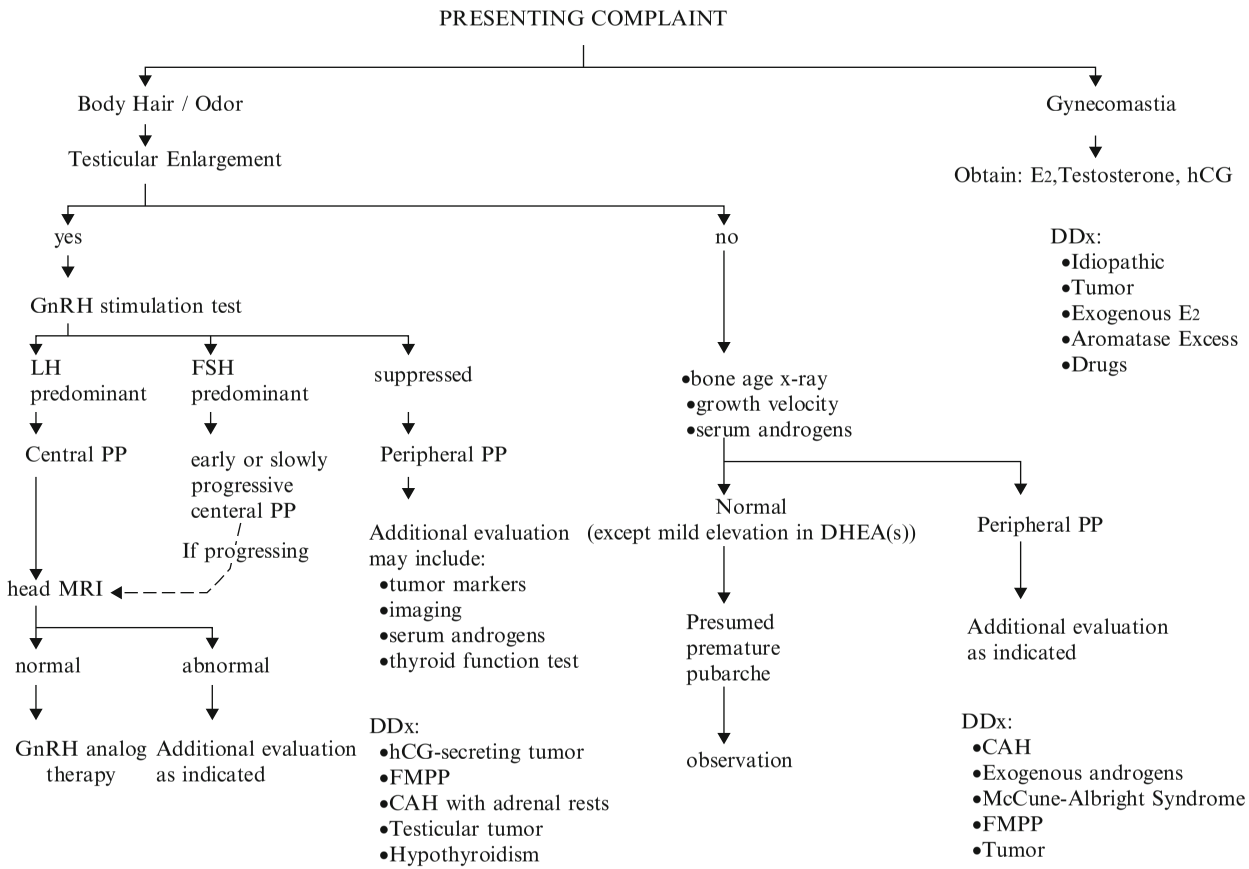


Fig. 23.8 Diagnostic approach to evaluation of precocious puberty in boys (Adapted from Eugster EA, Pescovitz OH. Endocrinology. 4th ed. WE Saunders Co.; 2000. p. 2011–21; with permission)

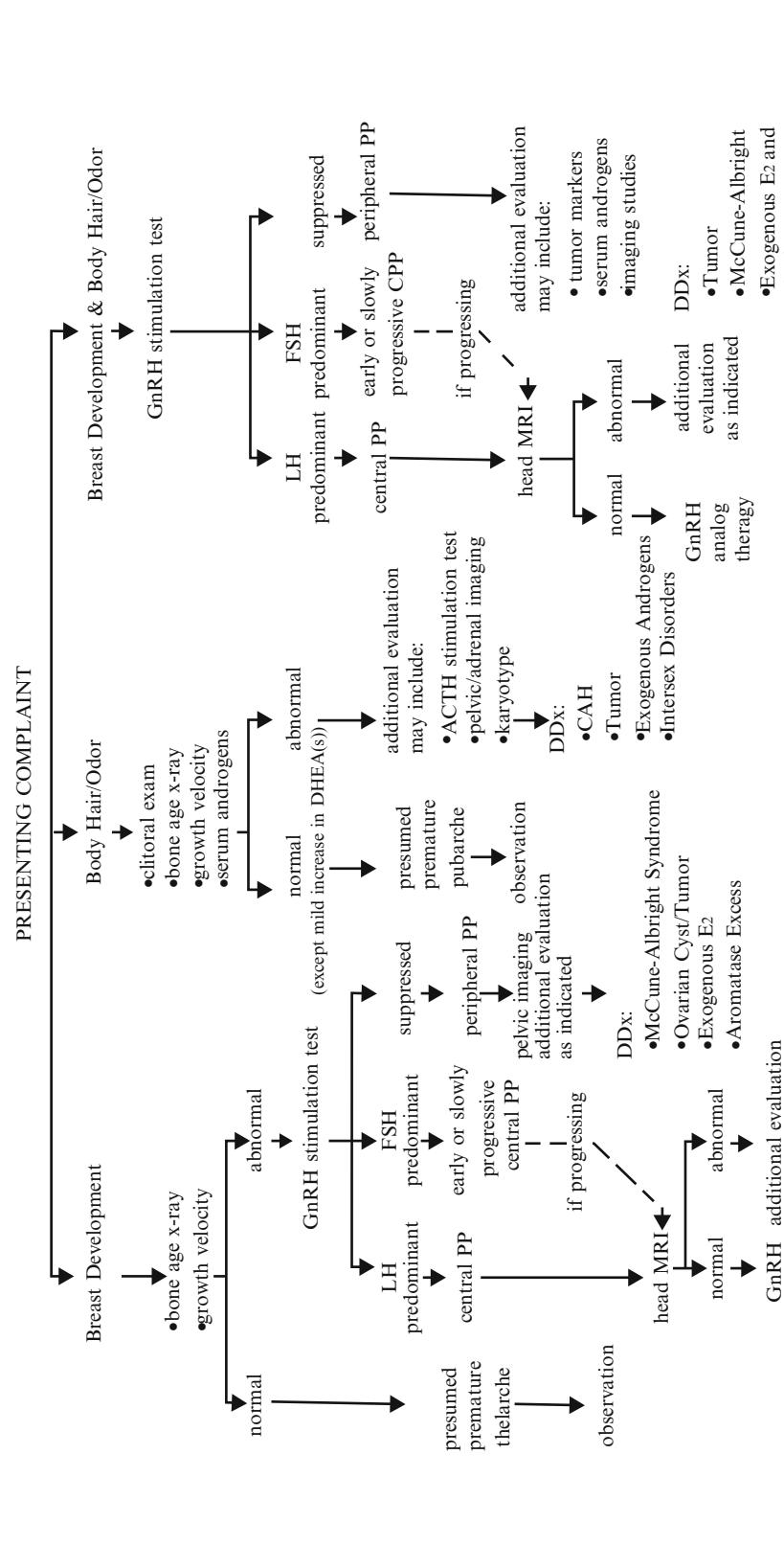


Fig. 23.9 Diagnostic approach to evaluation of precocious puberty in girls (Adapted from Eugsrer EA, Pescovitz OH. Precocious puberty. In: Endocrinology. 4th ed. WB Saunders Co.; 2000. p. 2011–21; with permission)

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. 23.8 and 23.9, 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 [151–154]. Follow-up of these selected patients suggests that therapeutic intervention may not be warranted [41, 155, 156]. 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.

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Management of Infants Born with Disorders of Sex Development

24

Indrajit Majumdar and Tom Mazur

Abstract

The aims of this chapter are the following: (1) provide a concise update on the mechanisms controlling normal and abnormal sexual differentiation, (2) provide a protocol used at our institution for practical management of infants born with DSD, (3) provide information to guide the physician on making a DSD diagnosis and 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. A discussion of infants who are born with DSD with normal-appearing genitalia is also included.

Keywords

Disorders of sex development • Sexual differentiation • Clinical approach • Challenges in medical management • Gender change • Gender dysphoria

New Nomenclature

The medical management of an infant born with disorders of sex development (DSD) has undergone significant change since this text was initially published in 2003. The European Society for Pediatric Endocrinology (ESPE) and the Pediatric Endocrine Society (PES), formerly known as the Lawson Wilkins Pediatric Endocrine Society (LWPES), issued a consensus statement on management of intersex disorders in 2006. The term "disorders of sex development" or DSD replaces the traditional terms hermaphroditism, pseudohermaphroditism, intersex, sex errors of the body, and ambiguous genitalia which not only were confusing to patients and professionals alike but also considered derogatory by some. DSD was hailed as the uniform alternative terminology and is defined as "congenital conditions in which development of

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Table 24.1 Example of disorders of sex development (DSD) classification

Sex chromosome DSD	46, XY DSD	46, XX DSD
45,X (Turner syndrome and variants) 47,XXY (Klinefelter syndrome and variants) 45,X/46,XY (MGD, ovotesticular DSD) 46,XX/46,XY (chimeric, ovotesticular DSD)	Disorders of gonadal (testicular) development: 1. Complete gonadal dysgenesis (Swyer syndrome) 2. Partial gonadal dysgenesis 3. Gonadal regression 4. Ovotesticular DSD Disorders in androgen synthesis or action: 1. Androgen biosynthesis defect (e.g., 17-hydroxysteroid dehydrogenase deficiency, 5αRD2 deficiency, StAR mutations) 2. Defect in androgen action (e.g., CAIS and PAIS) 3. Luteinizing hormone receptor defects (e.g. Leydig cell hypoplasia and aplasia) 4. Disorders of anti-Mullerian hormone and anti-Mullerian hormone receptor (persistent Mullerian duct syndrome)	Disorders of gonadal (ovarian) development: 1. Ovotesticular DSD 2. Testicular DSD (e.g., SRY and duplicate SOX9) 3. Gonadal dysgenesis Androgen excess: 1. Fetal (e.g., 21-hydroxylase deficiency, 11β-hydroxylase deficiency) 2. Fetoplacental (aromatase deficiency, POR [P450 oxidoreductase]) 3. Maternal (luteoma, exogenous, etc.) Others (e.g., cloacal exstrophy, vaginal atresia, MURCS [Mullerian, renal, cervicothoracic somite abnormalities], other syndromes)

Although consideration of karyotype is useful for classification, unnecessary reference to karyotype should be avoided; ideally, a system based on descriptive terms (e.g., an;drogen insensitivity syndrome) should be used wherever possible. StAR indicates steroidogenic acute regulatory protein (reproduced with permission from American Academy of Pediatrics [1])

chromosomal, gonadal, or anatomical sex is atypical" [1]. DSD is further classified as 46,XY DSD, 46,XX DSD, and chromosomal DSD (Table 24.1). The current karyotype-based classification highlights the importance of chromosomal analysis in diagnosis and management of individuals with DSD. Additionally, the consensus report emphasized best practice models that focused on "patient-centered" care provided by a multidisciplinary team for DSD infants and their families. Also discussed were topics of ongoing debate such as gender assignment and genital surgery [1, 2]. This chapter reflects the recommendations found in the consensus report.

Purpose

The aims of this chapter are the following (1) provide a concise update on the mechanisms controlling normal and abnormal sexual differentiation, (2) provide a protocol used at our institution for practical management of infants born with DSD, (3) provide information to guide the physician on making a DSD diagnosis and 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. A discussion of infants who are born with DSD with normal-appearing genitalia is also included.

Mechanisms Involved in Sexual Differentiation: Tissues, Genes, and Hormones

The Bipotential Gonad

The bipotential gonad is destined to become either a testis or an ovary depending on sex chromosome constitution of the germ cells

Table 24.2 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) and 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

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(classified as gonadal or primary sex) as well as other critical sex-determining genes that control male vs. female pathway of sex development (Table 24.2). At conception, the genetic sex (XX or XY) of the fetus is determined when a Y- or X-bearing sperm fertilizes the ovum. During the next few weeks, the developing germ cells migrate from their point of origin near the anal region to their final destination within the primitive gonad.

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. The second mechanism is supported by clinical observations of autosomal recessive inheritance in SRY negative 46,XX males. 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 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. 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 [3–5].

SRY mutations are not responsible for all cases of sex reversal in 46,XY females nor does it 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. SRY is present in only 10% of individuals with 46,XX ovotesticular DSD and 10% of 46,XX DSD. In contrast, among 46,XX males with normal male genitalia and testes, a majority have been found to be 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 [3].

The importance of certain autosomal genes in testicular differentiation has become more

Table 24.3 Causes of 46,XY disorders of sex development (DSD)

1. XY gonadal dysgenesis
2. Denys-Drash syndrome—WT1 mutation, also nephropathy, and Wilms' Tumor
3. Frasier syndrome—WT1 mutation, also nephropathy, and gonadoblastoma
4. XO/XY mosaicism—mixed gonadal dysgenesis
5. Campomelic dysplasia—SOX9 mutation
6. DAX-1 duplication (DSS Syndrome)
7. Wnt-4 duplication
8. SF-1 (NR5A1 gene) mutation
9. Deletion of 9p−, 10 q−
10. Duplication: Xp+

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obvious (Table 24.3). The most notable among these are the SOX genes (SRY-homeobOX-like genes). One member of this family, SOX9, is essential for Sertoli cell differentiation from precursor in interstitium of the primitive gonad. 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 [4, 6]. 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 [7, 8]. SOX9, in turn, mediates Sertoli cell development and testes differentiation [6] along with initiation of anti-Mullerian hormone (AMH) expression [9]. Consequently, SOX9 is an autosomal gene which plays a pivotal role in male sexual development (Fig. 24.1). Two additional genes, steroidogenic factor 1 (SF1) and Wilms' tumor 1 (WT1), have a synergistic role in testicular development [10]. SRY is transactivated by the WT1 gene product [11]. In the testis, SRY and SF1 cooperatively upregulate SOX9 [12].

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 [13, 14] and the synergy between WT1 and SF1 [10], blocking subsequent testicular development. Recently, mouse models have additionally demonstrated a "pro-testis" effect of DAX1 [15]. The animal models indicate that a precise dose of DAX1 is important for testicular development, "too much or too little can prevent cord formation" [16].

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 the 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 testes 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 in females inactivates SRY [13, 14] and SOX9 function, thereby blocking testicular development and anti-Müllerian hormone (AMH) expression [9]. 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 [17, 18]. 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 [19, 20], which in turn appears to inhibit testicular vascular development, stabilize oocytes, and induce Müllerian development [21]. Deletion of Wnt-4 leads to masculinization in 46,XX individuals and degeneration of Müllerian duct derivatives [22]. Wnt-4 appears upstream of and in concert with DAX1. In 46,XY individuals,

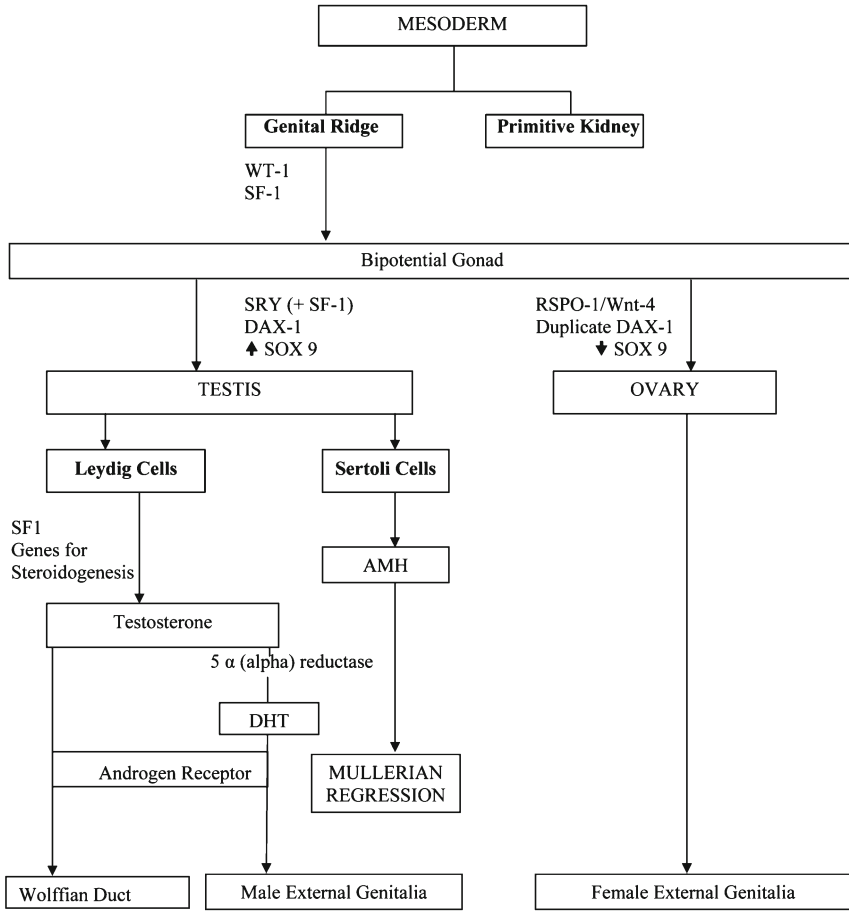

Fig. 24.1 Pathway of sexual differentiation (Reprinted with permission from Springer)

duplication of Wnt-4 causes upregulation of DAX1 in the testes and suppression of SOX9, resulting in a female phenotype [23]. Hence, duplication of either DAX1 or Wnt-4 cause dosage-sensitive 46,XY sex reversal.

Genes which support ovarian development have not been completely identified. However, they are probably located on the X chromosome and may have dosage-sensitive functions since 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 SF1and SOX9.
2. Granulosa cells develop from the supporting cells while Sertoli cell differentiation is blocked.
3. Wnt-4 induces differentiation of Müllerian structures into 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. 24.1).

Other mechanisms have been shown to cause sex reversal. Duplication of SOX9 is an autosomal cause of sex reversal in 46,XX individuals [24], while mutations in the SF1 gene and other sex-determining genes are also known to cause 46,XY gonadal dysgenesis and female phenotype.

The Paired Internal Ducts: Wolffian and Mullerian Ducts

Wolffian Ducts

The differentiation of the Wolffian duct into 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 [25, 26], which is initially regulated by human chorionic gonadotropin (hCG) [27]. Genetic males who are agonadal exhibit involution of the Wolffian ducts and also have Müllerian ducts because AMH is not produced [25, 28]. Normally, AMH is produced by the Sertoli cells in the fetal period, and the production continues until the age of 8–10 years in boys. Hence, AMH is a marker of Sertoli cell function in patients with DSD [29].

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 knockout mice fail to develop Müllerian structures and lose oocytes [22]. Additionally, they manifest hypersecretion of ovarian androgens derived from Leydig cell precursors in the interstitium and exhibit masculinization of the Wolffian ducts [30]. 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.

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 nonfunctional testes, or if androgen function is defective as in androgen insensitivity due to genetic defects.

Male External Genitalia

Development of male external genitalia depends on the adequacy of testosterone (T) and 5-α(alpha)-dihydrotestosterone (5-α(alpha)-DHT) production as well as functional androgen receptors (AR). The testis is formed by 6–7 weeks of fetal life and the Leydig cells are initially stimulated by hCG from the placenta in the first trimester [27] and by fetal pituitary LH in the second trimester [31]. 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(alpha)-DHT; the enzyme responsible for this conversion is 5-α(alpha)-reductase which is present in genital tissue. 46,XY infants with 5-α-reductase deficiency have normally functioning testes; the predominantly female phenotype in early childhood is due to defective conversion of T to 5-α(alpha)-DHT during early fetal life. In many societies where rates of 5-α(alpha)-reductase deficiency are high, spontaneous gender reassignment to male usually

occurs in puberty. A number of reasons have been suggested for this transition. 5-α(alpha)-DHT is not an essential androgen for adult male muscular development, whereas it is critical for normal external male genital differentiation in fetal life and virilization of males during puberty [32–34]. 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 the language [57].

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) homozygous or heterozygous mutations in both aromatase genes which leads to a deficiency of aromatase and failure to convert androgens to estrogens, (4) ovotesticular DSD, or (5) mixed gonadal dysgenesis [35–37]. 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 urogenital sinus with a large phallic-appearing clitoris that resembles a hypospadic penis. Rarely, extreme virilization causes complete labioscrotal fusion and a "penile urethra" (Prader stage 5). In the last example, the external genitalia appear normal, and the only clue to the possibility of a genetic female is the absence of palpable gonads which is mistakenly thought to represent cryptorchidism.

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 protocol we follow at our institution when a baby is born "in-house" or "is transferred in" from a regional hospital (see Fig. 24.2). Our team consists of pediatric endocrinologists, a pediatric psychologist with extensive endocrine expertise, pediatric urologists, geneticists, radiologists, the child's primary care physicians, and other specialists as required.

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

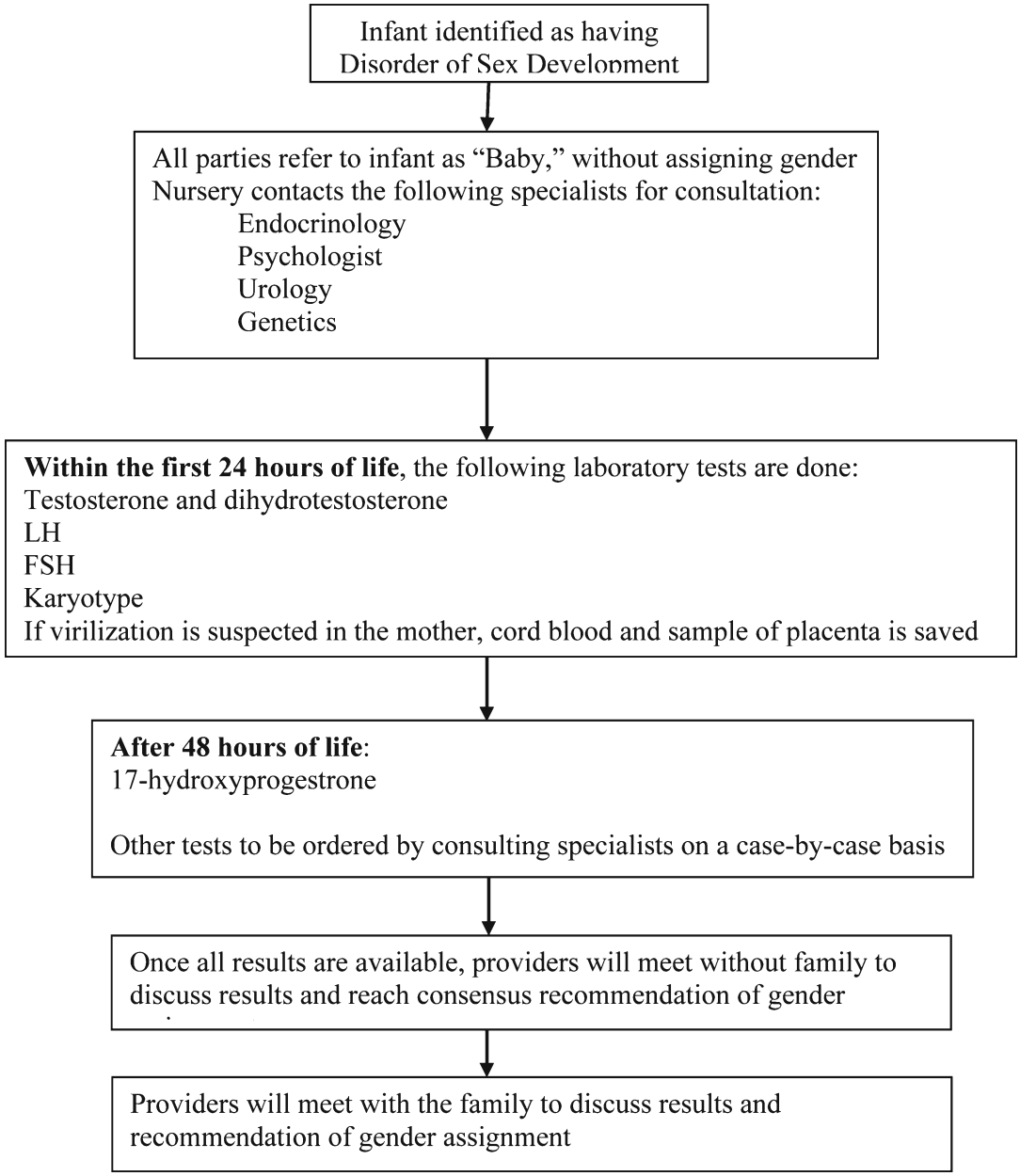

Fig. 24.2 DSD protocol: an outline of protocol we follow at our institution when a baby with DSD is identified

examines the infant. However, in some instances, the psychologist serves in this role. The team leader provides the parents the differential diagnosis, details about radiologic studies and biochemical tests which 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 also informed that all the 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 which 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 the development of gender identity development is also discussed. Diagrams and current source materials [2, 38] are helpful, and repetition is essential. Examining the infant with the parents helps them to understand the changes that have led to the genital appearance. 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 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 The Medical Management of DSD section). Diagnostic studies include hormone assays, genetic studies, ultrasound examinations of the pelvic and the abdominal structures (uterus, gonads, renal anatomy), vaginogram, and a voiding cystourethrogram.
5. Once all the tests results are available, the DSD team meets to review the results of the diagnostic studies, arrives at a consensus on the diagnosis, recommends the most suitable gender assignment, and decides 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.
6. The designated team members meet with the family for the following purposes:
   - 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.
   - Pros and cons of gender assignment as male or female and the consequences of each choice.
   - Give outcome information regarding fertility, need for hormonal therapy, and psychosexual data if known.
   - 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 than the team's recommendation.
   - Gender assignment is made and the name of the baby is announced.
   - Informed that a summary of their infant's condition along with important time periods to remember, i.e., hormone treatment will be given to them.
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 about what and how to inform their child about their 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 [39].

The Medical Management of DSD

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 46,XX chromosomes 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 which helps in the final diagnosis. Special attention is given to the history of maternal drug or medication use (body-building steroids or androgens in oral contraceptives), general health and endocrine status (maternal hirsutism or virilization), family history (including a history of infertile non-menstruating maternal female relatives with scant or absent pubic and axillary hair which suggests androgen resistance with a X-linked recessive inheritance pattern [40]), and consanguinity in the parents (for autosomal recessively inherited conditions like 5-α(alpha)-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 ultrasound genital findings (no phallus) may be an early evidence of androgen resistance or aphallia.

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 is (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 since 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 [41, 42]. If the stretched length is less than 2 cm, the diagnosis of microphallus is made [43]. The width of the erectile tissue and its consistency should also be recorded. Hypospadias is defined by the location of a single urethral opening and is classified as first degree (asymmetric on "glans"), second degree (mid-phallic shaft), third degree (junction of phallus with scrotum), or fourth degree (perineal, closer to 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.

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. The severity of posterior perineal fusion of the labioscrotal folds may be slight 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 a fatal condition in 46,XY infants who have striking skeletal dysplasia and female phenotype [6].

Diagnostic tests:

The following are tests which are informative:

1. Karyotype (may include genetic studies SRY, 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. Radiologic services:
   Ultrasound of pelvis/inguinal canal to identify the presence of uterus and gonads
   Ultrasound of kidneys
   Urethrogram/vaginogram/voiding cystogram—position of urethra, reflux, and presence of vagina

Challenges in Diagnosis and Treatment of DSD

An algorithm which links the diagnostic tests to clinical diagnosis is provided in Fig. 24.3.

The most common cause of DSD in a 46,XX genetic female with the diagnosis of CAH is 21-hydroxylase 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 [35]. Many 46,XX CAH individuals with 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 [44]. All were assigned as males at birth. Ten of the 12 individuals had always lived as a male. Two were reassigned female 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 "propose consideration of male assignment for 46, XX patients who have fully developed male genitalia" [44, 45]. In a third paper, Lee and Houk [46], report on other cases, although rare, of individuals who were assigned male and in adulthood had established a male gender identity. 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 micropenis, either isolated or associated with hypospadias. The testes may be normal size or hypoplastic. Partial androgen insensitivity is a concern because male genital growth is permanently compromised. In the past, 46,XY infants with micropenis, with or without hypospadias, were reassigned as female [47, 48]. This recommendation was based on the belief that it was easier to make a functional vagina than a functional

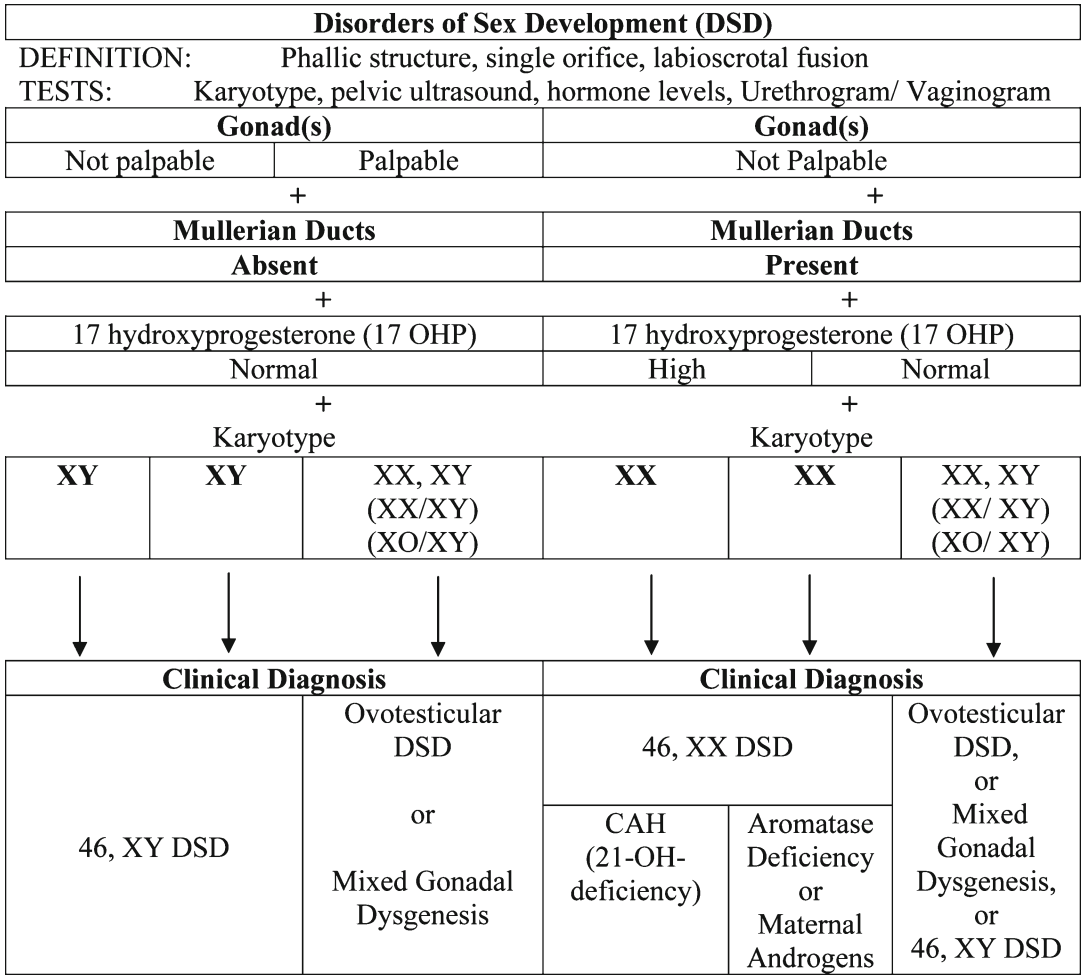

Fig. 24.3 Differential diagnosis of disorders of sex development (DSD): the main features included in this diagram are the presence or absence of external gonads, the structure of Mullerian duct derivatives (uterus, etc.), the serum level of 17-hydroxyprogesterone, the karyotype, and possible clinical 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-a(alpha)-hydroxylase deficiency, 46,XX males and 46,XY females) (Reprinted with permission from Springer)

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 [49]. 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 expressed

concerns about their penile size [50–52]. At present, many pediatric endocrinologist believe that 46,XY infants with isolated micropenis and fused scrotum should receive a short course of depot testosterone (25 mg im. q month for 3 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, increase in penile growth following testosterone treatment facilitates the 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 [50–52]. In the most severely affected 46,XY males with negligible phallic tissue, the urologist often advise the team and parents that penile reconstruction is not possible.

Gender assignment is an "imperfect art" [2] 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 [53]. 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 the intravaginal sensory responses or the ability to lubricate, will not likely result in satisfactory adult female sexual function. The reconstructive vaginoplasty may be associated with scarring of introitus further affecting sexual satisfaction. Additionally, it may carry the risk of neoplasia [54].

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 [2]. For example, early surgery may be medically indicated for infants with 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; however, this is now debated [55, 56]. Because female patients must actively participate in postoperative 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 additionally being promoted [2].

Some young women complain of discomfort resulting from prior genital surgery. Further, data measuring outcome parameters like vaginal width, depth, and comfort during intercourse may help resolve the debate on early vs. later vaginal surgery. The variability of postsurgical and psychosexual outcome 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 and the 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 [41, 57] and may

prove to be a diagnostic challenge. These include 46,XY males who have complete androgen insensitivity syndrome (CAIS) or 17-α(alpha)-hydroxylase (P450C17 or CYP17) deficiency who 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-(alpha)-hydroxylase deficiency will result in sexual hair growth, distinguishing them clinically from individuals with CAIS.

New Knowledge in DSD

Gender Change and Gender Dysphoria

Do individuals establish in adulthood a gender identity concordant with their initial gender assignment? This is an important question for parents and the medical team when faced with making the best decision possible for an infant. Recent review articles provide insight to this question. Gender change from initial gender assignment does occur in congenital adrenal hyperplasia [58], 5-α(alpha)-reductase deficiency, 17β(beta)-hydroxysteroid dehydrogenase deficiency [59], partial androgen insensitivity syndrome [60], and 46,XY persons with penile agenesis, cloacal exstrophy of the bladder, or penile ablation who are raised female [61].

Several conclusions can be drawn about gender identity and gender change in these 46,XY DSD conditions. First, the prevalence of individuals who undergo gender change varies markedly between syndromes. 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 exclusion of others, but suggest an indirect influence of androgens on such development since gender change appears more common in conditions with relatively high androgen exposure [61].

Gender change was not found in three reviews: (a) complete androgen insensitivity syndrome (CAIS) [60], (b) subjects with micropenis [60], and (c) subjects with Mayer-Rokitansky-Kuster-Hauser (MRKH) syndrome [62].

In a large series reviewing 156 cases of CAIS, all the reported individuals established an adult female gender identity [60]. Since this review, three cases of gender change were reported in CAIS [63]. Individuals with micropenis established gender identity concordant with their initial gender assignment [60], although some were assigned males at birth ($N=89$) and a few were assigned females ($N=10$). In MRKH syndrome, which is characterized by congenital absence of the uterus and vagina in 46,XX individuals with functioning ovaries, all 999 individuals in a recent review article established a female gender identity [62]. While it appears that the best predictor of an adult gender identity is initial gender assignment in these conditions, 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. However, until further evidence proves otherwise, we recommend an initial male gender assignment as previously stated.

Shift in Research Focus

Much of the early research on DSD focused on the influence of hormones, prenatal androgens in particular, on gender identity development, and gender role. 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, there is not a sufficient amount to draw any meaning conclusions. They also do not focus on the influence of genes on brain sexual differentiation and development, an area which is only beginning to be explored [64].

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. [65] and Stout et al. [66] on CAH, Schonbucher et al. [67] 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 [68], and Bean's review of MRKH [62]. Recent studies have also focused on the effects of having a child with a DSD on parenting characteristics [69, 70].

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 [71]. In essence, the patient (when old enough) and family become part of the team interacting in open communication with each other. To further "articulate and put into action the roadmap for quality improvement in clinical care and research encouraged by the Consensus Statement," a symposium was held at the University of Michigan in April 2009 [72]. A nonprofit organization (Accord Alliance) was created to assist institutions in establishing successful interdisciplinary teams [73]. This organization also maintains a web-based clearinghouse (www.accordalliance.org) for educational tools and information about living with and caring for those with a DSD which includes *Handbook for parents* [38] and *Guidelines for the Management of Disorder of Sex Development in Childhood* [2].

Conclusion

In the past, gender assignment decisions were hurried because physicians wished to shorten the period of anxiety for parents. Parental involvement was kept minimal during gender assignment decision process, often with incomplete disclosure about the precise genetic or anatomic diagnosis. Current recommendations have moved away from this practice, promoting 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 the molecular studies and other diagnostic tests because they realize the enormity of this decision on their child's future. 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. Besides a critical reevaluation as to the decision-making process in gender assignment, there is also debate about the timing of genital surgery, should it wait until the child can consent or not, its effects on sexual arousal, and even if it should be performed. We presented new data on gender identity in selected DSD diagnoses and a focus on

quality of life. Knowledge is still incomplete and gender identity development and differentiation remains a complex and incompletely understood phenomenon. The challenge for practitioners, working with limited knowledge, is how to maintain open, honest communication with parents and eventually the child in order to build a foundation of trust which can only result in the best care possible.

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Menstrual Disorders and Hyperandrogenism in Adolescence

25

Sara A. DiVall and Robert L. Rosenfield

Abstract

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.

Keywords

Delayed puberty • Amenorrhea • Anovulation • Hyperandrogenism • Polycystic ovary syndrome

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 year earlier in overweight girls and in non-Hispanic Black girls, with Mexican-American girls being intermediate. In addition, because of 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 [2–4]. This "physiologic adolescent anovulation" accounts for the greater menstrual irregularity and longer average intermenstrual length in the early post-menarcheal

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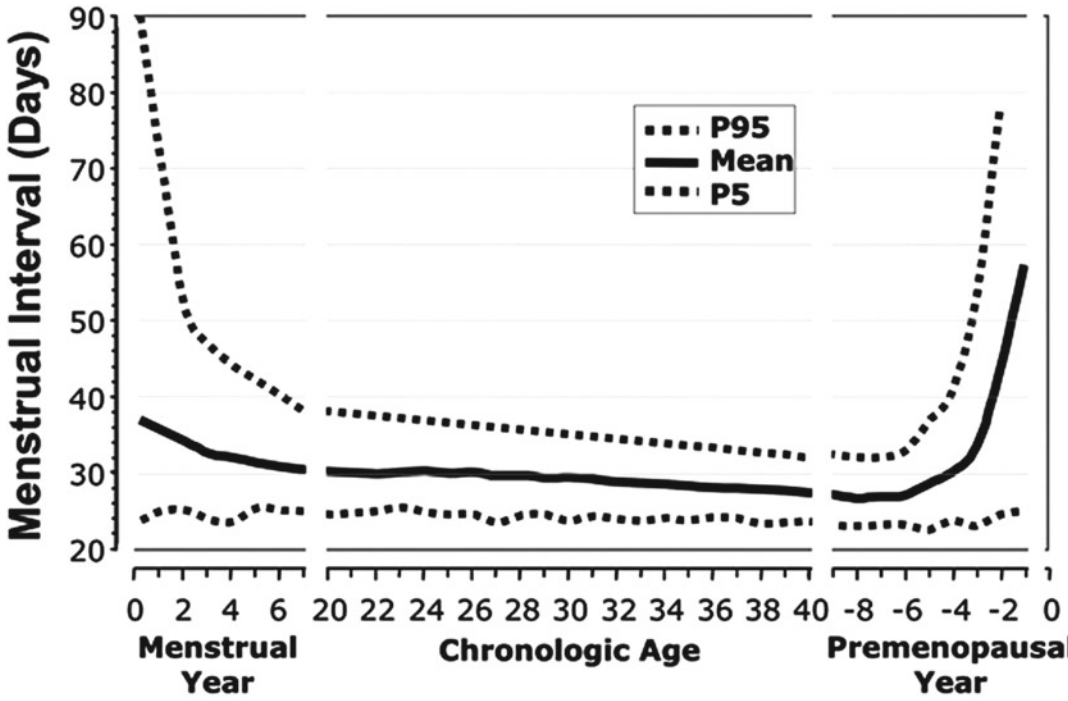

Fig. 25.1 Menstrual cycle lengths throughout reproductive life from menarche to menopause. Tenth, fiftieth, and ninetieth percentiles are shown (Reproduced with permission from Treloar et al. [5])

years than in adults (Fig. 25.1) [5]. However, about half of these seemingly "anovulatory" menstrual cycles are of normal length by adult standards, so menstrual regularity is greater than ovulatory frequency would suggest. In contrast, menstrual irregularity always indicates ovulatory irregularity.

The menstrual disorders 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* (less than eight menstrual periods a year, an average of >45 days between menses), and *dysfunctional uterine bleeding* (anovulatory bleeding that occurs more often than at 21-day intervals or is excessive, as indicated by bleeding for more than 7 days or requiring pad or tampon changes more than every 1–2 h) [4]. Because these menstrual patterns are statistically abnormal within the first year after menarche (occurring in less than 5% of adolescents), they are more appropriately considered to represent "symptomatic" rather than "physiologic" adolescent anovulation, and evaluation may be required. A two-thirds risk of persistent menstrual irregularity exists if symptoms persist 2 years beyond menarche; thus, evaluation is recommended if symptoms persist for 2 years after menarche [6].

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 etiology of menstrual disorders is given in Table 25.1 [6].

Table 25.1 Causes of adolescent menstrual disorders[a]

Abnormal genital structure
Aplasia[b]
Hymenal
Vaginal
Müllerian
Intersex
Endometrial adhesions
Ambiguous genitalia
Intersex
Pseudointersex
Anovulatory disorders
Hypoestrogenism: FSH elevated
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
Gonadotropin deficiency
Congenital
Acquired
Organic
Functional (nonorganic)
Delayed puberty
Constitutional delay[b]
Growth-retarding disease
Primary ovarian insufficiency
Complete, if BA < 11 year[b]
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

[a]Adapted from Rosenfield et al. [6] with permission from Elsevier
[b]Cause only primary amenorrhea

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 Rokitansky–Kustner–Hauser syndrome [7]. This syndrome occurs as a single-gene defect or as an acquired teratogenic event which sometimes affects differentiation of the urinary tract. A subtype due to *Wnt4* gene defects is associated with hyperandrogenism [8]. Obstruction of the genital outflow tract—most commonly due to imperforate hymen—causes hydrocolpos, which results in a protruding vaginal mass, 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 [9].

Abnormal bleeding, on the other hand, can result from genital tract trauma or infection. The most common examples of these are sexual abuse and foreign body. Bleeding may also result from genital tract tumors.

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 is present prior to the onset of neuroendocrine puberty, it causes sexual infantilism. If hypogonadism is slightly less severe or is manifested 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 dysfunctional uterine bleeding or with excessively frequent periods due to short luteal phase.

Hypogonadism can be categorized according to whether or not gonadotropins, particularly serum follicle-stimulating hormone (FSH) levels, are elevated (Table 25.1). Hypoestrogenism with elevated FSH indicates primary ovarian failure (hypergonadotropic hypogonadism). The causes include both hereditary and acquired disorders. Gonadal dysgenesis due to deficiency of genes on the X chromosome causing Turner syndrome is the most common cause of primary ovarian failure, with an incidence of about 1 in 2,500 live-born girls. About 5% of Turner syndrome patients present with secondary amenorrhea, even though they have congenitally dysgenetic ovaries [10]. Fragile X-chromosome permutation is associated with some cases of X-linked premature ovarian failure [11, 12]. Premature ovarian failure may result from hereditary gonadotropin resistance: LH receptor and FSH receptor mutations cause autosomal-recessive gonadotropin resistance [13]. These have been associated with a spectrum of defects ranging from primary amenorrhea to oligomenorrhea. Partial gonadotropin resistance is common in the Albright osteodystrophy form of pseudohypoparathyroidism because of the generalized defect in G-protein signal transduction [14]. Bioinactive gonadotropins on rare occasions may simulate primary gonadal failure [15, 16]. Steroidogenic blocks in estradiol biosynthesis can also cause secondary amenorrhea [17]. Acquired ovarian failure commonly results from irradiation, chemotherapy, trauma to the ovary, galactosemia, or autoimmune disease. Unexplained acquired ovarian failure has an autoimmune basis less than one-third of cases [12].

Hypoestrogenism without elevated FSH levels usually indicates secondary ovarian failure (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 occur in association with cerebral, hypothalamic, or pituitary dysfunction or as an isolated defect. Congenital hypopituitarism may be due to a chromosomal disorder (such as in Prader–Willi syndrome), single-gene mutations in the gonadotropin-releasing hormone (GnRH) signaling [18], or pituitary development cascades [19] or be associated with congenital brain defects of unknown origin. Congenital isolated gonadotropin deficiency may result from autosomal-recessive disorders, of which GnRH receptor deficiency is more common in women than the anosmia-associated Kallmann's syndrome [20].

Acquired gonadotropin deficiency may be organic or functional (nonorganic). Organic acquired gonadotropin deficiency can be a consequence of tumors, trauma, autoimmune hypophysitis [21], degenerative disorders involving the hypothalamus and pituitary [22], irradiation [23], or chronic illness of virtually any organ system [24]. Functional hypogonadotropism is commonly caused by eating disorders [25]. Anorexia nervosa is the prototypic form, but bulimia nervosa, the binge eating/purging variant, is easily overlooked because the weight is often normal and vomiting surreptitious. Hyperprolactinemia can also cause functional gonadotropin deficiency, as discussed below.

Gonadotropin deficiency can be mimicked by primary ovarian failure in two circumstances. The most common is in children who are too young to have undergone neuroendocrine puberty, as indicated by a bone age less than about 11 years. Gonadotropin levels may also not be elevated in incomplete or early premature ovarian insufficiency because gonadotropin levels may be normal as the ovary begins to fail during

the menopausal transition [12, 26]. Suppression of gonadotropins and estrogens occurs in frankly virilizing disorders.

Menstrual disturbance in the presence of adequate estrogenization is probably the single most common problem that is encountered. Pregnancy, hypothalamic anovulation, with its diverse causes including hyperprolactinemia, and hyperandrogenism are the considerations.

Hypothalamic anovulation occurs in patients who secrete sufficient gonadotropin tonically to estrogenize normally but have disorders which interfere with the ability to produce a midcycle surge of luteinizing hormone. In this group of disorders, there are disturbances of cyclic or pulsatile GnRH release that interfere with the positive feedback mechanism. Functional hypothalamic amenorrhea is often seen in the setting of weight loss, hyperathleticism, or psychogenic stress. Even when these factors are not obvious, similar mechanisms seem to underlie idiopathic functional hypothalamic amenorrhea [27]. 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 workup is required.

Chronic disease of virtually any organ system can mimic gonadotropin deficiency or hypothalamic anovulation. Obesity may cause amenorrhea via the overproduction of estrogen from plasma precursors in adipose tissue [28] or via a direct effect [29]. Glucocorticoid excess causes amenorrhea by multiple mechanisms, prime among which is interference with gonadotropin responsiveness to GnRH [30, 31]. Thyroid disorders are well-known causes of menstrual irregularity [32].

Hyperprolactinemia requires special consideration since it varies greatly in its presentation. This is because it engenders variable degrees of gonadotropin deficiency. Prime among the multiple mechanisms is disruption of GnRH pulsatility [33, 34]. Galactorrhea is present in about half of the patients, particularly those with residual estrogen production. The causes of hyperprolactinoma are diverse and include hypothalamic or pituitary disorders, drugs, hypothyroidism, renal or liver failure, peripheral neuropathy, stress, and idiopathy [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), dysfunctional uterine bleeding, or a hyperandrogenic picture.

Hyperandrogenism is the most frequent cause of anovulation, after pregnancy, so it is considered in more detail next.

Hyperandrogenism

Hyperandrogenemia is of ovarian origin in the vast majority of cases. It occasionally is of adrenal origin; in a few cases, it appears to be caused by abnormalities in the peripheral formation of androgen, and it is rarely caused by tumors or by self-administration. The causes of hyperandrogenism are listed in Table 25.2 [36].

Polycystic Ovary Syndrome

PCOS accounts for 85% or more of androgen excess presenting at or after the onset of puberty. The classic syndrome originally described by Stein and Leventhal is characterized by various combinations of amenorrhea, hirsutism (defined as excessive male-pattern hair growth), obesity, and polycystic ovaries. PCOS is now defined as otherwise unexplained hyperandrogenic oligoanovulation ("National Institutes of Health criteria") [37]. If anovulatory symptoms are lacking, it is now widely accepted that a polycystic ovary is an alternative criterion for the diagnosis ("Rotterdam criteria") [38, 39]. However, a polycystic ovary in isolation is a normal variant, found in about 20% of healthy women [40, 41]. Consequently, there is not uniformity of agreement about the alternative Rotterdam criterion of anovulatory symptoms and a polycystic ovary in the absence of hyperandrogenism [38].

About two-thirds of patients with classic PCOS have hirsutism (or the hirsutism equivalents, acne vulgaris, or pattern alopecia), two-thirds have anovulatory symptoms (which vary from amenorrhea to dysfunctional uterine bleeding to unexplained infertility), and half are obese. Thus, only about a third of otherwise classic

Table 25.2 Causes of hyperandrogenism[a]

Functional gonadal hyperandrogenism
Primary (dysregulational) functional ovarian hyperandrogenism[b]
Secondary polycystic ovary syndrome
• Poorly controlled classic congenital adrenal hyperplasia
• Syndromes of severe insulin resistance
• Ovarian steroidogenic blocks
Adrenal rests
Disorder of Sexual Differentiation
Chorionic gonadotropin related
Functional adrenal hyperandrogenism
Primary (dysregulational) functional adrenal hyperandrogenism[c]
Congenital adrenal hyperplasia
Prolactin or growth hormone excess
Dexamethasone-resistant functional adrenal hyperandrogenism
• Cushing's syndrome
• Cortisol resistance
• Apparent cortisone reductase deficiency
Peripheral androgen overproduction
Obesity
Idiopathic hyperandrogenism
Tumoral hyperandrogenism
Androgenic drugs

[a]Adapted from Rosenfield 1997 [36], with permission from Elsevier
[b]Common form of PCOS
[c]Uncommon form of PCOS

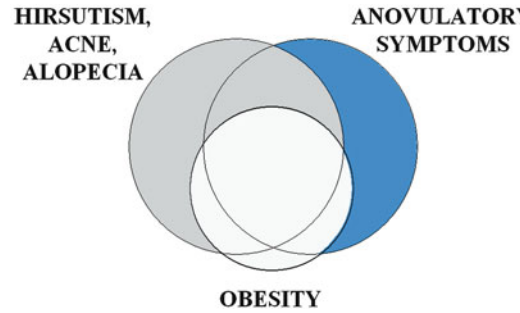

Fig. 25.2 Manifestations of polycystic ovary syndrome in approximate proportion to their relative incidence and coincidence. Cutaneous symptoms include hirsutism, acne, or acanthosis nigricans. Anovulatory symptoms include amenorrhea, oligomenorrhea, dysfunctional uterine bleeding, and infertility (Reproduced from Rosenfield [36] with permission from Elsevier)

cases have the full-blown clinical picture (Fig. 25.2). While obesity and insulin resistance are not necessary or diagnostic features of PCOS, they are common and appear to play a role in pathogenesis of many cases. Acanthosis nigricans, a sign of insulin resistance, may be prominent.

PCOS in adolescents has clinical and endocrine features similar to that of adults [41]. There may be an antecedent history of congenital virilization, premature pubarche, or syndromic obesity (pseudo-Cushing's syndrome or pseudo-acromegaly) [42]. Ovarian dysfunction is often found in the perimenarcheal phase of development, but it may not be demonstrable until 3 years after menarche [43].

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 favor the concept that the essence of PCOS is intrinsic functional ovarian hyperandrogenism (FOH) that is closely linked to the metabolic disorder [44, 45].

The theory that PCOS is a fundamentally neuroendocrine disorder is rooted in the observation that baseline LH levels and responses to GnRH are elevated in about half of PCOS patients. 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 large subset of women with PCOS who do not have elevated LH levels. 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

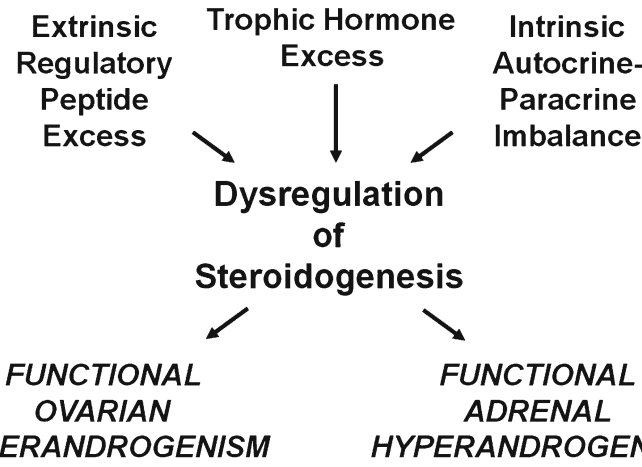

Fig. 25.3 Model of factors causing the common types of functional ovarian and adrenal hyperandrogenism. A mild degree of androgen excess can arise from excess trophic hormone (LH or ACTH) stimulation. Disturbances either extrinsic or intrinsic to these endocrine glands can amplify the effect of normal levels of trophic hormones. Extrinsic regulatory peptide excess is exemplified by hyperinsulinemia. Intrinsic peptides capable of inappropriately upregulating steroidogenesis include IGFs (Reproduced from Rosenfield [45] with permission from Elsevier)

in response to increased LH levels, but the downstream androgenic response to LH is limited [41, 46, 47]. 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 may be explained by abnormal sex steroid feedback [29]. 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 progesterone on LH pulse frequency.

We favor the concept that the hyperandrogenism of PCOS is caused by intrinsic defects in steroidogenesis. The 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. Put another way, these women have "escaped" from normal desensitization to LH. Overexpression of the steroidogenic enzyme cytochrome P450c17 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 [48, 49].

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 the 17-ketosteroid dehydroepiandrosterone (DHEA) and its precursor, 17-hydroxypregnenolone, without evidence of a steroidogenic block in response to ACTH stimulation, as would occur in congenital adrenal hyperplasia. This pattern is termed functional adrenal hyperandrogenism (FAH). FAH had earlier been mistaken for nonclassic 3ß-hydroxysteroid dehydrogenase deficiency, which is now known to be a rare disorder, and was considered to be "exaggerated adrenarche."

These steroidogenic defects have been postulated to result from dysregulation of steroidogenesis. This dysregulation is, in turn, postulated to result from imbalance among various intrinsic and extrinsic factors involved in the modulation of trophic hormone action (Fig. 25.3). Defects within ovarian theca cells causing overexpression of steroidogenic enzymes lead to hyperrespon-

siveness to normal or excessive LH stimulation and resultant excessive androgen production.

Obesity and insulin-resistant hyperinsulinemia are common features of PCOS, [50, 51]. The degree of obesity is inversely correlated with LH levels [29]. Insulin resistance is out of proportion to the degree of obesity, and compensatory hyperinsulinemia appears to be an important factor in the pathophysiology of PCOS. Women with PCOS have a high incidence of metabolic syndrome and are predisposed to type 2 diabetes mellitus [52]. Treatments which lower insulin levels reduce the androgen excess. 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 co-activator complex [53]. In adipocytes, KLF15 also stimulates lipogenesis. 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.

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 at least in part caused by intraovarian androgen excess, but an independent defect cannot be ruled out [47, 54].

Pathogenesis. The cause of PCOS is unknown. Like type 2 diabetes mellitus, PCOS likely arises because of interaction between genetic predisposing factors with environmental factors [42]. 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 our adolescents with PCOS have a parent with metabolic syndrome, indicating a close relationship to obesity, insulin resistance, and diabetes [52]. Environmental influences that promote obesity and associated hyperinsulinemia are aggravating and possibly precipitating pathogenetic factors. Any treatment, including weight loss, that lowers insulin levels improves ovarian dysfunction and ovulation [55–59].

Prenatal androgen excess is a distinct predisposing factor [42]. 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 [60]. Other proposed precipitating factors include intrauterine growth retardation, premature adrenarche, and heterozygosity for congenital adrenal hyperplasia [61].

Other Causes of Functional Ovarian Hyperandrogenism

Other functional causes of FOH can mimic PCOS (Table 25.2). 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 causes. Excessive stimulation via the LH receptor is a rare cause of hilus cell hyperplasia or chorionic gonadotropin-related hyperandrogenism during pregnancy [62, 63]. All known forms of extreme insulin resistance, including the hereditary cases which are due to insulin receptor mutations, as well as acromegaly [64], 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 from true hermaphroditism. PCOS has also been reported as a complication of the impaired steroid metabolism which occurs as a complication of portasystemic shunting [65]. The antiepileptic drug valproic acid causes hyperandrogenism [66, 67].

Other Causes of Functional Adrenal Hyperandrogenism

Less than 10% of adrenal hyperandrogenism can be attributed to the well-understood disorders listed in Table 25.2. Congenital adrenal hyper-

plasia 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 nonclassic ("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 the nonclassic disorder 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 [68]. Nonclassic 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. Apparent cortisone reductase deficiency is a rare autosomal-recessive form of functional adrenal hyperandrogenism [69].

Dexamethasone-resistant forms of hyperandrogenism such as Cushing's syndrome and cortisol resistance are even more unusual than nonclassic congenital adrenal hyperplasia. Prolactin excess causes adrenal hyperandrogenism [70]. Cushing's and hyperprolactinemia sometimes occur in association with polycystic ovaries [71, 72].

Other Causes of Hyperandrogenism

In approximately ten percent of hyperandrogenic patients, a gonadal or adrenal source of androgen cannot be ascertained after thorough testing. Many of these cases meet PCOS criteria and seem to be due to obesity. In the absence of menstrual dysfunction, this is termed idiopathic hyperandrogenemia. Obesity may explain some of these cases because adipose tissue has the capacity to form testosterone from androstenedione. Other idiopathic 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.

Differential Diagnosis

Workup for primary amenorrhea should be undertaken if spontaneous menses have not occurred by 15.0 years of age or earlier if menses have not occurred 3 years after breast budding. A diagnostic approach to primary amenorrhea is shown in Fig. 25.4 [6]. The history should include a search for clues to chronic disease or eating disorders. 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 [73, 74], 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 pregnancy test, plasma 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 workup, as shown in Fig. 25.5 [6]. However, because breast development persists even if hypoestrogenism ensues, the presence of a mature breast stage does not preclude the possibility of 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 [75].

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. 25.6) [6]. Dysfunctional uterine bleeding is an alternate presentation of anovulatory cycles. If present, other causes of dysfunctional uterine bleeding, such as sexual abuse, bleeding disorder, genital tract tumor, or feminizing cyst, should also be considered. The history of a woman with anovulatory symptoms should be carefully reviewed for evidence of the

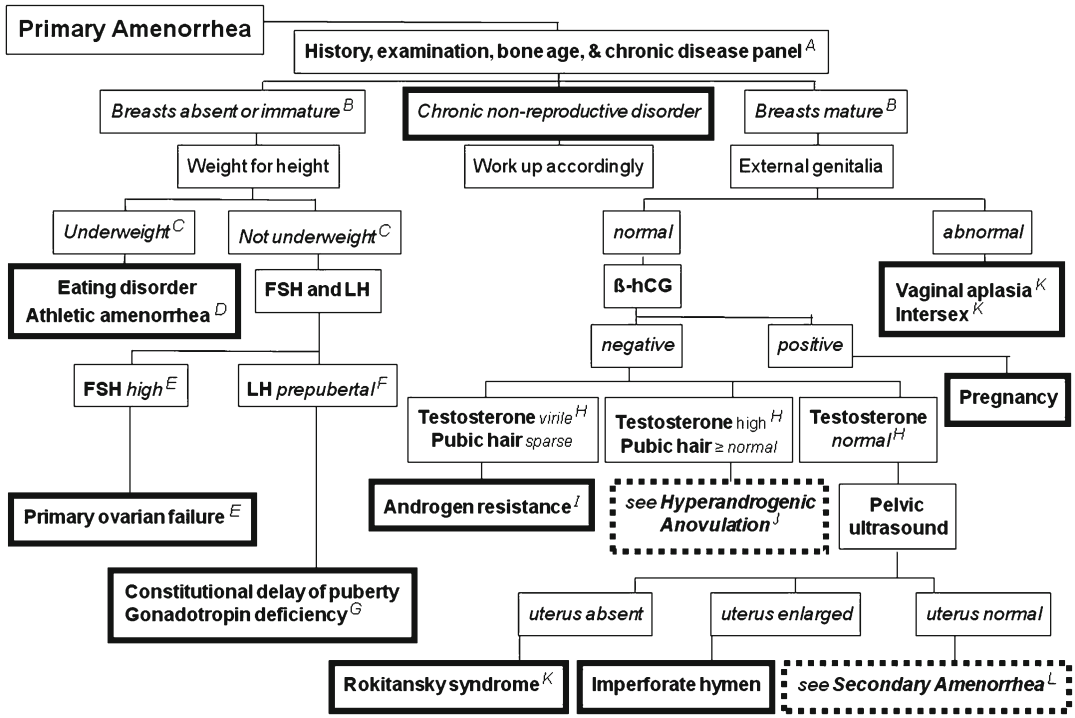

Fig. 25.4 Differential diagnosis of primary amenorrhea (Modified with permission from Rosenfield, RL. Primary amenorrhea and abnormal genital anatomy. In: Hochberg Z, editor. Practical algorithms in pediatric endocrinology. 2nd ed. Basel. S. Karger AG; 2007) [A]Prime among the causes of primary amenorrhea are growth-retarding or growth-attenuating disorders. In the absence of specific symptoms or signs to direct the workup, laboratory assessment for chronic disease typically includes bone age radiograph if the adolescent is not sexually mature and a chronic disease panel (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. 25.5 and 25.6). [C]BMI < 10th percentile generally corresponds to body composition <20% body fat, which is the critical factor. [D]BMI may not accurately reflect body fat in serious athletes (who have a disproportionately greater muscle mass) or bulimia nervosa. [E]FSH is preferentially elevated over LH in primary ovarian failure. The most common cause of primary amenorrhea due to primary ovarian failure is gonadal dysgenesis due to Turner syndrome, but acquired causes must be considered (such as cytotoxic therapy). The workup of primary ovarian failure is considered in detail in the next algorithm (Fig. 25.5, secondary amenorrhea and oligomenorrhea). Lack of FSH elevation virtually rules out primary ovarian failure 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's 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 midpubertal children. The GnRH test, measuring the gonadotropin response to a 50- to 100-μg bolus, in the premenarcheal teenager strongly suggests gonadotropin deficiency if the LH peak is less than 4.2 IU/L by monoclonal assay. However, the GnRH test has limitations because of overlap between hypogonadotropic and normal teenager responses. GnRH agonist testing (e.g., leuprolide acetate injection 10 μg/kg SC) may discriminate better. It may not be possible to definitively establish the diagnosis of gonadotropin deficiency until puberty fails to begin by 16 years of age or progress at a normal tempo. [H]Plasma total testosterone is normally 20–60 ng/dL (0.7–2.1 nM) in women and 300-1,200 ng/dL in men but varies somewhat among laboratories. Plasma free (or bioavailable) testosterone is about 50% more sensitive than total testosterone in detecting hyperandrogenemia. However, there are many pitfalls in testosterone assays at the low levels of women, reliable testosterone assays are not available to many physicians, and assaying the free testosterone introduces other potential sources of error. Therefore, it is reasonable to begin the evaluation with a total

nutritional disorders and the physical or emotional stress that are common causes of the menstrual symptoms. 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. 25.6). GnRH testing is indicated in confirmed hypoestrogenic cases. GnRH agonist testing yields similar results and in addition allows assessment of gonadotropin reserve in gonadotropin deficiency and also permits assessment of ovarian responsiveness to gonadotropins [76–78]. Imaging of either the ovaries or the brain is usually indicated.

Hyperandrogenism is the final consideration in the differential diagnosis of menstrual disorders (Fig. 25.7) [79]. The diagnosis may be difficult to establish [80, 81]. 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 approximately one-third of 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.

Unfortunately, dependable testosterone assays are not available to many practitioners. Validated testosterone assays are also not widely available. The automated assays of total testosterone, available as part of multichannel immunometric panels, are very inaccurate at the relatively low testosterone levels of women and children; thus, they give misleading information about androgen status in women. Determination of free or "bioavailable" testosterone is 50% more sensitive than total testosterone for the detection of hyperandrogenemia, as sex hormone-binding globulin (SHBG), the main determinant of the bioactive portion of serum testosterone, is often low in hyperandrogenic women. Direct assays of free testosterone are inaccurate. The most reliable method for total testosterone uses a chromatographic purification step before quantification by radioimmunoassay or mass spectrometry and measures women's testosterone levels with a precision and accuracy of about 12-30% [82]. The serum free testosterone is then calculated as the product of the total testosterone and the function of testosterone binding to SHBG (i.e., free testosterone concentration = total testosterone concentration × percent free testosterone).

It is reasonable to begin the evaluation with a total testosterone determination by a reliable method, as suggested above and in Figs. 25.4 and 25.6. 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. The routine assay of other androgens is probably of little utility for the detection of hyperandrogenemia in most populations. DHEA sulfate (DHEAS) may be useful if cystic acne is a major symptom or there is a high suspicion for a virilizing tumor. DHEAS 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

Fig. 25.4 (continued) testosterone determination if a free testosterone test in a reliable specialty laboratory is not available to the practitioner. [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 a later algorithm (Fig. 25.7). [K]Vaginal aplasia in a girl with normal ovaries may be associated with uterine aplasia (Rokitansky–Kustner–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 (intersex). [L]Secondary amenorrhea differential diagnosis is presented in Fig. 25.5

morning plasma free testosterone level performed in a reliable specialty laboratory.

If hyperandrogenemia is documented, we advise further diagnostic evaluation, as detailed in Fig. 25.7. An ultrasonographic examination is important to exclude tumor although the prevalence is only 0.2% and to reassure patients who have polycystic ovaries that the "cysts" are benign. While the presence of polycystic ovaries is supportive of a diagnosis of PCOS, this finding is not specific for PCOS nor are polycystic ovaries necessary for the diagnosis of PCOS [68, 71, 72]. Hyperandrogenic anovulation, in the absence of other causes of anovulation (Figs. 25.4, 25.5, and 25.6) including hyperprolactinemia, thyroid dysfunction, Cushing's syndrome, nonclassic congenital adrenal hyperplasia, and virilizing neoplasm, fulfills widely accepted criteria for the diagnosis of PCOS [38, 81].

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. For example, a history of rapid virilization, clitoromegaly, or rapid progression would be indications for a more extensive workup. It is our practice to initiate further workup for rare disorders according to the algorithm presented in Fig. 25.8 [79, 80]. An early morning blood sample for free testosterone and steroid intermediates and 24-h urine for 17alpha-hydroxysteroids are obtained [69], followed by a dexamethasone androgen-suppression test. We reserve ACTH testing, which is expensive if comprehensive, for the subset of patients with dexamethasone-suppressible androgen excess. 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 nonclassic congenital adrenal hyperplasia cannot be documented unless one of the steroid intermediates rises over 5 SD above average in response to ACTH [83–85]. 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 [41]. If no source for the androgen excess can be found, one is dealing with idiopathic hyperandrogenemia.

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. In many cases, surgical removal of dysgenic gonadal tissue may be necessary.

In some cases, treatment of hypogonadism is achieved without hormone replacement. The treatment of choice for prolactinoma is dopaminergic agents. Hyperprolactinemia will be maximally suppressed within one month and menstrual cycle normalized within 3 months by an effective regimen. Cabergoline 0.5-1.0 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 [86].

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

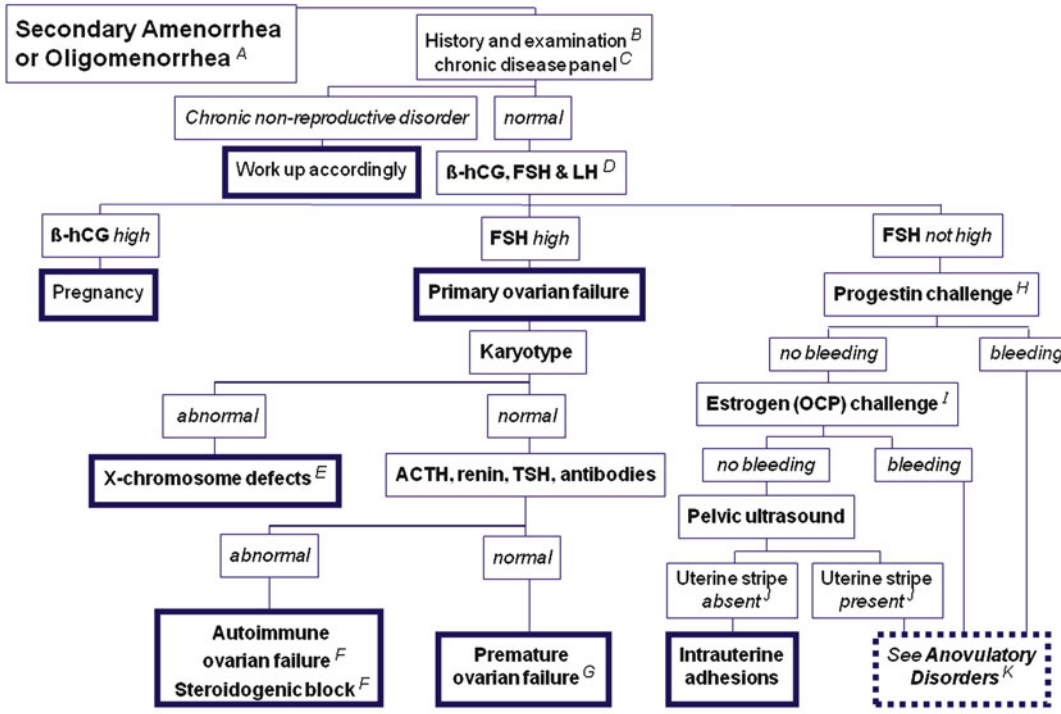

Fig. 25.5 Differential diagnosis of secondary amenorrhea or oligomenorrhea (Modified with permission from Rosenfield RL. Secondary amenorrhea or oligomenorrhea. In: Hochberg Z, editor. Practical algorithms in pediatric endocrinology. 2nd ed. Basel. S. Karger AG; 2007) [A]Mature secondary sex characteristics 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's 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]"Pediatric" gonadotropin assays sensitive to ≤0.15 U/L are critical to the early diagnosis of many anovulatory disorders. [E]Patients missing only a small portion of an X chromosome may not have the Turner syndrome phenotype. Indeed, 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, special studies for fragile X premutation and autoimmune oophoritis should be considered. [F]Autoimmune ovarian failure 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. LH is disproportionately high in steroidogenic defects or autoimmune disease specifically affecting theca cells. [G]The history may provide a diagnosis (e.g., cancer chemotherapy or radiotherapy). Other acquired causes include surgery and autoimmunity. Chromosomal causes of premature ovarian failure include X-chromosome fragile site and point mutations. Other genetic causes include gonadotropin-resistance syndromes such as LH or FSH receptor mutation and pseudohypoparathyroidism. A pelvic ultrasound that shows preservation of ovarian follicles carries some hope for fertility. [H]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. [I]A thin uterine stripe suggests hypoestrogenism. A thick one suggests endometrial hyperplasia, as may occur in polycystic ovary syndrome. [J]A single cycle of an OCP containing 30–35 μg ethinyl estradiol generally suffices to induce withdrawal bleeding if the endometrial lining is intact. [K]The differential diagnosis of other anovulatory disorders continues in Fig. 25.6

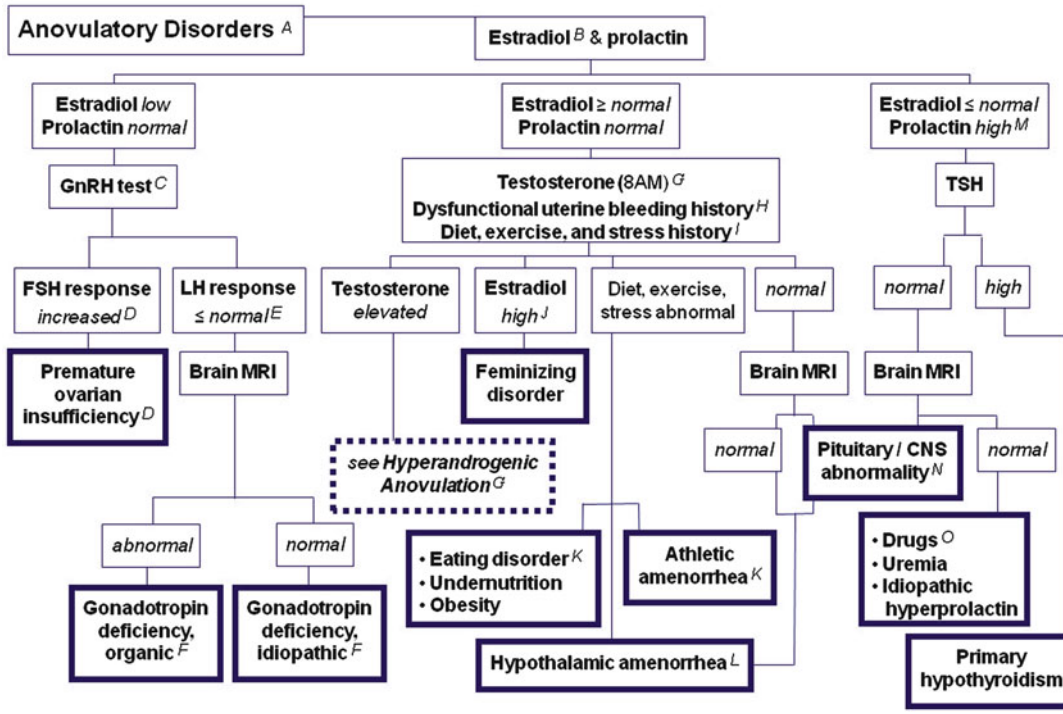

Fig. 25.6 Differential diagnosis of anovulatory disorders (Modified with permission from Rosenfield, RL; Bourguignon, J-P. Anovulatory disorders. In: Hochberg Z, editor. Practical algorithms in pediatric endocrinology. 2nd ed. Basel. S. Karger AG; 2007) [A]Anovulatory disorders should be considered in 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. 25.5 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 a "pediatric" assay sensitive to <10 pg/mL. However, a single estradiol level may be misleading because of cyclic or episodic variations. [C]Gonadotropin-releasing hormone (GnRH) testing is usually performed by assaying LH and FSH before and 0.5 h after the administration of 1 mcg/kg GnRH intravenously. GnRH agonist testing may alternatively be 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 the ovary begins to fail, as in early menopause, but an exaggerated FSH response to GnRH and subnormal E2 response to the gonadotropin elevation induced by acute GnRH agonist challenge are characteristic. The further workup is shown in Fig. 25.5. [E]LH responses to GnRH 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's syndrome. Kallmann's 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's syndrome. [G]Plasma free (or bioavailable) testosterone is about 50% more sensitive than total testosterone in detecting hyperandrogenemia. However, there are many pitfalls in testosterone assays at the low levels of women, reliable testosterone assays are not available to many physicians, and assaying the free testosterone introduces other potential sources of error. Therefore, it is reasonable to begin the evaluation with a total testosterone determination if a free testosterone test in a reliable specialty laboratory is not available to the practitioner. Simultaneous assay of 17-hydroxyprogesterone is indicated in subjects at high risk for congenital adrenal hyperplasia, such as Ashkenazi Jews. Differential diagnosis of hyperandrogenic evaluation is outlined in Fig. 25.7. [H]Dysfunctional uterine bleeding or menorrhagia not controlled

issues [87]. Menses resume when psychotherapy is effective and body fat is restored to normal. Estrogen treatment of patients with eating disorder will mask the psychopathology which underlies the menstrual disturbance and does not yield the recovery of bone loss that occurs with weight gain [88].

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. If maximizing height potential is patient important, substantial benefit can only be expected if treatment is initiated long before the induction of puberty; this is seldom realistic if initiated after 14 years of age. Panhypopituitary patients require replacement of growth hormone and cortisol deficits to obtain a normal degree of breast development. Further discussion of growth hormone therapy 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 [89]. However, as intramuscular estradiol at the low doses required to mimic physiology at puberty start is most accurately provided with the support of a compounding pharmacy, transdermal E2 6.25 μg daily is a reasonable alternative [10]. We deliver this dose by applying a 25-mcg patch continuously for 7 days monthly. Equivalent starting doses are 0.25 mg micronized E2 by mouth daily or 5 mcg ethinyl estradiol (one-fourth of the smallest available tablet) by mouth daily for three weeks out of four. The dose is increased every 6 months over a span of 2 years to adult replacement doses of transdermal estradiol 75-100 mcg daily, ethinyl estradiol 20 mcg, or conjugated estrogen 0.625 mg PO daily for 3 weeks out of four. Although conjugated equine estrogen has been effectively used to induce feminization, doses as low as 0.325 mg daily inhibit

Fig. 25.6 (continued) 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. [I]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. [J]The normal range for estradiol over the menstrual cycle is wide: values >95 pg/mL usually indicate the preovulatory or luteal phase but are compatible with a feminizing disorder. [K]Mild forms of stress disorders associated with low body fat (anorexia nervosa, bulimia nervosa, and athletic amenorrhea) may cause acquired hypothalamic amenorrhea rather than frank gonadotropin deficiency. 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 weight loss begins, indicating an important psychological component to the etiology. Obesity is also associated with anovulatory cycles and raises the possibility of Cushing's syndrome. [L]Hypothalamic amenorrhea is a diagnosis of exclusion. It is a form of partial gonadotropin deficiency in which baseline estrogen secretion is normal but 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 idiopathy. It may be difficult to distinguish from hyperandrogenemia. [M]Hyperprolactinemia is heterogeneous in its presentation. Some have normoestrogenic anovulation, which may be manifested as hypothalamic anovulation, hyperandrogenism, dysfunctional uterine bleeding, or short luteal phase. On the other hand, some are hypoestrogenic; these do not have galactorrhea. [N]Large hypothalamic–pituitary tumors or other types of CNS injury cause variable pituitary dysfunction, which may include complete or partial gonadotropin deficiency and various manifestations of hypopituitarism (including secondary hypothyroidism). If they interrupt the pituitary stalk, hyperprolactinemia ensues. Hyperprolactinemia may also be caused by prolactinomas. [O]Drugs, particularly neuroleptics of the phenothiazine or tricyclic type, may induce hyperprolactinemia

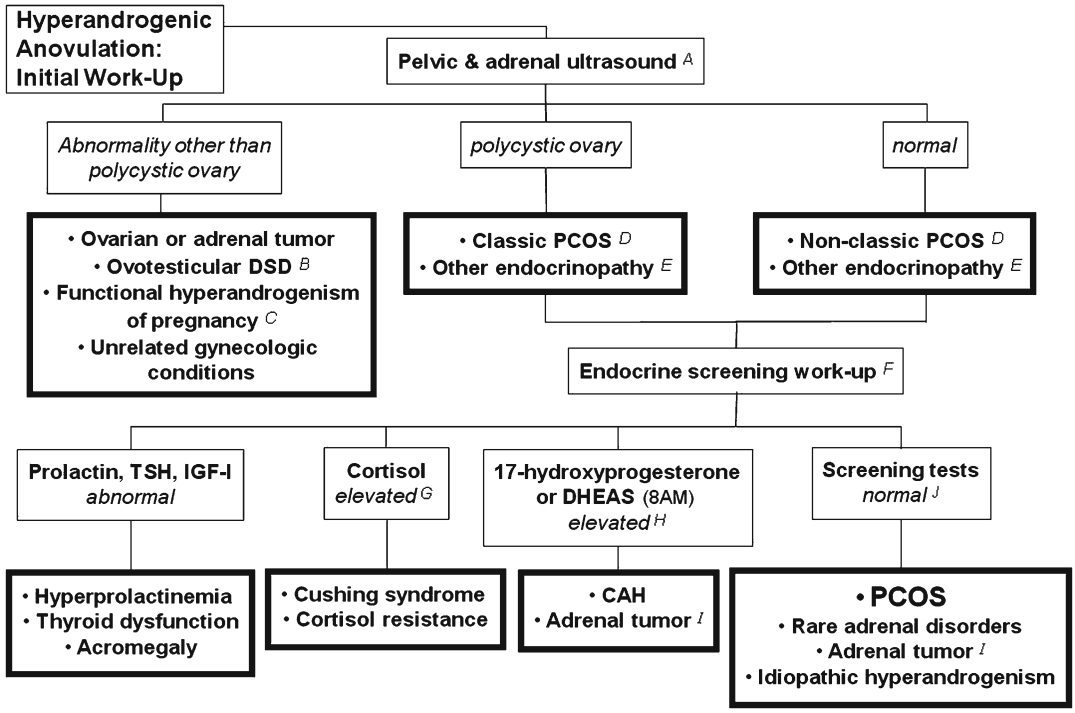

Fig. 25.7 Initial workup of hyperandrogenemic anovulation. Polycystic ovary syndrome (PCOS) accounts for the vast majority of cases; its most widely accepted definition is currently otherwise unexplained hyperandrogenic anovulation ("National Institutes of Health criteria") (Adapted with from Buggs and Rosenfield [79] with permission from Elsevier) [A]Ultrasonography is the initial study that detects polycystic ovaries and excludes ovarian pathology other than polycystic ovaries. The abdominal ultrasound that is indicated for pelvic ultrasonographic imaging in virginal adolescents can be used to screen for adrenal enlargement/mass. A polycystic ovary has been defined by international consensus as an ovary with a volume ≥10.5 cc (10.8 cc in adolescence) and/or containing ≥12 follicles (equivalent to ≥10 follicles in the maximum plane). [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]"Classic" PCOS is here used synonymously for those cases that have a polycystic ovary and supports the diagnosis, but a polycystic ovary is not necessary for diagnosis. [E]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. [F]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 mimics PCOS. [G]Plasma cortisol <10 mcg/dL essentially rules out endogenous Cushing's syndrome. [H]8 a.m. 17-hydroxyprogesterone >170 ng/dL is approximately 95% sensitive and 90% specific for detecting common-type (21-hydroxylase deficient) nonclassic 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). [I]Computed tomographic scanning of the adrenal gland is a more definitive study for identifying adrenal tumor than is ultrasound. [J]Exclusion of the preceding disorders in a hyperandrogenic patient with menstrual dysfunction meets National Institutes of Health criteria for PCOS with approximately 95% reliability. However, this workup does not identify rare adrenal disorders (e.g., some types of CAH, cortisone reductase deficiency), rare testosterone-secreting adrenal tumor, or, most commonly, idiopathic hyperandrogenism (here used to signify hyperandrogenism of unknown origin, which can arise from obesity or possibly metabolic abnormalities).

growth [90]. 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 days monthly. In the setting of estrogen replacement at adult doses, the

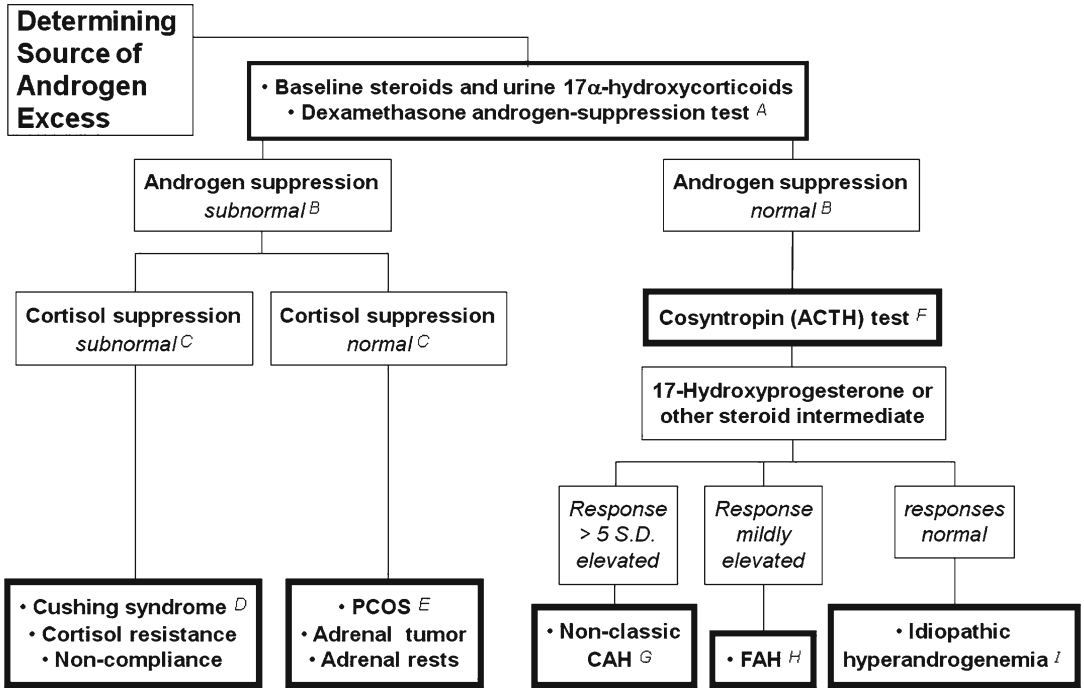

Fig. 25.8 Differential diagnosis of hyperandrogenism and hirsutism (Adapted from Buggs and Rosenfield [79] with permission from Elsevier). [A]After obtaining an early morning blood sample for baseline steroid intermediates (e.g., 17-hydroxypregnenolone, 17-hydroxyprogesterone (17OHP), androstenedione, dehydroepiandrosterone) and a 24-h urine for 17 alpha-hydroxycorticoids, a dexamethasone androgen-suppression test is performed. This consists of a 4-day course (7 days if body weight ≥100 kg) of dexamethasone 0.5 mg four times daily. A normal result is defined as suppression of adrenal androgens below the normal range and by at least 75%. [B]Androgen suppression is normal if level of 17OHP < 50 ng/dL (1.5 nmol/L), total testosterone <28 ng/dL (1.0 nmol/L), and DHEAS < 80 mcg/dL (2.1 μmol/L) (>75% fall); free testosterone falls below 8 pg/mL (27.7 pmol/L) in our hands. [C]Normal corticoid suppression results in cortisol <2.0 mcg/dL (54 nmol/L). [D]If both androgen and cortisol are not normally suppressed, Cushing's syndrome and cortisol resistance should be considered. Poor suppression can result from noncompliance with the dexamethasone regimen. [E]A subnormal suppression of testosterone and 17OHP and a normal suppression of cortisol and DHEAS are characteristics of PCOS, but the rare virilizing adrenal tumor or adrenal rests should be considered on the basis of clinical factors. Computed tomography is the most definitive test for adrenal tumor. [F]A cosyntropin (ACTH) stimulation test is appropriate if androgen suppression by dexamethasone is normal. [G]The diagnosis of congenital adrenal hyperplasia (CAH) is suggested if the steroidogenic intermediate response to ACTH is >5 SD above the average norm: for 17OHP, this is >1,000 ng/dL (30 nmol/L) and for 17-hydroxypregnenolone >5,000 ng/dL (158 nmol/L). [H]Primary functional adrenal hyperandrogenism (FAH) (suggested by a modest rise in 17-hydroxypregnenolone or 17OHP that does not meet the criteria for the diagnosis of CAH) is sometimes the only demonstrable source of androgen excess in PCOS. [I]Idiopathic hyperandrogenemia (distinct from idiopathic hirsutism) is the diagnosis when the source of androgen excess remains unexplained after intensive investigation (approximately 10% of cases). It is most commonly associated with obesity. Cortisone reductase deficiency is a rare consideration, in which the elevated urinary corticoids consist primarily of cortisone metabolites

addition of progesterone is necessary to lower the risk of endometrial hyperplasia and endometrial carcinoma [91].

Hypoestrogenism in sexually mature girls may be managed by administration of an oral contraceptive (OCP). OCPs are the most convenient option. Although the current generation of OCPs carries very little risk of venous thromboembolic disease [92] and the progestational component protects against endometrial hyperplasia, emerging evidence in postmenopausal women suggests that transdermal estradiol is slightly safer [93]. The lowest estrogen dosage currently available in combination OCPs in the

United States is 20 mcg ethinyl estradiol (e.g., with 1.0 mg norethindrone acetate in Loestrin 20 1/21®, 3 mg drospirenone in Yaz ®, 0.15 mg desogestrel in Mircette®). Higher estrogen doses are available, such as 30 mcg ethinyl estradiol (e.g., with 3 mg drospirenone in Yasmin®). Obese patients tend to require higher doses of estrogen. However, patients sensitive to estrogen because of such conditions as hypertension, migraine, or lymphedema are best advised to use a more physiologic type of therapy, estradiol itself in a form delivered systemically, bypassing the liver. Preparations of estradiol that are not oral include depot estradiol given intramuscularly with medroxyprogesterone acetate (Lunelle®) or estradiol patches. When using estrogen alone, a progestin is administered for the last 7-10 days of each course of estrogen (e.g., micronized progesterone 100-200 mg 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. 25.4), 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.

An acute episode of dysfunctional uterine 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. Premarin® in a dose of 25 mg intravenously every 3–4 h for 3–4 doses is customary. 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; and 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). Obtaining adrenal steroids while on treatment is necessary to monitor for adrenal suppression. Control of androgens in congenital adrenal hyperplasia may not suffice unless nocturnal progesterone excess is also controlled [94]. This treatment will typically normalize the menstrual pattern in nonclassic congenital adrenal hyperplasia, but the effect in classic congenital adrenal hyperplasia is more problematic, since PCOS complicates many of these cases, apparently as the result of congenital or perinatal masculinization [68].

The management of PCOS is directed toward treating symptoms and monitoring for associated disorders. 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 [95]. 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 [51]. 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 [52].

Treatment of menstrual irregularities in PCOS is recommended to prevent amenorrhea and the associated risk of endometrial hyperplasia and carcinoma. The combination OCP containing estrogen and progestin is the first-line treatment to induce regular menstrual cycles, especially in patients with hirsutism or its cutaneous equivalents. The estrogen component decreases bioactive testosterone by suppressing LH secretion, ovarian androgen production, and serum SHBG. The decrease in bioactive testosterone, assessed 3 months after start of therapy, is associated with an improvement in hyperandrogenic cutaneous symptoms. All estrogen–progestin combinations generally suffice for women with acne or mild hirsutism, in combination with cosmetic measures. OCPs containing non-androgenic progestins such as norgestimate (Ortho-Cyclen®) or ethynodiol diacetate (Demulen 1/50®) generally have favorable risk–benefit ratios and optimize lipid profiles. Those with antiandrogenic progestins in low dose may confer an additional benefit: drospirenone is available in the USA with 20 mcg (Yaz®) or 30 mcg (Yasmin®) 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.

Hirsutism and its cutaneous equivalents 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.

As an adjunct to cosmetic treatments, OCPs, by decreasing bioavailable testosterone, can improve acne within 3 months and arrest progression of hirsutism within 9-12 months in most PCOS patients. Androgen-lowering 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 [67]. 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 [67]. Spironolactone has been shown to be effective to the extent of lowering hirsutism score by about one-third [96], 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, 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; fatigue and hyperkalemia at higher doses may limit its usefulness, however. It is potentially teratogenic to fetal male genital development and may cause menstrual disturbance; 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 [67].

Weight loss improves ovulation, acanthosis nigricans, androgen excess, and cardiovascular risk in PCOS patients [58, 59, 97], while antiandrogens have only a modest effect on metabolic abnormalities [98]. 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 insu-

lin-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 [99, 100]. 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 [101]. 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 [102]. Thiazolidinediones also increase insulin sensitivity [57], 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 will require special care by a reproductive endocrinologist.

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Contraception

26

Helen H. Kim and Amy K. Whitaker

Abstract

Approximately 750,000 teenagers become pregnant each year in the United States, with a pregnancy rate of 71.5 per 1,000 women aged 15–19 years. The pregnancy rate among women in this age group who had ever had intercourse was over twice as high, at 152.8 pregnancies per 1,000 women. Adolescents in the United States have among the highest pregnancy rates of all developed countries, even though they do not have higher levels of sexual activity. Given the magnitude of the problem, contraception needs to be addressed with all adolescents to prevent unplanned pregnancies. The large majority of pregnancies, 82%, for adolescent women are unintended. Thus, preventing unintended pregnancy among young women is a vital public health concern.

Keywords

Adolescent • Teen • Contraception • Contraceptive use • Contraceptive device • Pregnancy prevention

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Introduction

Approximately 750,000 teenagers become pregnant each year in the United States, with a pregnancy rate of 71.5 per 1,000 women aged 15–19 years. The pregnancy rate among women in this age group who had ever had intercourse was over twice as high, at 152.8 pregnancies per 1,000 women [1]. Adolescents in the United States have among the highest pregnancy rates of all developed countries, even though they do not have higher levels of sexual activity [2]. Given the magnitude of the problem, contraception needs to be addressed with all adolescents to prevent unplanned pregnancies. The large majority of pregnancies, 82%, for adolescent women are unintended [3]. Thus, preventing unintended pregnancy among young women is a vital public health concern.

Birth rates for adolescents aged 15–19 years in the United States increased by 5% from 2005 to 2007. Although it is encouraging that the birth rate for the US adolescents did decline again (2% from 2007 to 2008), the 2-year increase contrasts sharply with the preceding 14 years, in which rates consistently declined each year, with a total decrease of 34% from 1991 to 2005 [4, 5]. Use of contraception played a primary role in the declining adolescent pregnancy rates during these years, with a significantly larger proportion of the decrease attributable to increased contraception use compared to abstinence [6]. Adolescents use a variety of contraceptive methods, with the oral contraceptive pill being the most common, followed by condoms (Fig. 26.1).

Unplanned and teen pregnancies have important public health consequences, including prematurity and low birth weight [7], and may be particularly deleterious in women with endocrine disease. Pregnancy increases thyroxine requirements in many women with primary hypothyroidism [8], and hypothyroidism during pregnancy has been associated with both maternal and fetal morbidity [9] and a lower IQ in the children [10]. There is also evidence that good metabolic control of diabetes at the time of conception reduces the risk of spontaneous abortions [11] and fetal malformations [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 26.1), each contraceptive method has its own set of advantages and limitations (Table 26.2). In choosing a contraceptive method, important considerations include effectiveness (Table 26.3), side effects, contraindications, fre-

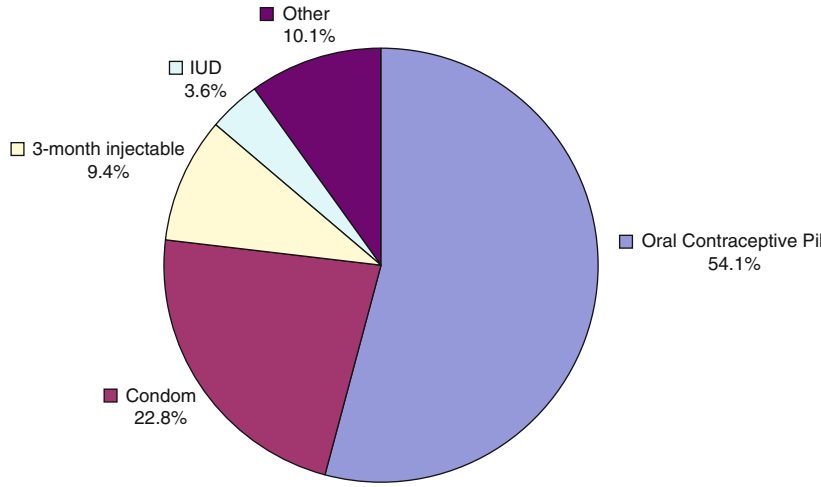

Fig. 26.1 Percentage of adolescents aged 15–19 years using selected method as their most effective method of contraception (source: Mosher [108])

Table 26.1 Available reversible contraceptive methods

Long acting (years)	
Intrauterine devices (IUDs)	Copper IUD (ParaGuard®)
	Levonorgestrel IUS (Mirena®)
Progestin-only contraception: implantable contraception	Subdermal implant (Implanon®)
Intermediate acting (months)	
Progestin-only contraception: injectable contraception	Depo-Provera®
Shorter acting (days to weeks)	
Combined hormonal contraception	Combined oral contraceptives
	Transdermal patch (Ortho Evra®)
	Vaginal ring (NuvaRing®)
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

quency of patient responsibility (e.g., daily, weekly, monthly, or longer acting), as well as the necessity of interrupting sexual spontaneity. 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 experience higher rates of contraceptive failure compared to older women [13].

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, and improvement of premenstrual dysphoric disorder symptoms. Hormonal contraceptives have also been shown to reduce the risk of endometrial, ovarian, and colorectal cancers [14].

The potential prevention of sexually transmitted infections (STIs) may be an important consideration for young patients who are not in long-term stable relationships. Condoms are the recommended method for preventing transmission of STIs [15] but are not a highly effective method of contraception, particularly among younger users [13]. Thus, dual method use should be encouraged for adolescents at risk for the transmission of STIs.

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 2010 Center for Disease Control (CDC) U.S. Medical Eligibility Criteria (MEC) for Contraceptive Use 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 [16].

Nonhormonal Methods of Contraception

Condoms

Latex condoms reduce the transmission of STIs [15]. It should be emphasized to adolescents that prevention of STIs is a critical step for the preservation of future fertility. Pelvic inflammatory disease (PID) usually results from ascending passage of bacteria from the lower reproductive tract into the tubal lumen [17]. It has been clearly

Table 26.2 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
Injectible 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 [16]

Table 26.3 Contraceptive failurea during first year of use

Method	Perfect use (percent)	Typical use (percent)
Subdermal implant	<1	<1
Intrauterine devices	<1	<1
Depo-Provera	<1	3
Combination hormonal contraceptives	<1	8
Progestin-only pill	<1	8
Male condom	2	15
Diaphragm (with spermicide)	6	16
Sponge:		
Nulliparous women	9	16
Parous women	20	32
Female condom	5	21
Fertility awareness-based methods	3–5	25
Withdrawal	4	27
Spermicides	18	29
No method	85	85

Adapted from Hatcher 2007 [20]
[a]Note that these failure rates are not specific to adolescents

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 two episodes, and in 54.3% of women after three episodes [18].

The male condom represents a mechanical barrier to fertilization and is the most frequently used method of contraception used at first intercourse, used by 68% of females and 82% of males at this time. Although 95% of sexually active

female adolescents report having ever used condoms, only 52% use them with every act of intercourse [19]. Numerous different types of 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 a 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% [20]. 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, as high as 17.4% in the first 12 months of use. 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 [15]. The CDC MEC recommends correct and consistent use of male latex condoms for reduction of STIs, with consideration of the female condom if the male condom cannot be used properly for infection protection [16]. 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 [15]. 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. Dual method use among adolescents has increased over the last decade, with only 8% of adolescent girls reporting use of both a condom and a hormonal method of contraception at last sexual intercourse in 1995, increasing to 20% in 2002 and 21% in 2008 [19].

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 mucus 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.

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 29% so that spermicidal agents can be recommended only as an adjunctive measure to increase the efficacy of barrier methods [20]. 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 [16].

Other Barrier 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 [21], but no more than 24 h, due to concern for Toxic Shock Syndrome (TSS) [22]. 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 [21]. Additional spermicide is inserted prior to each act of coitus. With perfect use, the failure rate has been estimated at 6% [20], but with routine use, the failure rate has been reported to be three times as high (16%) during the first year of use [20]. Both the diaphragm and the cervical cap are associated with an increased risk of urinary tract infections [21], presumably as a result of alterations in vaginal flora [23, 24]. 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 it appears to be twice as effective in the nulliparous woman with a similar failure rate in this group as the diaphragm and cervical cap, 16%, but a failure rate of 32% in parous women [20].

The combination of inadequate protection against pregnancy without the proven STI protection of condoms makes these 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 the "Spermicides" section above.

Hormonal Methods of Contraception: Overview

Hormonal contraceptive methods are highly effective. In the past, there had been concern that use of hormonal contraception in adolescents might adversely impact on 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 [25]. 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 (OCPs) (Table 26.1). Additionally, the levonorgestrel-releasing intrauterine system (Mirena®) is a hormonal method of contraception that acts via local release of a progestin (discussed in the section on intrauterine devices). OCPs are taken daily and are available as progestin-only pills (POPs), also known as "minipills," 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®) and the contraceptive vaginal ring (NuvaRing®), which require weekly and monthly administration, respectively. Longer acting hormonal contraception is available as progestin-only contraception 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 etonorgestrel for a 3-year period (Implanon®).

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 [26]. Progestins also produce an atrophic endometrium in which embryo implantation would be unlikely [27]. 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 [28]. It is also possible that exogenous hormones may impair fertilization and early embryo development by altering Fallopian tube secretion and peristalsis [29].

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 [30]) whereas today's low-dose combination oral contraceptive pill contains 20–35 mcg of ethinyl estradiol [31]. 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 [31].

Cardiovascular Effects

With the decreasing estrogen content of COCs over the past years, their cardiovascular safety has improved dramatically [32, 33]. 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 [34]. The CDC recommends that the proven or theoretical risks of combination hormonal contraception usually outweigh the advantages in women 35 years of age and older who smoke [16] whereas healthy, nonsmokers can continue to use CHC as long as they remain at risk for pregnancy [25]. 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 guidelines 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 [16].

Atherosclerotic heart disease and coronary artery disease are associated with increased triglycerides, increased LDL cholesterol, and decreased HDL cholesterol [35, 36]. Hormonal contraceptive agents have been shown to induce both favorable and unfavorable changes in the serum lipid profile [35, 36]. It is not clear, however, whether any of these changes in serum lipids increases the risk for cardiovascular disease, particularly in the adolescent.

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 [16]. Studies have not demonstrated significant changes in blood pressure with contraceptive methods containing only progestational agents [16] so that POPs, Implanon®, and the 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, [16].

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 [35–37]. 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 (20–35 mcg), 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, containing either levonorgestrel or norethindrone alone, have minimal effects on carbohydrate metabolism [36].

Deterioration of glucose tolerance has also been demonstrated in healthy users of DMPA [38, 39]. 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 [40]. Implanon® appears to induce mild insulin resistance without a significant change in serum glucose levels [41].

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 [42]. Similarly, glucose tolerance remained within normal limits for DMPA users after 12 months [38]. The most recent Cochrane review suggests that for women without diabetes, hormonal contraceptives have minimal effect on carbohydrate metabolism [43]. Even in previous gestational diabetics, the use of a low-dose COC is accompanied by a minimal risk of impaired glucose tolerance [44] and does not appear to influence their risk of developing diabetes [45]. CDC guidelines do not consider a history of gestational diabetes to be a restriction for the use of any form of hormonal contraception [16].

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 [46–48]. Current data suggests that modern COCs have little effect on glycemic control or the insulin requirements of diabetic women [46, 47]. Furthermore, neither current, past, or duration of COC use was associated with current gycosylated hemoglobin or the development of diabetic sequelae, such as retinopathy, nephropathy, and hypertension [49, 50]. Similarly, the levonorgestrel IUD appears 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 [51].

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 (102.7–112.9 mg/dl) while no change was found in the users of the copper T IUD [52]. 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 guidelines 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 [16].

Risk of Venous Thromboembolism

The most serious risk for adolescent users of CHC is venous thromboembolism (VTE), including pulmonary embolism. The increased risk appears to be related to estrogen, which increases hepatic production of coagulation factors in a dose-dependent manner [53, 54]. Accordingly, the risk of VTE has decreased along with decreases in the estrogen content of the COCs. Nevertheless, users of modern COCs, containing

30–35 mcg of ethinyl estradiol, still have a three- to fourfold elevated relative risk of VTE compared with nonusers [34]. Although combination OCPs with 20 mcg of ethinyl estradiol do not appear to have an effect on clotting factors [53], there is no evidence that lowering the estrogen content below 30 mcg further reduces the risk of VTE [33, 55]. It is important to remember, however, that venous thromboembolic events occur less frequently in users of combination OCPs (9 cases per 100,000 woman-years for women aged 20–24 years and 18 per 100,000 woman-years for women aged 40–44 years) than during pregnancy (60 cases per 100,000 woman-years) [56].

In 1995–1996, several European studies suggested that the progestin component of combination OCPs may also contribute to the risk of VTE, noting a small increase in VTE among users of COCs containing the newer progestins, gestodene and desogestrel [57–63]. Since then, newer studies have revealed biases in these original reports [62]. More recent studies that controlled for preferential prescribing and duration of use have found the risk of VTE with the new progestins to be equivalent to other COCs [64–68]. A similar phenomenon recently occurred with the newest progestin, drospirenone. Several early studies showed a small increase in VTE among users of drospirenone-containing COCs, but subsequent, better controlled, studies have found no association [69, 70].

Use of combination OCPs particularly increases the risk of VTE in women with thrombophilic disorders, such as the factor V Leiden mutation, which occurs in approximately 3–4% of the European population [62]. 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 [62]. 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% [71]. 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 [25, 62]. Even among women with factor V Leiden mutation, the vast majority will never get a VTE, and most VTE occur in women without a thrombophilic disorder [25].

Due to the increased risk of thromboembolism, CDC guidelines 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 [16]. Because prolonged immobilization is also a risk factor for VTE, CDC guidelines recommend that COCs should be discontinued when major surgery with prolonged immobilization is anticipated [16]. For all progestin-only methods of contraception, the CDC 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 [16].

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 aged 13–21, 50% reported that they would not accept a contraceptive method if it were associated with a 5-pound weight gain [72]. In fact, 41% of adolescents listed weight gain as the primary reason for discontinuing contraception with long-acting progestin [73].

Theoretically, both the estrogenic and progestational components of the COCs can contribute to weight gain. Progestins are thought to directly stimulate hypothalamic center to increase appetite [74]. Progestins have also been shown to increase insulin levels, which can be associated with symptoms of hypoglycemia and increased appetite [35]. Estrogen can result in increased subcutaneous fat deposition [35]. Additionally, fluid retention has been associated with both the estrogen and progestin component of COCs [35].

Despite the theoretical concerns about weight gain related to CHC use, the existing data does not support this concern. Several prospective studies have demonstrated that

OCP use was not associated with significant weight gain [75–78]. In another study, only 5% of women cited weight gain as a reason for discontinuing OCPs [79]. It appears that as many women lose weight as gain weight while taking COCs [35]. A 2008 review of randomized clinical trials found no association between CHC and weight gain [80].

Studies of Implanon® also do not show significant weight gain during use. In the largest US study of Implanon®, only 12% of women reported weight gain, with an average BMI gain of 1.7 kg/m [2] over 2 years. Importantly, only 3.3% of women discontinued Implanon due to concerns about weight [81]. Of note, all participants in this study were at least 18 years old, and there are no studies specifically addressing weight gain in the adolescent population.

In contrast to CHC and Implanon®, it appears that DMPA may be associated with significant weight gain. The FDA package labeling for DMPA states that 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 3,857 women [82]. Later studies, however, suggest that weight gain should not deter women from considering DMPA as a contraceptive method. 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 maintain their body weight in retrospective studies [83, 84].

Comparative studies in adults, using nonhormonal IUD users as controls, have yielded mixed results. One study found that users of both types of contraception gained a similar amount of weight over the 120-month study period: 10.9 kg for DMPA users and 11.2 kg for the IUD users [85], while another found a significantly higher weight gain in DMPA users: 4.3 kg for DMPA users and only 1.8 kg for IUD users over 5 years [86]. Obese adolescents 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 [87].

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 order to avoid unnecessary restriction of contraception use, the World Health Organization (WHO) held two meetings of experts in 1994 and 1995 to guide practitioners in a variety of health care settings around the world [88]. Using evidence-based criteria, these experts assessed the risk of pregnancy versus contraceptive use in the setting of various medical conditions, and identified only a few medical conditions that precluded the use of hormonal contraception. Additionally, with the exception of blood pressure measurement, a physical exam was not a prerequisite to hormonal contraception. In 1996, another 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 [25].

Because the adolescent population is at extremely low risk for cardiovascular disease, screening may be of limited utility. Nevertheless, the initial assessment is to identify contraindications to hormonal contraception use. In addition to those discussed above, other contraindications to the use of combined hormonal contraception include migraine headaches with aura, systemic lupus erythematosus with antiphospholipid antibodies, and active viral hepatitis [16].

A follow-up visit may be beneficial, especially for adolescents who have high rates of

incorrect use and discontinuation of contraceptive methods. However, inability to attend a follow-up visit 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. For IUD users, a follow-up visit is recommended 1 month after insertion to ensure that the IUD is still in place and that there are no signs of infection [20]. At a follow-up visit, a blood pressure measurement should be obtained for users of CHC. There is evidence from older formulations that use of combination OCPs increases the risk of malignant hypertension [89]. However, low-dose pills rarely cause a clinically significant increase in hypertension, and when changes do occur, they are reversible within 3–6 months of stopping COCs [20, 90]. If blood pressure elevations are identified, another method of contraception should be considered.

Obtaining a weight before beginning a method of hormonal contraception may be helpful. Although no method of contraception is contraindicated in obese women, efficacy of oral contraceptives may be impaired in overweight and obese women, although the data is conflicting, and the magnitude of the effect is small. There is some evidence that the contraceptive patch is less effective for women who weigh ≥90 kg. Implanon® has never been studied in women over 130% of ideal body weight. Obesity does not appear to affect the efficacy of the contraceptive vaginal ring, the IUD, or DMPA [91]. Because weight gain is common in reproductive-age women, and only DMPA has been associated with weight gain beyond that which would be expected with increasing age, 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.

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 [35]. 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 [46]. In particular, the CDC MEC indicate that the risks outweigh the benefits for combined hormonal methods with use of certain anticonvulsants (phenytoin, carbamezapine, barbiturates, primidone, topiramate, oxcarbazepine, and lamotrigine monotherapy), antibiotics (rifampicin or rifabutin only), and certain antiretrovirals [16]. These guidelines suggest that DMPA and the contraceptive implant may be used with these medications.

The use of hormonal contraception may also modulate the effects of other medications. Steroids weakly inhibit hepatic drug oxidation but enhance glucuronosyltransferase activity [35]. 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 [35] 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 [92]. 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 [16].

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 [93]. To the contrary, several studies have even demonstrated a protective effect of hormonal contraception against some types of reproductive cancer [93, 94]. 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.

Despite numerous studies, it is not clear whether there is any association with COC use and breast cancer [25]. 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 [95, 96]. 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 [95]. Compared to non-users, 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 had similar findings, either finding no increase in breast cancer risk or if a risk was found, the effect disappeared after discontinuing COCs [93].

Similarly, results for DMPA have been reassuring. DMPA was approved for use in several countries in the 1970s but did not receive FDA approval for contraceptive use in the United States until 1992 [97] due to concerns about breast cancer. A large case–control study by the WHO, 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 [98]. These results were interpreted to suggest that rather than cause new tumors, DMPA enhances growth of already existing tumors [97].

Some have suggested, however, that the timing of exposure to hormonal contraception is an important consideration [93]. 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 [99]. 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 [100]. 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 also been demonstrated in long-term users of COCs, but the risk decreases after discontinuation [93]. The major cause of cervical neoplasia is infection with particular types of human papilloma virus (HPV). It is likely that users of OCPs are more likely to be exposed to HPV since they probably do not use barrier methods of contraception, but there is also evidence that COCs may enhance expression of HPV genes [93]. 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 demonstrate a protective effect of COCs against ovarian and endometrial cancers [93, 101, 102]. Furthermore, the benefits of COCs are enhanced with duration of use and persist years after OCPs are discontinued [25]. 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 [103]. Even modern formulations, with ≤35 μg EE, appear to be protective against ovarian cancer [93]. The CASH study also found combination OCPs to be similarly protective against endometrial cancer [104, 105]. Most studies found that the protective effect against endometrial cancer persisted for up to 20 years after discontinuing COCs [93]. 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 [106] and may even reduce the risk further [107].

Combined Hormonal Contraception

Overview of Methods

Until 2002, the only method of CHC available to the US women was the OCP. However, in 2001, the Food and Drug Administration (FDA) 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 which, consequently, 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 2006–2008, 54.1% of 15–19-year-olds and 48.0% of 20–24-year-olds used the OCP as their most effective method. Only 7% and 11% of teens had ever used the birth control ring or patch, respectively [108]. 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 [109].

Efficacy of CHC

With perfect use, all CHC methods are extremely effective contraception with a failure rate of 0.3% in the first year of use for OCPs, the patch, and the vaginal ring [20]. In actual use, however, OCPs are associated with a much higher failure rate of 8.7% [13]. Adolescents are at high risk for inconsistent use and for discontinuation of OCPs. On average, adolescents miss up to three 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% [110]. In addition to inconsistent use, discontinuation of CHC among adolescent girls is common. Within 6 months, up to 75% of DMPA and patch users and >50% of pill and ring users will discontinue method use [109]. The most common reason given for OCP discontinuation is the presence of side effects, including nausea, irregular bleeding, breast tenderness, and mood changes [110].

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 [79]. The more common side effects (bleeding irregularities, nausea, weight gain, 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 [35]. 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.

Hormone Formulations and Dosing Regimens

CHC methods contain both estrogenic as well as progestational agents. The pills differ in their total estrogen content, the progestational agent used, and in the ratio of estrogen to progestin. In monophasic preparations, the dose of estrogen and progestin are 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, at doses of 20–35 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 [31]. The metabolism of ethinyl estradiol varies between individuals so that the same dose can cause side effects in one woman and none in another [30, 111]. The con-

traceptive 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 [31]. 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) [62]. 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-keto-desogrestrel, also known as etonogestrel, which is the progestin used in both the contraceptive vaginal ring (NuvaRing®) and contraceptive implant (Implanon®) 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 (Ortho Evra®).

Drospirenone is a newer progestin, which may have a pharmacologic profile more closely related to endogenous progesterone [112]. Drospirenone is an analogue of spironolactone, the aldosterone antagonist, and like progesterone, has mild antimineralocorticoid activity [113]. 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 [112]. In contrast to the other progestins, drospirenone has no androgenic activity and, in fact, appears to have mild anti-androgenic activity like spironolactone [112].

Combined hormonal contraception (pills, patch, or vaginal ring) has been traditionally administered for 21 days. After 21 days, the hormones are stopped for 7 days to allow a withdrawal bleed to occur. Because the synthetic steroids in CHC are not completely eliminated from the body within 24 h, there is a cumulative build-up over several days, and maximal effectiveness is not achieved until after several days [35]. A backup contraceptive method is usually recommended during the first 7 days of a CHC cycle, but immediate protection from pregnancy is thought to be present if CHC is started with the first day of the menstrual cycle [35].

Over time, the 21-day schedule and the necessity of a monthly withdrawal bleed have been questioned [114]. During the placebo week, some CHC users can experience hormonal withdrawal symptoms, such as pelvic pain and bleeding [115]. 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. Some COC preparations have placebo or ethinyl estradiol-only tablets four 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 an year [114]. 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 [116]. Although some women can take COCs continuously without placebo tablets, many experience breakthrough bleeding [117]. It has been suggested that women may individualize their COC regimen and begin a 7-day hormone-free interval when they begin to have breakthrough bleeding or at whatever interval is convenient.

Side Effects of CHC

Although the progestins are used at such low doses that the differences in their biologic effects should be negligible, different combination OCPs 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, and androgenic (Table 26.5). The hormone content of the COCs can differentially effect the endometrium as well [35].

The estrogenic effects of a particular CHC formulation are 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 [35]. Progestins, however, also reduce the biological activity of estrogen by acting as estrogen antagonists [35]. So 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 or estrogen excess or deficiency, a CHC preparation with different estrogenic activity can be tried (Tables 26.4 and 26.5). The contraceptive vaginal ring has been shown to have the lowest estrogen exposure while the transdermal patch has the highest [118].

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 [35]. 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 [35]. Nevertheless, all of the CHC methods exert an anti-androgenic 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 [119]. 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 [35]. 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 [35], and drospirenone appears to compete with androgen to exert an anti-androgenic effect [112].

Endometrial activity is measured by the ability of the method to prevent irregular bleeding while the active hormone pills are being taken [35]. 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 tenth day is usually a result of a deficiency in the progestational activity [35]. 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 (Table 26.4). 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 (Table 26.5).

Progestin-Only Contraception

Progestin-only contraception is useful for women who either cannot tolerate estrogenic

Table 26.4 Biological activity of selected monophasic and progestin-only oral contraceptive formulations

Formulation EE (mcg) + progestin (mg)	Endometrial activity[a]	Estrogenic activity[a]	Progestational activity[a]	Androgenic activity[a]
Lo/Ovral EE (30) Norgestrel (0.3)	9.6	25	0.8	0.46
Ovcon 35 EE (35) Norethindrone (0.4)	11.0	40	0.4	0.15
Desogen/Ortho-cept EE (30) Desogestrel (0.15)	13.1	30	1.5	0.17
Nordette EE (30) Levonorgestrel (0.15)	14.0	25	0.8	0.46
Ortho-Cyclen EE (35) Norgestimate (0.25)	14.3	35	0.4	0.18
Loestrin 21 1.5/30 EE (30) Norethindrone acetate (1.5)	25.2	14	1.7	0.80
Alesse EE (20) Levonorgestrel (0.1)	26.5	17	0.5	0.31
Loestrin 21 1/20 EE (20) Norethindrone acetate (1.0)	29.7	13	1.2	0.53
Micronor/NorQD: Norethindrone (0.35)	42.3	0	0.35	0.13
Yasmin EE (30) Drospirenone (3)	14.5	30	1.5	0.00
Yaz EE (20) Drospirenone (3)	13.8	20	1.5	0.00

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[a]Activities are defined as follows:
Endometrial is percent of users with irregular bleeding in third cycle or use
Estrogenic refers to ethinyl estradiol (mcg) equivalents per 28 days
Progestational refers to norethindrone (mg) equivalents per 28 days
Androgenic refers to methyltestosterone (mg) equivalents per 28 days

side effects or have contraindications to estrogen use. These methods are available as POPs, a 3-month depot intramuscular injection of DMPA (Depo-Provera®), or as a subdermal implant that releases etonorgestrel for a 3-year period (Implanon®).

Progestin-Only Pills

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 "NorQD." Unlike COCs, they

Table 26.5 Relation of side effects to hormonal biologic activity

Estrogen excess	Progestin excess	Androgen excess
Breast cystic changes	Cervicitis	Acne
Hypermenorrhea\Menorrhagia	Decreased withdrawal bleed	Cholestatic jaundice
Breast enlargement	Moniliasis	Hirsutism
Mucorrhea	Appetite increase	Libido increase
Bloating	Depression	Oily skin
Dizziness, syncope	Fatigue	Rash and pruritis
Edema	Hypoglycemic symptoms	Edema
Headache (cyclic)	Libido decrease	
Irritability	Hypertension	
Leg cramps	Leg vein dilation	
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		
Estrogen deficiency	**Progestin deficiency**	**Androgen deficiency**
Absence of withdrawal bleed	Irregular bleeding (days 10–21)	Libido decrease
Irregular bleeding (days 1–9)	Delayed withdrawal bleed	
Irregular bleeding (continuous)	Hypermenorrhea\Menorrhagia	
Pelvic relaxation symptoms	Bloating	
Atrophic vaginitis	Dizziness, syncope	
Nervousness	Edema	
Vasomotor symptoms	Headache (cyclic)	
	Irritability	
	Leg cramps	
	Nausea, vomiting	
	Visual changes (cyclic)	
	Weight gain (cyclic)	

Permission to reprint has been granted by EMIS, Inc. Medical Publishers, Fort Collins, CO. 80527. Tables have been adapted from Managing Contraceptive Pill/Drug Patients—14th edition, 2010, by Richard P. Dickey, MD, PhD; Table 11: Side Effects Related to Hormone Content (147–148) [35]

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 [120] and occurs in approximately half of oral progestin users [28]. Thus, the mechanism of action is the POP's effect on endometrium and cervical mucus [121]. With perfect use, the failure rate for POPs is 0.3% [20], but perfect use is difficult. Meticulous pill taking every 24 h appears critical for contraceptive efficacy, and a backup contraceptive method is recommended if a pill is taken more than 3 h late [121]. For this reason, POPs are not usually used in the adolescent population.

DMPA Injectable Contraception (Depo-Provera®)

DMPA received FDA approval in 1992. DMPA use is more popular among adolescents than in any other age group. In 2006–2008, 9.4% 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 [108]. 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 [35]. 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 [35]. Administration at other times in the cycle is acceptable if pregnancy can be ruled out and the patient can use a backup method of contraception for 7 days [122]. The dose of progestin is high enough to block the LH surge and ovulation [97]. 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 [97].

DMPA is one of the most effective contraceptive methods for adolescents, with a failure rate of 0.3% with perfect use [20]. With typical use, the failure rate after 12 months is 6.7% for the general population [13]. 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 [97]. Although 50% of women will conceive within 10 months of the last injection, some women will not conceive until after 18 months [97].

The side effects of DMPA are related to estrogen deficiency or progestational excess [35]. DMPA reliably and consistently suppresses estrogen levels [32]. Because estrogen levels may reach the postmenopausal range [123], 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, and [73]. In a multicenter US study, 46.0% of women reported amenorrhea, and 46.2% of women reported irregular bleeding after 3 month of use [124]. 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 [124]. In another study, 73% of users were found to be amenorrheic after 12 months [125]. 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 [126].

An estimated 55% of adolescents will discontinue DMPA within 1 year of starting [127]. 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%) [73]. The FDA package insert mentions depression as a side effect of DMPA [35], but published studies indicate that DMPA does not cause depression [97]. 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 [128]. The CDC guidelines indicate that DMPA can be used without restriction in women with depressive disorders [16].

The most controversial topic regarding the use of DMPA by adolescents is its effect on 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 [129–131]. In November of 2004, the US 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 [132].

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 [133]. Peak bone mass is reached by the age of 16–22 years, and this measure is related to the risk of osteoporosis [134]. 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 towards regaining BMD after cessation of use [135–137]. 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 WHO [138] and the American College of Obstetricians and Gynecologists [46] have supported the cautious use of DMPA in the adolescent population. The CDC guidelines also indicate that the advantages of using DMPA for women under the age of 18 years exceeds the risks [16]. Certainly, users of DMPA should be counseled about appropriate intake of calcium (1,200 mg/day), vitamin D, and weight-bearing exercise, which have all been shown to have positive effect on bone density [139].

Etonorgestrel Implantable Contraception (Implanon®)

The etonorgestrel 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. Because it was introduced to the US market so recently, we do not yet have data on the number of adolescents who use this method. This implant is highly effective at preventing pregnancy for up to 3 years of use. Several large studies have reported no pregnancies over 2–3 years of Implanon use [81, 140, 141]. Because there is almost no opportunity for user error, the perfect-use and typical-use failure rates should be nearly identical. Like DMPA, Implanon® is easy to use, 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 most common reason for discontinuation of the etonogestrel implant is irregular vaginal bleeding. Although there is a tendency towards amenorrhea (14–20% incidence) and infrequent bleeding, with an overall decrease in annual blood loss, the vaginal bleeding pattern with Implanon® is irregular and unpredictable. However, menstrual blood loss is not greater than that experienced with regular menses [142]. 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 [81, 143].

One potential advantage to use of Implanon® 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 Implanon® users compared to users of a nonhormonal IUD, there was no reduction in BMD in implant users [144].

Of note, there is little published data on Implanon use for adolescents. One study of 73 postpartum adolescents using Implanon found longer continuation and fewer pregnancies compared to OCP or DMPA users [145]. Another study of 48 postpartum adolescents reported no pregnancies or discontinuations after 1 year of use [146]. Although it should be considered as a safe and highly effective method for this age group, and the CDC guidelines indicate that it can be used without restriction in adolescent women [16], more research is needed on the use of Implanon® in adolescents.

Intrauterine Devices

The 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. Because of their ease of use, perfect-use and typical-use failure rates are nearly identical. From 2002 to 2006–2008, use of the IUD among young US women increased markedly, from 2.0 to 5.5% of current contraceptive users. Among teens, 3.6% of contraceptive users utilized the IUD in 2006–2008 [108]. There are two types of IUDs currently marketed in the United States. The copper T 380A (TCu380A, Paragard®) is approved for use by the FDA for up to 10 years and contains no hormones. Its failure rate is 0.7 per 100 women in the first year, with a cumulative 7-year failure rate of 1.4 per 100 women. The other IUD available in the United States is the levonorgestrel-releasing intrauterine system (LNG-IUS, Mirena®), approved for use by the FDA for up to 5 years. Its failure rate is 0.14 per 100 women in the first year, with a cumulative 7-year failure rate of 1.1 per 100 women. These failure rates rival those of permanent surgical sterilization. Continuation rates at 1 year for adult users of the TCu380A and LNG-IUS are similar at 78% and 80%, respectively [20].

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 is near 90%, with approximately 20% of users experiencing amenorrhea, after 1 year of use [20].

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 [147]. 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 LNG-IUS protected against upper genital tract infection, compared to a copper IUD [148]. A case–control study of 1,895 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 [149].

In December 2007, the American College of Obstetricians and Gynecologists Committee on Adolescent Health Care issued a committee opinion encouraging health care 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 [150]." A systematic review of IUDs for adolescents found reassuring results and also supported offering IUDs to adolescents as a first-line contraceptive option [151].

Non-contraceptive Benefits of 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 [152]. Hormonal contraceptive methods also offer protection from several benign conditions and are also used therapeutically.

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) [14]. The symptoms of both PMDD and PMS were improved by COCs, containing 30 μg of ethinyl estradiol with drospirenone [153], and the NuvaRing was found to be effective for treating PMS [154]. 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 [14, 35]. For heavy menstrual bleeding, a Cochrane review found that the levonorgestrel IUD may be more effective than COCs [155].

In addition to increased iron stores [156], studies of users of COC [157], Implanon [142], and Nuva Ring [154] have demonstrated improvement in dysmenorrhea. Hormonal contraception, including COCs, DMPA, Implanon, and the levonorgestrel IUD, has been shown to decrease the severity of dysmenorrhea and reduce the pelvic pain associated with endometriosis [14]. 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 [35, 158]. Oral progestins can be used to treat endometriosis as well but at much higher doses than in POPs [159]. Medroxyprogesterone (20–30 mg daily) or norethindrone (15 mg daily) is commonly used.

Hyperandrogenism can be associated with anovulation (sometimes called polycystic ovary syndrome), 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 [160, 161]. In combination, estrogens and progestins are anti-androgenic [14]. 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 shown to decrease adrenal androgen secretion [162]. Additionally, COCs restore cyclic endometrial shedding and protect against the endometrial hyperplasia that is associated with chronic anovulation due to hyperandrodogenism [119].

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 [163]. Hormonal preparations for contraception, as well as hormone preparations designed for postmenopausal women, have been used for hormone replacement in this setting. There is emerging evidence that postmenopausal preparations may be superior to COCs for hormone replacement, but younger patients may prefer contraceptive preparations due to familiarity and to be similar to peers [164]. It should also be noted that for unclear reasons, hormonal contraception may not be effective in suppressing ovulation in women with premature ovarian insufficiency [164], and if pregnancy is not desired, another form of contraception will be necessary.

Conclusions

Women under 30 years of age experience higher rates of contraceptive failure than their older counterparts [13]. Hormonal contraceptive methods are more effective than nonhormonal ones. 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.

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 appears to improve with pretreatment counseling. Additionally, hormonal contraception does not offer any protection against STIs so that a barrier method must also be recommended to individuals who are not in monogamous relationships.

In addition to providing reliable contraception, these hormonal methods also provide many non-contraceptive health benefits, including protection from certain cancer and benign medical conditions, such as PID, ectopic pregnancy, and benign breast disease. 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 appear to be particularly well suited for the adolescent population. Adolescents generally are not at risk for cardiovascular disease and do not have contraindications to hormonal contraception. Hormonal contraceptive methods appear to be the most efficacious in this age group, providing reliable contraception with numerous non-contraceptive health benefits.

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Part VII

Metabolic Disorders

Hypoglycemia

27

Diva D. De León and Charles A. Stanley

Abstract

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 strategy for diagnosis and therapy which includes not only hormonal disorders but also metabolic defects, as well as drugs and toxins. This chapter presents an approach to disorders of hypoglycemia based on the metabolic and endocrine systems involved in successful adaptation to fasting.

Keywords

Hypoglycemia • Hyperinsulinism • Neonate • Hormones • Glucose • Insulin

Introduction

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 strategy for diagnosis and therapy which includes not only hormonal disorders but also metabolic defects, as well as drugs and toxins. 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 problems with fasting adaptation. Since the integrity of these various systems is reflected in plasma levels of critical 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. These specimens of plasma and urine are known as the "Critical Samples" or the "Didja Tubes" ("Didja remember to order a _____ test?") and should be routinely obtained

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immediately prior to beginning treatment of the hypoglycemia.

Definition of Hypoglycemia

We recommend the use of the same plasma glucose thresholds for neonates than for children and adults, as there is no evidence that the brain is more resistant to effects of hypoglycemia in neonates than in older children [1]. These thresholds include the following: physiologically normal levels = 70–100 mg/dL, detectable neurophysiologic signs of hypoglycemia = 50–70 mg/dL, activation of glucose counter-regulatory systems = 65–70 mg/dL, diagnostic threshold to obtain critical sample < 50 mg/dL, and therapeutic goal = >70 mg/dL.

Lower limits for defining hypoglycemia have traditionally been applied in neonates (e.g., as low as 20 mg/dL for "low-birth-weight" neonates). However, it must be remembered that these values reflect only a statistical definition of hypoglycemia on day of life one and were based on infants fasted for what would now be considered exceptionally long times of 12 h or more immediately after delivery on the first day of life.

Diagnostic Approach: The Fasting Systems

In infants and children, hypoglycemia, with very few exceptions, always means fasting hypoglycemia. The physiology of normal successful fasting adaptation provides a useful framework that encompasses the diagnosis and treatment of all the potential hypoglycemia disorders. This "Fasting Systems Approach" was originally developed by Dr. Lester Baker at the Children's Hospital of Philadelphia [2].

The Fasting Systems

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

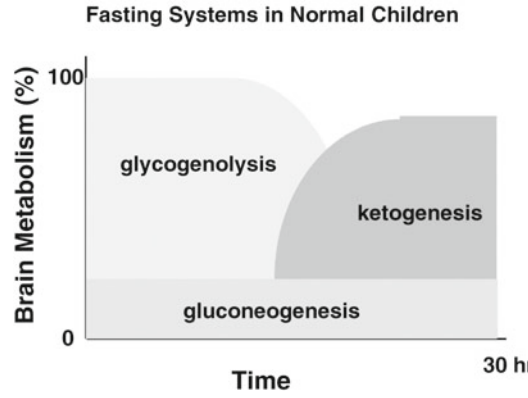

Fig. 27.1 Contribution of major fasting systems to brain metabolism over time in a typical normal infant. Note that glycogen stores are depleted by 8–12 h and that ketogenesis becomes the major source for brain substrate by 24–36 h (Reprinted with permission from Springer)

Hormonal Control of Fasting Systems

	Glycogenolysis	Gluconeogenesis	Lipolysis	Ketogenesis
Insulin	-	-	-	-
Glucagon	+	+		
Epinephrine	+		+	+
Cortisol		+		
Growth Hormone			+	

Fig. 27.2 Hormonal regulation of fasting metabolic systems (Reprinted with permission from Springer)

by the (4) endocrine system, consisting of suppression of insulin (the most important endocrine response to fasting, since insulin suppresses all three metabolic systems) balanced by a relatively redundant set of counter-regulatory hormones that activate one or more of the three metabolic systems: cortisol (gluconeogenesis), glucagon (glycogenolysis), epinephrine (glycogenolysis, gluconeogenesis, ketogenesis, and suppression of insulin), and growth hormone (ketogenesis via increased lipolysis) (Figs. 27.1 and 27.2).

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. 27.1, 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, supple-

mented 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 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 Critical Samples (Didja Tubes)

The circulating levels of certain key fuels and hormones at the time of hypoglycemia reflect the integrity of the metabolic and hormonal systems of fasting. As shown in Fig. 27.3, 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 have tripled (1.5–2.0 mM) and β-hydroxybutyrate, the major ketone, has risen 50- to 100-fold (between 2 and 5 mM); and (4) insulin has declined to essentially undetectable levels (<2 μU/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. These "critical" samples can be obtained during a formal fasting test, but are also extremely useful when obtained during a spontaneous episode of hypoglycemia. Therefore, pediatricians and residents should be trained to get these "Didja Tubes" whenever a child is treated for hypoglycemia ("Didja remember to order a _____ test?").

Categories of Hypoglycemia Based on the Critical Samples

Since it is easily obtained, we begin with the serum bicarbonate at the time of hypoglycemia to segregate the hypoglycemia disorders into four groups (Fig. 27.4), with and without acidemia (HCO_3 <15–17 mEq/L vs. >16–18 mEq/L). Additional tests (listed in parentheses) can then be selected within each of the groups to distinguish specific defects (Fig. 27.5):

1. *Acidemia due to lactate* typifies defects in hepatic gluconeogenesis: Glucose-6-phosphatase deficiency (type 1 GSD), GLUT2 deficiency, fructose-1,6-diphosphatase deficiency, normal neonates on day 1 of life, and ethanol ingestion (lactate and glucose responses to glucagon, galactose, fructose tolerance test, enzyme assays, mutation identification, etc.).
2. *Acidemia due to ketones* typifies normal children, ketotic hypoglycemia (probably also normal, but with shortened fasting tolerance), defects in glycogenolysis (GSD types 0, 3, 6, 9), growth hormone, and/or cortisol deficiencies (GH and cortisol assays, glucose and lactate responses to oral glucose, liver biopsy for enzyme assays, mutation identification, etc.).
3. *No acidemia with ketones and free fatty acids both suppressed*: Congenital hyperinsulinism, exogenous administration of insulin, oral hypoglycemics, SGA and birth asphyxia, and neonatal hypopituitarism (insulin, C-peptide, mutation identification, etc.).
4. *No acidemia with suppressed ketones, but elevated free fatty acids*: Genetic defects in fatty acid oxidation and ketogenesis (serum acylcarnitine profile, urinary organic acids, assays in cultured cells, mutation identification, etc.). Please note: if a disorder of fatty acid oxidation is suspected, a serum acylcarnitine profile should be obtained before fasting the child, since it may obviate exposing the patient to the potentially hazardous fasting test.

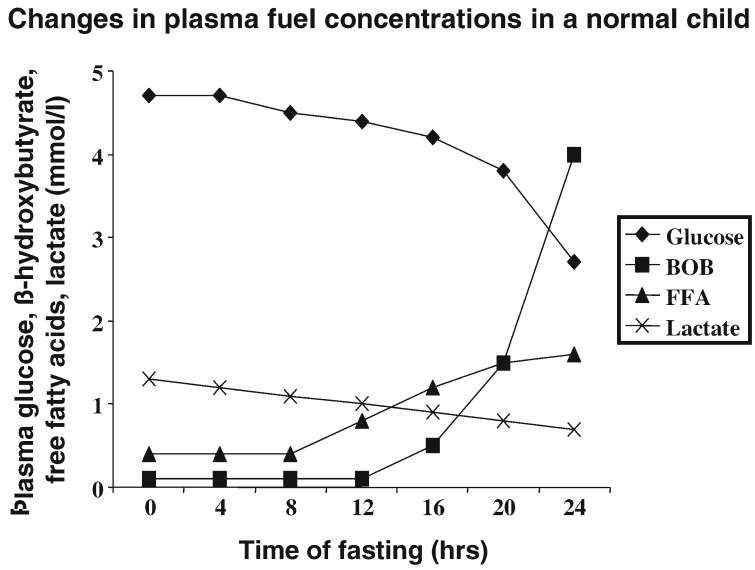

Fig. 27.3 Changes in plasma concentrations of major substrates during the course of fasting in a normal child (Reprinted with permission from Springer)

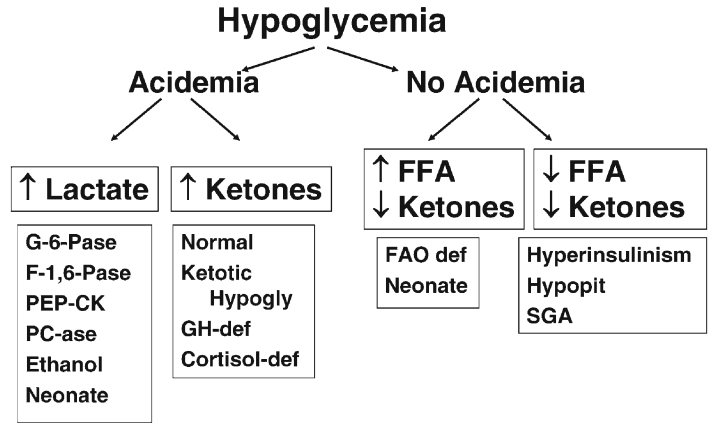

Fig. 27.4 Algorithm for diagnosis of hypoglycemia based on specimens obtained at time of fasting hypoglycemia (Reprinted with permission from Springer)

Specific Disorders

A. Acidemia owing to lactate

1. Examples include glucose-6-phosphatase deficiency (type 1a and type 1b glycogen storage disease) and fructose-1,6-diphosphatase deficiency (see Fig. 27.6). These often do not present with symptoms in the newborn period since the elevated levels of lactate provide an alternative fuel for the brain when glucose is low.
2. Normal neonates: Gluconeogenesis and ketogenesis are both poorly developed at birth, probably contributing to the high risk of hypoglycemia in the first 12–24 h of life in all groups of newborns. Both of these systems appear to mature quickly, perhaps accelerated by fat feeding, by 12–24 h of age. (NB: It is also possible that normal neonates, as well as SGA infants, have immaturity of insulin regulation as the underlying risk factor for hypoglycemia.)
3. Ethanol intoxication: Ethanol metabolism shifts the hepatic NADH/NAD+ redox potential to a more reduced state and blocks gluconeogenesis by diverting pyruvate to lactate. Hypoglycemia ensues if liver glycogen stores are depleted.

Hypoglycemia: The Critical Samples

	Hours	Lactate	BOB	FFA	Response to glucagon
G-6-Pase	2-4	↑	± ↑	↑	-
Debrancher	4-8	↓	↑	↑	-
F-1,6-Pase	8-12	↑	↓	↑	-
MCAD	12-16	N	↓	↑	-
Hyperinsulinism	0-?	N	↓	↓	↑
Hypopituitarism	12-16	N	± ↑	± ↑	-
Neonate	0-8	↑	↓	↑	-

Fig. 27.5 Differential diagnosis of hypoglycemia disorders based on the "Critical Samples" obtained at a time of fasting hypoglycemia (Reprinted with permission from Springer)

B. Acidemia due to ketones
   1. Examples include glycogen synthase, debrancher, liver phosphorylase, or phosphorylase kinase deficiencies (types 0, 3, 6, 9 glycogen storage disease) (see Fig. 27.6) and growth hormone and cortisol deficiencies (e.g., hypopituitarism, adrenal insufficiency). *NB: Neonatal presentation of hypopituitarism may mimic hyperinsulinism.*
   2. 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 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 and a few cases have been shown to have deficiency of hepatic glycogen synthase (GSD type 0).

C. No acidemia with ketones and free fatty acids both suppressed
   1. Congenital hyperinsulinism (HI) [4, 5]. Fasting tolerance: nil to 12+ h (depends on severity), low lactate, ↓ free fatty acids (FFA), ↓ beta-hydroxybutyrate (BOB), and positive glycemic response to glucagon stimulation (>30 mg/dL raise in glucose) [3]. NB: Contrary to common assumption, insulin levels are rarely very high in children with congenital hyperinsulinism. Thus, any detectable insulin at time of hypoglycemia is considered diagnostic. It is often necessary to make the diagnosis of hyperinsulinism based on the evidence of excessive insulin effects on the three fasting metabolic systems. Clinical features and treatment depend on the specific type (see Fig. 27.7):
      (a) Recessive mutations of KATP-channel genes (*ABCC8*, encoding SURI, sulfonylurea receptor; *KCNJ11*, encoding Kir6.2, ion pore) [6–9]. Severe neonatal onset, LGA birth weight, diazoxide unresponsive, very high glucose requirement (up to 20–30 mg/kg/min). Therapy: Diazoxide is unlikely to work (because it acts by binding to SUR1); octreotide is effective acutely, but tachyphylaxis may make it inadequate for long-term therapy, 95% subtotal pancreatectomy, and glucagon via continuous infusion (for identical phenotype in focal disease, see below). Activating mutations in the KATP-channel encoding genes are now

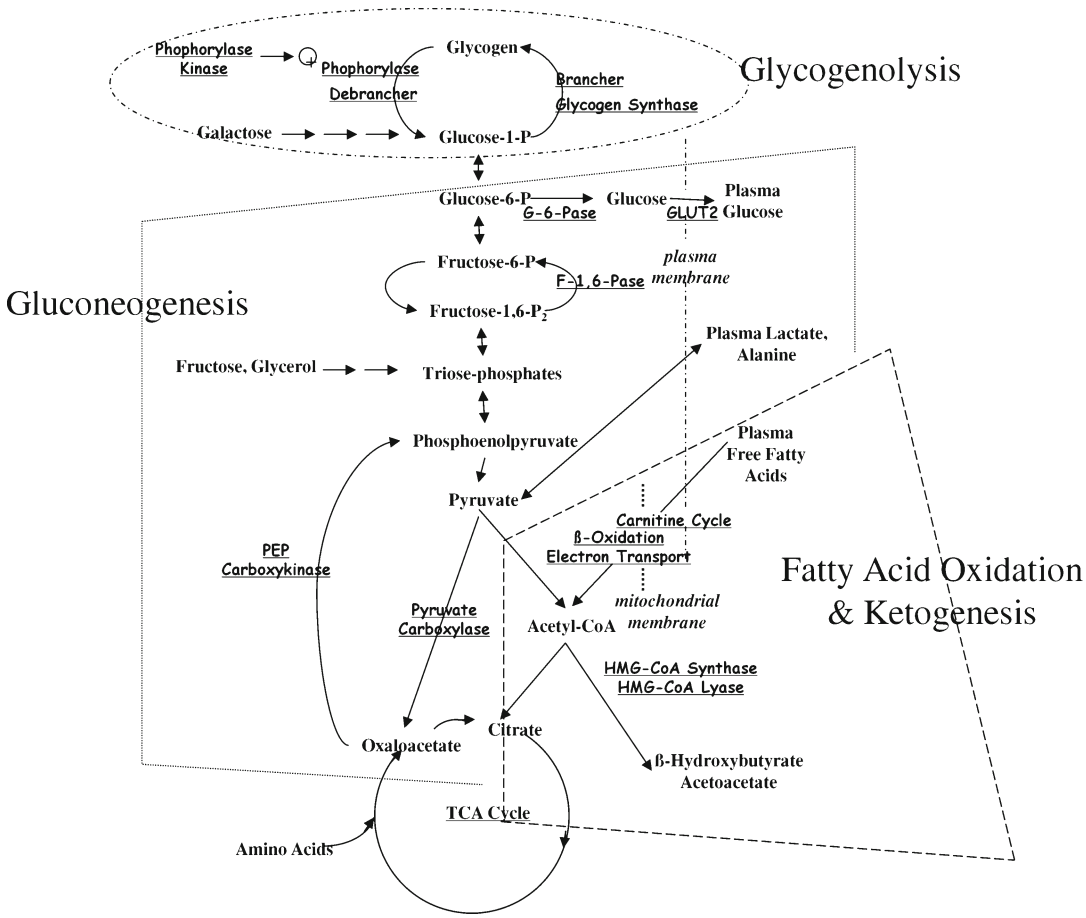

Fig. 27.6 Metabolic pathways of fasting adaptation. Sites of genetic defects are *underlined* (Reprinted with permission from Springer)

known to be responsible for the majority of cases of neonatal diabetes.

(b) Dominant mutations of glutamate dehydrogenase (*GLUDI*): Hyperinsulinism/hyperammonemia (HI/HA) syndrome [10–14]. Milder, later onset, diazoxide responsive, protein/leucine sensitive hypoglycemia. Associated with persistent mild hyperammonemia (plasma ammonium, 50–200 μM). Mutations cause a gain of GDH enzyme function with excessive enzyme activity in liver, in addition to β-cells.

(c) Dominant mutations of glucokinase (GK) [15]. Also milder, later onset. Diazoxide responsiveness is variable; some children may require a combination of diazoxide and continuous glucose through an intragastric tube. This form of hyperinsulinism is caused by mutations that lower the GK Km for glucose and reduce the glucose threshold for insulin release. Loss of function mutations of GK occur in MODY2 (maturity onset type diabetes of the young type 2).

(d) Dominant mutations of *ABCC8* and *KCNJ11* [16]. Unlike the severe disease often associated with recessive mutations of SUR1, hypoglycemia in these cases is milder and responsive to treatment with diazoxide. In addition to fasting hypoglycemia, dominant mutations are associated with protein-induced hypoglycemia [17]. (Some dominant mutations of *ABCC8* are not responsive to diazoxide.)

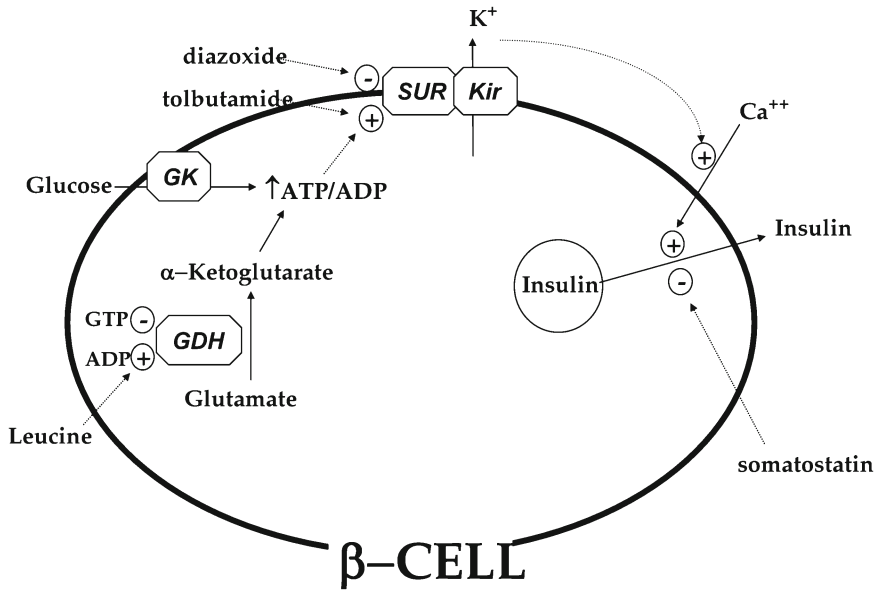

Fig. 27.7 Pathways of pancreatic beta-cell insulin secretion. Glucose stimulates insulin secretion via an increase in ATP/ADP ratio which leads to inhibition of plasma membrane ATP-dependent potassium channels, membrane depolarization, and activation of voltage-gated calcium channels with the subsequent 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) (Reprinted with permission from Springer)

(e) Focal hyperinsulinism [18–20]. Associated with focal loss of heterozygosity for maternal 11p and expression of a paternally transmitted recessive K_{ATP}-channel mutation (either *ABCC8* or *KCNJ11*). Phenotype identical to recessive (diffuse) K_{ATP}-channel disease. May account for 40–60% of cases of severe, neonatal onset diazoxide unresponsive hyperinsulinism. Preoperative localization of the lesion by 18 F-fluoro-L-DOPA PET scan [21, 22] and resection of the lesion can result in cure of the hyperinsulinism without the risk for diabetes associated with a near-total pancreatectomy.

(f) Recessive mutations of *HADH* (encoding SCHAD, the mitochondrial enzyme short-chain-3-hydroxyaxyl-CoA dehydrogenase) [23–25]. Mild hyperinsulinism responsive to diazoxide therapy. The biochemical hallmark, in addition to markers of increased insulin action, is increased levels of 3-hydroxybutyrylcarnitine in plasma and increased levels of 3-hydroxyglutarate in urine.

(g) Dominant mutations of hepatocyte nuclear factor 4 (*HNF4A*) that cause familial monogenic diabetes later in life (formerly known as MODY3) can present in the neonatal period with hypoglycemia due to hyperinsulinism[26]. The hyperinsulinism is mild and responsive to diazoxide.

2. Transient neonatal hyperinsulinism

(a) Infant of diabetic mother (IDM). Fetal hyperinsulinism secondary to maternal hyperglycemia. Features include LGA birth weight. Usually resolves in 1–2 days. Rx: early feeds, IV dextrose.

(b) Perinatal stress-induced hyperinsulinism [27–29]. Mechanism unknown. Associated with SGA birth weight, birth asphyxia, maternal toxemia; median age of resolution is 6 months. Clinically undistinguishable from the congenital forms with high glucose requirements to maintain euglyce-

mia (up to 20–30 mg/kg/min). Rx: diazoxide, glucagon, continuous IV dextrose.

3. Neonatal hypopituitarism: Features may mimic hyperinsulinism, including high glucose requirement, low FFA and BOB, glycemic response to glucagon. Suspect espe-cially with midline malformations of face, microphthalmia, and micropenis. May be associated with laboratory features of cholestatic liver disease. Rx: replacement therapy for deficient hormones.

D. No acidemia with suppressed ketones, but elevated free fatty acids
   1. Examples presenting usually beyond the newborn period include genetic fatty acid oxidation and ketogenesis defects (medium-chain acyl-CoA dehydrogenase, MCAD, deficiency is the most common of these) (see Fig. 27.4) [30–33]. 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 [34]. 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 [35].
   2. Normal neonates: Gluconeogenesis and ketogenesis are poorly developed at birth, accounting, in part, for the high risk of hypoglycemia in the first 12–24 h of life in all groups of newborns. The defect in ketogenesis has been shown to involve developmental delays in both CPT-1 and HMG-CoA synthase for the first 12 h of life: first feedings containing fat may aid in CPT-1 development through induction of transcription by long-chain free fatty acids [36, 37]. It is possible that insulin dysregulation may also contribute to the risk of hypoglycemia in normal neonates.

Rare postprandial hypoglycemia disorders in pediatrics: The following is the sole exception to the rule that all of hypoglycemia in pediatrics is fasting:

1. Post-fundoplasty hyperinsulinemic hypoglycemia: This occurs frequently in infants following surgery for gastroesophageal reflux [38]. It may be severe enough to cause seizures and brain damage. 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 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 [39]. This is the sole circumstance in pediatrics that warrants an oral glucose tolerance test to demonstrate the exaggerated glucose swings. Rx: frequent feedings, reduced high glycemic index foods, inhibitors of gastric motility; the alpha glucosidase inhibitor, acarbose, may be useful as a means to delay digestion and absorption of complex carbohydrates [40].

Therapeutic Goals for Managing Hypoglycemia

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 [4]. Delays in development of fasting systems make low glucose common in the first day of life. However, as noted above, the therapeutic targets for blood glucose should not be set lower in neonates than in older children. Ideally, treatment should maintain normoglycemia on a normal feeding schedule for age. Neonates who are suspected to have hypoglycemia persisting beyond the first day after birth should be tested for ability to fast >10–12 h: older infants for >16–20 h. It is advisable to periodically reassess efficacy of treatment of any form of hypoglycemia by a formal fasting study on treatment.

Selected Drugs for Hypoglycemic Disorders

- Dextrose (emergency Rx): IV 0.2 g/kg bolus (2 mL/kg of D10%), followed by D10% continuous. Children with hyperinsulinism may need infusion rates of glucose as high as 20–30 mg/kg/min.
- Glucagon (emergency Rx only in case of insulin-induced hypoglycemia): 1 mg SQ or IV can be used as a continuous intravenous infusion, a temporary measure to reduce glucose requirements in the hospital.
- Diazoxide: 5–15 mg/kg/day divided into two oral doses. Start with at least 10 mg/kg/day to test efficacy and increase to 15 mg/kg/day, if necessary. Responders usually require 10 mg/kg/day (10 mg/kg/day) or less. Fasting tolerance should be assessed after 5 full days of therapy (since the half-life is 24–36 h). The major side effect is fluid retention. Concomitant use of a diuretic should be considered, especially in infants receiving intravenous fluids.
- Octreotide: 5–15 μg/kg/day SQ divided q 6–8 h or continuous IV. Tachyphylaxis is commonly encountered, unfortunately [41]. Because of recent concerns about necrotizing enterocolitis in neonates treated with octreotide [42] and given the lack of lasting response in most cases, we advise against its use in young infants with severe hyperinsulinism that will require surgery anyway (i.e., K_{ATP} hyperinsulinism).

Figure 27.8 depicts the management approach for children with hyperinsulinism and other causes of hypoglycemia.

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 (NB: Standard bedside glucose meters are not accurate enough). The fast usually begins with the 8 pm 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 closely (e.g., every 3 h until <70 mg/dL, every 1 h until <60 mg/dL, then every 30 min to end): Obtain additional "critical" specimens for key fuels and hormones every 4–6 h and, especially, at the end of fast. End the test at plasma glucose <50 mg/dL or 36 h (24 h if <1 year old) or any worrisome symptoms (can end early if bedside measurement of BOB indicates values >2.5 m Mor urinary ketones = "large" in 2 subsequent occasions). If considering hyperinsulinism, it may end with glucagon stimulation test, 1 mg IV, to test liver glycogen reserve (at plasma glucose <50 mg/dL, appropriate glycemic response <30 mg/dL within 15–30 min after glucagon; glycemic response above 30 mg/dL is consistent with hyperinsulinism) [3]. Critical specimen assays should include lactate, FFA, BOB, and insulin. Include additional blood or urine tests depending on suspected diagnosis, e.g., serum HCO3, plasma GH and cortisol, plasma NH3, plasma acyl-carnitine profile, plasma total and free carnitine, and urinary organic acid profile. *CAUTION*: Fasting tests are potentially hazardous provocative tests that, like water deprivation tests, must be closely monitored for patient safety; sudden deaths during fasting tests have been reported in patients with fatty acid oxidation defects. The latter patients 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.

Other Tests

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. MCAD deficiency is particularly common (1/5,000) and easily detected by this method [35].
2. Genetic testing: 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. Genetic

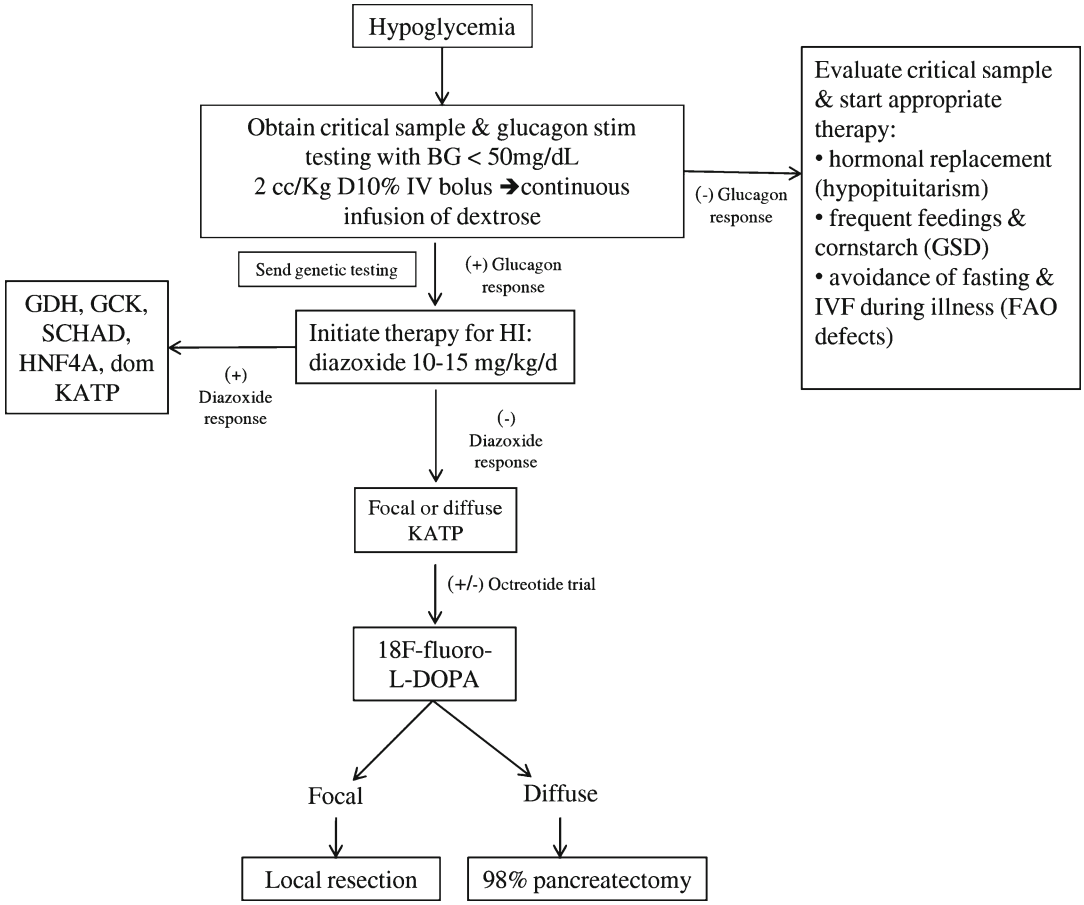

Fig. 27.8 Management approach to the child with hypoglycemia

testing for the most common forms of hyperinsulinism (*ABCC8*, *KCNJ11*, *GCK*, *GLUD1*) is available in commercial laboratories and should be obtained as soon as the diagnosis is confirmed in cases that may require surgery.

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.

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Diabetes Mellitus in Children and Adolescents

28

Kristin A. Sikes and William V. Tamborlane

Abstract

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 metabolic derangements linked to diabetes, including hyperglycemia. Hyperglycemia in turn, plays a key role in the microvascular and macrovascular complications of diabetes.

Keywords

Type 1 diabetes • Type 2 diabetes • Insulin pump • Hypoglycemia • Diabetic ketoacidosis • Insulin therapy

Introduction

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 metabolic derangements linked to diabetes, including hyperglycemia. 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, particularly in the high-risk population of overweight/obese adolescents of Hispanic, Native American, and African-American descent.

T1D occurs when pancreatic β-cells are destroyed in an autoimmune response that is currently the focus of many research studies. This autoimmune-mediated cellular destruction ultimately leads to a complete absence of endoge-

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Table 28.1 Diagnosis of diabetes

Fasting plasma glucose ≥126 mg/dL (7.0 mmol/L)
or
Plasma glucose ≥200 mg/dL (11.1 mmol/L) at 2 h on an oral glucose tolerance test
or
Random plasma glucose ≥200 mg/dL (11.1 mmol/L) *with* classic symptoms of hyperglycemia
or
A1c ≥6.5 %—this test should be performed using a method, that is certified by the National Glycohemoglobin Standardization Program and is standardized to the Diabetes Control and Complications assay

nous insulin secretion. Children with T1D are completely 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 beta cells are progressively destroyed in the autoimmune attack. Once the critical mass of β-cells falls below a given threshold, children with T1D typically present with acute symptoms of polyuria, polyphagia, and weight loss and, if these symptoms go unrecognized, ketoacidosis.

T2D results from impairments in systemic sensitivity to insulin due to obesity, ethnicity, and puberty in association with progressive β-cell dysfunction. This complicated pathogenesis results in some retention of endogenous insulin secretion, but the levels are low, especially in relation to ambient glucose levels. 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.

Diagnosis

Regardless of the type of diabetes, the current guidelines from the American Diabetes Association for the diagnosis of diabetes are in Table 28.1 [1].

Treatment of T1D

The treatment of T1D in children and adolescents presents unique challenges to pediatric healthcare 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. 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 patient 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 diabetic ketoacidosis. 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. Frequent phone follow-up is an important aspect in the care of newly diagnosed patients, and we speak with our newly diagnosed families on a daily basis for the first 2–3 weeks after diagnosis.

Once out of the newly diagnosed phase, regular follow-up visits approximately every 3 months are recommended [2]. 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 hemoglobin A1c (A1c) provide a means of evaluating glycemic control. A point of care method (such as the Affinion or DCA) 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 in as a diabetes "report card" to provide feedback on 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.

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 reduced the risk for retinopathy and the development of microalbuminuria [3–5]. These findings were supported and extended by the follow-up of the DCCT cohort in the EDIC study. Current standards of care mandate that glycemic control be as "close to normal as safely possible" [1], and we follow ISPAD recommendations which set an A1c goal of <7.5 % across all age groups in pediatrics. Nevertheless, it is important to individualize the treatment plan to meet the specific needs of each child. Intensive treatment places extra burdens on patients and their families and practical considerations such as acceptability of and compliance to the treatment regimens must be balanced appropriately in order to achieve treatment goals.

Insulin Management

Once so simple, the choice of insulin has become much more complicated. Current insulin options include standard human regular, neutral protamine Hagedorn (NPH), the newer analogs, and premixed combinations of these insulins. Insulin is available in a standard concentration of 100 U/mL (U-100). Regular insulin is also available in a U-500 concentration for patients (usually with T2D) who require 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.

Currently there are three rapid-acting analogs: aspart (NovoLog ®), lispro (Humalog®), and glulisine (Apidra®). The 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 insulins give better control of postprandial glucose surges and lower rates of late, post-meal hypoglycemia [6].

There are also two long-acting analogs: glargine (Lantus®) and detemir (Levemir®). 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 the subcutaneous interstitial fluid. This leads to precipitation of glargine following subcutaneous injection which delays its absorption into the circulation, creating a "peakless" long-acting insulin [7]. 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 action profile and lower dose-to-dose variability than either NPH or glargine [8].

NPH is the only remaining intermediate-acting insulin on the market today. It is an insulin suspension. 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 [7].

Finally, there are premixed combinations of both human regular and NPH (e.g., Humulin 70/30®) and of rapid-acting analogs and NPH (e.g., NovoLog 70/30® or Humalog 50/50®). These types of insulin may not be as effective in the treatment of type 1 diabetes as it is difficult to achieve the necessary 24-h insulin coverage.

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 insulin management with multiple daily injections of insulin could be used to optimize blood glucose control [3, 5]. 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 BG levels must be assessed in order to determine the regimen which 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.

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 insulin concentration. 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, 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 when compared to NPH-based regimens [7]. A drawback to this type of regimen is that the flat time-action profile of basal insulin makes it imperative that patients take their pre-meal boluses. In this type of regimen, hungry adolescents can expect to take upward of 6–8 injections per day for basal and meal/snack coverage. This can quickly become a problem as the difficulty of daily administration of pre-meal injections accounted for the findings in a study in which adolescents who were randomized to glargine-based basal–bolus regimen had a higher A1c than those randomized to the insulin pump [9].

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 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 [10].

To achieve rapid improvements in glucose control, most patients are started on a total daily dose of 1 U per kilogram body weight per day. During the 2–3 weeks following discharge, insulin doses are titrated toward target pre-meal glucose values of 70–120 mg/dL during daily telephone contacts. The patients are 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.

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 and leads to a loss of endogenous insulin secretion, blood glucose levels become more labile. 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. 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 [11], and extra physical activity during the day contributes to low glucose concentrations on the following night.

Insulin Pumps

Educational materials regarding continuous subcutaneous infusion (CSII) pump therapy are provided during initial diabetes education, and some families express interest in switching to pump therapy after only 2–3 months of injection therapy. In other patients, the decision to switch to CSII occurs later, commonly due to increasing difficulties in maintaining adequate glycemic control, which is usually reflected by increasing variability of prebreakfast and predinner blood glucose levels. Readiness for insulin pump therapy is assessed by our clinicians and both the clinician and family play a role in the decision to switch 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 10 years that there has been a substantial increase in pump use in pediatrics. Insulin pumps use only one type of insulin, most commonly the rapid-acting analogs as they have been associated with better blood glucose control, particularly postprandially, and fewer episodes of hypoglycemia than human regular insulin [12]. Current technology allows for the delivery of doses as small as 0.025–0.05 U of insulin.

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. 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.

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.

The second method of insulin delivery is the "bolus" or immediate burst of insulin that the patient delivers at mealtimes to correct hyperglycemia. There are two types of boluses: the meal bolus, designed to cover the carbohydrate content

of a meal, and the correction bolus, designed to return blood glucose levels to their 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. These bolus calculators only suggest the dose of insulin based on meal size and blood glucose values; it is then up to the patient or caregiver to determine whether this amount should be adjusted based on previous or anticipated exercise or on overall blood glucose trends.

When the pump's 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., 1 U/10 g of carbohydrates) 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 [13]. However, delivery of the bolus after 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 [14].

For a correction bolus, the pump's computer is programmed with correction or sensitivity factor (e.g., 1 U drops the BG 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. In the case of a value that is above target, additional insulin is given to lower the high reading. In the case of a value that is below target, insulin is subtracted from a meal or snack bolus in order to raise the low reading. The actual blood glucose value is entered into the pump either manually or by wireless transmission from the glucose meter into the pump. The pump will then recommend a dose of insulin to return the blood glucose level to the target range.

Insulin pump therapy is associated with improved glycemic control and improved quality of life for children with T1D and their families. However, it is important to remember that prolonged interruption of insulin delivery from a pump, on the order of several hours, may result in the development of ketones and progression to ketoacidosis [15]. Therefore, children and their families must be made aware of the pros and cons of insulin pump therapy. In our clinic, patients typically transition to insulin pump therapy whenever glycemic control warrants or when patients and their families express a need for an improvement in quality of life. When discussing pump therapy, the age of the child is of only minor importance. In fact, we prefer to use CSII in the very young infant and toddler as we have demonstrated durable improvement in both A1c and hypoglycemic risk over 2–4 years of treatment [16]. 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.

Adjusting Insulin Doses

Regular self-monitoring of blood glucose (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 5–10 % of laboratory measurements [17, 18]. 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 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 [19].

We recommend that children test their blood glucose levels at least four times daily, before meals and at bedtime. The results should be kept

Table 28.2 Continuous glucose monitoring

Improved overnight control
Hypoglycemia alarms
Retrospective data to optimize overnight basal insulin needs
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

in either a written logbook or an electronic form for regular review by patients and parents. Typical targets for SMBG include 80–120 mg/dL pre-meals and <180 mg/dL 2 h after meals. However, these targets may be altered based on individual need. Regular SMBG allows the family and clinicians to keep up with the ever-changing insulin needs of children and adolescents. It also allows for dose-to-dose corrections for measurements that are outside of target range.

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 [20]. 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 1–5 min which can then be used for in the moment or retrospective changes to the diabetes regimen. See Table 28.2 for possible uses of CGM in day-to-day management of T1D.

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 use of CGM than with SMBG alone [21, 22]. 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 [23]. The JDRF CGM RCT also showed that youth who wore the sensor almost every day achieved the same benefits as adult with respect to changes in A1c [21, 23]. Unfortunately, many fewer pediatric patients were able to use CGM consistently enough to receive these benefits over the long run [21]. The take home message from these trials is that in order to obtain benefits from CGM, it must be worn on an almost daily basis. Education is the cornerstone for success with this technology. 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.

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 [3]. Further, adolescence itself was an independent risk factor for severe hypoglycemia [4]. Most severe hypoglycemic events occur in the overnight hours, likely due in part to sleep-induced defects in counter-regulatory hormone responses to hypoglycemia [11]. Hypoglycemia represents the most 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 with 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 exogenously supplied insulin cannot be adjusted in response to falling glucose levels and they lose the ability to secrete glucagon in response to hypoglycemia. Thus, patients with T1DM 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 as any plasma glucose of 70 mg/dL or less, whether accompanied by symptoms or not [1], but 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 a patient has sufficient cognitive impairment at 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 3–4 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 injection of glucagon (0.5–1 mg) or IV glucose infusion may be required.

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.

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 [24]. 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 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, an insulin dose is 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 and often varies throughout the day. 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 require an individual-

ized 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.

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 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 [25]. 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 [26]. 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 [27]. Dropping overnight basal rates on "active days" may also help ward off the specter of nocturnal hypoglycemia. Serious athletes may need to reload on carbohydrates following intense and/or prolonged activity in addition to adjusting their insulin doses.

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 which need to be considered when designing the diabetes management plan Thus, trial and error is still a key component in the management of exercise and glucose levels in children with diabetes.

Sick Day Management

Children with intercurrent illnesses, such as infections or vomiting, should be closely monitored for elevations in blood glucose levels and ketonuria. On sick days blood glucose levels should be checked every two hours, and the urine should be checked for ketones with every void. 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, 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 at least 50 % and may also need to temporarily discontinue it and instead give small doses of rapid-acting insulin every 2–3 h. Doses of rapid-acting insulin can range from 10 to 20 % of total daily dose, depending on level of hyperglycemia and ketonuria [28]. 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.

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 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 traditionally meant achieving optimal control. Increasingly however, optimizing quality of life has also become a measure of successful adaptation [29]. 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, presence of maternal depression, and age can all impact a family's ability to cope with diabetes and to achieve successful outcomes [29]. 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.

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 [30]. 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 [31]. 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 women with diabetes are more likely than their counterparts without diabetes to suffer from disordered eating as well as more serious eating disorders [32]. The withholding of insulin is a technique that is unique to people with diabetes, as a way of purging excess calories. 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 [33]. Women who chronically use this method of purging are at risk for both the acute complication of diabetic ketoacidosis as well as more chronic complications associated with long-standing poor glycemic control. The first step in 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 and strict exercise regimens, and potentially repeated problems with ketonuria and/or ketoacidosis [32]. 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.

Associated Autoimmune Conditions

When treating a child for type 1 diabetes, care should also include evaluation for several other autoimmune conditions. Autoimmune thyroiditis is the most common co-condition with type 1 diabetes. According to the International Society for Pediatric and Adolescent Diabetes, up to 25 % of children with diabetes will exhibit antithyroid antibodies [34]. Current recommendations for evaluation in all children with T1D include obtaining antithyroid peroxidase and anti-thyroglobulin antibody levels at time of diagnosis with diabetes as well as measurement of thyroid stimulating hormone levels every 1–2 years or whenever there is a change in a child's growth and development or signs and symptoms of thyroid disease [1].

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 [34], most youngsters do not have any symptoms. Current guidelines recommend screening for celiac shortly after diagnosis with either antiendomysial or tissue transglutaminase [1] antibodies. A total IgA should be a part of the assessment as children with low circulating levels of IgA will have a false negative screening result. Children with a positive screening test for celiac should be referred to a pediatric gastroenterologist where a small bowel biopsy will confirm the diagnosis. 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 or adrenal insufficiency.

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.

Currently hypertension is defined as three consecutive blood pressures higher than the 95th percentile based on age, gender, and height, and prehypertension 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.

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 been normalized. For those children without a 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. If after these treatments the LDL remains >130 mg/dL, pharmacological treatment with a statin in children older than 10 years is indicated. For the younger age group, it is acceptable to consider referral to a pediatric lipid disorder specialist for further treatment.

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. As with retinopathy guidelines, evaluation for microalbuminuria, an early indication of nephropathic changes, does not need to begin until the child with T1D is 10 years old and has had diabetes for at least 5 years. Screening microalbuminuria should be done with a spot urine for albumin-to-creatinine ratio. An initial positive result is repeated at least two more times on separate occasions, and if remains persistently elevated, treatment with an ACE inhibitor is warranted.

Treatment of T2DM

In recent years, T2D has become an increasing concern for the health-care providers of children and adolescents. Current estimates put the incidence of T2D between 8 and 12 % for children ages 10–19, with some high-risk populations such as Native American adolescents approaching incidence rates of 50 % [35]. Risk factors for the development of T2DM 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 Native American background.

Insulin resistance (IR) and the inability of β-cells to fully compensate by increasing insulin secretion is 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 those with T2D, the β-cells are unable to meet the increased demand from the body. Genetic, environmental, and physiologic factors all contribute to the development of insulin resistance. Most children with T2DM are diagnosed during adolescence, which itself is a time of natural insulin resistance. This natural state coupled with the abnormal insulin-resistant state of prediabetes overburdens the β-cells and in some cases can lead to beta cell burnout, further pushing apart insulin supply from demand. Acanthosis nigricans, a darkening and thickening of the skin which can be found on the body in areas of folds and creases (e.g., neck, axillae, and groin), is a cutaneous indication of increase 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.

The first step in treating T2D is to identify the diabetes as quickly as possible, preferably by screening. For those patients who fall into a high-risk category, screening for diabetes should be a part of routine health care. Current recommendations for screening are to begin at age 10 years (or with the onset of puberty) in any overweight children with at least two of the following risk factors: history of T2D in a first- or second-degree relative, signs of IR, from one of the high-risk racial or ethnic backgrounds, or their mother was diagnosed with gestational diabetes [6]. Screening may be done with an A1c, a fasting plasma glucose, or an oral glucose tolerance test.

Treatment principles for T2D in children involve glycemic control, weight loss, increased physical activity, and control of comorbid conditions [36]. Initial techniques to achieve normoglycemia are dependent upon the severity of hyperglycemia at time of presentation. Children who have marked hyperglycemia, with or without ketosis, at time of presentation require insulin in order to correct metabolic abnormalities and limit glucotoxic effects of hyperglycemia on β-cell function. For those patients who present asymptomatically, initial treatment often involves a dual-prong approach involving behavior modifications and oral medications.

Behavior modifications are aimed at reducing caloric intake while simultaneously increasing caloric expenditure. Current dietary recommendations include elimination of sugar-containing beverages, eating breakfast every day, limiting portion sizes to those appropriate for age, reducing frequency of meals taken at fast food restaurants, and limiting screen (computer, TV, and video games) time to 2 h daily [37]. Exercise recommendations are for at least 60 min of moderate activity on a daily basis. It is important to remember that a stepwise approach incorporating the needs and desires of the patient and family is more likely to be successful than attempting to incorporate all behavior modifications at the same time. Further, a multidisciplinary approach involving professionals from medicine, nursing, mental health, nutrition, and exercise physiology is essential in order to provide patients with the best opportunity for success.

Although there are numerous oral agents available to treat T2DM in adults, relatively few of them are used in pediatric patients. The most commonly used agent, and the only one to have FDA approval for use in pediatrics, is metformin. This drug has the ability to decrease hepatic glucose production, has a favorable impact on weight loss or reduced weight gain, has a favorable safety profile, and is inexpensive. In one study, gastrointestinal side effects of cramping, nausea, and diarrhea were seen in up to 25 % of subjects but tend to only appear with initiation of the medication and subside both with time and with slow titration from 500 mg/day to a total daily dose of 2,000 mg/day [38].

Other oral medications in use in pediatrics include thiazolidine (TZD) class of peripheral insulin sensitizers (i.e., pioglitazone). The TODAY study is a multicenter randomized control trial being completed throughout the USA which is comparing use of behavior modification, metformin, and metformin with rosiglitazone in the treatment of T2DM in children. Once the data from this study are evaluated, we will have a better understanding of best treatment options for children with T2D.

As with T1D, it is important to evaluate all patients for comorbidities and to manage these conditions as well. In fact, there are several other conditions that are associated with the insulin resistance that is as the core of T2DM in children. These conditions include obesity, dyslipidemia, hypertension, ovarian hyperandrogenism, nonalcoholic fatty liver disease, and micro-/macroalbuminuria [36]. Evaluation of the study population of the TODAY trial reveals that more than one quarter of the adolescents with T2D also had borderline high blood pressure (>90th percentile for age, height, gender), 13 % showed signs of microalbuminuria, and nearly 80 % showed low levels of high density lipoprotein with or without high triglycerides [39]. Evaluation for these comorbidities should be done at time of diagnosis and at least annually thereafter. Management of these conditions is complex and involves a multifaceted approach incorporating lifestyle changes, behavior modification, and pharmacological interventions. Treatment of T2DM in children and adolescents is quickly evolving, and novel treatment options such as use of insulin pumps or bariatric surgery are fast coming to the forefront.

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Type II Diabetes Mellitus and Obesity in Youths

29

Cosimo Giannini and Sonia Caprio

Abstract

Type 2 diabetes (T2D), once considered an illness restricted to adults, is progressively affecting more and more adolescents as population rates of obesity increase. Estimates suggest that T2D represents 20–25% of new-onset cases in adolescents and that certain ethnic or racial groups are disproportionately affected. Its onset during adolescence represents a serious health burden as T2D shortens life expectancy and is associated with serious medical complications. Thus, effective treatments are urgently needed for youths who face the possibility of experiencing these complications at an earlier age than their adult counterpart. Therefore, the complete characterization of the pathophysiology of the disease represents a key element for assessing its risks and determining factors.

Keywords

Type 2 diabetes • Childhood obesity • Pathophysiology of type 2 diabetes • Insulin resistance • Beta-cell function • Therapy for T2D • Therapy for childhood obesity

Introduction

Diabetes mellitus is one of the leading chronic diseases of childhood affecting 1.82 out of every 1,000 young people in the United States [1]. Although until a few decades ago, type 1 diabetes mellitus (T1D) accounted for almost all cases of diabetes in childhood, relevant changes in the prevalence of type 2 diabetes mellitus (T2D) have recently emerged in parallel with the worldwide "obesity epidemic" that has included both the developed and the developing nations [2–4]. Although obesity and in particular obesity with ectopic fat accumulation is a major risk factor, other important contributing factors such as genetic, gender, ethnic background, pubertal stage, and the "toxic environment" are also important triggers for T2D in youths. In this chapter, we review the most recent studies pertinent

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to the epidemiology and pathophysiology of T2D in youths. In addition, practical approaches to diagnosis and treatment of T2D in obese youths are discussed, and final considerations on how to prevent this twin epidemic are offered.

Worldwide Epidemiology of T2D in Youths

As outlined in the American Diabetes Association (ADA) position paper on T2D in children and adolescents [5], classification of diabetes type in youths is not straightforward. Individuals with clinically diagnosed T1D may lack evidence for diabetes-related autoimmunity, and individuals with a clinical diagnosis of T2D may have positive diabetes-related autoantibodies [6, 7]. Due to these challenging issues, the existing data on the prevalence of T2D may lack precision and thus underestimate ultimately the true prevalence of the disease. Limited population-based studies in youths are reported; thus, most of the existing epidemiologic information is mainly from case series or hospital studies particularly for the American children and adolescents.

Indeed, the available data to date clearly support the finding that prevalence and incidence estimates for T2D are consistently higher in the USA and Asian countries compared to Europe and in nonwhite populations. The SEARCH for Diabetes in Youth Study found a prevalence of T2D of 0.19, 1.05, and 1.74 per 1,000 among 10–19-year-old non-Hispanic whites, African-Americans, and American-Indians, respectively [1]. However, these studies relied on physician-diagnosed cases only [1]. In Pima Indians from Arizona and in First Nations from Manitoba, T2D was diagnosed in 22.3–50.9/1,000 among 10–19-year-old adolescents [8]. In addition, in a US cohort of eighth-grade students who were predominantly a minority (52.7% Hispanic whites, 23.2% African-Americans), the prevalence of impaired glucose regulation and T2D reached 40.5/1,000 [9].

In the SEARCH study [6], the incidence rate (per 100,000 person-year) of T2D among children and adolescents varied greatly by ethnicity, with the highest rates observed among 15–19-year-old minority populations. In particular, the reported incidence rate was 49.4 for Native Americans, 22.7 for Asian/Pacific islanders, 19.4 for African-Americans, 17 for Hispanics, and 5.6 for non-Hispanic whites [6].

T2D risk in youths has also been described worldwide, including Asiatic and European children and adolescents. Relevant information on the prevalence of T2D in the Asiatic population has been reported by the two largest studies to date available in youths conducted in Japan and Taiwan. In these studies, ~9.2 and ~2.9 million school children were screened for glycosuria. Interestingly, the reported annual incidence rates were 2.55/100,000 in the Tokyo metropolitan area [10] and 6.5/100,000 in Taiwan [11].

Although for most European countries, population-based data on T2D prevalence or incidence in youths are not available, less alarming reports have been extracted by several studies in contrast to the data reported in the USA or in Asia. In particular, a recent survey conducted in German youths estimated a T2D prevalence of 0.02/1,000 in the age range from 0 to 20 years accounting for nationwide cases of about 500 adolescent T2D in total [12]. In addition, the combined prevalence of T2D and impaired glucose regulation was reported to be 25/1,000 [13] in a cross-sectional survey among school-leaving students (mean age 15.5 years) of below-average socioeconomic status and above-average weight in Dusseldorf (Germany). Similar data have been described in white youths in the UK showing an estimated annual incidence of T2D of 0.35/100,000 [14]. It should be noted that most of the described T2D subjects in youths in the UK are among adolescents from Southeast Asia, again pointing out a clear ethnic difference in this disorder that clearly affects more certain ethnic groups.

Pathophysiology of Altered Glucose Metabolism in Childhood

T2D represents the end of a spectrum of altered glucose metabolism that includes at least two prediabetic conditions: impaired glucose tolerance

(IGT) and impaired fasting glucose (IFG) [15, 16]. As not all those with a prediabetic condition progress to develop T2D, the prevalence of these prediabetic conditions is much greater than that of overt diabetes.

In adults, the progression rate of IGT to diabetes is estimated at 5–8% per year [17]; little is known about the natural history of IGT in children. As well, due to gradual characteristic of the phenomenon, a time gap of 5–10 years has been reported [17, 18]. The breakdown of the physiological balance between insulin sensitivity (related to the insulin activity in the peripheral tissues) and insulin secretion (related to the beta-cell function) appears to be the key pathophysiological factor necessary for the development of IGT as well as T2D [19]. However, although both insulin resistance and beta-cell secretion are the two primary steps in the natural course of T2D, to date the debate is still open on their temporal relationship in the course of the disease. In particular, studies are still needed, both in adulthood as well in childhood, to completely define the strict sequence of development of these abnormalities as well as the causes of failure of beta-cells or the nature of the signals from the insulin-resistant tissues that fail to induce an appropriate beta-cell response.

Insulin Resistance: Importance of Ectopic Fat

Obesity represents the major and most common cause of insulin resistance in the pediatric age-group, regardless of ethnicity [20]. In fact, 55% of the variance in insulin sensitivity among Caucasians [21] has been shown to be explained by obesity [21]. In addition, it accounts for the 29.1% of the variance in homeostasis assessment model of IR (HOMA-IR) constituting the major risk factor for IR independent of age, gender, or ethnicity in the National Health and Nutrition Examination Survey (NHANES) 1999–2002 data [22].

The presence of insulin resistance occurs early and is present in obese adolescents with IGT as well as adolescents with T2D. However, the reduction in insulin sensitivity is more pronounced in those with full-blown diabetes when compared with their obese nondiabetic peers [23]. Therefore, these data probably suggest that obese children and adolescents develop worsening insulin resistance during the transition from normal to IGT. Importantly, the distribution site of adiposity storage rather than the degree of obesity per se represents the most important determinant of the degree of insulin resistance [24–28]. In particular, by comparing equally obese (defined by a body mass index and percent body fat within the same range) youths with normal and with IGT, the differences in peripheral insulin sensitivity were accounted for by altered partitioning of fat in the abdominal cavity and by the increased intramyocellular lipid content [29]. Although the reciprocal role of visceral and subcutaneous fat in the development of insulin resistance needs still to be completely clarified [24], Cruz et al. [28] showed a direct impact of visceral fat accumulation on insulin sensitivity and secretion, independent of total body adiposity, in obese children with a family history of T2D. Interestingly, in obese adolescents with high proportion of visceral fat and relatively low abdominal subcutaneous fat, a significant increase in 2-h glucose and insulin resistance (homeostasis model assessment) and decrease in insulin sensitivity (Matsuda index) have been described [24]. Therefore, adolescents at risk for developing alterations in glucose metabolism are not necessarily the most severely obese, but are characterized by an unfavorable lipid partitioning profile. Of particular importance is the lipid accumulation in the liver that is often present in subjects with T2D, suggesting perhaps a critical role of the liver in the development of this metabolic disease [30, 31]. Fat accumulation in the liver is becoming a common complication in pediatric obesity and is strongly associated with the alterations in glucose and lipid metabolism, possibly because of the presence of insulin resistance [32]. The mechanisms responsible for the interrelationships between fatty liver disease and insulin resistance are not clearly understood; in fact, it remains unclear whether hepatic steatosis is a consequence or a cause of derangements in insulin

sensitivity. As recently shown by our group, the severity of fatty liver, independent of obesity, is associated with the presence of prediabetes, with an impairment of beta-cell function and with rising levels of insulin resistance in obese adolescents [31]. Of note is the fact that in those studies, fatty liver accumulation rose in parallel with increasing visceral fat as well as intramyocellular fat [31]. Therefore, from those earlier studies, it was virtually impossible to assess the independent contribution of the liver to the development of insulin resistance, since both visceral fat and intramyocellular fat are also known to modulate insulin sensitivity [32, 33]. Studies by D'Adamo et al. [34] examined the exclusive role of fatty liver in the alteration of insulin sensitivity and beta-cell function in two groups of obese adolescents, differing for the amount of hepatic fat content (%HFF), but characterized by the same distribution of abdominal and muscle fat and overall degree of obesity. In the present study, we found that obese adolescents with high liver fat content, independent of visceral and IMCL, had (1) impaired insulin action in the liver (reduced basal hepatic insulin sensitivity and during the low-dose insulin infusion), in the muscle (reduced insulin-stimulated glucose disposal), and adipose tissue (reduced basal adipose tissue sensitivity index); (2) early defects in beta-cell function, as shown by the low disposition index; and (3) low adiponectin levels [34].

These results strongly suggest that the liver has a central role in the complex phenotype of the insulin resistance state in obese adolescents with fatty liver. Results from our study are consistent with those from Fabbrini et al., showing in adult obese subjects that intrahepatic fat, not visceral fat, is linked with metabolic complications of obesity [30]. We expand on this theme by not only showing that the visceral fat may just be a marker of insulin resistance but that IMCL lipid content may be also an innocent bystander.

Beta-Cell Function Across the Spectrum of Glucose Tolerance in Obese Youths

The progressive decline in beta-cell function has been also shown to be one of the main determinants in the development of T2D [19]. In fact, until the beta-cell fails to compensate appropriately for the peripheral insulin resistance state, diabetes as well as prediabetes do not develop. The ability of the betacell to balance its secretion in response to the ambient level of insulin sensitivity is finely regulated by multiple factors, including beta-cell mass as well as beta-cell secretory capacity [35]. Both genetic and environmental factors are likely also strong regulators of beta-cell function [36]. Therefore, preexisting as well as genetically determined risks significantly influence the progressive loss of beta-cell function stressed by different metabolic derangements such as insulin resistance or lipotoxicity [19, 36].

Available studies evaluating beta-cell function in using the hyperglycemic clamp and including control groups with matched body fat composition showed that obese adolescents with T2D have a marked reduction in both first-phase and second-phase insulin secretion, as shown in Figs. 29.1 and 29.2 [23, 37]. Therefore, similar to those observed in adults subjects [19], almost ~80% of the beta-cell function is reduced or lost already at diagnosis [37]. In addition, differences in beta-cell function have been described in various prediabetic conditions seen in obese adolescents, such as IFG or IGT or the combined IFG/IGT states (Fig. 29.3). Cali et al. documented that IFG, in obese adolescents, is primarily linked to alterations in glucose sensitivity of first-phase insulin secretion. In contrast, those adolescents with IGT are affected by a more severe degree of peripheral insulin resistance and reduction in first-phase secretion. Interestingly, the association between IFG and IGT resulted in a new additional defect in glucose sensitivity of second-phase insulin secretion and by a profound insulin resistance [38]. Data obtained from a longitudinal study further support the presence of a "preexisting" beta-cell dysfunction risk in obese adolescents with normal glucose tolerance [39]. In fact, in a group of obese adolescents with NGT who have repeated serial OGTT over a period of ~3 years (20), those who progress to IGT had a lower beta-cell function at baseline than those who did not progress. Furthermore, the development of IGT was characterized by progressive decline in the DI.

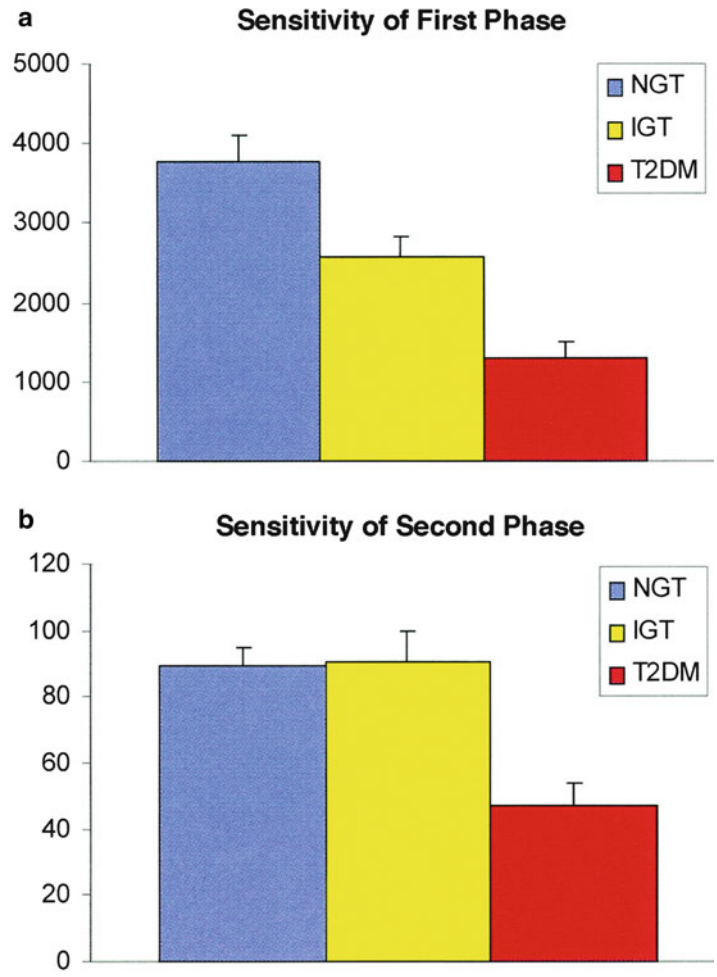

Fig. 29.1 Glucose sensitivity of the first- and second-phase insulin response in NGT, IGT, and diabetic subjects. (**a**) Glucose sensitivity of beta-cell first-phase secretion in adolescents with NGT (*blue*), IGT (*yellow*), and type 2 diabetes (T2DM) (*red*). (**b**) Glucose sensitivity of beta-cell second-phase secretion in adolescents with NGT (*blue*), IGT (*yellow*), and type 2 diabetes (*red*). $p<0.01$ for IGT vs. NGT; $p<0.02$ for type 2 diabetes vs. IGT NGT= Normal Glucose Tolerance; IGT= Impaired Glucose Tolerance (Copyright 2005 American Diabetes Association. From Weiss R. et al. Diabetes, 2005;54: 1735–1743. Reprinted with permission from the American Diabetes Association)

Thus, obese adolescents who progress to IGT manifest lower values of DI and a further progressive impairment of its levels than those who do not experience a worsening of glucose tolerance supporting the role of preexisting beta-cell dysfunction risk [39]. In addition, in adolescents with normal fasting plasma glucose, the impairment in beta-cell function relative to insulin sensitivity is apparent even within the nondiabetic fasting plasma glucose range in children [40]. In fact, dividing young subjects according to fasting plasma glucose into three categories (≤90, >90 to <100, and ≥100 to <126 mg/dl), beta-cell function defined by the glucose disposition index (given by the product of first-phase insulin and insulin sensitivity) decreased significantly across the three categories as fasting plasma glucose increased [40].

Although further studies are needed in order to completely define the changes in beta-cell throughout the development of T2D, its alterations seem to play a pivotal role in the risk of the disease. Therefore, markers of beta-cell dysfunction should be revealed early in the course of the natural history of T2D in obese adolescents. A further and complete characterization of these risk factors represents therefore a critical element for the prevention of diabetes in youths. In addition, the early presentation of T2D in youths raises the possibility of an accelerated pathophysiological process in these youngsters, compared with adults, thus shortening the

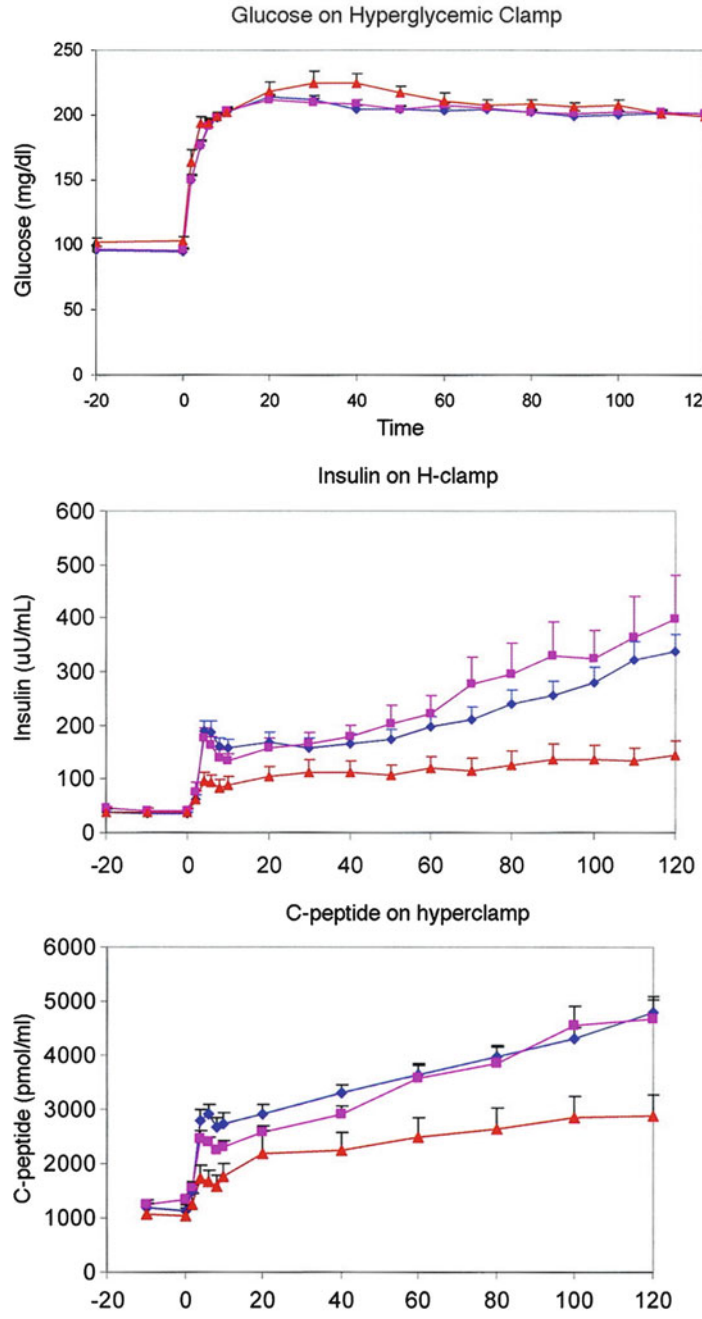

Fig. 29.2 Glucose, insulin, and C-peptide during the hyperglycemic clamp. *Filled diamond*, NGT; *filled square*, IGT; *filled triangle*, type 2 diabetes (T2DM). NGT= Normal Glucose Tolerance; IGT= Impaired Glucose Tolerance (Copyright 2005 American Diabetes Association. From Weiss R. et al. Diabetes, 2005; 54:1735–1743. Reprinted with permission from the American Diabetes Association)

transition time between IGT and diabetes. Therefore, the complete characterization of the pathophysiology of the different transitional phases from normal glucose tolerance to T2D represents an important element for defining those subjects at increased risk and who would benefit most from early interventions.

Available Therapy for T2D and Obesity in Childhood

The characterization of the pathophysiology and determinants of T2D will ultimately guide a more targeted therapy. Fundamental to the eventual prevention and elimination of this metabolic

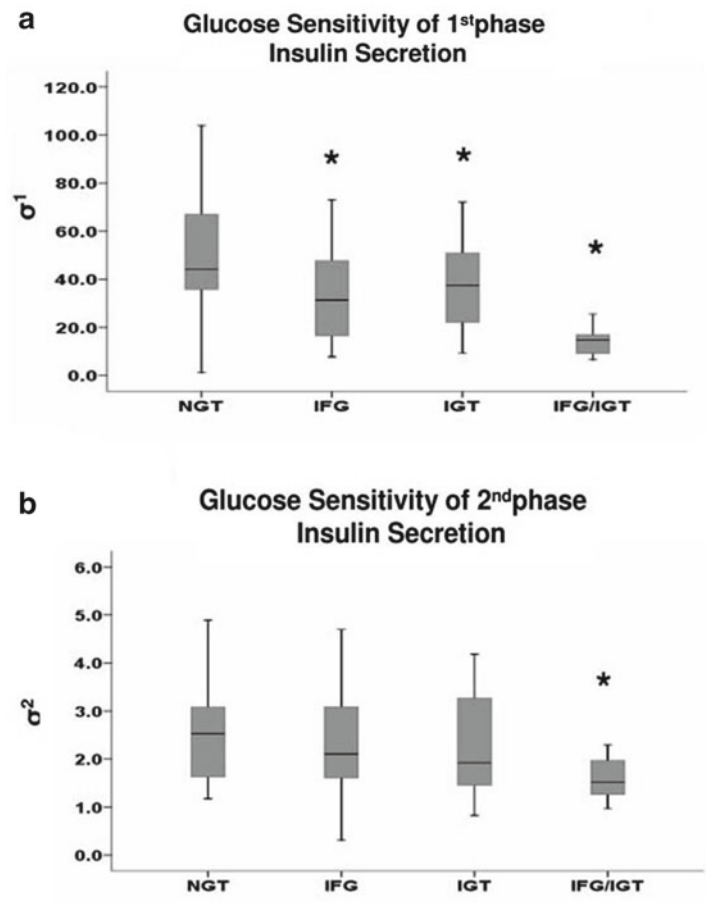

Fig. 29.3 (**a**) Glucose sensitivity of first-phase insulin secretion (σ^1); *$p=0.004$ IFG vs. NGT; *$p=0.04$ IGT vs. NGT; *$p=0.0001$ IFG/IGT vs. NGT adolescents. (**b**) Glucose sensitivity of second-phase insulin secretion (σ^2). NGT= Normal Glucose Tolerance; IFG= Impaired Fasting Glucose; IGT= Impaired Glucose Tolerance (Reprinted with permission from Cali A. et al. J Clin Endocrinol Metab 2008; 93; 1767–1773. Copyright 2008, The Endocrine Society)

disorder in youths will ultimately rely on the elimination of the relevant risk factors that are at the heart of this problem, namely, obesity.

In particular, treatment goals include weight management (by preventing or contrasting factors associated to weight gain), increasing physical activity, achieving glycemic control, and managing comorbidities such as dyslipidemia and hypertension.

Prevention Strategies for T2D and Obesity in Youths

Prevention, especially in young people, is universally viewed as the best approach to reverse the rising global prevalence of obesity with indirect relevant effects on T2D prevention. However, evidence about the most effective means of prevention of obesity development in children is scarce. Preventions should be firstly directed to early life factors that may modulate risk for obesity and development of T2D in youths. Developmental or fetal overnutrition as a result of gestational diabetes or maternal obesity might have contributed to the obesity epidemic [41] and to the elevated risk for T2D [42]. Similarly, a meta-analysis documented a U-shaped association between birth weight and incidence of T2D [43], with a similar degree of excess risk conferred by low birth weight and high birth weight. In addition, breast-feeding appears to be protective against development of obesity and T2D in youths, mediated in part by current weight status in childhood [44].

The American Diabetes Association recommendations indicate that although direct evidence is not available for youths, lifestyle interventions

(i.e., modest weight loss through dietary modification and physical activity) shown to reduce risk for T2D in adults are appropriate for youths, as long as proper allowance is made for normal growth and development [45]. Interestingly, Butte and Ellis calculated that an energy deficit of more than 250 kcal per day is needed to prevent further weight gain in 90% of overweight children; this deficit is equivalent to a child walking an additional 1–2 h per day at 1·9 km/h or consuming roughly a fifth fewer calories than usual per day [46]. Whole-grain intake has been associated with greater insulin sensitivity and lower BMI in adolescents [47]. Fiber in particular also attenuates postprandial glycemic excursions and has beneficial effects on insulin sensitivity, adiposity, and pancreatic function; furthermore, it promotes satiety [48]. Children should be encouraged to add at least five fruits and vegetables per day, eat a healthy breakfast, and minimize high-fat and high-calorie food items [49]. Sweetened beverages should also be minimized or eliminated [49]. If weight loss is not achieved with these interventions, structured meal plans should be planned in conjunction with a nutritionist or dietician [49]. The Diabetes Prevention Program [50] and the Finnish Diabetes Prevention Study [51] provide clear evidence for the efficacy of moderate weight loss to reduce risk for T2D in adult populations. Key dietary goals in these trials included reduced energy intake consistent with moderate weight loss and reduced intake of fat as a percent of energy. Increased physical activity was also a key focus of the interventions. Clinical trials of lifestyle approaches to facilitate weight management via diet and physical activity and improve insulin sensitivity in youths at high risk for T2D have been successful on a small scale. Recent successful intervention approaches have included a school-based program [52], a family-based program [53], and a physician-directed program [54].

Most randomized prevention trials on obese youths have taken place in schools since they are viewed as a universal catchment setting for children. The core features of most prevention programs are to change the caloric content of school meals and encourage physical activity. Gonzalez-Suarez et al. identified 19 high-quality trials of school-based interventions and reported reduced odds of overweight or obesity in intervention compared with control groups (pooled odds ratio 0.74 [95% CI 0.60–0.92]) [55]. Although initiatives have also been aimed at children in kindergarten or nurseries, the few controlled trials in this setting have not yet been systematically reviewed [56]. Some crucial periods during childhood present both challenges and windows of opportunity for obesity prevention because they are associated with notable changes in adiposity accrual or obesity-related behavior. These periods are the first year of life [57], during adiposity rebound (ages 3–7 years), and menarche [58]. The transition from childhood to adolescence is a time of striking behavioral changes, including an abrupt reduction in physical activity [59].

In addition, physical activity has been show to have a relevant impact in improving insulin sensitivity mainly through improved insulin-independent glucose uptake, upregulation of glucose transport-4 (GLUT-4) expression and translocation to the cell membrane, enhancement of glycogen synthesis, increase in oxidative capacity, and the promotion of mitochondrial biogenesis and increased lipid oxidation and turnover [60, 61]. Studies in adolescents have shown that physical activity is positively associated with improved glucose metabolism and resting energy expenditure [62] and negatively associated with insulin resistance-associated metabolic parameters [63]. To promote physical activity, screen time (television and computer) should be reduced to less than 2 h per day in children older than the age of 2 and avoided completely in children under the age of 2 [49].

In a systematic review of randomized controlled trials of treatments for childhood obesity, investigators identified 64 trials, 54 of which assessed non-pharmacological lifestyle interventions. Although some limitations related to the small sample size (16–218 participants, with 70% including fewer than 30 participants) as well as substantial methodological limitations and short-term follow-up, this analysis showed encouraging and provides useful guidance for treatment of

obese children. In particular, this review showed that family-based, lifestyle interventions, with a behavioral program aimed at changing diet and physical activity and thinking patterns, provide significant and clinically meaningful decreases in overweight in both children and adolescents in the short and the long term [64].

Non-pharmacological and Pharmacological Approaches to T2D in Youths

As for adults with T2D, treatment goals for youths with the disease include glycemic control as close to normoglycemia as possible while avoiding episodes of hypoglycemia and reducing other risk factors for long-term complications of diabetes (e.g., hypertension, dyslipidemia, and albuminuria) [65]. Treatment initiated at the time of diagnosis will vary according to clinical presentation, which can range from asymptomatic hyperglycemia to diabetic ketoacidosis [5]. In particular, non-pharmacological measures (diet and physical exercise) represent the first step in approaching youths with T2D. If results achieved are not within the guideline targets for the specific age range, introduction of approved drugs is needed. In particular, insulin and metformin represent the two pharmacological approaches approved for children and adolescent with T2D, though other oral hypoglycemic agents have been proposed.

Insulin therapy is essentially suggested when a child presents with severe hyperglycemia (>200 mg/dl, HbA1c 1 8.5%, and/or ketosis) in order to rapidly achieve metabolic control. Thus, metformin with lifestyle intervention should be started as soon as ketosis and rehydration have been achieved and especially once the diagnosis of T2D is made unequivocally (absent pancreatic autoantibodies) [66]. Accelerated deterioration in beta-cell function may occur in some youths with T2D needing therefore, in some highly selected patients, the early introduction of insulin to achieve metabolic control [67]. Therefore, the aim of preserving beta-cell function represents important additional potential uses of insulin [68]. Metformin represents the main therapy for young people with T2D. In particular, metformin inhibits endogenous (liver) glucose production, mainly gluconeogenesis, and improves insulin-stimulated glucose uptake in peripheral tissues [69] leading to relevant antihyperglycemic effects. In addition, the reported effects on the modest weight loss in overweight T2D patients, on the improved lipid profile, on the increased fibrinolysis [70, 71], and on the decreased transaminases in patients with nonalcoholic steatohepatitis [72] represent useful ability of this drug for the care of children and adolescent with T2D. Metformin gained approval for its use in pediatrics based on a randomized, double-blind, placebo-controlled trial that evaluated the efficacy and safety of the medication, at dosages up to 1,000 mg twice daily in 82 children aged 10–16 years. The participants were treated up to 16 weeks. Metformin significantly improved glycemic control and HbA1c values with no cases of lactic acidosis and minimal side effects [69]. Metformin treatment is therefore prescribed in nonketotic patients starting with a low dose followed by a progressive increase of it during a phase of a 1–3-week period to the final therapeutic dosage of 1,000 mg twice a day. The presence of renal impairment or hepatic or cardiopulmonary insufficiency, or if a patient is undergoing evaluation with radiographic contrast materials (because it may precipitate lactic acidosis), represents important contraindications for its adoption. In addition, alcohol consumption as well as the presence of ketosis represents two very important additional warning advices to be evaluated, especially in adolescents. The most frequently encountered side effect is mild gastrointestinal discomfort which rarely necessitates drug discontinuation.

There are no other oral hypoglycemic agents that have been approved for use in the pediatric population, though rosiglitazone, a potent insulin sensitizer, was evaluated in juvenile-onset T2D. The study included 195 obese T2D children (age range 8–17 years) in a 24-week double-blind, randomized, metformin-controlled, parallel group design. Participants were randomized to rosiglitazone, maximum dosage

of 4 mg twice a day, or metformin, maximum dosage of 1,000 mg twice a day. Median reductions in HbA1c from baseline (rosiglitazone group −0.25%, $p=0.027$; metformin group −0.55%, $p<0.0001$) and from screening (rosiglitazone group −0.5%, $p=0.011$; metformin group −0.5%, $p=0.0037$) to week 24 were statistically significant in both groups. Differences between groups were not statistically significant. The rosiglitazone group gained ~3 kg at 24 weeks [73]. Sulfonylurea (e.g., glimepiride, glyburide, glipizide), which increases both basal and meal-stimulated insulin secretion, has been used in the treatment of T2D in adults for more than half a century. Results from a single-blind, 26-week active-controlled, multinational study randomized 263 obese youths with T2D to receive glimepiride (1–8 mg once daily) or metformin (500–1,000 mg twice daily) for 24 weeks are available [74]. In this study, authors showed no significant difference in HbA1c reduction between the two groups. However, there was a difference in weight gain (kg) (glimepiride, change from baseline 1.97 Kg and 0.26 kg/m^2; metformin, 0.55 Kg and –0.33 kg/m^2; P=0.003 and P=0.005, respectively [74]. During the last years, novel and promising treatment opportunities have been developed. In particular, recent studies in adults with T2D including GLP-1 analogs (exenatide) or amylin analogs (pramlintide) may prove to be beneficial in youths.

Relevant pediatric trials are also underway in order to clarify the effectiveness of some of the available drugs available for the treatment of T2D in youths. In particular, the Treatment Options for Type 2 Diabetes in Adolescents and Youth (TODAY) is a 15-center clinical trial sponsored by the National Institute of Diabetes and Digestive and Kidney Diseases (start date, 2003; projected end date, 2012) that is examining the comparative efficacy of three approaches to the treatment of T2D in youths ages 10–17 years. To date, 1,092 youths (64% female) have been screened; 9 and 704 of these youths have been randomized to one of the three treatment arms: metformin alone, metformin plus rosiglitazone, or metformin plus an intensive lifestyle intervention called the TODAY Lifestyle Program (TLP). Although the overall design of the TODAY trial has been described elsewhere, this chapter details the development of the TLP, a family-based behavioral weight-loss program. To our knowledge, the TLP is the first program of its kind designed to take a comprehensive, continuous care approach to lifestyle modification with severely overweight youths with medical comorbidities (i.e., the median BMI for the screening sample was 34.9 kg m^{-2}, and the median BMI percentile was 98.9, with duration from diagnosis of T2D less than 2 years).

Developing effective treatments for youths with T2D and obesity is especially relevant given that children and adolescents experience the medical complications of these diseases at earlier ages and for longer periods of time than their adult counterparts.

Conclusions

T2D in children and adolescents is a multisystem disease manifesting with early hyperglycemia yet accompanied by a myriad of cardiovascular risk factors. The disease is tightly related to obesity in childhood, specifically to altered lipid partitioning manifesting as increased lipid deposition in the muscle, liver, and visceral compartments. The combination of ectopic lipid deposition, an adverse adipocytokine profile, and possibly low-grade inflammation can tip the delicate balance of insulin sensitivity and secretion to a point beyond which compensation is inadequate and hyperglycemia ensues. Once the compensatory mechanisms against insulin resistance, namely, increased insulin secretion and decreased insulin clearance, are depleted, overt hyperglycemia develops. IGT seems to be the critical point in which diabetes may be prevented, yet the "window of opportunity" is rather narrow as deterioration of glucose metabolism in youths seems to be faster compared to adults. Primary prevention of obesity in childhood and tailored conservative and/or pharmacologic interventions for obese youths with prediabetic conditions hold the promise of halting the rising prevalence of T2D in the pediatric age-group.

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Diagnosis and Treatment of Dyslipoproteinemias in Children and Adolescents

30

Peter O. Kwiterovich Jr. and Kathleen Hawke Byrne

Abstract

This chapter is intended to be a resource for pediatric endocrinologists and other physicians and health care providers on the clinical and genetic presentation, diagnosis, and treatment of disorders of hyperlipoproteinemia and hypolipoproteinemia. The major long-term goal in children and young adults with atherogenic dyslipoproteinemias is to prevent the development of the early lesions of subclinical atherosclerosis and future cardiovascular disease (CVD). In youths with profound abnormalities in triglyceride metabolism, the focus of treatment is to prevent pancreatitis. Those with rare disorders of hypolipoproteinemia may require treatment beyond standard dietary and drug interventions.

Keywords

Hyperlipoproteinemia • Hypolipoproteinemia • Atherosclerosis • Lipoprotein • Dyslipidemia

This chapter is intended to be a resource for pediatric endocrinologists and other physicians and health care providers on the clinical and genetic presentation, diagnosis and treatment of disorders of hyperlipoproteinemia and hypolipoproteinemia. The major long-term goal in children and young adults with atherogenic dyslipoproteinemias is to prevent the development of the early lesions of subclinical atherosclerosis and future cardiovascular disease (CVD). In youths with profound abnormalities in triglyceride metabolism, the focus of treatment is to prevent pancreatitis. Those with rare disorders of hypolipoproteinemia may require treatment beyond standard dietary and drug interventions.

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Overview of Plasma Lipid and Lipoprotein Metabolism

Human plasma lipoprotein metabolism is complex but basically consists of three interrelated major pathways. These include the exogenous (intestinal) pathway, the endogenous (hepatic) pathway, and reverse cholesterol transport

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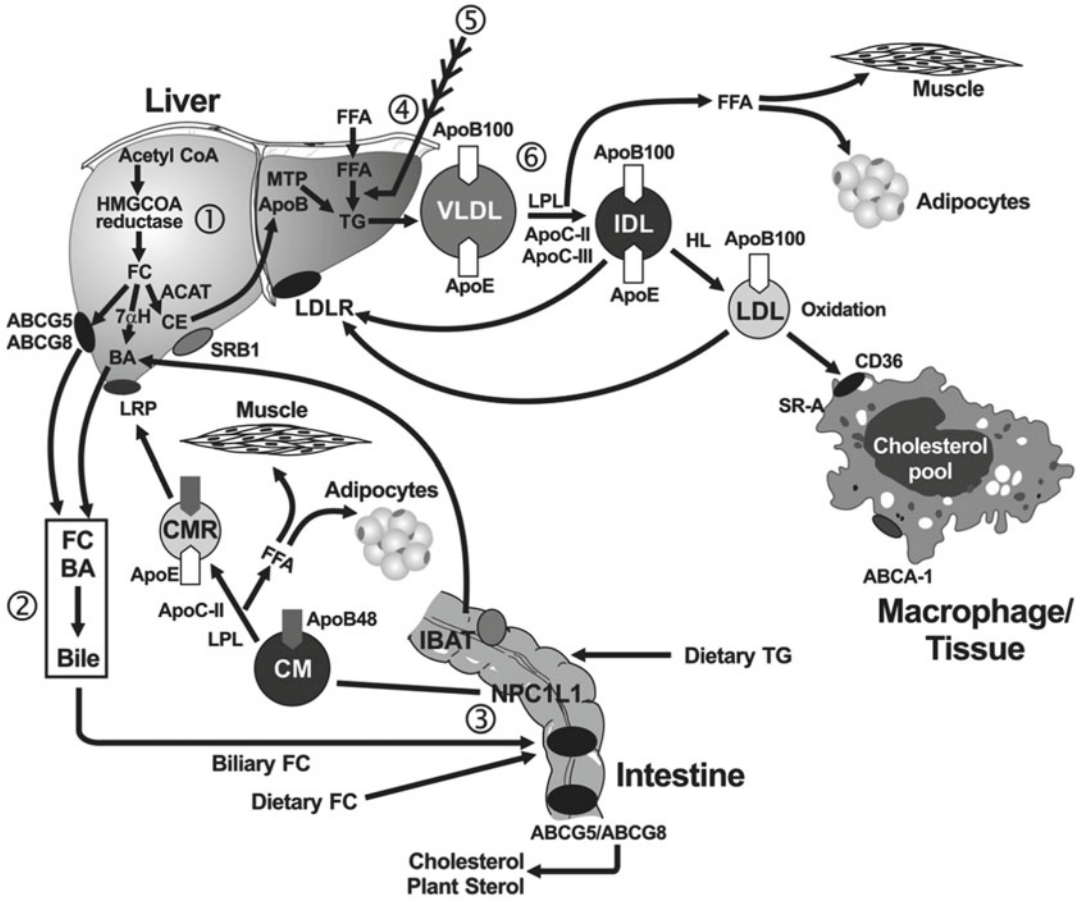

Fig. 30.1 Pathways of exogenous (intestinal) and endogenous (hepatic) lipoprotein metabolism. Chylomicrons (CM) transport lipids of dietary and hepatic origin. Cholesteryl esters (CE) and triglycerides (TG) are emulsified by bile acids (BA), hydrolyzed by pancreatic lipases, absorbed by the small intestine, and resynthesized and packaged by microsomal triglyceride transport protein (MTP) and apoB-48 into chylomicrons (CM), which are then secreted. TG in CM are hydrolyzed by lipoprotein lipase (LPL) and apoC-II, producing free fatty acids (FFA), which can be taken up by adipocytes or muscle. The CM remnant (CMR) is then taken by the low-density lipoprotein-like receptor (LRP) in liver. BA are reabsorbed through the ileal bile acid transporter (IBAT) and recycled to the liver. UC is synthesized in the liver through HMG-CoA reductase and can be excreted from the liver into bile by ATP-binding cassette (ABC) proteins G5 or G8. Unesterified cholesterol (UC) can also be converted into BA by 7 ά-hydroxylase (7άH) or esterified by acyl cholesterol acyltransferase (ACAT) into CE. CE interact with apoB-100, reducing its proteolysis, and TG are added by microsomal triglyceride transfer protein (MTP), producing VLDL, which contains one molecule of apoB-100 that is required for its secretion. The TG on VLDL are hydrolyzed by LPL and apoC-II, producing FFA and intermediate-density lipoprotein (IDL). Some IDL is removed by the interaction of apoE with the hepatic LDL receptor (LDLR), while the rest is converted into LDL by hepatic lipase (HL). ApoC-III inhibits both LPL and apoE-mediated IDL uptake. LDL is normally removed by the LDLR; excess LDL can be oxidized in the vascular wall and taken up by the scavenger receptors, CD 36 and SR-A on macrophages, promoting CE storage. The sites of actions of the six major classes of drugs are depicted as follows: (1) HMG-CoA reductase inhibitors, (2) bile acid sequestrants, (3) cholesterol absorption inhibitors, (4) niacin, (5) omega-3 fish oils, and (6) fibric acid derivatives (Reproduced with permission from Kwiterovich PO. Lipid, apolipoprotein, and lipoprotein metabolism. In: Kwiterovich PO, editor. The Johns Hopkins textbook of dyslipidemia. Wolters Kluwer/Lippincott Williams & Wilkins; p. 1–21)

(Figs. 30.1 and 30.2). An understanding of normal lipid, apolipoprotein, and lipoprotein metabolism is of paramount importance to make the correct diagnosis of the dyslipoproteinemia present, to select the appropriate dietary and drug treatment, and to interpret the efficacy of treatment.

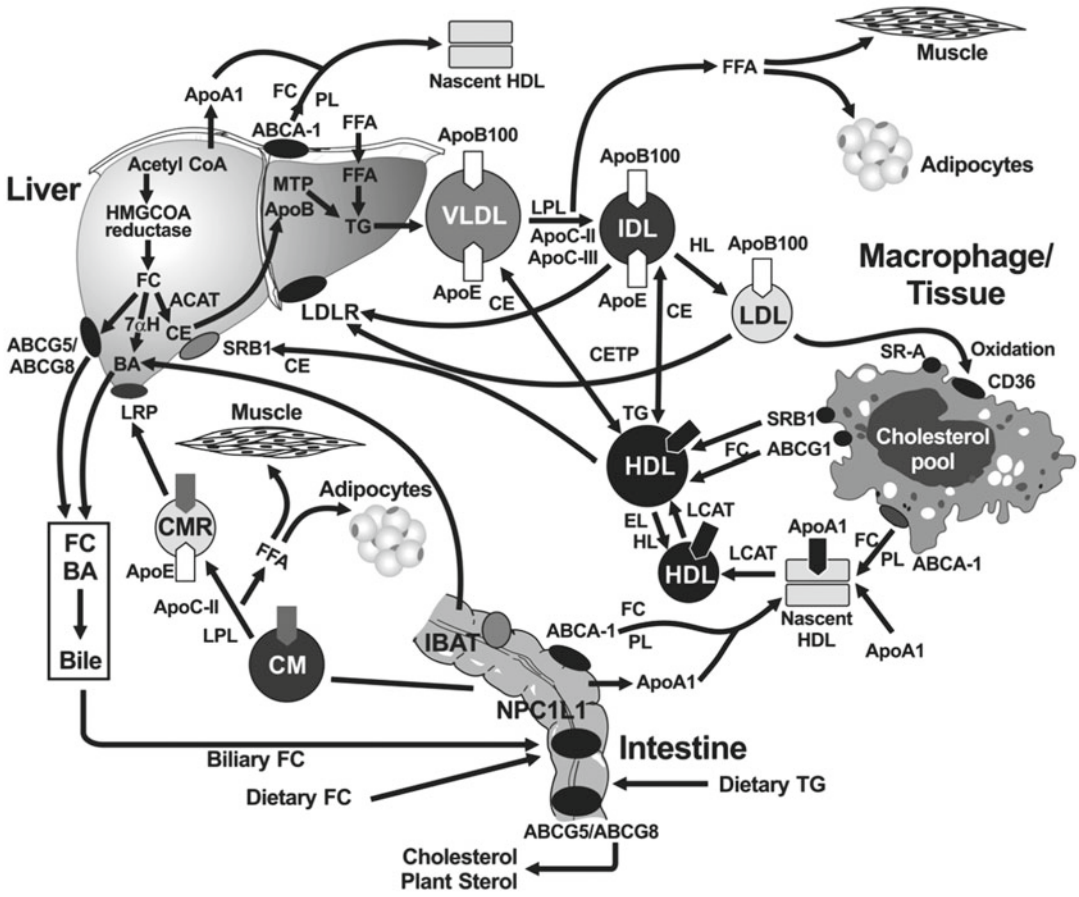

Fig. 30.2 The reverse cholesterol transport pathway and its interaction with the exogenous (intestinal) and endogenous (hepatic) pathways. ApoA-I is synthesized and secreted by intestine and liver, after which it interacts with ATP-binding cassette (ABC) protein A1, promoting the egress of UC and phospholipids (PL) and the formation of the nascent HDL particle. Lecithin cholesterol acyltransferase (LCAT) and apoA-I catalyze the formation of CE by adding a FFA from the PL to UC, producing a spherical HDL particle, which becomes larger through LCAT activity. The mature, larger HDL exchange some of its CE for TG from the apoB-containing lipoproteins, which are then removed by the LDLR. The CE on large HDL can also be delivered to the liver by specific uptake of the scavenger receptor class B type I (SR-BI) receptor. Once inside the liver, cholesterol must be excreted into bile directly through ABCG5/ABCG8 or converted into BA by 7ά́H to complete the reverse cholesterol transport pathway (Reproduced with permission from Kwiterovich PO. Lipid, apolipoprotein, and lipoprotein metabolism. In: Kwiterovich PO, editor. The Johns Hopkins textbook of dyslipidemia. Wolters Kluwer/Lippincott Williams & Wilkins; p. 1–21)

Lipoprotein Structure

The structure of plasma lipoproteins consists of hydrophilic phospholipids (PL), apolipoproteins, and unesterified cholesterol (UC) on the outer surface and hydrophobic triglycerides (TG) and cholesteryl esters (CE) in the core [1]. The major plasma lipoproteins are classified according to their hydrated density, electrophoretic mobility, or chemical composition (Table 30.1). Chylomicrons and very low-density lipoproteins (VLDL) are the major carriers of TG, while low-density lipoproteins (LDL) and high-density lipoproteins (HDL) transport most of the CE.

Apolipoproteins in Lipoproteins

The protein component of lipoproteins, apolipoproteins, have one or more functions, such

Table 30.1 Density, electrophoretic mobility, and chemical composition of the major human plasma lipoproteins

			Surface components			Core lipids	
Class	Density (g/ml)	Electrophoretic mobility	Cholesterol	Phospholipids	Apolipoproteins	TG	Cholesteryl esters
Chylomicrons	<0.95	Remains at origin	2	7	2	86	3
VLDL	0.950–1.006	Pre-β lipoproteins	7	18	8	55	12
IDL	1.006–1.019	Slow pre-β lipoproteins	9	19	19	23	29
LDL	1.019–1.063	Beta lipoproteins	8	22	22	6	42
HDL-2	1.063–1.125	Alpha lipoproteins	5	33	40	5	17
HDL-3	1.125–1.210	Alpha lipoproteins	4	35	55	3	13
Lp (a)	1.040–1.090	Slow pre-β-lipoproteins					

HDL-2 and HDL-3 are the two major subclasses of HDL. Lp (a) consists of a molecule of LDL covalently attached to a molecule of apo (a), a glycoprotein homologous to plasminogen. Its lipid composition is similar to that of LDL. Compositions are given in % (by weight)
VLDL very low-density lipoproteins, *IDL* intermediate-density lipoprotein, *LDL* low-density lipoproteins, *HDL* high-density lipoproteins

Table 30.2 Characteristics of major human plasma apolipoproteins

Apolipoproteins	Major tissue sources	Functions	Molecular weights
ApoA-I	Liver and intestine	Removes cell cholesterol via ABCA1 onto nascent HDL; cofactor LCAT; facilitates uptake of cholesteryl esters from HDL, LDL, and VLDL by SR-B1	29,016
ApoA-II		Not known	17,414
ApoA-IV		Activates LCAT; helps form chylomicrons	44,465
ApoA-V		Stimulates proteoglycan-bound LPL	39,000
ApoB-48	Intestine	Secretion of chylomicrons from intestine	240,800
ApoB-100	Liver	Secretion of VLDL from liver; binding ligand of LDL to LDLR	512,723
ApoC-I	Liver	Activates LCAT; inhibits CETP and SR-B1	6,630
ApoC-II		Cofactor LPL	8,900
ApoC-III		Inhibits LPL and binding of IDL to LDLR	8,800
ApoD	Many sources	Promotes reverse cholesterol transport	19,000
ApoE	Liver	Ligand for uptake of chylomicron remnants and IDL by LRP and LDLR	34,145

The entire sequence of the gene for each apolipoprotein listed in Table 30.2 is known (http://www.ncbi.nlm.nih.gov/sites/entrez)

ABCA1 ATP-binding cassette transporter 1, *LCAT* lecithin cholesterol acyltransferase, *HDL* high-density lipoproteins, *LDL* low-density lipoproteins, *VLDL* very low-density lipoproteins, *SR-BI* scavenger class B type I receptor, *LPL* lipoprotein lipase, *CETP* cholesteryl ester transfer protein, *IDL* intermediate-density lipoprotein, *LDLR* low-density lipoprotein receptor, *LRP* low-density lipoprotein-like receptor protein

as structural proteins for packaging lipoproteins, cofactors for enzymes, and ligands for lipoprotein receptors [1]. The nomenclature for apolipoproteins is based on an alphabetical scheme (Table 30.2). Apolipoprotein A-I (apoA-I) constitutes about 70% of the apolipoproteins of HDL. The full-length apolipoprotein B (apoB-100) is made in liver. One molecule of apoB-100 is present on VLDL, VLDL remnants, LDL, and intermediate-density lipoproteins (IDL). ApoB-100 is also a major component of lipoprotein (a), or Lp (a) lipoprotein. ApoB-48 is a truncated product of the same gene as apoB-100, due to posttranslational modification. ApoB-48 is made in intestine and is found in chylomicrons (CM) and CM remnants (Table 30.2).

Exogenous Lipoprotein Metabolism

The exogenous pathway transports lipids of dietary origin (Fig. 30.1). Dietary lipids are emulsified by bile acids and hydrolyzed by pancreatic lipases into their component parts, monoglyceride, free fatty acids (FFA), and UC. After absorption into the intestinal cells, FFA and monoglycerides are synthesized into TG and incorporated with cholesterol into CM by microsomal triglyceride transport protein (MCT). CM contain apolipoproteins A-I, A-IV, and B-48 [2] (Table 30.2). About 90% of the lipid in CM is TG (Table 30.1). CM are secreted into the thoracic duct, a process that requires apoB-48. Thereafter, CM enter the peripheral circulation, where they acquire apoE and apoC-I, apoC-II,

Table 30.3 Key enzymes and transfer proteins affecting plasma lipid transport

Enzyme	Major tissue source	Functions	Molecular weight
Lipoprotein lipase (LPL)	Adipose tissue Striated muscle Macrophages	Hydrolyzes TG and phospholipids of chylomicrons and large VLDL	50,394
Hepatic lipase (HL)	Liver	Hydrolyzes TG and phospholipids of small VLDL, IDL, and HDL-2	53,222
Endothelial lipase	Endothelial cells	Hydrolyzes phospholipids and a small amount of TG *especially of HDL*	56,795
Lecithin cholesterol acyltransferase (LCAT)	Liver	Transfers a free fatty acid from phosphatidylcholine on nascent (prebeta) HDL to form cholesteryl esters and mature HDL	47,090
Cholesterol ester transport protein (CETP)	Liver, spleen, and adipose tissue	Transfers cholesteryl esters from HDL to apoB-containing lipoproteins Converts α-HDL to pre-β HDL	74,000
Phospholipid transfer protein (PTP)	Placenta, pancreas, adipose tissue, lung	Transfers the majority of phospholipids in plasma Converts α-HDL to pre-β HDL	81,000
Scavenger class B type I receptor	Liver, adrenal, gonads, endothelium, macrophages	Mediates the selective uptake of cholesteryl ester from the core of lipoproteins, including HDL, LDL, and VLDL	82,000

and apoC-III from HDL. When CM enter the capillaries of skeletal muscle and adipose tissue, they are exposed to lipoprotein lipase (LPL), a lipolytic enzyme attached to the surface of the endothelial cells (Table 30.3, Fig. 30.1). LPL along with its cofactor, apoC-II, hydrolyzes TG into FFA, which then enter muscle and adipose tissue (Fig. 30.1). Chylomicron remnants (CMR), containing TG, cholesterol, apoB-48, and apoE, are taken up into the liver by a receptor-mediated endocytosis involving the low-density lipoprotein-like receptor protein (LRP) [3] (Fig. 30.1). In liver, cholesterol is used for lipoprotein synthesis, cell membrane structure, or excreted into bile, either as UC or as bile acids derived from cholesterol. The cholesterol on the CMR can therefore be derived from either diet or liver (Fig. 30.1, left lower part).

Endogenous Lipoprotein Metabolism

The endogenous pathway involves the hepatic production and metabolism of the TG-rich VLDL (Fig. 30.1). The major apolipoproteins of VLDL are apoB-100, apoE, and apoC (I, II, III) (Table 30.2).

Biosynthesis of VLDL

TG, CE, and apoB-100 in liver are required for VLDL synthesis (Fig. 30.1). Hepatic FFA are normally activated (fatty acid CoA) and then oxidized or incorporated into TG or CE. ApoB-100 is made constitutively in liver. The expression of *APOB* is not regulated. The quantity of apoB-100 in liver is regulated by proteolysis. When CE interact with apoB-100, apoB-100 is less likely to be degraded, leading to increased production of apoB-100. MTP catalyzes the incorporation of TG into this complex, producing VLDL (Fig. 30.1, left upper part). The synthesis of VLDL is thus influenced by the amount of TG, CE, and cholesterol produced in liver.

Secretion and Metabolism of VLDL

ApoB-100 is required for secretion of VLDL into plasma. VLDL-TG are transported to tissue capillaries, where they are hydrolyzed by LPL and apoC-II, releasing FFA. A large VLDL remnant is produced; the TG are further hydrolyzed, generating IDL (Table 30.1) (Fig. 30.1). A portion

of IDL is cleared from the circulation by hepatic uptake through the binding of apolipoprotein E (apoE) on IDL to the LDL receptor (LDLR) (Fig. 30.1). The TG on the remainder of IDL are hydrolyzed by hepatic lipase (HL) (Table 30.3), producing LDL, the final product of endogenous VLDL metabolism (Fig. 30.1, central upper part).

LDL Binding and Internalization

The LDLR pathway was elegantly discovered by Goldstein, Brown, and colleagues [4]. After synthesis in the endoplasmic reticulum (ER), LDLR is glycosylated in the ER and Golgi. LDLR is directed toward clathrin-coated pits where the ligand-binding domain of the LDLR is exposed to apoB-100 or apoE. ApoB-100 on LDL and apoE on IDL bind to the LDLR with high affinity, promoting their internalization and cellular metabolism (Fig. 30.1 central upper part). These receptor–ligand complexes are internalized within coated vesicles by endocytosis and delivered to endosomes with the help of an adaptor protein, called autosomal recessive hypercholesterolemia (ARH) [5].

LDL Degradation

In the acidic environment of the endosomes, LDL is displaced from the LDLR, permitting release of LDL into the endosomes and recycling of the LDLR to the cell surface [4]. The released LDL is subsequently degraded in the lysosome [4]. A protease called proprotein convertase subtilisin-like/kexin type 9 (PCSK9) can be secreted from the liver, interact with the LDLR, and promote its degradation through an incompletely understood mechanism [5, 6].

Regulatory Effect of Cholesterol Derived from LDL Degradation

In lysosomes, the apoB-100 of LDL undergoes proteolysis, and CE are hydrolyzed into UC and FFA [4]. The UC derived from LDL can be used for membrane or lipoprotein synthesis or as a precursor for steroids, sex hormones, or bile acids. The UC from LDL decreases both HMG-CoA reductase and LDLR activity by inhibiting the sterol regulatory element-binding protein (SREBP) pathway [7]. Cholesterol regulates the proteolytic release of SREBPs, a family of transcription factors, from the ER [7]. This effect occurs through the SREBP cleavage-activating protein (SCAP) that is both a sensor of sterols and an escort of SREBPs. As the cholesterol content of the hepatocytes increases, the transcription of the LDLR and HMG-CoA reductase genes decreases, and vice versa [7].

Scavenger Receptors for LDL

LDL can also be removed by scavenger receptors such as SR-A, CD36, LOX-1, and scavenger receptor class B type I (SR-BI), which take up chemically modified, oxidized, or glycated LDL [8] (Fig. 30.1, right upper part). Scavenger receptors are not regulated by intracellular cholesterol levels. In peripheral tissues, such as macrophages, excess cholesterol accumulates within the plasma membrane and is esterified to CE by the enzyme, acyl-CoA cholesterol acyltransferase (ACAT). Cytoplasmic lipid droplets are then formed, and the cells are then converted into foam cells (an early stage of atherogenesis) (Fig. 30.1). In liver, SR-B1 also functions as a lipoprotein receptor promoting the uptake of CE and UC from HDL, LDL, and VLDL (see below).

The level of LDL in plasma is the result of the rate of LDL production and removal. If LDL-C is <100 mg/dL, it is mostly removed through the high-affinity LDLR pathway. The higher the LDL level >100 mg/dL, the greater the amount of LDL that is removed by the scavenger receptor pathway.

High-Density Lipoproteins and Reverse Cholesterol Transport

Reverse cholesterol transport (Fig. 30.2) refers to the process by which UC is removed from extrahepatic tissues and ultimately transported to the

liver, from which it can be excreted into the intestine, either as UC or as bile acids.

Synthesis of HDL

ApoA-I is released as a lipid-free protein from intestinal and liver cells (Fig. 30.2). ApoA-I interacts with ABCA1, on the basolateral membranes of hepatocytes, enterocytes, and macrophages, acquiring PL and UC to form a more stable nascent HDL particle [9, 10] (Fig. 30.2). The nascent HDL particle is transformed into a spherical "mature" HDL through the esterification of UC by LCAT and its cofactor, apoA-1, creating CE for the hydrophobic core (Fig. 30.2). The subsequent addition of cellular cholesterol to the HDL particle occurs in macrophages and other peripheral cells through the action of ABCG1 and SR-BI, molecules that prefer larger HDL as acceptors [9] (Fig. 30.2 right part). HDL also acquire lipids from chylomicrons and VLDL during hydrolysis of TG by LPL.

Transfer of Lipid Between HDL and the ApoB-Containing Lipoproteins

CE from HDL are exchanged for TG from apoB-containing particles by the cholesteryl ester transfer protein (CETP) [7] (Table 30.3, Fig. 30.2 central part). The phospholipid transfer protein (PLTP) (Table 30.3, Fig. 30.2) is structurally similar to CETP and mediates the transfer of unsaturated fatty acids on PL from the apoB-containing lipoproteins to HDL.

Reverse Cholesterol Transport

CE in spherical HDL can be transported back to liver by two mechanisms. First, CE from HDL are transferred by CETP to the apoB-containing lipoproteins, which are then cleared by the LDLR. Second, CE and UC may also be delivered directly to the liver through SR-BI, also called the "HDL receptor" [11]. CE are hydrolyzed into UC and FFA; UC can be excreted directly into bile by ABCG5/ABCG8 or converted into bile acids by 7 ά-hydroxylase, both reactions promoting the excretion of sterols into the stool (Fig. 30.2). Reverse cholesterol transport is postulated to explain, at least in part, the protective effect that HDL and apoA-I have against the development of atherosclerosis.

Other Protective Effects of HDL

In addition to their participation in reverse cholesterol transport, HDL may be cardioprotective by their antioxidant, anti-inflammatory, and antithrombotic effects [9, 10]. HDL impede LDL oxidation by metal ions, an effect that may be due to the influence of several molecules on HDL, including apoA-I, platelet-activating factor acetylhydrolase, and paraoxonase [9, 10].

HDL Catabolism

The cellular uptake of an HDL particle for intracellular catabolism is incompletely understood [9, 10]. SR-B1, the "HDL receptor," removes CE from the core of HDL for uptake by liver, but the entire HDL particle is not removed by SR-B1 [11]. HL on liver hydrolyzes phospholipids and TG on larger HDL producing a smaller particle. Endothelial lipase (EL) (Table 30.3, Fig. 30.2) hydrolyzes mostly phospholipids on larger HDL, again producing a smaller particle. Accumulation of large HDL-2 (Table 30.1, Fig. 30.2), thought to be the most cardioprotective of the HDL subclasses, is favored by estrogens, which negatively regulate HL. In contrast, progesterone and androgens, which positively regulate HL, lead to increased production of small HDL-3 (Fig. 30.2).

Lipid, Lipoprotein, and Apolipoprotein Levels

The levels of the lipid-related parameters result from the complex interactions of the biochemical reactions involved in lipoprotein metabolism

Table 30.4 Acceptable, borderline, and high plasma lipid; lipoprotein; and apolipoprotein concentrations for children and adolescents

Category	Low	Average	High[a]
TC	<120	160	≥200
LDL-C	<65	100	≥130
Non-HDL-C	<75	115	≥144
ApoB	<60	80	≥110
TG			
0–9 years	<30	60	≥100
10–19 years	<35	70	≥130
HDL-C	<35	50	>70
ApoA-I	<110	130	>170

Values for plasma lipid and lipoprotein levels are from the National Cholesterol Education Program (NCEP) Expert Panel on Cholesterol Levels in Children [12]. Non-HDL-C values from Bogalusa are equivalent to NCEP pediatric panel cutoff points for LDL-C [13]. Values for plasma apoB and apoA-I are from the National Health and Nutrition Examination Survey III (NHANES III) [14]

[a]The cutoff points for a high or low value represent approximately the 95th and 5th percentiles, respectively [12–14]

(Figs. 30.1 and 30.2). Diagnostic guidelines for hyperlipoproteinemia and hypolipoproteinemia are selected using cut points, based on percentiles from the general population of youth. High and low levels of lipids, lipoproteins, and apolipoproteins are defined as the upper 95th and lower 5th percentiles, respectively [12–14] (Table 30.4).

Primary vs. Secondary Dyslipidemia

Secondary Dyslipidemia

Before considering dyslipidemia to be a primary abnormality, secondary causes of dyslipidemia must be excluded (Table 30.5). If dyslipidemia persists after treatment of the secondary disorder, the patient will require dietary and, if indicated, drug treatment (see below for guidelines).

Metabolic and Genetic Disorders of Hyperlipoproteinemias

Disorders of Exogenous Lipoprotein Metabolism

The two classic disorders of exogenous lipoprotein metabolism are defective or missing LPL and defective apoC-II, the cofactor for LPL (Fig. 30.1). Both involve decreased removal of chylomicrons. Several more recently reported disorders involving chylomicron catabolism include homozygous loss-of-function mutations in *APOA5*, deficiency in glycosylphosphatidylinositol-anchored high-density lipoprotein-binding protein 1 (GPIHBP1), and frameshift mutations in the cell surface protein caveolin-1 [1–52].

Lipoprotein Lipase Deficiency

Familial LPL deficiency is a rare, autosomal recessive disorder that results from a variety of mutations in the LPL gene and affects one in one million children [15]. Parents are often consanguineous. Classic LPL deficiency presents in the first months of life with striking hypertriglyceridemia, often ranging from 5,000 to 10,000 mg/dL with a TC from 500 to 1,000 mg/dL. This disorder is often suspected because of creamy plasma on the top of a hematocrit tube, eruptive xanthomas, hepatosplenomegaly, persistent colic, or lipemia retinalis. Usually only CM are elevated (type I phenotype) (Table 30.6), but occasionally both CM and VLDL are elevated (type V phenotype). Because CM replace water (volume) in plasma, sodium levels decrease between 2 and 4 meq/L for each 1,000 mg/dL increase of plasma TG. Later in childhood, the development of abdominal pain suggests the presence of pancreatitis, a life-threatening complication. Premature atherosclerosis in adulthood is unusual, because CM are too large to enter the vascular wall.

Table 30.5 Causes of secondary dyslipidemia in children and adolescents

Exogenous	*Storage diseases*
Alcohol	Cystine storage disease
Oral contraceptives	Gaucher disease
Prednisone	Glycogen storage disease
Anabolic steroids	Juvenile Tay–Sachs disease
13-*cis*-Retinoic acid	Niemann–Pick disease
Endocrine and metabolic	Tay–Sachs disease
Acute intermittent porphyria	*Acute and transient*
Type I and type II diabetes	Burns
Hypopituitarism	Hepatitis
Hypothyroidism	*Others*
Lipodystrophy	Anorexia nervosa
Pregnancy	Cancer survivor
Renal	Heart transplantation
Chronic renal failure	Idiopathic hypercalcemia
Hemolytic-uremic syndrome	Kawasaki disease
Nephrotic syndrome	Klinefelter syndrome
Hepatic	Progeria (Hutchinson–Gilford syndrome)
Benign recurrent intrahepatic cholestasis	Rheumatoid arthritis
Congenital biliary atresia	Systemic lupus erythematosus
Alagille syndrome	Werner syndrome

The diagnosis is confirmed by a test for post-heparin lipolytic activity (PHLA). Following an overnight fast, blood is drawn at baseline and 10 and 45 min after intravenous injection of heparin (60 units per kg), which releases the membrane-bound LPL and HL (Table 30.3) into the blood. The total lipolytic activity and that due to HL is measured and LPL activity determined by subtraction of HL activity from the total activity. The mass of LPL released can also be assessed, using an ELISA assay. Parents of LPL-deficient patients often have LPL activity halfway between normals and their LPL-deficient child. The parents may be moderately hypertriglyceridemic or normal.

Treatment is a diet very low in fat (10–15% of calories) [1–52]. Affected infants and children must get at least 1% of their calories from the essential fatty acid, linoleic acid.

Lipid-lowering medication is ineffective. Affected infants can be given a special formula enriched in medium-chain TG (MCT), which are absorbed directly into the portal vein and do not require the formation of chylomicrons. A subset of LPL-deficient patients with unique, possibly posttranscriptional, genetic defects respond to therapy with MCT oil or omega-3 fatty acids by normalizing fasting plasma TG; a therapeutic trial with MCT oil should, therefore, be considered in all patients with LPL deficiency [13]. MCT oil can also improve the palatability and caloric content of a very low-fat diet.

Table 30.6 Lipoprotein phenotypes of hyperlipidemia

Lipoprotein phenotype	Elevated lipoprotein
Type I	Chylomicrons
Type IIa	LDL
Type IIb	LDL, VLDL
Type III	Cholesterol-enriched IDL
Type IV	VLDL
Type V	Chylomicrons, VLDL

ApoC-II Deficiency

Marked hypertriglyceridemia (TG >1,000 mg/dL) can also present in patients with a rare autosomal recessive disorder affecting apoC-II, the cofactor for LPL. The disorder is often delayed into adulthood and can be suspected in children and adolescents who have very cloudy or creamy plasma or recurrent bouts of pancreatitis. Affected homozygotes have very high TG ranging from 500 to 10,000 mg/dL. A type V lipoprotein phenotype (Table 30.6) is typical. Eruptive xanthomas and lipemia retinalis may be present, but those with apoC-II deficiency usually do not get premature atherosclerosis.

The PHLA test shows very low LPL activity. Addition of normal plasma containing apoC-II to the PHLA test restores normal LPL activity. Using an ELISA assay, apoC-II is very low or undetectable. ApoC-II deficiency is caused by a variety of mutations [15]. Obligate heterozygous carriers of apoC-II mutants usually have normal plasma lipid levels, despite a 50% reduction in apoC-II.

The treatment of patients with apoC-II deficiency also requires a very low-fat diet. Infusion of normal plasma in vivo into an affected patient also decreases TG.

Disorders of Endogenous Lipoprotein Metabolism

These disorders are heterogeneous from both a metabolic and genetic perspective.

Disorders of VLDL Overproduction

Familial Hypertriglyceridemia

Patients with familial hypertriglyceridemia (FHT) most often present with elevated levels of TG but normal LDL-C levels (type IV lipoprotein phenotype) (Table 30.6). FHT has been associated with mutations in the apolipoprotein A5 gene (*APOA5*) (Table 30.2), the lipase I gene (LIPI), and with a polymorphism in the RP1 gene [15].

FHT may be autosomal dominant with reduced penetrance in children. The diagnosis is suspected by finding only the type IV phenotype in multiple family members. VLDL levels can increase to a considerable degree, leading to hypercholesterolemia as well as marked hypertriglyceridemia (>1,000 mg/dL) and occasionally to hyperchylomicronemia (type V phenotype) (Table 30.6), usually due to the effects of obesity and type 2 diabetes mellitus. Adults with FHT often manifest hyperuricemia, glucose intolerance, obesity, and peripheral vascular disease; a family history of premature CAD may or may not be present. Pancreatitis may occur in the rare FHT patient with a type V lipoprotein phenotype.

The metabolic defect in FHT appears to be due to the increased hepatic production of TG that is not accompanied by a corresponding increase in the production of apoB-100. This results in the enhanced secretion of very large VLDL particles that apparently are not hydrolyzed at a normal rate by LPL. Thus, LDL-C and apoB levels remain normal, or low normal, throughout a range of levels of TG. In some cases of FHT, one or more molecular variants in *APOA5* lead to decreased *s*timulation of proteoglycan-bound LPL by apoA-V [15].

Diet, particularly reduction to ideal body weight, is the cornerstone of therapy in both children and adults with FHT. For adult-sized adolescents with TG >200 mg/dL and adults with persistent hypertriglyceridemia above 500 mg/dL, treatment with fibric acid derivatives, niacin, or omega-3 fish oils may reduce the elevated TG by up to 50%.

Familial Combined Hyperlipidemia and the Small, Dense LDL Syndromes

Clinical Presentation

Patients with familial combined hyperlipidemia (FCHL) can present with elevated LDL-C alone (type IIa phenotype), normal LDL-C but elevated TG (type IV phenotype), or with both LDL-C and TG elevated (type IIb phenotype) (Table 30.6). The diagnosis of FCHL requires the finding of one, or preferably more, first-degree family members, who have a different lipoprotein phenotype from the proband. Characteristics of FCHL include an elevated apoB level and an increased number of *small*, *dense LDL particles*, which link FCHL to hyperapobetalipoproteinemia (hyperapoB) (increased apoB with normal LDL-C), LDL subclass pattern B, and familial dyslipidemic hypertension [17]. In addition to hypertension, patients with FCHL and the small, dense LDL syndromes often manifest hyperinsulinism, glucose intolerance, low HDL-C, and increased visceral obesity (syndrome X of Reaven).

From a clinical prospective, FCHL and other small, dense LDL syndromes are the most commonly recognized disorders in families with premature CAD. FCHL has a delayed clinical expression, but in lipid clinics it is not uncommon for children or adolescents from families with premature CAD to express disparate Type IIa, type IIb, or type IV phenotypes.

FCHL: Metabolic Derangement

There are three metabolic defects that have been described in both FCHL and hyperapoB patients: (1) hepatic overproduction of VLDL and apoB-100, (2) slower removal of chylomicrons and chylomicron remnants after a fat load, and (3) abnormally increased FFA levels [17, 18].

The abnormal FFA metabolism likely reflects the primary defect in these patients. Decreased inhibition of hormone-sensitive lipase by insulin in adipocytes leads to increased plasma FFA. Increased delivery of FFA to liver may drive hepatic

overproduction of TG and apoB-100. FFA and glucose compete as oxidative fuel sources in muscle. Increased concentrations of FFA inhibit glucose uptake in muscle and contribute to insulin resistance. A cellular defect in adipocytes of some hyperapoB patients prevents the normal stimulation of FFA incorporation into TG by a basic, small molecular weight protein, named the acylation stimulatory protein (ASP) [19, 20]. A defect in a basic protein receptor involved in phosphorylation and signal transduction appears to be present in a subset of patients with hyperapoB and premature CAD [21].

FCHL: Genetic Defects

Efforts to demonstrate even one single, well-delineated molecular defect in this heterogeneous disorder have not succeeded to date. A number of genes (oligogenic effect) appear to influence the expression of FCHL and the small, dense LDL syndromes [17, 22].

FCHL: Treatment and Prognosis

Treatment starts with reduction of body weight, regular aerobic exercise, and a diet reduced in saturated fat, trans fat, and cholesterol as well as simple sugars. The low-fat diet reduces the burden of postprandial CM and the atherogenic CM remnants. Reduction to ideal body weight often improves insulin sensitivity and decreases VLDL overproduction. Regular aerobic exercise burns calories and increases the basal metabolic rate. Acanthosis nigricans, often associated with insulin resistance in obese, FCHL children and adolescents, can improve or disappear after such therapeutic lifestyle changes (TLC). Children and adolescents 10–17 years of age from a family with FCHL and premature CAD and an LDL-C >160 mg/dL after TLC may be treated with a statin to lower LDL-C to <130 mg/dL.

Lysosomal Acid Lipase Deficiency: Wolman Disease and Cholesteryl Ester Storage Disease

Clinical Presentation

Historically, Wolman disease is fatal with a very short lifespan, usually under 1 year [23]. Persistent and forceful vomiting marked abdominal distension, watery stools, severe anemia, and failure to thrive start in the first weeks of life. Hepatosplenomegaly is invariably present and may be massive. The most striking feature is calcification of the adrenal glands. Circulating vacuolated lymphocytes and foam cells in bone marrow are almost constant findings.

In contrast to Wolman disease, cholesteryl ester storage disease (CESD) is characterized by a relatively variable and mild phenotype [24]. The principal and sometimes only sign, hepatomegaly, may be evident at birth or in early childhood, increases with time, and eventually leads to hepatic fibrosis. Acute or chronic liver failure and jaundice have been observed. Recurrent abdominal pain occurs frequently. Patients with CESD can survive for longer periods of time [24], and some adults with CESD develop premature atherosclerosis.

Metabolic Derangement

Both Wolman disease and CESD are autosomal recessive disorders due to mutations in lysosomal acid lipase (LAL). LAL is an important lysosomal enzyme that normally hydrolyzes LDL-derived CE into UC. In LAL deficiency, CE are not hydrolyzed in lysosomes and are markedly increased in liver. Low UC levels result, leading to the upregulation of cholesterol synthesis and LDLR activity (see also above). The enhanced synthesis of cholesterol contributes to increased VLDL synthesis, leading to increased secretion of VLDL which is subsequently converted to LDL.

Treatment

Lovastatin reduced both the rate of cholesterol synthesis and the secretion of VLDL, leading to significant reductions in TC, LDL-C, and TG in CESD [24]. Infants with Wolman's disease respond either to transplantation of unrelated HLA-mismatched umbilical cord blood-derived stem cells, which restored normal LAL activity before permanent end-organ damage [25], or to hematopoietic cell transplantation [26].

Disorders of LDL Removal

Familial hypercholesterolemia (FH), the prototype of these disorders, provided the initial insights into

Table 30.7 Major monogenic diseases that cause decreased LDLR activity and marked hypercholesterolemia (Modified from Rader, Cohen, and Hobbs [5])

Disease	Defective gene	Prevalence	LDL-C	Metabolic defect
Autosomal dominant				
FH Heterozygous FH Homozygous FH	LDLR	1 in 500 1 in 1×10^6	3X 5X	Decreased LDL clearance (1^0) Increased LDL production (2^0)
FDB Heterozygous FDB Homozygous FDB	APOB	1 in 1,000 1 in 4×10^6	2X 3X	Decreased LDL clearance
FH3 Heterozygous FH3	PCSK9	<1 in 2,500	3X	Unknown
Autosomal recessive				
ARH Sitosterolemia	ARH ABCG5 ABCG8	<1 in 5×10^6 <1 in 5×10^6	4X 1X to 5X	Decreased LDL clearance Decreased cholesterol excretion(1^0) Decreased LDL clearance (2^0)

X indicates the mean LDL cholesterol (LDL-C) level in normals
ARH autosomal recessive hypercholesterolemia, *FDB* familial ligand-defective apoB-100, *FH* familial hypercholesterolemia, *PCSK9* proprotein convertase subtilisin-like kexin type 9, *ABCG5 and ABCG8* ATP-binding cassette half transporters G5 or G8

the role of LDL in *human* atherosclerosis. Five monogenic diseases due to decreased LDL removal will be discussed: FH, familial ligand-defective apoB-100 (FDB), heterozygous FH3, autosomal recessive hypercholesterolemia (ARH), and sitosterolemia (Table 30.7). While their underlying molecular defects may differ, the end result is reduction of LDLR activity and markedly elevated LDL-C.

FH: Clinical Presentation

FH is an autosomal codominant disorder that presents in heterozygotes as a two- to threefold elevation in TC and LDL-C levels [5]. FH is completely expressed at birth and can be reliably detected by 1 year of age [27]. FH leads to premature CAD, and by age 50, about half of untreated heterozygous FH males and 25% of affected females will develop CAD. Heterozygotes develop tendon xanthomas in adulthood, often in the Achilles tendons and the extensor tendons of the hands. FH homozygotes usually have cholesterol levels between 600 and 1,000 mg/dL and planar xanthomas by the age of 5 years, notably in the webbing of fingers and toes, knees, and buttocks, and often develop life-threatening supravalvular aortic stenosis and CAD in the second decade [1].

FH: Metabolic Derangement and Genetics

FH is one of the most common inborn errors of metabolism seen in pediatrics, affecting 1 in 500 children worldwide [5] (Table 30.7). FH has a higher incidence in Afrikaners, Christian Lebanese, Finns, and French Canadians due to founder effects. FH results from one of more than 900 different mutations in *LDLR* [5]. About one in a million children inherit two mutant alleles in *LDLR*, presenting with a four- to eightfold increase in LDL-C. Most FH homozygotes inherit two different mutant alleles in *LDLR* (genetic compounds), but some have two identical *LDLR* mutations (true homozygotes). Prenatal diagnosis of FH homozygotes can be performed. Mutant LDLR alleles can cause (1) failure to produce any LDLR protein, (2) deficient intracellular transport of LDLR between ER and Golgi, (3) defective binding of LDL by LDLR, (4) abnormal internalization of normally bound LDL, and (5) defects in the normal recycling of LDLR back to the cell surface [4, 5].

Table 30.8 Guidelines for use of pharmacologic agents to lower LDL-C in children and adolescents [12]

Risk factors for CVD	Postdietary LDL-C level	LDL-C treatment goal
None	> or =190 mg/dL	Minimum <130 mg/dL
		Desirable <110 mg/dL
(1) Positive family history for premature CVD	> or ≥160 mg/dL	Minimum <130 mg/dL
(2) Two or more other CVD risk factors		Desirable <110 mg/dL

FH: Treatment

The dietary treatment of children with FH can be safely supplemented with plant sterols or stanols (usually purchased as commercially available margarines) to decrease cholesterol absorption. Most FH heterozygote children require higher doses of more potent statins to lower LDL-C sufficiently. The addition of a bile acid-binding sequestrant (BAS) or a cholesterol absorption inhibitor (CAI) to a statin is often necessary to lower LDL-C <110 mg/day (Table 30.8). Decreased LDLR activity can also lead to moderate hypertriglyceridemia (due to decreased binding of apoE on TG-enriched to LDLR) and to low HDL-C. FH homozygotes respond somewhat to high doses of statins (with a fall in LDL-C of between 100 and 200 mg/dL). Niacin can lower LDL-C in FH homozygotes about another 25%. Both statins and niacin decrease production of hepatic VLDL, leading to decreased production of LDL. Ezetimibe, a CAI inhibitor, lowers LDL-C another 25% in FH homozygotes. Such triple-lipid-altering therapy in FH homozygotes can lower the LDL-C into a range found in FH heterozygotes. FH homozygotes inevitably require weekly LDL apheresis to lower LDL-C into a less atherogenic range. If LDL apheresis cannot be performed, then hepatic transplantation may be considered. In the future, ex vivo gene therapy for FH homozygotes may become the treatment of choice.

Phenocopies of FH Homozygotes

The other primary disorders affecting LDLR activity (Table 30.7) can also present with planar, tendon, or tuberous xanthomas in such homozygous affected children and adolescents, so can adolescents with the dominant form of dysbetalipoproteinemia (see below). Patients with secondary disorders of dyslipidemia accompanied by xanthomas include biliary cirrhosis, congenital biliary atresia, Alagille syndrome, myelomas, and Wolman disease [5]. These disorders have other clinically salient findings to distinguish them from FH homozygotes.

Familial Ligand-Defective ApoB

Heterozygotes with FDB may present with normal, moderately elevated, or markedly increased LDL-C [5, 28] (Table 30.7). Hypercholesterolemia is usually not as markedly elevated in FDB as in FH heterozygotes. About 1 in 20 of affected patients with FDB has tendon xanthomas and more extreme hypercholesterolemia. This disorder represents a small fraction of patients with premature CAD, i.e., no more than 1%.

In FDB patients, there is delayed removal of LDL from blood despite normal LDLR activity, but the clearance of VLDL remnants and IDL TG-enriched particles is not affected.

The most commonly recognized mutation in FDB is a missense mutation (R3500Q) in the LDLR-binding domain of apoB-100 [28]. The frequency of FDB heterozygotes is about 1 in 1,000 in Central Europe [5] but appears less common in other populations (Table 30.8).

Dietary and drug treatment of FDB is similar to that used for FH heterozygotes.

Heterozygous FH3

The clinical phenotype of heterozygous FH3 is indistinguishable from FH heterozygotes [5, 6]. FH3 does not segregate with *LDLR* and results from mutations in PCSK9 [5, 6]. PCSK9 facilitates the degradation of LDLR [6], but the exact mechanism(s) is not known. Gain-of-function

mutations that increase PCSK9 activity decrease LDLR activity, producing a phenotype similar to FH. Conversely, loss-of-function mutations that decrease PCSK9 activity increase LDLR and produce levels of LDL-C <80 mg/dL and decreased CAD (see also below). Drugs that inhibit PCSK9 activity are being developed and have promise for lowering LDL-C, either alone or in combination with a statin.

Autosomal Recessive Hypercholesterolemia

ARH is a rare autosomal recessive disorder that usually presents with LDL-C levels in between those in FH heterozygotes and FH homozygotes [5]. ARH patients often have large tuberous xanthomas. Their onset of CAD is often later than that of FH homozygotes. Most of the families reported to date have been Sardinian or Lebanese. LDL-C in the parents is usually normal but can be elevated. Strikingly, in ARH there is normal LDLR activity in fibroblasts, but it is defective in lymphocytes. To date, at least ten mutations have been described in the ARH gene [5].

Fortunately, patients with ARH respond quite dramatically to treatment with statins and a CAI. A BAS may also be added to the statin to effect a further reduction in LDL-C.

Despite this therapy, some ARH patients, especially those with CAD, may also require LDL apheresis.

Sitosterolemia

Patients with this rare, autosomal, recessive can present with normal to markedly elevated TC and LDL-C levels, tendon and tuberous xanthomas, premature CAD, and aortic stenosis [5]. Homozygotes manifest abnormal intestinal hyperabsorption of plant (sitosterol, campesterol, and stigmasterol) and shellfish sterols and of cholesterol. In normal humans, very little plant sterols are absorbed, and plasma plant sterol levels are low (0.3–1.7 mg/dL) constituting <1% of plasma total sterol. The levels of total plant sterols (13–37 mg/dL) in sitosterolemics are very elevated and represent 7–16% of the total plasma sterols. Patients often present in childhood with striking tuberous and tendon xanthomas, despite normal or FH heterozygote-like LDL-C levels. The diagnosis is made by documenting elevated plant sterols using gas–liquid chromatography. The parents and obligate heterozygous siblings usually have normal LDL-C and only slightly higher plant sterol levels.

Two ABC half transporters, ABCG5 and ABCG8 [5], normally limit the intestinal absorption of plant sterols and cholesterol and promote their excretion (Figs. 30.1 and 30.2). Sitosterolemia is caused by two mutations in either of the two adjacent genes that encode ABCG5 or ABCG8 (Table 30.7), thereby enhancing absorption of dietary sterols. This leads to an increased hepatic content of cholesterol and plant sterols, suppression of *LDLR*, inhibition of LDLR synthesis, and elevated LDL-C.

Dietary treatment is paramount in sitosterolemia and consists of a diet very low in *both* cholesterol and plant sterols. Thus, in contrast to the standard low-cholesterol, low-saturated fat diet, plant foods with high-fat, high plant sterol content such as oils and margarines must be avoided. BAS are particularly effective in lowering plant and LDL sterol levels. A CAI, ezetimibe, is also quite effective [29]. These patients respond poorly to statins.

Cholesterol 7a-Hydroxylase Deficiency

A few patients have been described with a deficiency in the rate-limiting enzyme of bile acid synthesis, cholesterol 7α-hydroxylase, which converts cholesterol into 7α-hydroxy-cholesterol (Fig. 30.1, upper left). The hepatic cholesterol pool can increase, decreasing LDLR and increasing levels of LDL-C and TG-enriched remnants. Both hypercholesterolemia and hypertriglyceridemia were reported [24]. As with patients with sitosterolemia, these subjects were relatively resistant to statin therapy [30].

Disorders of Endogenous and Exogenous Lipoprotein Transport

Dysbetalipoproteinemia (Type III Hyperlipoproteinemia)

Adults with dysbetalipoproteinemia present with elevations in both TC and TG, usually but not always above 300 mg/dL. The hallmark of the disorder is the presence of VLDL that migrate as

beta lipoproteins (β-VLDL), rather than prebeta lipoproteins (type III lipoprotein phenotype) (Table 30.6). β-VLDL reflects the accumulation of cholesterol-enriched remnants of both hepatic VLDL and intestinal chylomicrons (Fig. 30.1) [31]. These remnants result from the presence of a dysfunctional apoE, the ligand for the receptor-mediated removal of both chylomicron and VLDL remnants by the liver.

Premature atherosclerosis of the coronary, cerebral, and peripheral arteries in adults is often present. Xanthomas are common, especially planar lesions in the creases of the palms and tuberoeruptive xanthomas over the knees or buttocks. Occasionally, tuberous and tendon xanthomas are found. Hyperuricemia and glucose intolerance occur in up to half the patients with this syndrome.

Human apoE exists as three major isoforms (E2, E3, and E4), each of which is specified by an independent allele at the locus for the apoE gene [31]. One in 100 persons is homozygous for the apoE2 allele, which results in decreased affinity of the TG-enriched remnants to their hepatic receptors; however, because the prevalence of this disorder is only 1:10,000, other modifying factors such as hypothyroidism, low-estrogen state, obesity, or diabetes are necessary for full-blown clinical expression. This recessive form of dysbetalipoproteinemia has a delayed penetrance beyond childhood.

The diagnosis of dysbetalipoproteinemia is based on (1) demonstration of E2E2 genotype, (2) the presence of β-VLDL, and (3) a cholesterol-enriched VLDL (VLDL-C/TG ratio >0.30). LDL and HDL-C levels are low or normal [31].

Dysbetalipoproteinemia in Children and Adolescents

A dominant form of dysbetalipoproteinemia is caused by the expression of one of several rare variants of apoE that usually involve the substitution of neutral or acidic amino acid for basic ones in the region of apoE that interacts directly with the LDLR [31]. The dominant form can be expressed in childhood and does not require the presence of modifying factors. Affected adolescents often present with yellow creases in their palms (planar–palmar xanthomas).

The diagnosis of this rarer form of dysbetalipoproteinemia will require sequencing of the apoE gene.

Children or adults with dysbetalipoproteinemia are very responsive to a low-fat, low-cholesterol diet that decreases the burden of TG-enriched remnants. Fibric acid derivatives have traditionally been the treatment of choice, which normalize both the TC and TG levels. Niacin and statins are also quite effective.

Hepatic Lipase Deficiency

Patients with HL deficiency can present with features similar to dyslipoproteinemia (type III hyperlipoproteinemia), including hypercholesterolemia, hypertriglyceridemia, accumulation of TG-rich remnants (including β-VLDL), and planar xanthomas and premature cardiovascular disease [32]. Recurrent bouts of pancreatitis have been described.

HL shares a high degree of homology to LPL and pancreatic lipase. HL hydrolyzes both TG and PL in plasma lipoproteins and converts IDL to LDL and large HDL-2 to HDL-3 (Figs. 30.1 and 30.2). In HL deficiency therefore, LDL-C is usually low and HDL-C is often quite *high* (despite the hypertriglyceridemia).

HL deficiency is rare and inherited as an autosomal recessive trait. Obligate heterozygotes are normal. The diagnosis is made by a PHLA test to determine that HL activity is absent but LPL activity is normal.

Treatment includes a low-total-fat diet. In one report, the hypercholesterolemia and hypertriglyceridemia in HL deficiency improved dramatically on treatment with lovastatin, while gemfibrozil reduced TG but elevated cholesterol [32].

Disorders of Reduced LDL-C Levels

Abetalipoproteinemia

Abetalipoproteinemia is a rare, autosomal recessive disorder characterized by fat malabsorption, acanthocytes, and hypocholesterolemia in infancy [33]. Later in life, deficiency of fat-soluble vitamins leads to atypical retinitis pigmentosa, posterior column neuropathy, myopathy, and

coagulopathy [33]. Fat malabsorption in infancy is associated with symptoms of failure to thrive (poor weight gain and steatorrhea) and lipid vacuoles invading enterocytes that are visible on intestinal biopsy. Fat malabsorption is due to the inability to assemble and secrete chylomicrons from enterocytes. Symptoms of neurological problems begin during adolescence and include dysmetria, cerebellar ataxia, spastic gait, and axonal peripheral neuropathy mimicking vitamin E malabsorption or Friedreich ataxia. Anemia and arrhythmias may also present.

TC levels are exceedingly low (20–50 mg/dL). Total plasma apoB is undetectable, and thus the apoB-containing lipoproteins, i.e., chylomicrons, VLDL, or LDL, are absent. HDL levels are measurable but low. Vitamin E levels are extremely low. Parents have normal lipid levels.

The absence of plasma apoB was initially believed to be due to defects in *APOB*. However, the defect in synthesis and secretion of apoB-containing lipoproteins was secondary to absent MTP, which normally permits the transfer of lipid to both apoB-48 and apoB-100 [33]. MTP is a heterodimer composed of the ubiquitous multifunctional protein, protein disulfide isomerase, and a unique 97-kDa subunit. Abetalipoproteinemia is caused by mutations that lead to the absence of a functional 97-kDa subunit.

Treatment of Abetalipoproteinemia

The intake of fat is first reduced to 5–20 g/day to control steatorrhea, a step that results in marked clinical improvement and growth acceleration. The diet should also be supplemented with linoleic acid (e.g., 5-g corn oil or safflower oil/day). MCT as a caloric substitute for long-chain fatty acids may produce hepatic fibrosis, and thus MCT should be used with caution, if at all. Fat-soluble vitamins should be added to the diet. High-dose oral vitamin E (150–200 IU/kg/day) is essential to prevent or ameliorate neurologic and retinal complications. Vitamin E levels increase on treatment but remain low. Rickets can be prevented by normal quantities of vitamin D, but high dosages of vitamin A (200–400 IU/kg/day) may be required to raise the level of vitamin A in plasma to normal. Enough vitamin K (5–10 mg/day) should be given to maintain a normal prothrombin time.

Hypobetalipoproteinemia

The phenotype of hypobetalipoproteinemia (hypobeta) is characterized by notably low levels of LDL-C and apoB, usually defined as < the lower fifth percentile (Table 30.4). TC is low; VLDL-C and TG are low or normal. Hypobetalipoproteinemia can be primary or secondary to anemia, dysproteinemias, hyperthyroidism, intestinal lymphangiectasia with malabsorption, myocardial infarction, severe infections, and trauma.

Familial Hypobeta

Familial hypobeta is inherited as an autosomal dominant, which may or may not be associated with mutations in APOB. Affected individuals are usually asymptomatic, the prevalence of CVD is low, and often longevity is found. Those with a defect in APOB have decreased synthesis of apoB and reduced secretion of VLDL from the liver, which can lead to increased hepatic fat of about threefold. A relatively large number of mutations in the APOB gene cause familial hypobeta [34]. Almost all of the mutations are either nonsense or frameshift mutations that create a premature stop codon and a truncated apoB-100. Familial hypobeta has also been linked to a susceptibility locus on chromosome 3p21 and in some families is linked neither to APOB nor to chromosome 3p21 [34].

Familial Combined Hypolipidemia

Musunuru and colleagues [35] found that the presence of two nonsense mutations in the angiopoietin-like 3 gene (ANGPTL3) on chromosome 4 resulted in markedly decreased LDL-C that was accompanied by notably low TG and HDL-C, a phenotype they termed familial combined hypolipidemia. LDL-C and TG levels were inherited as codominant traits, while the low HDL-C was only present in the genetic compounds. This novel finding in this large family suggests a new mechanism for decreasing LDL-C in patients.

Loss-of-Function Mutations in PCSK9

The phenotype of hypobeta is also found in those with a loss-of-function mutation in the PCSK9 gene [6]. In this case, the low LDL results not from decreased production of VLDL but from enhanced LDLR activity due to the decreased

PCSK9 function [6] (see also above). Patients with this cause of familial hypobetalipoproteinemia also have a considerable lifelong reduction in CVD [36].

Homozygous Hypobetalipoproteinemia

The clinical presentation of children with this disorder depends upon whether they are homozygous for null alleles in *APOB* (i.e., make no detectable apoB) or homozygous (or compound heterozygotes) for other alleles, which produce lipoproteins containing small amounts of apoB or a truncated apoB [37]. Null-allele homozygotes are similar phenotypically to those with abetalipoproteinemia and may have fat malabsorption, neurologic disease, and hematologic abnormalities as their prominent clinical presentation and require similar treatment. However, the parents of these children are heterozygous for hypobetalipoproteinemia in contrast to parents of abetalipoproteinemia children who are normolipidemic.

Chylomicron Retention Disease

Chylomicron retention disease (CRD) or Anderson's disease is a rare genetic disease that causes malnutrition, failure to thrive, growth failure and vitamin E deficiency, and other complications [38, 39]. The diagnosis is based on a history of chronic diarrhea with fat malabsorption and very decreased but not absent LDL-C and apoB. In contrast to abetalipoproteinemia and homozygous hypobetalipoproteinemia, the TG are normal in CRD [39]. Fat-laden enterocytes and vitamin E deficiency are invariably present. Hepatic steatosis is common. Increased creatine kinase levels and cardiomyopathy may reflect the muscular complications. Neurological and ophthalmologic complications in CRD are less severe than in other types of familial hypocholesterolemia; for example, there is little acanthocytosis and no retinitis pigmentosa. The molecular defects in CRD are due to mutations in *SAR1B*, leading to a defective Sar1b protein, which prevents the normal transport of prechylomicrons from the endoplasmic reticulum to the Golgi apparatus [33]. No postprandial chylomicrons or apoB-48 are detected. On institution of a low-fat diet supplemented with lipid-soluble vitamins (A and E) and essential fatty acids, normal growth resumes with reduction of gastrointestinal symptoms. Departure from a low-fat diet results in rapid relapse and recurrence of symptoms. Essential fatty acid deficiency is especially severe early in life. Especially, large amounts of vitamin E are necessary to prevent neurological complications.

Disorders of Reverse Cholesterol Transport

Familial Hypoalphalipoproteinemia

Hypoalphalipoproteinemia is a relatively uncommon phenotype defined as a low level of HDL-C (<5th percentile, age and sex specific) in the presence of normal lipid levels [6, 40]. Patients with this syndrome can have a significantly increased prevalence of CAD but do not manifest the clinical findings typical of other forms of HDL deficiency. Low HDL-C levels of this degree are most often secondary to disorders of endogenous TG metabolism.

In some families, hypoalphalipoproteinemia behaves as an autosomal dominant trait, but the basic defect is usually unknown. It is likely that the etiology of primary low HDL-C levels is oligogenic, i.e., there are significant effects of several genes being expressed [40].

Apolipoprotein A-I Mutations

APOA1 exists on chromosome 11 as part of a gene cluster with *APOC3* and *APOA4* (Table 30.2). A variety of molecular defects in *APOA1* include gene inversions, gene deletions, and nonsense and missense mutations [6]. Homozygous gene deletions or nonsense mutations are rare and exhibit little if any biosynthesis of apoA-I by the liver and intestine (Fig. 30.2). The virtual absence of apoA-I is accompanied by marked decreases in HDL-C. Obligate heterozygotes as well as the homozygotes develop premature CVD. In addition to precocious CVD, homozygotes can manifest other clinical findings of peripheral cholesterol deposition, e.g., retinopathy, cataracts, and xanthomas. Missense mutations in *APOA1* have

been described in kindreds with low HDL-C levels. However, the relationship to premature CAD is less clear.

Tangier Disease

Tangier disease is an autosomal recessive disorder in which HDL-C levels are extremely low and of an abnormal composition (HDL Tangier or T). HDL-T are chylomicron-like particles which disappear when a patient consumes a low-fat diet [6, 41].

The classic findings in Tangier patients include enlarged orange-yellow tonsils, splenomegaly, and a relapsing peripheral neuropathy. The orange tonsils reflect the deposition of beta carotene-rich CE in foam cells in the lymphatic tissue. Foam cells can also occur in skin, peripheral nerves, bone marrow, and the rectum. Mild hepatomegaly, lymphadenopathy, and corneal infiltration (in adulthood) may also occur.

APOA1 in Tangier patients is normal. The underlying defect is a deficiency in *ABCA1* [6, 41]. The very low HDL-C is due to the lack of cholesterol efflux by the deficient ABCA1 to nascent HDL; this deficiency can be measured in fibroblasts from Tangier patients [41]. Some but not all patients with Tangier disease have premature CAD in adulthood [6, 41]. Treatment with a low-fat diet diminishes the abnormal lipoprotein species.

Lecithin Cholesterol Acyltransferase Deficiency

Lecithin cholesterol acyltransferase (LCAT), an enzyme located on the surface of HDL particles, is important in transferring fatty acids from the sn-2 position of phosphatidylcholine (lecithin) to the 3-β-OH group on cholesterol (Table 30.3, Fig. 30.2). In this process, lysolecithin and esterified cholesterol are generated (α-LCAT). Esterification can also occur on VLDL/LDL particles (β-LCAT).

Both α- and β-LCAT activity are missing in patients with classic LCAT deficiency [42], a rare, autosomal, recessively inherited trait. More than several dozen mutations have been reported in *LCAT*, which is located on chromosome 16. The diagnosis is suspected in patients presenting with low HDL-C, corneal opacifications, and renal disease (proteinuria, hematuria). The ratio of plasma UC to TC is measured, with a result >0.7 diagnostic of LCAT deficiency.

In *fish eye disease*, only α-LCAT activity is absent. Patients present with corneal opacifications but do not develop renal disease [42]. Variability in clinical presentations of fish eye disease, compared to LCAT deficiency, may be due to differences in total LCAT activity.

To date, there is no treatment of the primary defects. Patients usually die from renal disease, and atherosclerosis may be accelerated by the underlying nephrosis. Patients with LCAT deficiency, and other lipid metabolic disorders associated with renal disease, should be aggressively treated, including a low-fat diet. The secondary dyslipidemia associated with the nephrotic syndrome responds to statin therapy.

Cholesteryl Ester Transfer Protein Deficiency

The role of CETP in atherosclerosis is incompletely understood. CETP is upregulated in liver and peripheral tissues in response to either dietary or endogenous hypercholesterolemia. Elevated HDL-C due to deficiency of CETP was initially described in Japanese families [43]. Several mutations in *CETP* are known. The prevalence of CAD in CETP deficiency is not straightforward. In the Japanese kindreds, increased CAD was primarily observed for HDL-C of 41–60 mg/dL; when HDL-C was >60 mg/dL, men with and without CETP mutations had a low CAD prevalence [43]. These effects occur in spite of lower levels of apoB in CETP deficiency [44]. Thus, it has not been resolved whether a genetic CETP deficiency is an independent risk factor for CAD.

Due to its important role in modulating HDL levels, CETP inhibitors were developed to raise plasma HDL-C. However, many side effects, including increased death from CAD, attributed to interference with aldosterone metabolism, were found with the first CETP inhibitor (CP529, 414: torcetrapib) [45]. Other CETP inhibitors (anacetrapib, dalcetrapib), thought not to affect aldosterone levels, are currently under investigation.

Scavenger Receptor Class B Type I Receptor Deficiency

SR-BI is a functional lipoprotein receptor that participates in the selective uptake of cholesteryl esters (CE) from HDL [46], LDL [47], and VLDL [48] and is regulated by a number of factors. One of its major functions is to mediate the uptake of CE from the core of these lipoproteins. Single-nucleotide polymorphisms (SNPs) in the *SCARB1* gene are significantly associated with HDL-C levels [49]. Certain SNPs in *SCARB1* are significantly associated with subclinical carotid atherosclerosis [50].

Deficiency of Endothelial Lipase

Endothelial lipase (EL) is a member of the triglyceride lipase family of proteins that includes LPL and HL (Table 30.3). EL is a product of *LIPG* and primarily hydrolyzes PL with little TG lipase activity. EL hydrolyzes the lipids in HDL the most efficiently of all the lipoproteins, converting HDL from a larger to a smaller particle (Fig. 32.2). Rare loss-of-function EL variants produce a high HDL-C [51].

Elevated Lipoprotein (a)

Lipoprotein (a) (Lp (a)) consists of a glycoprotein, apo (a), covalently linked to apoB-100 of LDL through a disulfide bond [52]. Apo (a) is highly homologous to plasminogen but has no protease activity. Lp (a) levels are highly heritable and are almost entirely dependent on the apo (a) gene on chromosome 6q27. Lp (a) enters the vascular wall, and elevated Lp (a) is a causal risk factor for CVD [53]. Lp (a) also promotes thrombosis through the inhibition of apo (a) of the conversion of plasminogen to plasmin at the surface of endothelial cells [52]. Lp (a) also binds oxidized PL. The precise physiological function of Lp (a) is unknown.

Diagnosis and Treatment of Lp (a) Lipoprotein

The best method for diagnosis of elevated Lp (a) is an ELISA assay using a monoclonal antibody. The upper limit of normal <75 nmol/L Niacin and estrogen can effectively lower Lp (a) levels, while the statins and fibrates do not. Although clinical trial evidence is lacking regarding the benefit of specifically lowering Lp (a) on the prevalence of CVD, the recommended approach is to treat LDL-C more aggressively patients with CVD who also have elevated Lp (a). A statin is used to reduce LDL-C to <100 mg/dL, at a minimum. Niacin can be added to reduce Lp (a) and to increase HDL-C.

Guidelines for the Clinical Evaluation and Treatment of Dyslipidemia in Children and Adolescents

Screening for Dyslipidemia in Pediatric Endocrinology

Each child and adolescent seen by a pediatric endocrinologist optimally should have a lipoprotein profile performed after an overnight fast. This approach will detect secondary dyslipidemias often due to an endocrinological disorder (Table 30.5) as well as those who may have a separate primary disorder of lipoprotein metabolism. Screening should not simply be based on a family history of premature CVD or hypercholesterolemia, since this approach will fail to detect substantial numbers (from 17 to 90%) of children who have elevated lipid levels [54]. This approach permits the detection of children and adolescents with undiagnosed heterozygous FH or more marked FCHL, who will require more intensive treatment, including the possibility of drug therapy. This may also lead to the detection of unsuspected dyslipidemia in the parents and siblings. Adolescents including those with FH [27] may have a false-negative result due to the effect of puberty on lowering LDL-C levels. Thus, adolescents should be rescreened as they enter young adulthood.

What to Measure

A lipoprotein profile is measured after an overnight fast and includes TC, TG, LDL-C, HDL-C, and non-HDL-C. LDL-C is calculated from the Friedewald equation: LDL-C = TC − (HDL-C + TG/5). TG divided by 5 estimates very-low-density lipoprotein (VLDL)-C. If TG are more than 400 mg/dL, the patient is non-fasting, or type III hyperlipoproteinemia

(dysbetalipoproteinemia) (see above) is present; this formula is inaccurate. A direct LDL-C may be determined under these circumstances, but has more variability than most other lipid-related measures.

In a non-fasted subject, TC, HDL-C, and non-HDL-C can be measured accurately. Non-HDL-C is determined by subtracting HDL-C from TC. Non-HDL-C consists of the amount of cholesterol carried by the atherogenic apoB-containing lipoproteins (VLDL, IDL, LDL, and Lp (a) lipoprotein) (Table 30.1). In children, non-HDL-C is at least as good a predictor as LDL-C of future dyslipidemia and early lesions of atherosclerosis in young adults [55, 56]. Percentiles for non-HDL-C in children and adolescents are available from the Bogalusa Heart Study [13] (Table 30.4).

Well-standardized immunochemical methods are available for apoB and apoA-I measurements [14, 57], which can be of particular use in screening youths with premature CVD in parents [58, 59]. Cutoff points for apoB and apoA-I from the National Health and Nutrition Education Survey (NHANES) are used [14] (Table 30.4). Those with a low HDL-C and elevated TG but normal or borderline LDL-C (Table 30.4) should have an apoB measured. If the apoB is elevated in such children (Table 30.4), then the child probably has FCHL. The complete dyslipidemic expression of FCHL is often delayed until adulthood, although elevated apoB is the first expression of FCHL in adolescents and young adults [60]. ApoA-I can be measured in a child with a low HDL-C to determine the severity of the phenotype.

Advanced lipoprotein testing has been used in research studies to determine the subclasses of VLDL, LDL, and HDL in children and adolescents by nuclear magnetic resonance spectroscopy [61–63] or by vertical-spin density-gradient ultracentrifugation [64], but cutoff points derived from these methods for the diagnosis and treatment of dyslipidemia in youths are not currently available.

When to Sample for Dyslipidemia

Human plasma TC levels are lowest during intrauterine life and at birth [65]. TC and LDL-C increase rapidly in the first weeks of life and then gradually until 2 years of age. Screening for dyslipidemia is therefore generally recommended after 2 years of age when the lipid and lipoprotein levels become quite constant up to adolescence [12].

Definitions of Dyslipidemia

The cut points for abnormal plasma level of lipids, lipoproteins, and apolipoproteins are summarized in Table 30.4. An elevated level is >the 95th percentile, while a low level is <5th percentile.

Definition of the Metabolic Syndrome

There is no current consensus regarding the definition of the metabolic syndrome in youth, and that in those ages 12–17 proposed by Cook et al. [66] from the third NHANES survey is one of several. An adolescent is considered to have the metabolic syndrome if *three* or more of these following factors are present: (1) TG of 110 mg/dL or higher, (2) HDL-C of 40 mg/dL or lower, (3) waist circumference at the 90th or higher percentile, (4) fasting glucose of 110 mg/dL or higher, and (5) blood pressure at the 90th percentile or higher for age, sex, and height. One alternative to waist circumference may be a BMI higher than the 95th percentile for age and gender [67].

Obesity, Dyslipidemia, and the Metabolic Syndrome

Obesity is of critical importance in the development of dyslipidemia and the metabolic syndrome [66–73]. In the past 20 years, the prevalence of adolescents with a BMI above the 95th percentile has increased by more than 50% [73]. The prevalence of the metabolic syndrome increases with the severity of obesity and insulin resistance, as does the dyslipidemic triad (elevated TG, low HDL-C, and increased number of small LDL-P), elevated highly sensitive C-reactive protein, and decreased adiponectin [68]. Acanthosis nigricans is often a sign of underlying insulin resistance. Higher LDL-C levels and obesity [74] and higher blood

pressure levels [74, 75] increase carotid IMT in adulthood. The metabolic syndrome in youths predicts adult metabolic syndrome and CVD two to three decades later [71, 72]. If the LDL-C is <160 mg/dL after hygienic measures, metformin has been used in several studies of obese adolescents with the metabolic syndrome and hyperinsulinemia [76, 77].

Guidelines for Treatment of Dyslipidemia in Children and Adolescents

Dietary Therapy

Treatment and Follow-Up with Dietary Treatment

Youths with dyslipidemia are first treated with a diet reduced in total fat (<30% of calories), saturated fat (<7% of calories), trans fat (none), and cholesterol (<200 mg/day) [12]. Monounsaturated fat and polyunsaturated fat, such as found in canola and olive oil, can be used to make the diet more palatable. The intake of complex carbohydrates is increased, whereas that of simple sugars and fructose sweetened products is decreased, a change particularly important in the young patient with elevated TG and/or obesity. No decrease in total protein is recommended. Calories are sufficient to maintain normal growth and development. The NCEP pediatric panel recommended diet treatment after 2 years of age [12].

When to Initiate Treatment with Diet

If the first lipoprotein profile indicates that TC, LDL-C, non-HDL-C, or TG are elevated or that the HDL-C is low (Table 30.4), then another sample is obtained at least 3 weeks later to confirm the results from the initial testing. At that time, secondary causes of dyslipidemia (Table 30.2) are evaluated and dietary treatment begun. A registered dietician should perform the dietary instruction at the second baseline visit and then evaluate dietary compliance at the third visit in 6–8 weeks. Dietary therapy can be continued for another 6 months, at which time the clinician decides if the dyslipidemia is sufficiently marked to institute treatment with a lipid-altering drug (see below).

Safety and Efficacy of Dietary Therapy in Infants, Children, and Adolescents

Overall, a diet low in fat in children with dyslipidemias appears safe and efficacious when performed under supervision. Medical and nutritional support is necessary to reinforce good dietary behaviors and ensure nutritional adequacy. In STRIP, a low-fat diet was efficacious and safe in children from 7 months to 11 years of age [78–80]. In the Dietary Intervention Study in Children (DISC), starting at ages of 8–10 years throughout adolescence, a low-fat, low-cholesterol diet was safe [81–83]. Therefore, although normal growth is achieved and maintained on low-fat diets, attention needs to be paid to ensure adequate intake of calcium, zinc, vitamin E, and phosphorus [81–83]. Human milk remains the gold standard for infant feeding, and the higher TC in breastfed infants does not persist into childhood, adolescence, or adulthood [84].

The use of margarines (about three servings daily) high in either plant stanol esters [85, 86] or plant sterol esters [87] can reduce LDL-C an additional 10–15%, when added to a low-fat diet. Water-soluble fibers [88] such as psyllium [89, 90] may also provide an additional 5–10% lowering of LDL-C.

Effect of a Low-Fat Diet in Childhood on Future CVD in Adulthood

That a low-fat diet in childhood will prevent CVD in adulthood has only been inferred from epidemiological studies [12]. Insulin resistance is promoted in youths by obesity. A low-saturated fat counseling program starting in infancy in STRIP improved insulin sensitivity in 9-year-old healthy children [91], decreased obesity in girls [92], and enhanced endothelial function in 11-year-old boys, but not in girls, effects mediated in part by the diet-induced reduction in TC [80].

Pharmacological Therapy

Guidelines for the Institution of Drug Therapy

When to Start Drug Therapy

The primary use of drugs in pediatrics is to lower significantly elevated LDL-C [93] (Table 30.8). Drug treatment to lower LDL-C can be initiated at 10 years of age. The American Academy of Pediatrics [94] indicated that onset of treatment might be lowered to 8 years of age in children with marked elevations in LDL-C and a striking family history of premature CVD. A more conservative approach is to wait until Tanner stage II in males and after menstruation in girls.

Criteria for Instituting Drug Therapy

Pharmacologic treatment of elevated LDL-C in youth can be considered if the postdietary LDL-C is (1) >190 mg/dL and there is a negative or unobtainable family history of premature CVD or (2) >160 mg/dL and there is a family history of premature CVD or two or more risk factors for CVD are present [12, 93, 94] (Table 30.8).

LDL-C Goals for Drug Treatment

The minimum goal after drug treatment is an LDL-C <130 mg/dL (Tables 30.4 and 30.8). A desirable goal is an LDL-C <110 mg/dL, which is below a borderline elevated LDL-C of 110–129 mg/dL (Tables 30.4 and 30.8).

HMG-CoA Reductase Inhibitors (Statins)

The statins are generally the first class of drugs that are used to treat children 10–17 years of age with autosomal dominant hypercholesterolemia or significant FCHL. The statins inhibit the rate-limiting enzyme of cholesterol synthesis, hydroxymethylglutaryl-CoA reductase, thereby reducing hepatic cholesterol and releasing SREBP from the cytoplasm into the nucleus, where SREBP binds to the SRE of the promoter of *LDLR*, increases the number of LDLR, and decreases LDL-C [7] (Fig. 30.1).

A meta-analysis of a number of randomized, placebo-controlled trials of the statins [95] showed high efficacy for LDL-C and apoB lowering of 30–35% with a range of 25–50%. There was no increase in side effects with statins, compared with placebo [95]. Atorvastatin, fluvastatin, lovastatin, pravastatin, simvastatin, and rosuvastatin are approved by the FDA for use in FH children 10–17 years of age. The equivalent doses are about 5 mg rosuvastatin, 10 mg atorvastatin, 20 mg simvastatin, 40 mg lovastatin, 40 mg pravastatin, and 80 mg fluvastatin. Atorvastatin and rosuvastatin both have long half-lives of about 17 h. Thus, they can be taken in the morning or evening in contrast to other statins which should be taken at bed time because of their short half-lives. All except, fluvastatin, and rosuvastatin are available generically.

Efficacy of Statins on LDL-C Reduction: Consideration of Combined LDL-C-Lowering Agents

The ability of the statins to achieve LDL-C goals (Table 30.8) will be related to how high the baseline LDL-C is elevated. In one study, the average baseline LDL-C in FH heterozygotes was 232 mg/dL. Using a higher dose of 20 mg of a potent statin, rosuvastatin, the mean LDL-C fell 50%, but 40% of these patients did not achieve an LDL-C goal of <110 mg/dL [96]. Ezetimibe (see also below) has been combined with simvastatin to effect an additional 15–25% decrease in LDL-C in FH heterozygous children [97]. There is also a nonlinear dose–response relation when a BAS or niacin is added to statin [98] (see also below). Since both ezetimibe and BAS increase cholesterol biosynthesis, the effect of either of these agents is complementary when combined with a statin. Combination of another LDL-C-lowering agent to a statin should be undertaken in consultation with a lipid specialist.

Effect of Statins on Carotid Intima-Media Thickness

Wiegman et al. [99] found that a 24% reduction in LDL-C with pravastatin in FH heterozygotes 8–15 years of age significantly decreased carotid IMT compared with placebo. Younger age at statin initiation was an independent predictor of effect of treatment on carotid IMT in

this Dutch study [100]. Early statin therapy also restored endothelial function in children with FH [101]. In an open-label study in FH heterozygotes 10–16 years of age with fluvastatin 80 mg/day, median LDL-C fell 34% but there was no significant change in carotid IMT [102]. Early intervention with statins appears likely to reduce future atherosclerosis and CVD in those with FH.

Effect of Statins in Patients with Homozygous FH

Patients with homozygous FH have little or no LDLR activity. Use of a higher dose of a more potent statin will result in some modest reduction in LDL-C, due to their effect on decreasing the production of VLDL and consequently LDL [103]. Addition of ezetimibe produced another 25% decrease in LDL-C [103]. BAS may have a modest effect on LDL-C. Some FH homozygotes respond well to niacin (55–87 mg/kg/day in divided doses), which reduces hepatic production of TG leading to decreased VLDL and LDL levels [93]. Almost all FH homozygotes will require LDL apheresis in addition to drug treatment to effect a satisfactory reduction in LDL-C [103].

Side Effects of the Statins in Children and Adolescents: Liver and Muscle

Statins are generally well tolerated, especially in youths, and have an excellent safety profile with minimal side effects. In a meta-analysis [95], the prevalence of elevated alanine aminotransferase three times above the ULN in the statin group was 0.66% (3 per 454). Instances of asymptomatic increases (>10-fold) in creatine kinase, although unusual, have been reported in adolescents receiving statin therapy [95]. No cases of rhabdomyolysis have been reported [95–97].

Liver function tests (AST, ALT) should be monitored at baseline, following 6–8 weeks after initiating treatment and every 4 months for the first year. After that, patients on a stable dose of a statin can have their liver function tests monitored every 6 months. Consideration should be given to reducing the dosage of drug, or its discontinuation, should the liver function tests exceed three times the upper limits of normal. Creatine kinase (CK) should be measured at baseline and repeated if the patient develops muscle aches and cramps. The statin is discontinued if the CK is >5× the upper limit of normal in those with symptoms of myositis or >10× the upper limit of normal in asymptomatic patients. In adults, 1/500 to 1/1,000 patients may develop myositis on a statin, which can lead to life-threatening rhabdomyolysis [104]. Rhabdomyolysis is a rare event, occurring at an incidence of 1.2 per 10,000 patient-years [104]. Three statins, lovastatin, simvastatin, and atorvastatin, are metabolized by the CYP3A4 isozyme of the cytochrome P450 microsomal enzyme system and consequently have drug interactions with other agents metabolized by CYP3A4. Examples include erythromycin, verapamil, cyclosporine, HIV protease inhibitors, sertraline, and the fibric acid derivative, gemfibrozil.

Special Issues in Young Females

The statins are effective and safe in adolescent girls, with no significant adverse effect on growth and development or on gonadal and adrenal hormones [95].

Because of potential risk to a developing fetus, statins are contraindicated during pregnancy. Birth control is mandatory for those who are sexually active. Because of this concern, the long-term commitment to therapy, and the fact that CAD increases after menopause, some specialists believe that statins should not be used to treat adolescent FH females. Others do recommend treatment of adolescent FH females, especially those with a striking family history of premature CAD. Additional studies are needed to document the long-term safety of statins and to determine their effects on future CVD.

Bile Acid Sequestrants

BAS were the only class of drugs recommended by NCEP for pharmacologic lipid-lowering therapy because of their long track record of safety over three decades [12]. BAS do not enter the blood stream but bind bile acids in the intestine [105], preventing their reabsorption through the ileal bile acid transporter (IBAT)

(Fig. 30.1). Decreased return of bile acid stimulates the conversion of cholesterol to bile acids through 7 ά-hydroxylase, lowering the hepatic cholesterol content and inducing LDL receptors (Fig. 30.1). The BAS produce only a modest LDL-C reduction of about 15% [106, 107]. The first-generation BAS, cholestyramine and colestipol, suffered from significant tolerability issues including constipation, heart burn, bloating, decreased serum folate levels, and interference with the absorption of other drugs [105]. In one study, over 80% of FH heterozygous children discontinued BAS after an average of 22 months, secondary to gritty taste and gastrointestinal complaints [107]. The second-generation sequestrant, colesevelam (625-mg tablets, three or six per day), has a greater affinity for bile salts and can be used in a lower dose. Colesevelam is associated with less annoying side effects than cholestyramine, such as constipation and gritty taste, and does not interfere with the absorption of other drugs. Colesevelam, alone or combined with a statin, is approved by the FDA as an adjunct to diet and exercise to reduce LDL-C in boys and postmenarchal girls, aged 10–17 years with heterozygous FH. Colesevelam can be administered as 625-mg tablets or as an oral suspension [108].

Safety of Bile Acid Sequestrants

In randomized clinical trials, cholestyramine did not affect height velocity [106, 107]. Levels of fat-soluble vitamins were maintained, except the BAS group had significantly lower 25-hydroxyvitamin D than the placebo group. Low folate and high homocysteine levels have been reported on BAS [105–107].

Cholesterol Absorption Inhibitor

The CAI, ezetimibe, decreases the intestinal absorption of cholesterol derived from diet and from bile by about 50% [103] (Fig. 30.1), leading to decreased hepatic cholesterol, increased LDL receptor activity, and decreased LDL-C. Ezetimibe selectively inhibits a protein transporter, Niemann-Pick C1-like 1 (NPC1L1), localized at the brush border of enterocytes that normally moves cholesterol from mixed micelles into the cells of the jejunum [109] (Fig. 30.1). Ezetimibe lowers LDL-C by about 15–20% either alone or when combined with a statin [29, 97, 103]. Ezetimibe is not yet approved by the Food and Drug Administration (FDA) for use in children, except in very rare cases of sitosterolemia [29] or homozygous FH [103]. While there have been isolated case reports of possible ezetimibe-associated myopathy, there is no evidence from randomized clinical trials of increased myopathy or rhabdomyolysis with ezetimibe [105]. Other side effects include gastrointestinal upset, headache, and increased incidence (about 3%) of elevated liver function tests when combined with a statin. Ezetimibe is administered in one dosage only, 10 mg/day.

Niacin (nicotinic acid) is a water-soluble B-complex vitamin, which through its interaction with its receptor GPR109A [110] inhibits the release of FFA from adipose tissue, leading to decreased delivery of FFA to liver and reduced synthesis of TG and consequently VLDL and LDL [110] (Fig. 30.1). Niacin also inhibits the uptake of HDL through its catabolic pathway, prolonging the half-life of HDL and presumably increasing reverse cholesterol transport. Niacin is also the only lipid-altering drug that reduces LP (a) lipoprotein.

Niacin is not routinely used in pediatrics. There are little published data on the safety and efficacy of niacin in pediatrics. The single exception is the FH homozygous patient (see above). Children with either hemorrhagic or ischemic strokes have elevated Lp (a) lipoprotein [99], but there are no data on the safety or effectiveness of niacin in such children [111]. Niacin is associated with a number of side effects, including a cutaneous flush after administration, increased liver function tests, and precipitation of diabetes mellitus or gout in adults.

Fibrates

Fibric acid derivatives (fibrates) are agonists for the peroxisome proliferator-activated receptor alpha (PPAR alpha), which upregulate the gene for LPL and apoA-V and downregulate the gene for apolipoprotein C-III [112] (Tables 30.2 and 30.3, Fig. 30.1). Fibrates also upregulate the gene for apoA-I, which increase HDL-C levels. Use of a fibrate is usually reserved for that adolescent

with TG over 500 mg/dL, who may be at increased risk of pancreatitis. The most common side effects of fibrates are upset stomach, nausea, or vomiting. Abdominal pain is the second most common side effect. There is a slightly increased risk of gallstones. Gemfibrozil can potentiate drugs that prevent blood clotting (anticoagulants), causing bleeding.

Omega-3 Fatty Acids

Omega-3 fatty acids inhibit the production of TG in liver by several postulated mechanisms, including interfering with the incorporation of FFA into TG (Fig. 30.1). The omega-3 fatty acids are enriched in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) that are concentrated from fish oils to a 90% purified form that is available in a prescription formulation, Lovaza, but the prescription version of ω-3 fatty acids is not yet approved by the FDA for use in children. TG can be lowered up to 50% at a dosage of two 1-g capsules taken twice daily.

Treatment of Dyslipidemia Secondary to Other Diseases

Type I Diabetes

Youths with type I diabetes are at high risk for CVD as adults and already have increased carotid IMT [113]. After dietary therapy and the best achievable diabetic control, the American Diabetes Association strongly recommends the use of statins in those with LDL-C >160 mg/dL [113].

Nephrotic Syndrome

The dyslipidemia in children with the nephrotic syndrome can be marked, with both TC and TG that approach 300 mg/dL or higher [114]. Those patients who are unresponsive to steroids and have a postdietary LDL-C of more than 160 mg/dL may be at an increased risk for CVD [114] and warrant treatment with a statin, which is effective in this condition.

Polycystic Ovarian Syndrome

Polycystic ovarian syndrome (PCOS) presents in adolescence with menstrual disorders, acne, and hirsutism [115, 116]. Insulin resistance and dyslipidemia are often present. After diet and weight control, an estrogen/progesterone combination is often used [115]. Metformin can be considered, especially in those who are obese. Increased carotid IMT is present in young adults with PCOS [115, 116], and treatment with a statin can be considered in those with LDL-C >160 mg/dL.

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Part VIII

Other Endocrine Disorders

Autoimmune Endocrine Disorders 31

Jennifer M. Barker

Abstract

Autoimmune endocrine disorders are common conditions evaluated for and treated by pediatric endocrinologists. Recognition of the underlying autoimmunity associated with the disorders and disease associations is critical to providing appropriate care for these patients. Autoimmune endocrine disorders coexist in recognized syndromes known as the autoimmune polyendocrine syndromes (APS): APS-1 or autoimmune polyendocrinopathy candidiasis and ectodermal dystrophy (APECED) and APS-2. More rare autoimmune endocrine disorders include the immunodysregulation polyendocrinopathy enteropathy X-linked (IPEX) syndrome. This rare disorder presents in infancy with type 1 diabetes and enteropathy. In this chapter, we will discuss the pathophysiology of the autoimmune process, the underlying genetics and disease associations of the autoimmune polyendocrine syndromes, and treatment and screening protocols and briefly touch on rare autoimmune endocrine disorders.

Keywords

Autoimmune polyendocrine syndrome type 1 • Autoimmune polyendocrine syndrome type 2 • Hypothyroidism • Hyperthyroidism • Type 1 diabetes • Celiac disease • Addison's disease

Introduction

Autoimmune endocrine disorders are common conditions evaluated for and treated by pediatric endocrinologists. Recognition of the underlying autoimmunity associated with the disorders and disease associations is critical to providing appropriate care for these patients. Autoimmune endocrine disorders coexist in recognized syndromes known as the autoimmune polyendocrine syndromes (APS): APS-1 or autoimmune polyendocrinopathy candidiasis and ectodermal dystrophy (APECED) and APS-2. More rare autoimmune endocrine disorders include the immunodysregulation polyendocrinopathy enteropathy X-linked

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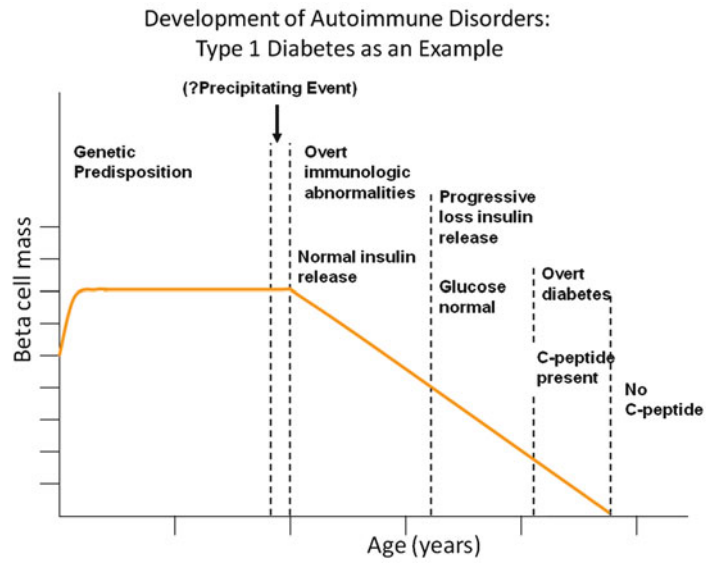

Fig. 31.1 Development of autoimmune disorders: type 1 diabetes as an example

(IPEX) syndrome. This rare disorder presents in infancy with type 1 diabetes and enteropathy. In this chapter, we will discuss the pathophysiology of the autoimmune process, the underlying genetics and disease associations of the autoimmune polyendocrine syndromes, and treatment and screening protocols and briefly touch on rare autoimmune endocrine disorders.

Natural History of Autoimmunity (Fig. 31.1)

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. This disease schema is a hypothesis of how autoimmune diseases develop and is obviously a simplification [1].

Much is known about the natural history of autoimmune disease through studies in infants and young children at risk for type 1 diabetes. Large-scale clinic trials such as the Diabetes Autoimmunity Study in the Young (DAISY) [2], the Finnish Diabetes Prediction and Prevention Project (DIPP) [3], and The Environmental Determinants of Diabetes in the Young (TEDDY) [4] studies enroll young children and infants who are at a heightened risk for the development of type 1 diabetes on the basis of family history of type 1 diabetes and/or presence of high-risk genetic markers for the disease prior to the development of autoimmunity associated with type 1 diabetes. The participants are then followed for diabetes-related autoimmunity and overt diabetes in an attempt to determine factors that are associated with disease development. These sorts of studies have increased our knowledge about the natural history of autoimmunity, and therefore, type 1 diabetes serves as a model for the development of other autoimmune diseases.

There are multiple genes that have been associated with the risk for autoimmune disease. The most consistent risk has been shown with the genes that make up the human leukocyte antigens (HLA) found on chromosome 6. Different HLA alleles have been associated with different autoimmune conditions. For example, the HLA DR3/DR4 has been associated with type 1 diabetes [5]. DR3 and DR4 are associated with autoimmune hypothyroidism [6] and DR3 with celiac disease [7], and DQ0602 is associated with protection from type 1 diabetes [8] and an increased risk for multiple sclerosis [9]. Other protective alleles for type 1 diabetes have also been identified including DP0402 in the setting of the high-risk DR3/

DR4 [10]. A particular allele of DR4, DRB1 0404, has been associated with Addison's disease [11]. 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). In addition to genetic risk, family history is known to play an important role in the development of autoimmune conditions. For example, in siblings of patients with type 1 diabetes, the presence of DR3/4 confers a greater risk for the development of type 1 diabetes compared with the risk in the general population with that HLA genotype. Moreover, 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% by 5 years of follow-up [12]. Thus, the risk for development of autoimmune disease can be additive.

Despite the strong genetic influence in development of autoimmune diseases, the genetic background does not tell the entire story related to the development of autoimmune 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. For example, it appears that timing of introduction to solid foods is an important risk for both type 1 diabetes, associated with early introduction of cereals, (<4 months) [13] and celiac disease associated with early introduction of gluten (<4 months) [14]. Vaccines have been shown to not be associated with the risk for type 1 diabetes [15]. Viruses and environmental toxins are also being investigated.

While the autoimmune destruction is thought to be mostly T cell mediated, the autoimmune process is marked by the presence of autoantibodies (antibodies against self-antigens) (Table 31.1). 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 [16], the persistence of the autoantibodies on multiple tests [17], and autoantibody level and affinity of autoantibodies for the antigens [18]. 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 antibodies [19]. Therefore, the autoantibodies are a marker of risk for disease, but the disease may develop over many years. T cell assays are currently under development. As our assays develop, we hope to be able to predict the development of autoimmune disease with greater accuracy.

Once the autoimmune process is initiated, progressive failure of the affected gland occurs. Markers of insulin release, insulin resistance, and glucose metabolism are associated with progression to type 1 diabetes once autoimmunity has occurred [20]. Hemoglobin A1c tends to rise within the normal range as diabetes develops in subjects with diabetes-related autoimmunity [21]. However, use of hemoglobin A1c to identify subjects at risk for type 1 diabetes as having normal glucose tolerance is not advised given that many people will have a normal hemoglobin A1c at the time of diabetes diagnosis based on oral glucose tolerance testing [22]. 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. In subjects with 21-hydroxylase autoantibodies, progressive deterioration in adrenal secretion of cortisol and aldosterone is noted [23]. In thyroid disease, patients may initially present with compensated hypothyroidism and be relatively asymptomatic

Table 31.1 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 Thyroglobluin autoantibodies	TSH Thyroid hormone levels
Hyperthyroidism	Thyroid stimulating immunoglobulin	TSH Thyroid hormone levels
Adrenal insufficiency	21-Hyroxylase 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

(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.

Autoimmune Polyendocrine Syndrome Type 1 (APS-1)/ Autoimmune Polyendocrinopathy Candidiasis and Ectodermal Dystrophy (APECED) (Table 31.2)

APS-1 is an autosomal recessive disorder historically defined by the presence of two of the following three conditions: hypoparathyroidism, adrenal insufficiency, and candidiasis. The disorder is rare but has an increased frequency in certain populations such as Iranian Jews (1:9,000), Sardinians (1:14,000), and the Finns (1:25,000) [24].

Over the last decade, the underlying genetic basis of APS-1 has been better defined. Mutations in the gene (located at 21q22.3) that encodes the AIRE protein are responsible for APS-1. The gene is a putative transcription factor and 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 an important transcription factor for the expression of self-antigens within the thymus and that this expression is important for the deletion of autoreactive T cells (negative selection) [24]. Therefore, in subjects who have inherited two defective copies of the AIRE gene, autoreactive T cells are released into the periphery and can precipitate the autoimmune destruction of the organ to which the T cells respond. The role of the AIRE gene in the mucocutaneous candidiasis and ectodermal dystrophy that is observed in patients with APS-1 continues to be defined.

Clinically, patients often present in infancy with chronic mucocutaneous candidiasis. Additional autoimmune diseases develop over time. Typically, the first autoimmune endocrine disorder that is identified is hypoparathyroidism which often presents in early childhood at a median of 6 years of age. The next disorder that develops is adrenal insufficiency, at a median of 10 years of age. However, it is important to note that the time from first disease component to the

Table 31.2 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		Diagnosed with TTG 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

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second component that would classify a patient as APS-1 can range from 2 to 20 years, thereby profoundly delaying 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 [15, 25, 26]. Table 31.2 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 syndromes. 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 [27]. Patients commonly develop autoimmune hepatitis, pernicious anemia, severe obstipation, and diarrhea. More rarely, patients can develop autoimmune hypophysitis with resultant pituitary hormone deficiency, autoimmune disease affecting the lung, rheumatoid arthritis, and nephritis. Asplenia can also be present and puts 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 involvement [15, 25, 26, 28].

The candidiasis associated with APS-1 generally is limited to the skin and mucosa. It is rarely systemic. The candidiasis can be difficult to control, and treatment with antifungals on a continuous

basis may be required. Patients may present with candidal esophygitis which may require endoscopy to diagnosis. Additionally, candida that is poorly responsive to treatment is a risk for carcinoma of the esophagus which has very high morbidity and mortality. Aggressive control of candidal infections is recommended [28].

Ocular disease can also develop in patients with APS-1. Approximately 20% of patients develop keratoconjunctivitis. Keratoconjunctivitis often presents in childhood and puts the patient at risk for blindness [29]. Ectodermal dystrophies are also present in patients with APS-1 and include enamel hypoplasia, nail dystrophy, and calcium salt deposits in the tympanic membrane. The underlying cause of these abnormalities is not known. The diagnosis can be made on a clinical, immunologic, and 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). Note that in subjects with a sibling with APS-1, 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. Recently, autoantibodies against interferon alpha and omega have been found in almost 100% of patients with APS-1 [27]. 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 [27]. Subjects with APS-1 require careful and close monitoring for the development of additional autoimmune diseases. Table 31.2 shows a proposed schema for follow-up and screening of patients with APS-1.

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 will 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.

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.

Autoimmune Polyendocrine Syndrome Type 2 (APS2)

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 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) have been described. 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 have been associated 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 [30]. Hypothyroidism is most commonly seen. Occasionally patients present with hyperthyroidism. The presence of thyroid-related autoantibodies is associated with a higher progression to thyroid disease [19]. 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 [31]. 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 [23, 32]. In this population, the risk for adrenal insufficiency is influenced by genes outside of the MHC (e.g., MIC-A), level of 21-hydroxylase autoantibody, gender, and presence of associated autoimmune conditions [23]. Patients with celiac disease are at an increased risk for the development of autoimmune thyroid disease, most commonly hypothyroidism [33, 34]. Conversely, patients with hypothyroidism are also at risk for the development of celiac disease [35].

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 thyroid-stimulating hormone (TSH). 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 screening for Addison's disease in the populations with type 1 diabetes [36]. 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. The specific screening 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.

Treatment focuses on treating the underlying autoimmune disease identified. Care should be taken related to the assessment for additional underlying autoimmune disease. For example, treatment of patients with undiagnosed adrenal insufficiency and hypothyroidism with thyroid medication 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. To date, no therapy is FDA approved outside of the research setting. 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 and abnormalities of glucose metabolism, not diagnostic of type 1 diabetes 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 have been established to identify subjects at risk for type 1 diabetes or

with newly diagnosed type 1 diabetes for randomized clinical trials in prevention of type 1 diabetes or preservation of c-peptide in patients newly diagnosed with type 1 diabetes [37].

The very earliest stages are usually found in infants and young children. Therefore, a primary goal is the safety of the treatment. Treatment trials include the use of hydrolyzed formulas at discontinuation of breast feeding [38] treatment with DHA (and other components of fish oil) and treatment with oral insulin [39]. 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 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.

Patients who have diabetes-related autoantibodies are already at an increased risk for the development of autoimmunity. Fewer subjects are needed to see an effect, and treatments can be slightly more toxic. 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 [40]. 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 [41]. 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. At this stage, treatments are generally targeted toward the immune system with the goal preservation of c-peptide production. Anti-CD3 is a T cell-depleting therapy, in which the T cell depletion is short term. 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. Treatment includes intravenous administration of the medication and has side effects related to the depletion of T cells. C-peptide production has been preserved 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 [42, 43]. Similarly, treatment with anti-CD 20 (a B cell-specific antibody) has shown preservation of c-peptide production for approximately 1 year after treatment [44]. The treatments appear to temporarily decrease the autoimmune process, but are not altering the underlying autoimmunity. It is possible that multiple or combination treatments will be required over a lifetime to permanently maintain c-peptide production.

Immunodysregulation Polyendocrinopathy Enteropathy X-Linked Syndrome (IPEX)

IPEX is a rare autoimmune endocrine disorder inherited in an X-linked fashion. The underlying genetic defect is in the FOXp3 gene [45]. 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 type 1 diabetes and severe enteropathy resulting in diarrhea and profound failure to thrive. Recent reports have shown that multiple autoantibodies can be present in these patients, suggesting a role for the CD25+/CD4+ T cells in regulation of B cells [46]. The association of these autoantibodies with development of autoimmune

diseases remains to be determined. As this is a rare condition, treatments are largely based on anecdotal evidence and have included immunosuppressive medications including sirolimus and bone marrow transplantation. A recent report of two patients with low-intensity non-myeloablative conditioning hematopoietic cell transplantation showed stable engraftment of the transplanted cells [47]. The authors proposed this method for preparation given that more intense regimens may be associated with significant toxicity in the already fragile infants.

Conclusion

Autoimmune endocrine disorders are common disorders in pediatric endocrinology. The autoimmune endocrine disorders 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 with a single-gene mutation responsible for disease. 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 associated with it, 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 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 thorough history and physical examination for signs or symptoms of autoimmune diseases. Routine screening with laboratory tests may be indicated for certain disorders.

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Multiple Endocrine Neoplasia Syndromes

32

Michael S. Racine and Pamela M. Thomas

Abstract

The multiple endocrine neoplasia (MEN) syndromes form a heterogeneous set of familial disorders featuring diverse neoplasias—hyperplasia, adenomas, even carcinomas—of endocrine glands. This collection of syndromes, made up of MEN types 1, 2A, and 2B, and the related familial medullary thyroid carcinoma (FMTC) are caused by mutations of discrete genetic loci inherited in an autosomal dominant pattern. Prior to the identification of the respective, relevant genes for MEN types 1 and 2 in the 1990s, all offspring of affected individuals were considered at risk and were subjected to annual biochemical and radiographic screening. The possibility of carrier status confirmation has obviated the need for routine biochemical screening in all such children.

Keywords

Parathyroid hyperplasia • Neuroendocrine tumor • Pituitary adenoma • Neoplasia syndromes • Pheochromocytoma • RET • MEN 1 • MEN 2 • Menin

Overview

The multiple endocrine neoplasia (MEN) syndromes form a heterogeneous set of familial disorders featuring diverse neoplasias—hyperplasia, adenomas, even carcinomas—of endocrine glands. This collection of syndromes, made up of MEN types 1, 2A, and 2B, and the related familial medullary thyroid carcinoma (FMTC) are caused by mutations of discrete genetic loci inherited in an autosomal dominant pattern. Prior to the identification of the respective, relevant genes for MEN types 1 and 2 in the 1990s, all offspring of affected individuals were considered at risk and were subjected to annual biochemical and radiographic screening. The possibility of carrier status confirmation has obviated the need for routine biochemical screening in all such children.

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Other syndromes of endocrine gland neoplasias (Von Hippel-Lindau disease, Carney complex, Peutz-Jeghers, and Cowden syndromes) and the recently described, rare MEN 4 [1] are beyond the scope of this review. This discussion is limited to the presentation, diagnosis, genetic basis, and treatment of MEN syndromes 1 and 2 as they pertain to children.

Multiple Endocrine Neoplasia Type 1

Introduction

Approximately 20 individuals affected by multiple adenomas of various endocrine glands had previously been described in Europe and the USA when, in 1954, Paul Wermer's paper entitled *Genetic Aspects of Adenomatosis of Endocrine Glands* appeared in the American Journal of Medicine [2]. The presentation of various collections of endocrine tumors in a man and four of his nine adult offspring was offered for consideration, and although the possibility of a familial disorder had been suggested by previous investigators, Dr. Wermer was the first to propose a single genetic defect with autosomal dominant transmission and a high degree of penetrance. In the 40 years which followed, Wermer's syndrome (now known as multiple endocrine neoplasia type 1) proved to fulfill perfectly the assertions made by Dr. Wermer in 1954.

With an estimated prevalence of one per 10,000–25,000 in the general population [3], MEN 1 is a rare familial syndrome of neoplastic transformation of variable combinations of endocrine glands, featuring the principle triad (recalled by the mnemonic "the 3 Ps") of primary hyperparathyroidism, pancreatic neuroendocrine tumors, and anterior pituitary adenomas [2, 4]. Carcinoid tumors, adrenocortical tumors, lipomas, facial angiofibromas, and skin collagenomas are occasionally present [5, 6]. Any of the three chief components of MEN 1 may be the initially presenting manifestation, and the specific constellation of tissue involvement varies between kindreds and between individuals within an affected family.

Primary Hyperparathyroidism

Overview

Primary hyperparathyroidism, characterized by hypercalcemia with an inappropriately normal or elevated plasma concentration of parathyroid hormone (PTH), develops in over 90% of individuals with MEN 1 by the 5th decade of life and, as such, exhibits the highest penetrance of the MEN 1 tumors [3, 7–9]. Asymmetric hyperplasia involving multiple parathyroids differentiates this syndrome histologically from the solitary adenoma of sporadic primary hyperparathyroidism.

Clinical Presentation

Primary hyperparathyroidism is usually reported to be the first disorder of MEN 1 to present, classically in the 3rd decade. Detection at a mean age of 19 years was demonstrated through careful prospective biochemical screening of adolescents at risk in a Swedish cohort [10]; however, it has been reported in a child as young as 5 years of age [11]. The hypercalcemia of MEN 1-related hyperparathyroidism may be relatively mild [12], and classic symptoms of hypercalcemia (polyuria, constipation, myalgias, abdominal pain, etc.) may be absent.

Diagnosis

Primary hyperparathyroidism is confirmed by a serum calcium concentration above 10.4 mg/dL (2.6 mmol/L), accompanied by a non-suppressed plasma PTH, measured by an amino-terminal or intact PTH assay, in the absence of chronic renal insufficiency.

Therapy

Bilateral neck exploration and total or subtotal parathyroidectomy, with near-total thymectomy, is indicated in symptomatic patients with a serum calcium concentration of 12 mg/dL (3.0 mmol/L) or higher, nephrolithiasis, or evidence of bone loss [6].

Two strategies have their respective proponents [13, 14]: total parathyroidectomy involves excision of all identified parathyroids, with reimplantation of a parathyroid autograft to the nondominant forearm. Subtotal parathyroidectomy involves excision of all but one-half gland, leaving a small residual in the neck with its native vascular supply. Persistent postoperative hypocalcemia is typically avoided when as little as 50 mg of parathyroid tissue remains [14]. Regardless of surgical strategy, rates of recurrent hypercalcemia are high, greater than 50% by 10 years in long-term follow-up series [13, 15], a reflection of the inexorable proliferative process of MEN 1.

Pancreatic Neuroendocrine Tumors

Overview

Multifocal neoplastic transformation of pancreatic islet cells, with or without associated syndromes of hormone excess, occurs in 30–80% of patients with MEN 1. Because of the risk of malignant transformation and metastasis, observed in up to one-half of patients, pancreatic neuroendocrine tumors (PNTs) represent the most pressing MEN 1-associated threat to life [9]. PNTs are classified according to the primary hormone secreted, if any, and on the clinical syndrome which results. "Nonfunctioning" PNTs secreting chromogranin A or pancreatic polypeptide (PP) are clinically silent unless they become large enough to cause mass effects. They may develop before functional PNTs do and have been reported in children as young 12 years of age [16]. Among functional PNTs, gastrinomas with subsequent Zollinger-Ellison syndrome (ZES) are the most common, followed by insulinomas. Tumors secreting somatostatin, vasoactive intestinal polypeptide (VIP), glucagon, and adrenocorticotropic hormone (ACTH) are far less common.

Clinical Presentation

Hyperplasia of the pancreatic islets may occur before hyperparathyroidism in some patients with MEN 1. Among seven new cases of MEN 1 identified in a prospective screening study of 80 at-risk individuals, three presented with isolated PNT, and two others presented with PNT simultaneously with primary hyperparathyroidism. Only two of seven cases presented with hyperparathyroidism alone [10]. The mean age at diagnosis of PNT in this series was 25 years, and the youngest patient was 16 years of age, demonstrating that carefully conducted regular screening of children at risk for MEN 1 allows for earlier diagnosis of these potentially malignant tumors.

Gastrinoma with ZES develops in approximately one-third of adults with MEN 1 and presents about 10 years earlier than sporadic ZES [17, 18]. In a large NIH review [19], the average age of onset was 33 years in MEN 1-associated cases of ZES, and the youngest patient was 12 years old. Gastrinomas as small as 1–2 mm often arise within the duodenal wall and are prone to metastasize to local nodes [20]. Non-gastrinoma PNTs are most commonly identified in the pancreatic body or tail. Insulin-secreting PNTs present similarly to sporadic insulinomas, with recurrent, often severe postabsorptive and fasting hypoglycemia. An MEN 1-related insulinoma has been reported in a child as young as 5 years of age [21].

Diagnosis

Hypersecretion of gastrin should be suspected by the presence of the classic symptoms of diarrhea, epigastric pain and heartburn, and/or by the presence of peptic ulcer disease. A fasting plasma gastrin level above 200 pg/mL (114 pmol/L) and a concurrent fasting gastric pH below 5.0 are diagnostic; in adults, the secretin stimulation test may provide confirmation when the fasting gastrin is under 200 pg/mL [18]. The presence of nonfunctional PNTs or of preclinical tumor formation and early pancreatic involvement may be detected by the analysis of plasma PP and gastrin response to ingestion of a mixed meal [22]. The second most common functional PNT, an insulinoma, should be suspected in a child at risk for MEN 1 with hypoglycemia fulfilling Whipple's triad. During a carefully monitored fast of 24–72 h, a plasma insulin concentration above

3 mcU/mL (18 pmol/L) and C-peptide above 0.60 ng/mL (200 pmol/L), concurrent with plasma glucose <45 mg/dL (2.5 mmol/L), provides confirmation [23]. Measurement of other pancreatic neuroendocrine hormones such as glucagon, PP, VIP, chromogranin A, and somatostatin may be indicated as necessary.

Therapy

The recognition that very small, 1–2 mm, gastrinomas are commonly hidden within the duodenal wall or scattered within the pancreas is consistent with the complete failure, historically, of surgical attempts at cure of MEN 1-related ZES. Medical management of ZES, conversely, is extremely effective; duodenal or gastric ulceration and perforation with massive bleeding, historically the commonest cause of death related to MEN 1, has been virtually eliminated by management of hypergastrinemia with proton pump inhibition and histamine-2 receptor blockade. Pharmacotherapeutic suppression of an insulin-secreting PNT is currently unavailable however, and localization and surgical excision are necessary. The risk of malignant transformation and metastasis of any PNT, moreover, may necessitate surgery, preceded by T1-weighted magnetic resonance imaging and/or endoscopic ultrasonography for preoperative localization. A combination of duodenotomy and subtotal pancreatectomy or, with the aid of intraoperative ultrasonography, focused enucleation of any identifiable PNT's may be indicated [17, 21, 24]. Long-term risks of subtotal pancreatectomy include iatrogenic insulin-dependent diabetes mellitus and exocrine pancreas dysfunction.

Tumors of the Anterior Pituitary

Overview

MEN 1-associated anterior pituitary adenomas are characteristically benign and have been reported to occur in 10–65% of MEN 1 subjects. Tumors secreting prolactin (PRL) are the most common pituitary manifestation of MEN 1 and are the third most common expression of the syndrome as a whole. Prolactinomas are followed in frequency by somatotroph and corticotroph adenomas.

Clinical Presentation

Clinical features of pituitary adenomas are dependent upon hormone hypersecretion, if any, and on the presence of mass effects, which may include headache, visual field defects, delayed puberty or hypogonadism, hypopituitarism, and central diabetes insipidus [25]. A prolactinoma may present with galactorrhea and amenorrhea in females or hypogonadism and gynecomastia in males. Growth hormone (GH)-secreting tumors produce accelerated linear growth, acromegalic changes (coarsened facial features, hyperhidrosis, interphalangeal synovitis, etc.), and headaches. Classic Cushingoid features, including weight gain and growth failure, are manifestations of corticotroph adenomas [25, 26]. MEN 1-associated pituitary tumors are frequently multicentric and may be locally invasive. Although pituitary involvement in children and adolescents with MEN 1 is uncommon, the appearance of a PRL- and GH-co-secreting macroadenoma associated with MEN 1 has been reported in a 5-year-old child [27], and a prolactinoma has been reported in a 16-year-old adolescent [28].

Diagnosis

In a child at risk for MEN 1, plasma PRL greater than 200 mcg/L (8,696 pmol/L) is highly suggestive of a prolactinoma [25]. A somatotroph adenoma should be suspected when plasma insulin-like growth factor I (IGF-I) is above the reference range for an age- and sex-matched population; however, biochemical confirmation is defined by a failure of plasma GH suppression to <1 mcg/L after 75 g anhydrous glucose given orally [29]. Measurement of midnight plasma or salivary cortisol and 24-h urine-free cortisol are reasonable initial tests when Cushing's disease is suspected on clinical grounds. Pituitary magnetic resonance imaging, before and after gadolinium

contrast enhancement, reveals an adenoma as a hypointense lesion on post-contrast images [30].

Therapy

Suppression of prolactin secretion with dopamine agonists is usually effective and can reduce tumor size markedly, though in our experience adolescents may have relatively insensitive tumors. Nausea and hypotensive effects common to bromocriptine may be minimized by beginning treatment at a low daily dose (1.25–2.5 mg) [25]. Alternatively, cabergoline, which is generally tolerated better than bromocriptine and may have higher efficacy, can be started at 2.5 mg p.o. twice weekly. Transsphenoidal surgery at a high-volume center is indicated for the treatment of somatotroph adenomas, corticotroph adenomas, large nonsecretory adenomas, or large prolactinomas which threaten the optic nerves or chiasm by local impingement or are unresponsive to dopamine agonist therapy [26, 30].

Genetics of MEN 1

MEN 1 is caused by a heritable germline loss-of-function mutation of *menin*, located on chromosome 11q13. First isolated in 1997 by positional cloning [31], *menin* encodes a 610 amino acid nuclear protein, menin, which appears to act as a tumor suppressor by regulating several cell-cycle functions including DNA replication, repair, and transcription [32–34]. The syndrome is expressed when a second, somatic mutation (a "second hit") leads to the loss of the wild-type, normal allele inherited from the non-affected parent. Over 80% of probands with a family history of MEN 1 are identified as harboring a germline mutation, whereas in individuals with simplex MEN 1 syndrome, a *menin* mutation is identified in about 65% (reviewed in 34). With rare exceptions [35], no apparent correlation exists between genotype and phenotype [28, 34].

As MEN 1 is inherited in an autosomal dominant pattern, each child of an individual affected with the syndrome has a 50% chance of inheriting the mutation. The risk of disease in relatives of a proband with no known family history of MEN 1 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 MEN 1. Early determination of the genetic status of any child with a family history of MEN 1 is required to differentiate those children in need of prospective, periodic biochemical and radiographic screening from those in whom screening is unnecessary.

Screening of Children and Adolescents at Risk of Developing MEN 1

Several groups have published recommendations for screening children at risk for MEN 1 [28, 34, 36]. Screening guidelines should apply to any child with a known mutation and to any child of an affected parent whose mutation cannot be positively identified. In general, clinical assessment for symptoms of the principal tumors and biochemical screening are recommended yearly, with radiographic screening every 3–5 years (see Table 32.1).

The recommended age for initiation of annual biochemical screening has decreased in recent years as reports of children as young as 5 years of age affected by MEN 1-related tumors have multiplied [11, 21, 27]. The relatively low risk of tumorigenesis before the second decade and the potentially large volume of blood required suggest a balanced approach of delaying screening until the child has reached 5–8 years of age. Parathyroid involvement is screened with annual measurement of plasma total calcium, with intact PTH added if the calcium concentration is high. The presence of PNTs should be sought by measurement of fasting plasma gastrin and PP yearly and by T1-weighted contrast-enhanced pancreatic MRI every 3 years. Pituitary involvement should be screened with annual measurement of plasma IGF-I and prolactin and with pituitary MRI every 3 years.

Table 32.1 Clinical surveillance for children with known or suspected MEN 1

Annual biochemical screening (beginning at 5–8 years of age)
Fasting serum total calcium (corrected for albumin) or ionized calcium (add intact PTH if calcium is elevated)
Fasting serum glucose, insulin, C-peptide
Fasting and/or stimulated serum gastrin
Serum PRL and IGF-I
Imaging every 3 years
Abdominal MRI (or CT, beginning at 10–15 years of age)
Head MRI (beginning at 5–8 years of age)
PTH, parathyroid hormone; PRL, prolactin; IGF-I, insulin-like growth factor I
MRI, magnetic resonance imaging; CT, computed tomography
Adapted from Ref. [34]

Multiple Endocrine Neoplasia Type 2

Introduction

Since the initial description of the MEN 2 syndromes in 1961 [37], a gradual advance in understanding of these syndromes of endocrine gland tumorigenesis has revolutionized clinical practice. Recognition of the clinical components and the autosomal dominant inheritance pattern comprised the most basic level of understanding and was followed by the development of biochemical methods for screening for the syndrome before overt symptoms became apparent [38]. The final step in this evolution has been the delineation of activating point mutations of the *RET* proto-oncogene as the initiating molecular defect for both the MEN 2 syndrome and the familial medullary thyroid carcinoma (FMTC) variant [39, 40]. The disease phenotype is now known to strongly correlate with mutation in specific RET codons (reviewed in 41). Genetic testing in members of families affected by MEN 2A has become a tool for the recognition and screening and even a guide to management of this disease of endocrine neoplasia and has replaced the previously used calcitonin-based stimulation testing.

The MEN 2 syndromes are composed of the following distinct categories: MEN 2A, MEN 2B, and FMTC. MEN 2A is the most common of the three and consists of medullary thyroid carcinoma (MTC), pheochromocytoma, and parathyroid hyperplasia [42]. The additional feature of a pruritic skin rash caused by the deposition of keratin-like peptides in the dermal–epidermal junction characterizes the variant of MEN 2A known as cutaneous lichen amyloidosis (CLA) [43, 44]. MEN 2B, which accounts for about 5% of all MEN 2 cases, has findings similar to those of MEN 2A, with the additional unique features of both a Marfanoid habitus (without the lens, palate, or cardiac anomalies associated with true Marfan's syndrome) and diffuse ganglioneuromatosis of the alimentary tract and ocular system (45, reviewed in 46). The unique features of MEN 2B, owing to the unsubtle presence of diffuse ganglioneuromatosis, may result in a characteristic appearance identifiable early in childhood. Parathyroid hyperplasia is almost universally absent in MEN 2B [42]. FMTC is a distinct entity, which occurs without other associated endocrinopathies [47]. The incidence of the clinical manifestations associated with each of these variants is summarized in Table 32.2.

Medullary Thyroid Carcinoma

Although only 20% of all cases of medullary thyroid carcinoma (MTC) are associated with MEN 2, more than 90% of those affected with the MEN 2 syndrome will develop MTC, often as the initial manifestation of disease [48]. MTC, a bilateral, multifocal proliferative process originating in the calcitonin-secreting parafollicular thyroid C cells, is the most common cause of death in those with the MEN 2 syndromes [49]. C cell hyperplasia has been demonstrated to be the precursor of MTC [50]. The time course for progression from hyperplasia through microscopic carcinoma to macroscopic disease and metastasis is unclear, but may involve years (reviewed in 47, 51). C cell hyperplasia has been demonstrated in a child with MEN 2A as early as 20 months of age [52], MTC as early as 3 years of age [53], and metastatic disease as early as 6 years of age [54]. In general, the MTC of MEN

Table 32.2 Incidence of clinical manifestations associated with MEN 2A, 2B, and FMTC

	MEN 2A	MEN 2B	FMTC
MTC	>90%	>90%	100%
Parathyroid hyperplasia	20%	~0%	0%
Pheochromocytoma	≤50%	50%	0%
Mucosal ganglioneuromatosis	0%	~100%	0%

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2B is more aggressive, with an earlier clinical presentation of neoplasia [55]; C cell hyperplasia has been demonstrated at birth in patients with this form of endocrinopathy [52].

MTC is prevented or cured by total thyroidectomy, and the satisfactoriness of the initial operation determines clinical success [36]. When possible, surgery for MTC is recommended before the age of possible malignant progression, although consensus in regard to the timing of prophylactic thyroidectomy is lacking. One suggested approach in children with a RET germline mutation is based upon the specific mutation present, with surgery recommended before 1 year in the highest risk group, by 5 years in the mid-risk level group, and before age 10 in the lowest risk level group [41, 56]. Due to the rare presentation of MEN 2, the complicated considerations surrounding the diagnosis, clinical care, surgical demands, postoperative decision-making, and the need for ongoing outcomes data, it is recommended that prophylactic thyroidectomy for children occurs in experienced tertiary care settings [57].

Pheochromocytoma

Less than 20% of patients with MEN 2-related pheochromocytoma present earlier than 20 years of age, and it is rarely detected before the onset of MTC [50]. As with MTC, the pheochromocytomas of MEN 2 are usually multicentric and are found in the context of diffuse adrenomedullary hyperplasia [44]. About half of pheochromocytomas are bilateral in the MEN 2 syndromes [58]. Malignant pheochromocytoma is a rare feature of the MEN 2 syndrome, with an incidence in the range of 0–8% [58, 59].

The classic history of facial flushing, episodic hypertension, and headache is not reliably present in MEN 2-related pheochromocytoma. Clinical manifestations are usually subtle, and more than one-half of individuals are asymptomatic and normotensive. Early clinical signs and symptoms of adrenal medullary hyperplasia or of pheochromocytoma in MEN 2 include palpitations, nervousness, and jitteriness. Initial biochemical findings include elevated plasma epinephrine concentrations, with an increase in the ratio of epinephrine to norepinephrine, in timed urine collections [60].

Primary Hyperparathyroidism

Approximately 20% of MEN 2A patients develop hyperparathyroidism as a later manifestation of the syndrome [36]. The usual pathologic findings are those of parathyroid adenomatous formation within a background of parathyroid hyperplasia involving multiple glands [60]. Pheochromocytoma is a rare cause of hypercalcemia but should be considered and excluded as well in those with MEN 2 [51].

Diagnostic Guidelines: Screening for the Presence of MEN 2

Genetics of MEN 2 Syndromes

All current discussions of screening paradigms for the presence of MEN 2 must be prefaced by an understanding of the molecular genetics of the disorder, since current standards of care are predicated on methods of DNA testing. The MEN 2 syndromes are either inherited as an autosomal

dominant disorder or they occur as new germline mutations in the absence of a family history for the syndrome. In 1987, the gene locus for MEN 2 was linked to the centromeric region of chromosome 10 [61, 62]. Single activating point mutations within the *RET* proto-oncogene were subsequently found to be associated with the range of clinical phenotypes of the MEN 2 syndrome [41]. The *RET* gene, which maps to chromosome 10q11.2, encodes a transmembrane protein of the receptor tyrosine kinase family and is expressed in derivatives of neural crest origin and their tumors [46, 63]. The gain-of-function missense mutations that cause MEN 2 are located in the extracellular cysteine-rich region involved in receptor dimerization and in the intracellular tyrosine kinase domain [41]. Codon 634 mutations are present in about 80% of individuals affected with the classic MEN 2A syndrome, although a small percentage of families harboring this mutation have FMTC only. MEN 2B is most commonly associated with a germline mutation of codon 918 and less commonly with a mutation of codon 883 [64]. The specific *RET* codon mutation present correlates with the phenotypic expression of MEN 2 [41, 65].

Screening for the Presence of MEN 2

Prior to the era of a molecular genetic screening for the MEN 2 syndrome, the preferred screening method for MTC was provocative testing of calcitonin release using pentagastrin stimulation [51]. The test is administered by giving pentagastrin 0.5 mcg/kg as an intravenous bolus over 5–10 s, with plasma calcitonin measurements at 2 and 5 min. Widely available DNA testing, however, has largely replaced biochemical screening [57].

There are clear advantages to the use of DNA testing in at-risk family members. DNA testing allows the possibility of detection of the MEN 2 syndrome prior to the development of C cell abnormalities, allowing for potentially curative early thyroidectomy. Secondly, DNA testing eliminates the need for repeated biochemical testing in those that do not harbor a *RET* mutation. 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 [64]. Screening for *RET* germline mutations can be performed at any age, even at birth or shortly thereafter, since only a small blood sample is required. Genotyping should thus ideally be performed before the age at which prophylactic thyroidectomy would be recommended if a mutation were discovered [66]. Six to eight percent of adults with sporadic MTC, i.e., those without apparent family histories of MEN 2, nonetheless harbor germline *RET* mutations and thus have the risk of familial transmission. It is prudent therefore to offer *RET* gene screening to individuals with apparently sporadic MTC [66]. Multiple centers now offer screening of genomic DNA obtained from peripheral blood samples for mutations of the *RET* locus (see endnote). Perhaps the greatest difficulty occurs in the rare situation where germline transmission of MTC is proven, but no *RET* mutations are identified, in which case it becomes necessary to identify a research laboratory that will analyze regions of the *RET* gene outside the most commonly mutated regions [67].

Screening: Pheochromocytoma and Hyperparathyroidism

Annual screening for pheochromocytoma, which should be initiated in all patients with MEN 2 syndromes beginning at around age 6 years, is accomplished by a thorough review of relevant signs and symptoms and measurement of urine or plasma catecholamine levels [68]. Hypertensive encephalopathy secondary to a pheochromocytoma in MEN 2A has been described in a 13-year-old child [69]. The presence of pheochromocytoma must be ruled out prior to any operative procedure.

It is currently recommended that screening for hyperparathyroidism in patients with MEN 2A consists of a measurement of serum calcium every other year after the age of 10 years. An elevated level should be followed up with parathyroid hormone measurements. Treatment is as discussed above for MEN 1-related primary hyperparathyroidism.

Management of MEN 2 Kindreds: Incorporating Genetic Data

The availability of the highly sensitive and specific DNA-based screening for identification of the MEN 2 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 MEN 2 syndromes, genetic identification of a *RET* mutation does not dictate prophylactic surgical adrenalectomy. Hyperparathyroidism occurs in the minority of patients and also does not have malignant potential. Therefore, recommendations for screening for these components of the syndrome remain unchanged by the recent 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 [52, 53]. Multiple studies have demonstrated that stage of disease at diagnosis most accurately predicts the length of patient survival (reviewed in 52). Current recommendations are for prophylactic thyroidectomy to occur in the early preschool years for those with positive DNA-based testing, typically before 5 years of age, and with some centers pursuing intervention at 3 years of age. For those with MEN 2B, due to the more aggressive form of disease, prophylactic thyroidectomy is recommended as early as possible [52]. Genetic diagnosis of the MEN 2 syndromes is readily and commercially available.

Conclusion

The multiple endocrine neoplasia syndromes are capable of producing significant morbidity, if not mortality, in adolescents and in preteen children. The era of molecular genetic analysis, by allowing conclusive identification of children at risk, has made possible the elimination of costly, needless surveillance for one-half of offspring of affected parents. At the same time, growing experience with screening of at-risk children has yielded added benefit in improved biochemical and radiographic screening protocols and has significantly enhanced the likelihood of early disease detection. The treatment of MEN-related tumors should be undertaken in centers with significant experience in the management of these challenging syndromes.

For information on genetic screening for MEN types 1 and 2, go to: www.genetests.com.

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The Endocrine Response to Critical Illness

33

Ari J. Wassner and Michael S.D. Agus

Abstract

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.

Keywords

Critical illness • ICU • Stress response • Adrenal insufficiency • Non-thyroidal illness

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. 33.1).

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

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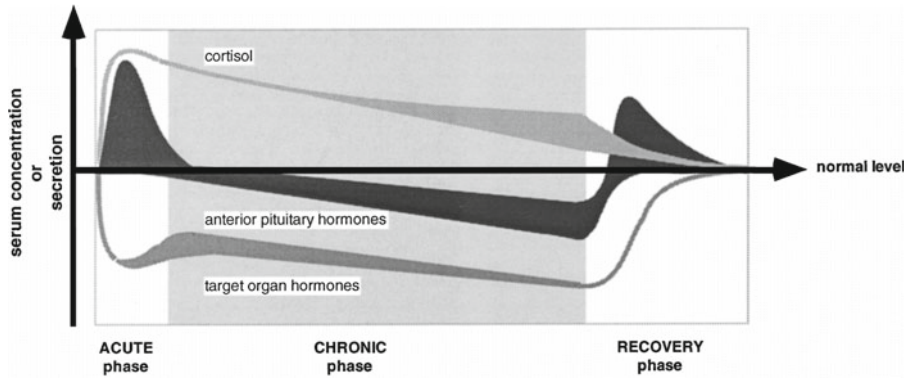

Fig. 33.1 Endocrine changes in critical illness. At the onset of illness, anterior pituitary hormones surge with an associated peripheral inactivation of target organ hormones. Once the chronic response has been engaged, the sensitivity to pituitary hormones is restored, but both remain low due to failure of the pituitary to resume normal secretory activity (Reproduced with permission from Van den Berghe G, de Zegher F, Bouillon R. Clinical review 95: acute and prolonged critical illness as different neuroendocrine paradigms. J Clin Endocrinol Metab 1998;83(6):1827–34. Copyright 1998, The Endocrine Society)

response (or components of it) is 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.

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 1856 [1]. The initial adrenal response is characterized by high serum ACTH and cortisol concentrations up to six times normal. 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 increases free water clearance and 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 protein catabolism and lipolysis. The result is a shunting of energy resources away from peripheral organs and toward the brain and heart. Finally, cortisol suppresses elements of the immune system, which may be important in preventing an overexuberant, and potentially destructive, immune response [2].

Once the patient enters the chronic phase of critical illness, ACTH levels decline, but cortisol concentrations remain elevated. Potential mediators of these effects include atrial natriuretic peptide, substance P, and endothelin. 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 cortisol production.

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 high intra-adrenal concentrations of cortisol (estimated at 50 times above circulating concentrations). These high intra-adrenal concentrations are only achievable if normal cortisol synthesis continues in the adrenal cortex [3, 4].

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 production is adequate to cope with the stress of critical illness. Several clinical studies have implicated such "relative" adrenal insufficiency as a risk factor for poor outcomes, primarily in the setting of septic shock [5]. In this context, relative adrenal insufficiency is often defined as failure of the serum cortisol concentration to increase by more than 9 mcg/dL 1 hour after stimulation with 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 [5, 6]. However, there is little consensus on precise diagnostic criteria for relative adrenal insufficiency, or even whether it is a meaningful clinical diagnosis.

Treatment

Data are conflicting as to whether treatment of "relative" adrenal insufficiency 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 (PRCT) demonstrated decreased mortality in patients with severe septic shock and relative adrenal insufficiency who were treated with hydrocortisone early in their clinical course [7]. On the other hand, a larger PRCT demonstrated no significant benefit of hydrocortisone in septic shock, even in patients with relative adrenal insufficiency [8]. It has been suggested that patients with more severe shock may be more likely to benefit from treatment of relative adrenal insufficiency [9], but 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 relative adrenal insufficiency [10]. Thus, there may be a role for treatment of relative adrenal insufficiency in certain clinical contexts, but further research is needed to clarify this issue. 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 [11].

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 function, 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 "euthyroid sick 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

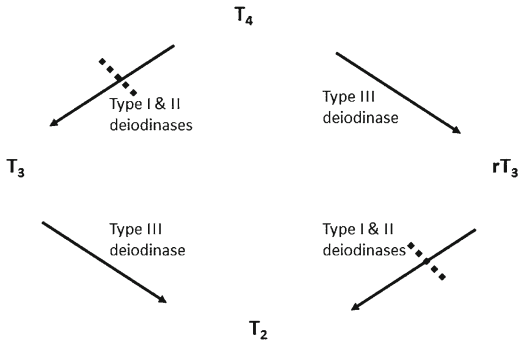

Fig. 33.2 Thyroid hormone metabolism. --- indicates downregulation of type I and II deiodinases in critical illness leading to a decreased T3, increased rT3, and having no direct effect on T4 (Reprinted with permission)

of acute illness or trauma, circulating T3 concentrations decline significantly, and the magnitude of the drop in T3 within the first 24 h reflects the severity of illness [12]. 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. 33.2) [13] and to increased turnover of thyroid hormones [14]. T4 uptake by the liver is also suppressed, reducing the available substrate for conversion to T3 [15].

Under normal conditions, 35–40% of the T4 produced by the thyroid is eventually deiodinated to T3, which accounts for almost all (80–90%) circulating T3 (the remainder is produced directly by the thyroid) [16]. 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 [17]. 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, allowing available resources to be allocated to critical functions in the brain, heart, and immune system.

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 [18, 19], 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 [20]. 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.

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 [21]. In children, pulmonary function and the ventilatory response to hypoxia are also diminished [22]. 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

[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.

the setting of altered thyroid hormone binding to 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 [23]. 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 [24].

Another significant consideration prior to instituting therapy is concomitant medications. Many of those commonly used in the ICU setting have profound effects on various aspects of the HPT axis and are listed in Table 33.1 [25].

Treatment

Non-thyroidal Illness

Direct supplementation with thyroid hormone in the setting of NTI currently does not appear beneficial in noncardiac adult patients, as demonstrated in two PRCTs [26, 27]. It cannot, therefore, be recommended as standard therapy for NTI in children with noncardiac critical illness. However, therapy may be justified if T3 is extremely low and important clinical sequelae of hypothyroxinemia are apparent (e.g., severely elevated SVR, severe hyponatremia, or uncontrolled coagulopathy), although no data are currently available regarding this issue.

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 [28]. This data has prompted several PRCTs in postoperative cardiac patients. In two adult trials, no benefit was demonstrated with an intravenous loading dose of T3 followed by a T3 infusion [29, 30]. PRCTs 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 [31–33]. The largest PRCT 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

Table 33.1 Drugs that influence thyroid function (reproduced with permission from [25] © 1995 Massachusetts Medical Society. All rights reserved)

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

seen in the youngest patients (<5 months) [34]. Thus, T3 treatment in children after cardiac surgery appears to have little risk and may have modest benefits in very young infants.

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 [27]. For patients with preexisting hypothyroidism not associated with NTI, T4 therapy should be continued at the usual dose while in the ICU. Due to incomplete absorption of T4, 75% of the enteral dose should be given intravenously to patients who cannot take the medication enterally [35].

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 [36].

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 [20]. 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 has yet been reported to support this approach.

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 3–5 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 [37]. 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 [38]. 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 [39]. 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 nonthyroidal 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 [40, 41]. Glucocorticoids acutely stimulate GH secretion, but beyond 12 h 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 [42–45]. 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 min of discontinuation of dopamine, however, and persist for at least a day [46, 47].

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.

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 0.18 mg/kg/week divided into daily subcutaneous doses. In any other critical care setting, 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 [48]. This issue will be discussed further in the final section of this chapter.

Gonadal Axis

Hypogonadotropic hypogonadism has been demonstrated in virtually all studies of sex hormones in critically ill adults, although there are currently no available data for children. 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 [49, 50]. As the patient enters the chronic phase of critical illness, she or he becomes hypogonadotropic, and the low sex steroid levels persist [51, 52]. 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. However, this hypothesis has not yet been confirmed experimentally with a gonadotropin-releasing hormone (GnRH) pulsatile infusion.

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.

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.

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 [53, 54]. If protein loss persists, it is associated with increased morbidity and mortality [55]. Limiting protein degradation and maximizing protein accretion is of particular importance in children because of their limited protein reserves and their requirement for growth and development. For instance, infants on extracorporeal life support (ECLS, also known as ECMO), who are among the most profoundly ill children in pediatric critical care, quantitatively demonstrate the highest rates of protein loss ever reported [56]. 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 [57].

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 [58]. Recombinant GH has since been investigated in a number of small clinical trials over a period of approximately 25 years. Small PRCTs in a variety of ICU adult patients, using a variety of GH doses, documented improvements in indices of protein turnover and clinical outcome [59–62]. One moderate-sized pediatric study ($N=72$) demonstrated decreased protein catabolism in burned children treated from ICU discharge until 1 year from burn [63]. The adult studies prompted a large multicenter PRCT 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 [48]. 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 [64].

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 3-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 [65]. These effects persist in the fed state, with glucocorticoid-induced catabolism [66], and were confirmed in a non-randomized fashion in stable, post-burn adults [67]. Unfortunately, three PRCTs failed to demonstrate a significant effect in ill postoperative adult patients [68–70]. In stable post-burn children, one PRCT demonstrated significant effects in mitigating the extent of protein catabolism using IGF-I in combination with IGF-BP3 [71]. IGF-I has been shown to be safe in a phase I clinical trial in critically ill adults [72]—further clinical data are imminent.

Anabolic sex steroid therapy in the critically ill with burns or trauma has produced several reports of positive results in adults [73–75] and one in burned children [63]. Oxandrolone, a non-aromatizable androgen, has been the therapeutic agent of choice due to its decreased virilizing potency and hepatotoxicity as compared to testosterone. Data from pediatric burn patients have demonstrated a doubling of the fractional synthetic rate of protein and a substantial improvement in net protein balance [63]. A PRCT in adult burn patients showed decreased length of stay in those treated with oxandrolone compared to placebo [76]. This finding was replicated in a large pediatric PRCT 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 [77]. 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 [78–81]. In burned adults, protein synthesis was stimulated, and wound healing was accelerated [82–84]. A single study in children demonstrated an 80% suppression of proteolysis in four stable, premature neonates but noted a significant rise plasma lactate during the insulin infusion [85].

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 [86–89]. Two large PRCTs 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 [90, 91]. 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 [92–97]. Furthermore, the most recent large PRCT demonstrated increased mortality in adults treated with intensive insulin management to near-normoglycemia, compared to those managed less aggressively [98]. The only pediatric PRCT of intensive glycemic control in the ICU resulted in shorter length of stay, fewer infections, and fewer deaths in the intensively treated group but was associated with an extraordinarily high rate of severe hypoglycemia (<40 mg/dL) at 25% of those treated and 44% of those less than 1 year of age [99]. 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, 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 [100, 101]. 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 [86]. 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 USA 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 [102]. Coadministration 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 [103]. However, further research investigating clinical outcomes using infusions of hypothalamic peptides is required to definitively address this issue.

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