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Bone Drugs in Pediatrics

Efficacy and Challenges

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To Joann, Adrienne, Andrew Howard
and Andrew George, and in memory
of my late mentors Ivor H. Mills (Cambridge)
and Jack W. Coburn (UCLA).

Contents

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Abbreviations

1/3Rad	Distal 1/3 radius
25(OH)D	25-hydroxyvitamin D
A	Area
aBMD	Areal bone mineral density
AI	Adequate intake
ALP	Alkaline phosphatase
BA	Bone area
BAP	Bone alkaline phosphatase
BMAD	Bone mineral apparent density
BMC	Bone mineral content
BMD	Bone mineral density
BMP	Bone morphogenetic protein
BMU	Basic multicellular unit
BP, BPs	Bisphosphonate(s)
BPCA	Best Pharmaceuticals for Children Act of 2002
BS	Bruck syndrome
BTT	Bone transmission time
BUA	Broadband US attenuation
CDER	Center for Drug Evaluation and Research
CF	Cystic fibrosis
CFTR	Cystic fibrosis transmembrane conductance regulator
Circ	Circumference
Cort	Cortical
CTX	C-terminal cross-linked telopeptides of type I collagen
CXSA	Cross-sectional area
D.Rad	Distal radius
DF	Distal femur
DMD	Duchenne muscular dystrophy
DPD	Deoxypyridinolin
DXA	Dual X-ray absorptiometry
EDS	Ehlers–Danlos syndrome

ELISA	Enzyme-linked immunosorbent assays
Endost	Endosteal
ERT	Enzyme replacement therapy
FA	Forearm
FD	Fibrous dysplasia
FDA	Food and Drug Administration
FGF23	Fibroblast growth factor 23
FN	Femoral neck
FOP	Fibrodysplasia ossificans progressiva
GCs	Glucocorticosteroids
GH	Growth hormone
GSD	Glycogen storage diseases
HPLC	High-performance liquid chromatography
Ht	Height
HTS	High throughput screening
HYP	Hydroxyproline
IGFBP	Insulin-like growth factor binding protein
IGF-I	Insulin-like growth factor
IOM	Institute of Medicine
ISCD	International Society for Clinical Densitometry
IU	International unit
LBM	Lean body mass
LS	Lumbar spine
LTM	Lean tissue mass
MFS	Marfan syndrome
MMP	Matrix metalloproteinase
NICHD	National Institute of Child Health and Human Development
NTX	N-terminal cross-linked telopeptides of type I collagen
OC	Osteocalcin
OI	Osteogenesis imperfecta
OPPG	Osteoporosis-pseudoglioma syndrome
Osx	Osterix
P.Rad	Proximal radius
Periost	Periosteal
PICP	Procollagen type I C-terminal peptide
PINP	Procollagen type I N-terminal peptide
PPSR	Proposed pediatric study request
pQCT	Peripheral quantitative computed tomography
PREA	Pediatric Research Equity Act of 2003
PTH	Parathyroid hormone
PYD	Pyridinoline
QUS	Quantitative ultrasound
RANKL	Rank ligand
RDA	Recommended daily allowance
rHGH	Recombinant human growth hormone

RIA	Radioimmunoassays
ROI	Region of interest
sc	Subcutaneously
SoS	Speed of sound
SSI	Strength–Strain Index
TB	Total body
TBSA	Total burn surface area
TGF-beta	Transforming growth factor beta
Thick	Thickness
Tot	Total
Trab	Trabecular
vBMD	Volumetric bone mineral density
VDR	Vitamin D receptor
WR	Written request

Chapter 1
Introduction

Gordon L. Klein

This book is an attempt to bring together in one volume existing information on the use of drugs to treat or prevent bone loss in children. Unless a child suffers from a genetic condition in which bone loss is flagrant the process of bone loss is often asymptomatic and if it occurs consequent to an underlying condition it does not attract medical attention. Therefore, this book undertakes to call to the reader's attention the drugs available to prevent or treat pediatric bone loss. Even though no drugs have current approval for this purpose from the United States Food and Drug Administration (FDA), these chapters contain evidence of both safety and efficacy of antiresorptive and anabolic medications that have been used off-label in a variety of pediatric conditions.

The outline of the book proceeds from the general to the specific, beginning with a chapter on the influence of age and development on drug pharmacokinetics (Chap. 2). This general chapter provides the justification for producing this book inasmuch as children may metabolize drugs differently than adults. Then, in order to provide at least a glimpse of the process of drug discovery, we examine how drug discovery may potentially play a role in studies on the mechanism of bone formation as Chap. 3 focuses on the search for a compound with anabolic potential to stimulate *osterix* in the differentiation of marrow stromal cells into mature osteoblasts.

Because the detection of bone loss requires a variety of diagnostic techniques Chap. 4 describes the physiologic basis for the tools used to diagnose bone loss and to monitor the efficacy of potential treatment.

Proceeding to the specific bone drugs used in children we have divided them into antiresorptives, more specifically the bisphosphonates, and anabolic drugs, at this time consisting of recombinant human growth hormone (rhGH) and oxandrolone.

While the mechanism of action of the bisphosphonates is briefly discussed in Chap. 5 on the use of these agents to treat osteogenesis imperfecta, the detailed

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mechanism of action of this class of drugs is already well described, even in the pediatric literature, in a clear and precise summary by Russell [1]. As the article is relatively recent there is no need to repeat it here.

Perhaps the most widely studied condition in which bisphosphonates have been used with success is osteogenesis imperfecta. The results of supporting studies summarized in Chap. 5 explain not only why these drugs are now a therapeutic mainstay for this set of conditions but also why they do not solve all the difficulties this group of conditions presents. While this chapter describes a significant amount of supportive data we now go to the other extreme attempting the formidable task of culling the literature as well as personal experience for cases of other more rare genetic diseases being treated with bisphosphonates in Chap. 6. The anecdotal nature of this evidence highlights the difficulty encountered when attempting to study drug efficacy and safety in a small and widely dispersed population. These contrasts are followed by Chap. 7, which describes still another type of use for bisphosphonates, preliminary but promising data on the limited use of bisphosphonates, a single dose or at most two doses separated by 1 week acutely following severe burn injury in order to entirely prevent resorptive bone loss for up to 2 years following the burn injury. Chapters 4 and 5 also list some of the concerns voiced in the pediatric community regarding the use of bisphosphonates in children.

In the section on anabolic drugs the various uses of rhGH in children are discussed, specifically its effects on bone (Chap. 8). One inference to be drawn from this chapter is that much needs to be clarified regarding the mechanism or mechanisms of anabolic action of rhGH, inasmuch as it stimulates both bone formation and bone resorption and yet in some conditions has been reported to increase bone mineral density. When used in burned children as described in Chap. 9, rhGH increases bone mineral content and bone area proportionately with increased bone resorption only occurring at the highest doses studied. It is also unclear how much of the anabolic effect of rhGH is secondary to skeletal loading resulting from its anabolic effect on muscle. Similar questions arise for oxandrolone, a non-aromatizable androgen that has also successfully increased bone mineral content following burn injury.

Next we consider vitamin D and calcium as bone drugs, and Chap. 10 carefully reviews the evidence supporting both efficacy and safety of these medications, including a comment on whether it is advisable to treat all disorders involving bone loss with vitamin D and calcium.

The great majority of this book, as well as the field of pediatric bone health in general, concerns cortical and trabecular bone. As a welcome addition, we have included a discussion of membranous bone (Chap. 11), most specifically disorders of craniofacial bones, and the role of pharmacotherapy in the management of these conditions. Of interest, special emphasis is given to osteonecrosis of the jaw as a consequence of bisphosphonate treatment.

Finally, we look into the future of bone drugs in pediatrics by examining the present array of drugs used in internal medicine. The discussion in Chap. 12 includes the bone drugs available to treat adults, their mechanisms of action, and the efficacy of combination therapy. The aim of this chapter is to stimulate further study of

the safety and efficacy of these newer drugs in children in the hope of offering the best selection of drugs for use singly or in combination to prevent or treat bone loss in pediatrics.

What you will encounter when reading these chapters is that the level of evidence supporting the use of these drugs is highly variable. Therefore, the information presented here must be taken as the best available but still far from acceptable. I shall have more to say on this subject at the conclusion of the volume.

Reference

1. Russell RG. Bisphosphonates: mode of action and pharmacology. Pediatrics. 2007;119 Suppl 2:S150–62.

Chapter 2
Developmental Pharmacokinetics: Drug Disposition Relative to Age

Michael D. Reed and Michelle L. Bestic

Introduction

The fundamental goal of drug therapy is to provide optimal efficacy for disease management without adverse event, with the most important factor in achieving this goal being drug dose. A medication's optimal dose is dependent on a number of chemical and patient-specific factors including desired target therapeutic effect(s) combined with a patient's age, body habitus, genetics, disease state(s), major organ function (e.g., kidney, liver, heart), and concurrent therapies. Once the drug is administered these variables modulate overall drug exposure and for the treatment of systemic disease, a sufficient amount of drug must be available to distribute and bind to its receptor for a sufficient period of time to elicit a therapeutic effect. This balance of systemic drug exposure, receptor binding, and therapeutic effect is dependent on the integration of a drug's pharmacokinetic (PK: drug disposition), pharmacodynamic (PD: mechanism of action), and pharmacogenomic (PG) characteristics. Pharmacokinetics describes a drug's overall disposition profile which is markedly influenced by patient age [1, 2]. Pharmacokinetics encompasses the processes of drug absorption, distribution, metabolism, and elimination—the integration of these processes relative to patient age is the focus of this chapter.

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Drug Absorption

In the absence of topical administration for local effect, a drug must be absorbed into systemic circulation to distribute to its site of action to elicit the desired therapeutic effect. The PK parameter describing drug absorption is termed bioavailability and routinely abbreviated as "F." Although drug F is often considered the amount of drug absorbed into systemic circulation, this PK parameter estimate also encompasses the rate of drug absorption. Thus, drug F is the composite of rate as well as overall extent of drug absorption. This distinction may seem minor but in fact is very important and clinically relevant. For example, if a drug's effect is dependent on the peak drug concentration (e.g., certain antibiotics: aminoglycosides) a drug with slow absorption characteristics may not achieve necessary peak concentrations for efficacy. Similarly for a drug that requires prolonged systemic exposure, a timed-release/sustained-release preparation might be the best formulation for optimal therapeutic effect.

Drug F is most often reported as a percent, i.e., the percent of the total amount of the drug dose administered that enters systemic circulation as the active drug. The absolute F for a drug is the ratio of the drug's systemic exposure, as determined by a drug's area under the serum (blood) concentration–time curve (AUC), after extravascular administration (e.g., topical, oral, intramuscular [IM]) relative to the AUC achieved after intravenous (IV) administration, i.e., AUCex divided by the AUCiv. Most importantly the drug concentration used in these assessments is the amount of *active drug* that reaches systemic circulation. This important distinction accounts for those limited number of drugs that are administered as a pro-drug, i.e., an inactive (minimally active) form of the drug requiring some form of in vivo alteration to liberate the active drug [3, 4]. The manufacturing of a drug as a pro-drug is a process pharmaceutical scientists use to chemically modify drugs that cannot be easily formulated. For some drug chemicals such alteration is required for the drug to be absorbed or to manufacture a better flavored liquid formulation. A pro-drug formulation may also be necessary to assure that a drug distributes to anatomic sites that if chemically unaltered could not distribute to its target site. Examples of drugs administered as pro-drugs include codeine (converted to active morphine), the anti-influenza drug oseltamivir (Tamiflu®), and prednisolone (converted to active prednisone). In addition to pro-drug administration, certain drugs undergo substantial "first-pass" metabolism, where a large amount of the drug dose may be absorbed into systemic circulation but after its "first-pass" through the liver, only a fraction of the dose remains as the active drug with the remainder as metabolites (note: the metabolite(s) may be therapeutically active or inactive and/or be responsible for adverse effects). The bioavailability evaluation could be for the parent drug, the active metabolite(s), or both. It is important to determine and know what drug moiety is included in the bioavailability data you are presented with.

It is important to recognize that topically or orally administered drugs may also be metabolized within the cells they traverse limiting the amount that reaches systemic circulation. Furthermore, intestinal cells and many anatomic sites, including

the blood–brain barrier, placenta, and others, contain drug transporters that can enhance or oppose drug absorption [1, 2, 5–9]. The importance of drug transporters is addressed in greater detail below.

Lastly, a drug's physicochemical characteristics influence the rate and extent of drug absorption. These physicochemical characteristics include molecular weight/size, degree of ionization under physiologic and pathophysiologic conditions, and degree of lipid solubility. The most favorable physicochemical characteristic for optimal drug F (and distribution—see below) is a small, highly lipid soluble molecule of low molecular weight that is un-ionized under physiologic and/or pathophysiologic conditions.

The importance of the factors outlined above cannot be overemphasized and patient age exerts many influences on the process of drug F. Blood flow characteristics at the site of absorption, e.g., the muscle for IM injections, intestine for oral meds, as well as the type, amount, and pH of intestinal contents combined with the extent and variability of gastric emptying and intestinal motility, will all influence a drug's F. In addition, maturity and functional capacity of drug metabolizing enzymes (e.g., cytochrome P450 isoenzymes—see below) and influx/efflux transporters located within cells will also impact on the amount a drug is absorbed. Thus, the ontogeny of gastric and intestinal circulation combined with cellular and organ function can and will dramatically affect a drug's F.

Drug Absorption: Physiologic Influences

For decades it has been believed that shortly after birth infants experience a relative period of achlorhydria. The original description of gastric pH by Miller in 1941 reported a gastric pH at birth of ~7, rapidly falling to pH 3 within the first few hours but slowly rising to >pH 4 [10, 11]. More recent data suggests that at birth gastric pH does rapidly decline to pH 2–3 but in fact fluctuates throughout the day and is not universally more alkaline, i.e., defined as gastric pH > 4 [11]. A better assessment of the ontogenic influences on gastric pH has been proposed to focus on the proportion of time the gastric pH peaks above 4 in a 24 h period. In preterm infants, this proportion of time has been reported to range from 46 to 70 % whereas for children up to 2 years of age the value approaches 51 % and in older children 34 % [11]. The higher proportion in younger children may partially be explained by the buffering effects of milk formula; older children are less frequently fed and receive more solid foods. In addition to gastric pH, age and diet will influence the rate of gastric emptying. Noting that most drugs are absorbed in the upper part of the small intestine the rate of an orally administered drug to transit from the stomach into the duodenum will influence drug F. Consumption of human milk and lower caloric substrates/formulas can increase (prolong) gastric emptying whereas feeds of higher caloric density or long-chain fatty acids may shorten gastric emptying [10, 12]. Lastly, the developmental pattern of bile acid synthesis and secretion can influence the absorption of lipophilic drugs which are poorly soluble in the aqueous digestive fluids [11].

Clinical relevance: With few exceptions, the maturational changes observed over the first year of life in gastrointestinal functions (as described above) have a limited effect on routine drug therapy in the care of premature, newborn, and young infants. Routine dose recommendations accommodate for these developmental processes. Nevertheless, these maturational processes are reflected in the much higher variability observed in drug F during this age period. Specific examples would include acid-labile compounds where F would be expected to be increased (e.g., penicillin g) and decreased F for weak acids (e.g., phenobarbital, ganciclovir) in premature and full-term infants as compared to adults. Also as discussed above, the type and quantity of enteral feedings and the magnitude of gastric emptying and intestinal motility can and will influence the rate and extent of oral drug F. With respect to drug F after intramuscular (IM) administration, drug absorption can be highly variable, particularly in the ill neonate and young infants, where cardiovascular function and, thus, blood flow dynamics can be compromised. For these reasons, the IV route for drug administration for ill premature and newborn infants is preferred. Nevertheless, if one is unable to establish IV access in an ill neonate/infant requiring prompt drug therapy, the IM route for drug administration, for a drug that can be administered IM, should be used initially until IV access becomes available.

Overall, these expected changes in drug F simply underscore the importance of close patient monitoring for dose–effect outcomes in each patient, that are best determined under steady-state conditions (see below). Furthermore and very important in pediatric practice is the drug formulation. The formulation can have great influence on the rate of absorption which is expected to be faster after administration of a liquid dosing formulation (liquid > suspension) compared with a solid formulation (capsule ≥ tablet > sustained-/delayed-release tablet). For a drug to be absorbed from any site it must be in solution before it is available to cross membranes and enter systemic circulation.

Drug Distribution

Once drug is absorbed into systemic circulation, a dynamic equilibrium is achieved between drug bound to plasma proteins and the nonprotein-bound fraction, commonly referred to as the "free" drug. It is the free drug that is capable to distribute outside the vascular compartment, it is the free drug that crosses cells/membranes, and it is only the free drug that will bind to its receptor(s) and elicit a pharmacologic and/or toxicological response(s). The extent to which a drug distributes throughout the body is dependent upon a number of drug- and patient-specific variables including the drug's physicochemical characteristics as noted above (i.e., molecular weight, degree of ionization at physiologic/pathophysiologic pH, and degree of lipid solubility), affinity for cellular transporters (see below), and degree of protein binding. As noted for drug F above, a small molecule un-ionized at physiologic/pathophysiologic pH that is highly lipid soluble is associated with wide body

Table 2.1 The developmental aspects of fluid compartment sizes

Patient age	Total body water[a]	Extracellular fluid[a]	Intracellular fluid[a]
<3-Month fetus	92	65	25
Term gestation	75	35–44	33
4–6 months	60	23	37
12 months		26–30	
Puberty	~60	20	40
Adult	50–60	20	40

Adapted from [2]

[a]Values expressed as percentage of total body weight

distribution or in PK terms, a drug with a large volume of distribution (V_d). The importance of the PK parameter V_d is that a drug's V_d is used to determine the size of an individual dose. A drug's V_d is influenced by a patient's size and age. As a drug must be in solution for absorption and distribution, the ontogeny of physiologic fluid compartments will influence a drug's V_d value. The developmental pattern of body fluid compartments is shown in Table 2.1 [13] and as a result, a drug's V_d is influenced greatly by the age of the pediatric patient. The importance of these developmental changes cannot be overemphasized as the volume of these fluid spaces will directly impact the absolute drug concentration and, thus, could directly influence the magnitude and time course of drug effect.

Understanding the body distribution characteristics for a drug is important when prescribing a drug and calculating the dose to be administered. For example, if target drug receptors are located within the central nervous system (CNS) the drug must distribute into the CNS for therapeutic effect. Although the absolute value of a drug's V_d does not correlate with any real physiologic volume (i.e., hence the formal name for V_d is *apparent* volume of distribution), knowledge of this PK parameter provides insight into the total amount of drug present in the body relative to its concentration in blood and, thus, tissue distribution. Clinically it can be speculated with a moderate degree of certainty that a drug with a very large V_d (e.g., 10 L/kg) can be assumed to distribute widely throughout the body (possibly even into the CNS, e.g., anesthetics), whereas a drug with a very low V_d value (e.g., 0.1 L/kg) might be expected to have limited body distribution. However, regardless of a drug's V_d numeric value, what really matters is the drug effect. Independent of a drug's V_d, what matters clinically is that a sufficient amount of drug distributes to and binds to the necessary receptor site to stimulate the desired pharmacologic effect(s).

Identification of the age-appropriate V_d for a drug can be obtained from most computer information/pediatric drug dosing references. The reported V_d value is unique to each individual drug and will change relative to age and possibly even by disease, particularly for those diseases that result in large volume shifts. A comparison of V_d values between neonates and adults for a few select drugs is shown in Table 2.2.

Table 2.2 Comparison of volume of distribution and elimination half-life for selected drugs in neonates compared to adults

	Neonate		Adult	
Example drug (brand name)	V_d	$t1/2$	V_d	$t1/2$
Amikacin (Amikin®)	0.6	8.4	0.3	2.3
Amoxicillin (Amoxil®)	0.7	3	0.2	1.7
Bumetanide (Bumex®)	0.22	6.5	0.13	1.5
Caffeine citrate (Cafcit®)	0.85	84	0.6	5
Caspofungin (Cancidas®)	0.43	8.3	0.25	13
Cefepime (Maxipime®)	0.43	5.0	0.26	2.1
Gentamicin (Garamycin®)	0.7	7.2	0.31	2.5
Levetiracetan (Keppra®)	0.89	9	0.6	6
Morphine	2.3	7.0	3.0	2
Pantoprazole (Protonix®)	–	3.1	–	1–1.5
Phenobarbital	0.71	108	0.54	60–80
Tobramycin (Nebcin®)	0.7	8.3	0.33	2.2
Vancomycin (Vancocin®)	0.57	6–10	0.39	6

The drug brand name noted is one example as many of the drugs listed may have multiple brand names

Data presented represent best estimate averages for comparative purposes and were obtained from published parameter estimates in premature and newborn infants usually during the first week of life

V_d, apparent volume of distribution presented in L/kg (kg body weight); $t1/2$, elimination half-life in hours (h)

Drug Distribution: Protein Binding

In addition to body fluid compartments, the amount (%) of drug binding to protein will influence the amount of drug distributed within the body. Drug bound to proteins (or other fractions) is not pharmacologically active or available for metabolism or excretion—it is only the free (unbound) fraction of the absorbed drug that is pharmacologically (also toxicologically) active, capable of diffusing outside the circulatory compartment/across cell membranes distributing to the site(s) of action, and available for body elimination, e.g., liver metabolism and/or renal excretion. Important differences exist between the degree of drug–protein binding in premature and newborn infants compared to adults and examples are outlined in Table 2.3. The concentration of plasma proteins is reduced in the immediate post-delivery period as well as there are select, endogenous circulating compounds found in a neonates circulation that may/will compete for plasma/albumin binding. The most important of these compounds is bilirubin for which controversy persists surrounding possible displacement of bilirubin from its albumin binding sites by drugs and possibly precipitating kernicterus. Although such an albumin–drug–bilirubin displacement interaction is possible, the magnitude of such an interaction depends on a number of variables but most notably, the absolute concentration of drug(s) and

Table 2.3 Percent (%) protein binding of select representative drugs in newborn infants compared to that in adults

Drug	Percent bound	
	Newborn	Adult
Ampicillin	10	18
Diazepam (Valium®)	84	99
Digoxin (Lanoxin®)	20	32
Ibuprofen (Caldolor®)	95	99
Micofungin (Mycamine®)	96.7	99.6
Morphine	35	45
Nafcillin (Unipen®)	69	89
Phenytoin (Dilantin®)	80	90
Phenobarbital	32	47
Theophylline	36	56

The drug brand name noted is one example as many of the drugs listed may have multiple brand names
Data presented represent best estimate averages for comparative purposes and were obtained from published parameter estimates in premature and newborn infants usually during the first week of life

bilirubin relative to the circulating albumin concentration, number of available albumin binding sites, and the presence of other albumin binding compounds that will compete for the same albumin binding sites. Fortunately, this combination of required events rarely occurs in clinical medicine. In fact more recent animal data suggests that the immaturity of the efflux pump, P-glycoprotein, in the blood–brain barrier may be the most important determinant of bilirubin-brain concentrations rather than a simple drug–protein displacement interaction [8]. Further complicating this controversy is the fact that our knowledge of the extent to which a drug is usually bound to albumin, e.g., 50 and 90 %, provides little to no dependable clinically relevant information as to the extent a drug might displace bilirubin from its albumin binding sites. For example, two drugs frequently used in the care of premature and newborn infants furosemide (Lasix®) and midazolam (Versed®) are both highly bound to plasma albumin [14] and unassociated with any drug–bilirubin–albumin displacement reaction.

Clinical relevance: Knowledge of the age-appropriate V_d in liters per kg body weight (V_d L/kg) for a given drug allows the clinician a simple yet accurate method to calculate the peak drug concentration achieved with the *first dose* of drug. The peak concentration obtained after the first dose can be calculated using the following relationship: peak drug concentration = drug dose (μg or mg) divided by drug V_d (l/kg) multiplied by the patient's body weight in kg. For example, if you order a 5 mg dose of a drug to be administered to an 800 g infant and the drugs V_d is 0.4 L/kg, the estimated peak concentration right after the full dose is administered would be peak = (5,000 μg dose) divided by (320—infant body weight (0.8 kg) multiplied by the drug V_d in milliliters—400 mL); thus it would be ~15.6 μg/mL. Conversely, if you want to determine the dose of a drug to achieve a specific target blood concentration the equation can be rearranged to: dose (μg or mg) = (desired blood level

in μg/mL) multiplied by (the drug V_d in mL/kg) (patient body weight (kg). The most common errors observed in these simple mathematical calculations are those related to converting units properly, i.e., mg to μg and kg to g. Note: the reason this simple calculation is only valid after the first dose is these equations assume no drug is present in the body at the time of the first dose. After multiple doses the amount of drug must be inserted into the calculation (subtracted from the peak concentration) to accurately determine a drug's V_d.

With respect to drug binding to plasma proteins the extent of binding for a specific drug is most often of limited to no clinical significance. The defined clinically used dosing strategy accounts for the amount of drug bound and the amount of free drug. However for certain drugs, the "target" serum drug concentration differs from that defined for infants as compared to older infants, children, or adults. This important clinical discrepancy is merely due to the reduced drug–albumin binding for various drugs in the neonate vs. older infants, children, and adults (see Table 2.3). The importance of this factor is that defined, target "therapeutic serum/plasma drug concentrations" determined in adults may be totally appropriate for older infants and children but may be different in the neonate underscoring the importance of drug concentration definitions for different post-conceptional ages (PCA). Table 2.3 provides some estimates in the percent of protein binding for select drugs used in the NICU.

Drug Metabolism

Most drugs are not excreted from the body unchanged, but rather undergo biochemical modification usually by specialized enzymatic systems in a process known as xenobiotic metabolism or biotransformation. These enzymes are found in most human tissues (e.g., lung, kidney) with the highest concentrations in the liver and small and large intestines. While the liver is considered the major source of drug metabolizing activity, the enzymes located within the epithelial cells of the small intestine initiate the biotransformation of most orally administered medications. As noted in absorption above, once drugs are intestinally absorbed they enter the portal circulation for the aforementioned "first-pass" through the liver. Any hepatically metabolized drug that "escapes" this initial pass through the liver eventually undergoes sufficient metabolism on subsequent passes through the portal circulation. One can avoid or minimize the impact of intestinal and/or hepatic first-pass drug metabolism by using the sublingual (e.g., nitroglycerine), nasal, and in some cases rectal routes for drug administration. Some drugs are not easily metabolized and, hence, remain in the body for longer periods of time whereas other drugs may not undergo any metabolism and will be eliminated from the body unchanged.

Historically, the enzyme systems responsible for drug biotransformation have been grouped into either Phase I or Phase II reactions. Phase I reactions are those involving oxidation, reduction, or hydrolysis reactions that will add or expose functional groups on the drug molecule, thus increasing the compound's polarity,

Table 2.4 Metabolic enzymes involved in human drug metabolism

Phase I metabolism	Phase II metabolism
Cytochrome P450 oxidases (CYPs)	UDP-glucuronosyltransferases (UGTs)
Flavin-containing monooxidases (FMOs)	Sulfotransferases (SULTs)
Monoamine oxidases	Glutathione-*S*-transferases (GSTs)
Alcohol/aldehyde dehydrogenase	*N*-acetyltransferases (NATs)
Peroxidases	Methyltransferases (MTs)

reducing its likelihood for absorption or reabsorption, and fostering its body elimination. A drug may undergo Phase I metabolism resulting in inactive metabolite(s) that may either be excreted or act as a substrate for Phase II metabolism. In some cases, a drug's Phase I metabolism may result in converting a pharmacologically inactive compound (a pro-drug) into a pharmacologically active one—pro-drug conversions were addressed specifically in absorption above.

Phase II metabolism consists of conjugation reactions, including glucuronidation, sulfation, acetylation, and methylation among others. Like Phase I reactions, the end product of a Phase II reaction is the genesis of a compound that is usually pharmacologically inactive but more water soluble (hydrophilic) promoting efficient body elimination. Occasionally Phase I and/or Phase II metabolism can result in the formation of a toxic metabolite, which can elicit an adverse reaction. An important example of such a reaction involves acetaminophen (e.g., Tylenol®), a drug that is metabolized via the cytochrome P450 enzyme system (CYP2E1) to the toxic metabolite, *N*-acetyl-*p*-benzoquinoneimine (NAPQI), in overdose or in situations of excess acetaminophen consumption. A list of the enzymes responsible for carrying out Phase I and Phase II metabolism can be found in Table 2.4.

Phase I Metabolism

The cytochrome P450 enzyme superfamily (or CYPs) are the primary enzymes involved in Phase I metabolism. While this family of enzymes do play an important role in the biotransformation of numerous endogenous chemicals in the body, they also account for the transformation of up to 75 % of exogenously administered compounds. The human genome project identified 57 genes divided into 18 families of CYPs. However, only a handful of CYPs (1A2, 2C9, 2C19, 2D6, 2E1, and 3A4) are responsible for the vast majority of drug metabolism (~90 %), with CYP3A4 being the most prominent in drug metabolism [15–18]. Most CYPs have very broad substrate specificity and can metabolize multiple compounds. This broad substrate specificity also means that many drugs can be substrates for and metabolized by multiple CYPs. The selectivity of the CYPs for certain compounds is determined by the inherent characteristics of the drug (i.e., lipophilic vs. hydrophilic compounds) and cannot be predicted by drug class. This is perhaps best illustrated by the antidepressant medications fluoxetine (e.g., Prozac®), paroxetine (e.g., Paxil®), and

sertraline (e.g., Zoloft®), all members of the class of selective serotonin-reuptake inhibitors (SSRIs)—fluoxetine and paroxetine are extensively metabolized by CYP2D6 whereas sertraline's metabolism does not appear to be affected by any specific or predominate CYP.

Phase II Metabolism

A large number of physiologic enzymes are involved in Phase II metabolism including, but not limited to, the UDP-glucuronosyltransferases (UGTs), sulfotransferases (SULTs), glutathione-*S*-transferases (GSTs), *N*-acetyltransferases (NATs), and methyltransferases (MTs). Among the most important of the Phase II enzymes are the UGTs and, like the CYPs, the UGTs possess broad substrate specificity. Unlike, the CYPs only two families of UGT enzymes exist (UGT1 and UGT2), with the vast majority of drug metabolism involving the UGT1 family.

Drug Metabolism: Physiologic Influences

The rate and extent of drug metabolism can vastly influence both the safety and efficacy of a drug. Should a compound be metabolized to an inactive entity too quickly, its therapeutic effectiveness may be greatly diminished. Conversely, drugs that are metabolized very slowly can accumulate in the body, potentially resulting in an adverse drug reaction. The factors controlling the extent and rate of metabolism are both complex and varied. Some of the known factors include age, disease, gender, environmental, drug dose, drug–drug interactions, diet, and genetics, among others. Some of these variables are discussed in more detail below.

Age

Important differences in the maturational pathways for both Phase I and Phase II drug metabolizing enzymes exist. These developmental changes can have significant impact on the efficacy and safety profile of drugs administered to infants and children. These changes are best exemplified by the administration of chloramphenicol to newborns, a lifesaving antibiotic once used but replaced by safer agents today. Glucuronyltransferase activity, which is greatly diminished at birth, is necessary for converting chloramphenicol to the inactive glucuronide metabolite (more water soluble) for renal excretion. The immaturity of the glucuronyltransferase enzyme leads to very diminished chloramphenicol metabolism with accumulation of unaltered chloramphenicol and subsequent cardiovascular collapse or "Grey Baby Syndrome." While not all drugs administered to infants and children will have such deleterious effects, it is nevertheless important to consider the age-related

changes in drug metabolizing enzymes. Huge variability in morphine metabolism is partially explained by variability in the UGT pathways. The importance of the maturation and activity variability relative to patient age is becoming increasingly important at the bedside for many Phase I- and Phase II-metabolized drugs.

The greatest amount of research into the ontogeny of drug metabolism has focused on the aforementioned cytochrome P450 superfamily of enzymes known as the CYPs [18]. As previously mentioned, CYP3A4 is the most common drug metabolizing enzyme in humans. However, this enzyme is expressed at very low levels at birth. Instead, CYP3A7, a CYP usually undetectable in adults, is the predominant CYP in fetal liver [17]. After birth CYP3A4 activity increases upwards of 60 % of adult levels within the first week of life and achieves full adult levels by a child's first birthday. Conversely, CYP3A7 peaks shortly after birth and then rapidly declines. Other distinct patterns of CYP developmental expressions have also been observed. CYP2C19 and CYP2E1 activities appear shortly after birth and gradually increase until adult levels are reached at ~6 months and 1 year, respectively. Some CYPs, such as CYP2D6 and CYP2C9, do not exhibit vastly different activity across the age spectrum. As would be expected, the maturational changes of each CYP enzyme potentially represent an altered PK profile for any substrate [15–18].

Unfortunately, less information is known about the ontogenic expression of the Phase II enzymes [16]. Studies with specific drugs have shown that individual UGT isoforms have, like the CYPs, unique maturational profiles which result in differing PK profiles of drugs dependent on these enzymes for elimination from the body. An example of this would be the case of morphine, a UGT2B7 substrate. The drug and its metabolites, morphine 3-glucuronide and morphine 6-glucuronide, are detected in various proportions with increasing age and body weight.

Genetics

Both Phase I and Phase II drug metabolizing enzymes are influenced by an individual's genetic makeup. Genetic variation in the expression and/or activity of enzymes can influence an individual's response to drug with respect to either therapeutic efficacy or adverse events. Patients may inherit variable numbers of gene copies that define the activity or functional capacity, of that enzyme. To date, patients are classified as to the expected activity of the enzyme based on their genotype, i.e., two nonfunctional (or "inactive") alleles—poor metabolizers (PM), two functional or "normal" copies or one functional allele and one reduced activity level allele—extensive metabolizers (EM), two reduced activity alleles or one functional and one reduced activity allele (significantly below capacity seen in PM)—intermediate metabolizers (IM), and patients with multiple copies of functional alleles—ultrarapid metabolizers (UM). In reality and based on our family tree, the entire spectrum from zero to extremely rapid activity is possible and can result in altered patient response [17].

Unfortunately, the clinical utility of genotyping test results has yet to keep up with the technology developed to identify various genotypes. Moreover, there are

Table 2.5 Selected list of known inducers of CYP enzymes

CYP1A2	Tobacco
CYP2C9	Rifampin
CYP2C19	Carbamazepine, prednisone
CYP2D6	Dexamethasone, rifampin
CYP3A4	Phenytoin, phenobarbital, rifampin, St. John's Wort

CYP cytochrome P450

apparent discrepancies in a patient's clinical response and the predicted response based on genotype results, often termed the "genotype–phenotype discordance." This discordance is primarily reflective of the involvement of multiple genes in overall drug disposition and/or the drug's pharmacologic effect rather than one polymorphism responsible for the observed phenotype [17]. In addition, multiple compensatory mechanisms are inherent to our genome which can be expressed to enhance or stop an encoded process thereby mitigating the effects of particular genotypes. Similarly, environmental factors (e.g., age, disease severity) will influence the ultimate phenotypic expression within an individual patient.

Enzyme Inhibition and Induction

Not only can drugs and endogenous compounds act as substrates for the various drug metabolizing enzymes, but they are also capable in some cases of altering the activity of the enzymes themselves. A drug that is known to either induce or inhibit enzymes may either enhance or diminish the metabolism of other drugs but also its own metabolism. Enzyme-inducing drugs include various anticonvulsants and anti-infective agents, among others. This effect is usually only noticed after repeated use and can take days to weeks to reach maximum activity levels. Patients receiving enzyme-inducing drugs may require much higher doses of concurrent medication(s) to achieve a therapeutic effect as is the case with patients receiving chronic phenobarbital therapy requiring higher warfarin doses to achieve adequate anticoagulation. As noted, a drug may also induce its own metabolism potentially leading to reduced therapeutic effectiveness of the inducing drug as well as concurrent medications. The classic example of a drug that induces its own metabolism and the metabolism of many others is carbamazapine. In such circumstances the dose of the inducing drug may also require adjustments as well as the affected co-administered drug(s). A partial list of drugs that may enhance drug metabolism is provided in Table 2.5.

Conversely, drugs that inhibit certain enzyme activity do so either via competitive substrate inhibition or via irreversible substrate-mediated enzyme inactivation and require less time, hours to days, to achieve this effect—enzyme stimulation requires time for the body to generate more enzyme whereas inhibition can occur immediately with the proper concentration. Enzyme inhibition may result in impaired elimination of a drug and potentiate its effects. This is especially true of

Table 2.6 Selected list of known inhibitors of CYP enzymes

CYP1A2	Fluvoxamine, ciprofloxacin
CYP2C9	Fluconazole
CYP2C19	Proton pump inhibitors
CYP2D6	Bupropion, fluoxetine, paroxetine
CYP3A4	Itraconazole, ketoconazole, erythromycin, clarithromycin, grapefruit juice

CYP cytochrome P450

drugs with narrow therapeutic indices as was the case with terfenadine (e.g., Seldane®, a second generation "nonsedating" antihistamine) leading to its removal from the market. Terfenadine requires metabolism by CYP3A4 to its active and safe metabolite fexofenadine (Allegra®). When terfenadine was co-administered with a CYP3A4 inhibitor (e.g., erythromycin), the cardiotoxic parent compound, terfenadine, accumulated leading to fatal cardiac arrhythmias prompting the drug to be withdrawn from the market. A partial list of drugs that may inhibit important enzymes is provided in Table 2.6.

Drug Transporters

All drugs undergo some form of passage through a cell membrane at various point(s) in the drugs' sojourn throughout the body. This is required for absorption, distribution, metabolism, and excretion. The process by which this occurs can vary according to, among other things, the cells involved and the inherent characteristics of a drug. These processes can happen passively, via simple diffusion, or require carrier-mediated transport. Carrier-mediated transport can require energy, as in active transport, or facilitated diffusion where no energy input is required, such as glucose's movement mediated via the GLUT4 transporter across a cell membrane. These mechanisms can be required for a drug to distribute to where it needs to go (absorption and distribution) or to remove it from the body (metabolism and excretion). Transporters play an important role in defining a drug's pharmacokinetic profile. The most pharmacologically important transporters usually fall into one of the two superfamilies, the ATP binding cassette (ABC) and solute carrier (SLC) transporters. ABC transporters are active transporters while the SLC group involves facilitated or secondary activated transporters [5–9].

Transporters, located in all cell membranes throughout the body, control the influx and efflux of endogenous, usually nutrients and waste products, and exogenously administered compounds like drugs. Because transporters can control the cell's exposure to a drug, they can be crucial in a drug's efficacy as well as toxicity profiles. Multiple transporters may work together to facilitate a drug's movement into and out of cells affecting the absorption, distribution, and elimination from the body.

Transporters in the Intestine: Absorption

Both ABC and SLC transporters are present in the apical membrane of the enterocyte that work to facilitate drug absorption or decrease a drug's bioavailability. The most extensively researched ABC transporter is P-glycoprotein (ABCB1). This protein transports drug from the enterocyte back into the intestinal lumen, thereby decreasing a drug's absorption into the body. Hence, P-glycoprotein and members of the ABC superfamily are often referred to as efflux transporters [5–9]. The efflux transporters have a diverse group of substrates and can also serve as a source of the ever important drug–drug interaction. Like the CYP drug metabolizing enzymes, P-glycoprotein activity may be affected by exogenously administered substrates and/or genetics. Therefore, drugs that inhibit the transporter's activity can result in increased absorption of a drug that would normally be removed from the enterocyte if the transporter was functioning at normal capacity. Likewise, if P-glycoprotein was induced or overactive, normally well-absorbed drugs may be "effluxed" out of the enterocyte thereby limiting drug absorption. Unfortunately, the data involving these findings in humans is incomplete and occasionally conflicting. This is also true of the SLC transporters whose role in drug absorption remains unclear. Most importantly, much more data is needed describing the ontogenic influences that influence transporter functional capacity along the age continuum and what effects, if any, are observed in clinical response.

Transporters in the Liver and Kidney: Elimination

Transporters play an important role in both the uptake of drug into the liver and removal of the drug from hepatocytes. The uptake of drug into the liver, when simple diffusion is not sufficient, is usually accomplished by the SLC transporters. This entry into the hepatocytes allows for the drug to be presented to drug metabolizing enzymes. Once the drug enters the hepatocyte, the ABC transporters can then facilitate the removal of the drug or its metabolites into the blood or bile, eventually to be removed from the body completely [6–8].

For drugs with little hepatic metabolism or biliary excretion and/or metabolites, the kidney represents the major process for drug elimination (see below). The secretion of drugs into the renal tubular lumen is usually a transport-mediated multi-step process mostly carried out in the proximal tubule. For the clinician, the most important aspect to remember about this process rather than how it occurs is that the process is saturable, modifiable, and subject to genetic mutations. Therefore, some drug's renal elimination may be reduced at high concentrations. Further, drugs can compete for a similar transporter, resulting in reduced elimination of one compound. This may not always be a disadvantage as exemplified by the historical maneuver of co-administering probenecid along with penicillin derivatives to extend the limited supplies of penicillin during World War II, soon after the discovery of

penicillin. Finally, genetic mutations can influence protein function or expression. However, the clinical influences of these findings are limited.

Clinical relevance: The metabolism for many drugs can involve multiple metabolic pathways, i.e., primary and possibly one or more secondary pathways. Knowing the developmental pattern of the drug metabolizing pathways allows one to accurately calculate repeat doses for ongoing drug therapy. If a primary pathway is compromised by enzyme inhibition or is modified by inheritance, i.e., genetics, a secondary pathway(s) may become important to the body elimination of a drug ultimately leading to no or only minimal change in the drug's overall body disposition and, thus, no observable change from that expected with the original routine drug dose. An example would include the drug amitriptyline (Elavil®), a tricyclic antidepressant used for the treatment of depression and sleep disorder in adults and ADHD in select children. Amitriptyline is metabolized by multiple CYP pathways but primarily by two polymorphically expressed cytochrome P450 isoenzymes, CYP2C19 and CYP2D6. The patient may be a CYP2D6PM but a CYP2C19EM or similar perturbations where the sum of the metabolic pathways results in the expected drug elimination and, thus, desired clinical response. This situation is often the basis for the discordance sometimes observed in patients receiving multiple medications and you expect a drug–drug interaction but it is not observed. This occurs as the metabolism of the drug or drugs has alternate pathways unaffected by the primary drug interaction. With the case of amitryptyline, if the patient is a CYP 2D6 and CYP 2C 19 PM or receives a drug or drugs that interfere with both CYPs the patient may experience increased toxicity as the drug elimination is slowed because of diminished to null activity in both metabolic pathways. Lastly, enzyme inhibition may reduce or ameliorate enzyme activity thereby making an individual with "normal" enzyme activity function as a poor metabolizer.

The converse is true for enzyme inducers. It is important to recall that enzyme induction requires time to generate additional enzyme and the timing of when one might observe the clinical effects from an enzyme induction drug interaction varies (often 5 days to 3 weeks) and that the effect will be gradual until full induction is achieved. If co-administered drug doses are increased to compensate for the observed induction one simply needs to recall that if the dose of the enzyme-inducing drug is decreased or the drug discontinued completely the doses of the other co-administered drugs can be reduced appropriately as well.

Drug Elimination

Drugs can be removed from the body by various processes of elimination, with the kidney and liver being the two most important organs involved. Drugs and their metabolites may also be eliminated from the body via sweat, saliva, bile, expired air, and other bodily fluids, though the kidney and liver represent the pathways for the majority of clinically used agents. Drugs may either be eliminated as a "whole" or otherwise unaltered as the parent compound or, as described above, may be

metabolized to various metabolites which are then eliminated from the body via one mechanism or another, typically the kidney. The PK parameter clearance (CL) best describes the rate of drug elimination from the body. This PK parameter comprises all routes of drug clearance from the body, i.e., hepatic, renal, lung, etc., and is usually described as CL = renal CL + nonrenal CL. Obviously a drug's CL is dependent on the functional capacity of the body to remove agents as well as any impact that the environment or disease may have on elimination routes.

Clearance is especially useful in designing a regimen for long-term drug administration as it provides the clinician insight into the subsequent or maintenance doses that should be administered in order to maintain drug concentrations within the therapeutic window and most importantly, achieve the desired therapeutic effect(s). When most drugs are eliminated from the body, they typically do so in a linear fashion. This means that the rate of elimination is the same, regardless of the dose and resultant plasma drug concentration—the CL is constant, a concept known as linear or first-order PK. However, for a few drugs, most notably ethanol, aspirin, and phenytoin (e.g., Dilantin®), the rate of clearance is not constant as the rate of elimination is proportional to the plasma concentration of the drug. These drugs are said to undergo nonlinear or "saturable" elimination. The importance of this PK characteristic is for these later drugs a small increase in dose can lead to a substantial increase in the amount of drug in the body (e.g., elevated plasma drug concentrations) and lead to toxic effects.

Another important PK parameter in relation to drug elimination from the body is the time it takes for the serum concentration of a drug to decrease by 50 %, otherwise known as the drug half-life ($t1/2$). This value is very helpful at the bedside in estimating the time to reach steady-state conditions, i.e., steady state is where the rate of drug administration equals the rate of drug elimination. For drugs that follow linear or first-order PK (proportional), it takes ~5 drug half lives to reach this state of equilibrium, steady state. Furthermore, the $t1/2$ can be helpful in determining when to restart drug dosing or initiate new drug therapy after temporary discontinuation of a medication regimen. Total elimination from the body is usually complete by ~10 $t1/2$ s but clinically relevant elimination (i.e., >90 %) is usually achieved after four drug $t1/2$ s. For drugs that follow nonlinear or saturation PK, the $t1/2$ is often unhelpful as the $t1/2$ continues to change as the drug's plasma concentration changes.

Clinical relevance: The functional capacity of renal function depends on gestational age as well as postnatal adaptations. Nephrogenesis begins as early as 9 weeks gestation and is nearly complete by ~36 weeks gestation. Postnatally, changes in renal and intrarenal blood flow lead to increases in glomerular filtration rate (GFR). GFR rates vary widely among different PCA. Term infants have decreasing vascular resistance with concomitant increases in cardiac output as the infant grows until adult GFR values are reached by ~1 year. Adult values of renal tubular secretion and reabsorption are invariably reached by ~6–9 months of age, despite being only 20–30 % of adult values at birth. For infants born preterm, there is tremendous variability in renal functional activities with relation to the infant's gestational and postnatal ages. The maturation of these activities is best correlated with the PCA

combined with any underlying disease state(s). These changes dramatically alter the CL of drugs or metabolites that undergo extensive renal elimination. A classic example would be the dosing regimens of aminoglycosides requiring every other day administration in preterm neonates but daily administration in term infants. Thus, the infant's age which correlates directly with renal functional capacity must be accounted for in designing drug dosing in premature and young infants with the same principles employed in older infants and children with varying degrees of renal dysfunction.

Conclusion

Pharmacokinetics describes the overall disposition of a drug in the body accounting for the dosage form and route of drug administration. The disposition characteristics are influenced by a number of important chemical as well as patient- and disease-specific variables. Age as it is reflective of major organ function and maturation is an important influence upon drug PK in the pediatric patient. Using knowledge of and by integrating a drug's pharmacokinetic profile with the drug's pharmacodynamic and pharmacogenomic profiles we can design more optimal drug dose regimens for patients, regimens with the greatest likelihood of prompt effectiveness with limited to no adverse effects.

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Chapter 3
Drug Development for Pediatric Diseases with Bone Loss

Chi Zhang

Current Treatment of Orthopedic Diseases with Bone Loss in Children

Osteoporosis is the most common metabolic bone disease, affecting about 44 million people in the USA. It is characterized by a marked decrease in bone mineral density and strength, resulting in fragility fractures associated with high morbidity and mortality. Osteoporosis is becoming a global public health concern and represents a considerable medical and socioeconomic burden for modern societies. According to National Osteoporosis Foundation, osteoporosis was responsible for an estimated two million fractures and $19 billion in costs in 2005. By 2025, it is predicted that osteoporosis will be responsible for about three million fractures and $25.3 billion in costs each year. Basically, osteoporosis is due to the unbalanced bone remodeling process with lower bone formation than bone resorption. Traditionally, osteoporosis is considered a well-known health problem affecting adults, especially the elderly. Unfortunately, pediatric osteoporosis is not yet widely recognized. Since doctors may not think of the risk for osteoporosis in children, the disease may go untreated. In severe cases of osteoporosis, a child will even develop fractures [1]. With less severe but more chronic forms of bone loss, a child may never reach his or her genetically determined peak bone mass. Pediatric patients may also be at greater risk for adult-onset osteoporosis because the child will enter adulthood with lower bone mass than would otherwise be expected. This chapter summarizes current treatment of orthopedic diseases with bone loss in children, for example, pediatric osteoporosis.

Approaches to prevent and treat pediatric osteoporosis are evolving. Doctors must take all risk factors into consideration by optimizing nutrition and activity

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within the constraints of the underlying disease. Regrettably, these general measures are sometimes overlooked. While the guidelines for osteoporosis treatment in adults are widely accepted, limited data for pediatric osteoporosis makes it more difficult to set accurate guidelines for children. No anabolic agent has been approved yet by the US Food and Drug Administration (FDA) as safe for children, although both recombinant human growth hormone (rhGH) [2] and oxandrolone [3] have been used in children who are catabolic following bone loss from burn injuries.

General Options of Treatments for Pediatric Osteoporosis in the USA

With regard to genetic diseases, few specific treatments are available short of gene therapy or stem cell transplantation. Exceptions to this include the use of intravenous bisphosphonates in osteogenesis imperfecta resulting in short-term benefit but with return of pain and fractures following treatment [4], and the use of phosphate and 1,25-dihydroxyvitamin D in XLH [5].

Currently no drugs used in the prevention or treatment of osteoporosis in children, anabolic or antiresorptive, are approved for these purposes by the FDA. Moreover, there has been a paucity of testing these drugs in children by the pharmaceutical industry.

The primary antiresorptives that have been used in children are the bisphosphonates, especially intravenously administered pamidronate. It has been used safely and with no adverse effects on growth in children with osteogenesis imperfecta [4] and it has been used safely and effectively in the first 10 days following pediatric burn injury to prevent both acute [6] and chronic [7] bone loss. Otherwise, clinical experience in pediatrics has been anecdotal.

Recommendation of Medications to Treat Pediatric Osteoporosis

Treatment for pediatric osteoporosis usually begins with a nutritional approach. It is well known that inadequate vitamin D and calcium intake can result in rickets, osteomalacia, and osteoporosis. Due to uncertainties regarding use of some drugs for pediatric osteoporosis (such as bisphosphonate), safe recommendation of medications to treat pediatric osteoporosis is the same as those for osteomalacia and rickets.

Vitamin D: Vitamin D is crucial for bone health and maintaining serum calcium and phosphate levels. Vitamin D treatments include intake of foods rich in vitamin D, increased sunshine, and the right amount of vitamin D products. Preventive doses of vitamin D deficiency will depend on the age of child patients, in general, 400–800 U/day. Treatment of rickets with osteoporosis can be oral vitamin D 2,000–4,000 U/day. If vitamin D cannot be taken orally or osteoporosis patients are

in serious conditions, an intramuscular injection of 20,000–30,000 U is needed, followed by a change in vitamin D treatments to preventive doses 3 months later. Along with high doses of vitamin D treatment, supplement calcium 800–1,000 mg/day is needed. Regular monitoring of serum calcium, phosphorus, and alkaline phosphatase (ALP) levels is required, and doses of vitamin D and calcium supplement need to be adjusted accordingly. If the patient's osteoporosis condition does not recover, the possibility of vitamin D-resistant rickets should be considered with osteoporosis. In that case, alternative selection of the treatments could be vitamin D2 capsules, vitamin AD products, etc.

Calcium supplement: Daily oral intake of calcium is essential for maintaining adequate homeostasis and facilitating bone remodeling and growth. Breastfeeding intake of calcium is about 225 mg/day for a baby age 0–1, and the appropriate intake (AI) is 400 mg/day. AIs are 600, 800, and 800 mg/day for children 1–3, 4–6, and over 7 years old, respectively. For children aged 11–14, AI can be 1,000 mg/day. AI is 800 mg/day for adults over 18 years of age. The intake of appropriate levels of calcium alone may not be enough on its own, but calcium product is an ideal supplement for osteoporosis treatments.

Other nutrients: Osteomalacia or rickets patients are often malnourished and suffer from various vitamin deficiencies. As needed for pediatric osteoporosis, patients should receive enough protein and multivitamins for health maintenance.

Other treatments: There are still uncertainties regarding bisphosphonate use for pediatric osteoporosis. Both the length of maximal bone mass gain and the drug's long-term effects and safety remain unclear. On the other hand, it is critical to treat the primary diseases leading to secondary osteoporosis. For example, tumor-caused osteoporosis patients should receive treatments for their tumor as early as possible; patients with high fluoride intake should be isolated from the fluorine source and receive fluoride removal treatment; patients with osteoporosis caused by drugs should consider discontinued use of those medications; patients with hypophosphatemic vitamin D-resistant osteomalacia or rickets should take oral neutral phosphate medications along with vitamin D and calcium supplements; patients with renal tubular acidosis need to receive enough HCO_3^- against excessive H^+ to correct acidosis.

Rationale for Developing New Anabolic Drugs for Treatment of Pediatric Osteoporosis

Raising a child's vitamin D level can rarely be achieved by nutrition alone. Although an improved diet and supplements are helpful in treatment of pediatric osteoporosis, they are not effective for all patients. Anabolic agents are not commonly used in children for the purpose of promoting bone density accrual or preventing bone loss. In fact, in the case of burn injury the anabolic agents available will not prevent bone

loss but will, if given daily over a 1-year period, increase bone mineral content and bone area proportionately so that the result is a bigger and hence a biomechanically stronger bone. There are two anabolic agents available for use in children: rhGH [2] and oxandrolone [3]. Both have been used without causing either premature epiphyseal closure or virilization.

The most effective anabolic agent in adults, recombinant human parathyroid hormone (rhPTH), is not approved by the FDA for use in children. In the USA, its use is expressly prohibited by the FDA because experimental data in rats has demonstrated an increased incidence of osteogenic sarcoma [8], a cancer predominant in children and young adults. This current ban is in effect despite the fact that use of PTH in larger animals has not produced the same increased incidence of osteogenic sarcoma. Furthermore, rhPTH is given to children who suffer from hypoparathyroidism [9], although long-term follow-up studies have not yet been carried out to assess the incidence of osteogenic sarcoma in this population. For conditions in which bone resorption is primary, treatment with an antiresorptive agent is the most appropriate option. For conditions in which the predominant finding is a lack of new bone formation, use of anabolic agents should be considered along with appropriate management of the underlying condition. In many conditions, the cause of pediatric osteoporosis is multifactorial. If nutritional supplements can help (e.g., in malabsorption), or the inflammatory response is due to recurrent infections (e.g., in cystic fibrosis) then appropriate antibiotic therapy is indicated. Similarly, for immobilization, either weight-bearing exercise or use of continuous vibration therapy should be considered. It is important to meet a child's caloric and protein needs when dealing with muscle wasting of malnutrition-associated diseases, and the development of newer, and safer, forms of cancer chemotherapy can hopefully spare the bone marrow as much as possible.

Distraction osteogenesis is a well-established surgical technique for limb lengthening and replacement of bone loss due to trauma, infection, or malignancies in children. Exogenous application of bone morphogenetic proteins (BMPs) can increase bone formation during distraction osteogenesis. BMP2 has been shown to accelerate bone formation in numerous clinical and preclinical reports; however, BMP2 has many drawbacks and long-term concerns. An alternative method for increasing the rate of bone formation is needed.

Basically, osteoporosis is due to the unbalanced bone remodeling process with lower bone formation than bone resorption. So far, therapeutic options for the treatment of osteoporosis comprise mostly antiresorptive drugs, aimed at inhibiting bone resorption. The limitations of these drugs are that they result in a low turnover state where bone formation continues to decrease with reduced bone remodeling activity. Thus, antiresorptive drugs alone are insufficient to achieve efficient gain in bone mineral density. To treat osteoporosis, it is also important to stimulate new bone formation. Anabolic drugs may be an excellent option to treat osteoporosis in both children and adults. Identification of novel anabolic agents that can stimulate bone formation in the treatment of osteoporosis has been recognized as a priority in the bone biology field.

Approaches for Drug Discovery

A rational and scientific approach to the drug discovery process is intended to define the specific molecular mechanism to be targeted according to biological and clinical observations. In principle, certain diseases can be considered as an abnormality at the mechanistic level. This abnormality results in a functional deficit that will cause the dysfunction of the cell or organ. These problems will spread and lead to secondary changes in the organism and cause symptoms and physiological changes that are used for the categorization of diseases. Based on this process, drug discovery strategies can be summarized at three different levels: mechanism-based approach, function-based approach, and physiology-based approach. Each approach comes with its own specific strengths and limitations, and the choice of approach depends on the actual drug developmental needs and preferences.

Mechanism-Based Screening

The mechanism-based approach aims to produce a therapeutic effect by targeting a specific mechanism. Novel targets are identified according to biological and clinical findings and validated according to gene or protein expression patterns and knockout mice phenotypes. The novel target can normally address the specific mechanism of biological activity. After the target is selected, a functional in vitro assay will be developed to measure the selectivity of compounds to the target. Then the high throughput screening (HTS) will be performed. This normally leads to the identification of many compounds, which probably belong to different chemical classes, with different degrees of effects on the target. In the lead identification process, small-scale analoging is performed around these structure classes to determine feasibility of reaching a selective compound with appropriate drug-like properties. In the lead optimization process, a number of analogues are produced around the lead structures and are screened for target selectivity as well as pharmacokinetic and metabolic properties. After the lead optimization process, candidate compounds can be tested in disease models in vivo for proof-of-principle. If the study is promising and reproducible, the compounds can be selected for further development. This mechanism-based approach has widely been used in drug discovery [10, 11].

Function-Based Approach

The function-based approach aims to induce a therapeutic effect by normalizing a disease-specific functional abnormality. It is supposed to identify compounds for their abilities to induce or normalize functional parameters in disease-relevant models, such as axonal transport, growth processes, hormone secretion, or apoptotic processes. Compared with mechanism-based approach, functional parameters provide a higher level of organism complexity because they involve integrated

action of many different mechanisms. In contrast to the physiology-based approach, the parameters of a function-based approach cannot be compared directly to the symptoms observed in patients. Examples of the function-based approach currently used in drug discovery are microdialysis and whole-cell or extracellular electrophysiology. A limitation of this approach is that the screening capacity of these methods is low, so library screening cannot be performed with these methods. Basically, the initial step is to find a functional deficit that is unique to the disease state. For example, identify a problem by comparing cells or tissue from patients, or transgenic animals carrying a human mutation with healthy controls. The second step is to validate the specificity of the unique dysfunction for the certain disease. The next steps are to transfer the assay to a format good for screening, to perform the screening assay, and to select candidate lead structures. Side-effect tests can be performed to ensure safety. In the end, the lead optimization can be started using the function assay to determine compound efficacy, and related assays will be carried out to examine pharmacokinetic and metabolic properties.

Physiology-Based Approach

The physiology-based approach aims to induce a therapeutic effect by reducing disease-specific symptoms or physiological changes. It is intended to identify compounds for these properties in animal models that usually mimic specific aspects of disease symptomatology, common treatment side effects, or characteristics of clinically effective compounds. The screening is normally conducted in isolated organ systems in some cases or in whole animals in other cases. In fact, the physiology-based approach was the first drug discovery paradigm and is still used extensively. However, it is limited by a very low screening capacity. Basically, the first step is to develop a disease model that mimics certain symptoms of the disease. The second step is to provide predictive validity by showing that clinically effective drugs are effective in the disease model. Compounds can then be screened in the disease model, including measuring their ability to increase therapeutic efficacy or improve a side effect. In the end, lead optimization can be performed using the disease model as well as different assays for pharmacokinetic parameters, side effects, and so on.

High Throughput Screening

Principle of the HTS Assay as an Approach of Mechanism-Based Screening

HTS has become an integral part of the mechanism-based, small-molecule drug discovery process. HTS is a relatively recent innovation, made feasible largely through modern advances in robotics and high-speed computer technology. This

drug discovery process is widely used in the pharmaceutical industry nowadays. It leverages automation to quickly assay the biological or biochemical activity of a large number of drug-like small molecules. Normally, HTS assays are performed in "automation-friendly" microtiter plates. HTS has the capability to support cellular and biochemical assays using absorbance, fluorescent kinetics, fluorescence anisotropy, time-resolved fluorescence, time-resolved fluorescence resonance energy transfer, bioluminescence (e.g., luciferase and green fluorescent protein), scintillation proximity, and cellular fluorescence imaging. Small molecules can be synthesized in high quantity and purity, as well as conveniently supplied or removed, giving them great potential to be useful for therapeutic and basic research applications. Permeable small molecules can control cellular processes by regulating different signal transduction pathways, gene expression, or metabolism and have been effectively used in drug discovery protocols. HTS of chemical libraries has become a critical tool in basic biology and drug discovery in universities or institutions. Use of this assay has led to the identification of several marketed drugs and natural compounds promoting short-term stem cell maintenance and compounds directing early lineage choice during differentiation. For example, HTS has been performed to identify novel small molecules that can support self-renewal of embryonic stem cells [12, 13] and the specification of cardiomyocytes [14] and neural progenitors [15].

New Developments in Bone Biology Related to Drug Discovery Target

Recent improvements in our understanding of the molecular mechanisms for regulation of bone formation provide important clues to help guide development of new specific anabolic therapeutic options for osteoporosis.

Regulation of Bone Formation

Bone formation includes two distinct processes. Most bones form by endochondral ossification with a cartilage template. A small number of skeletal elements, mainly craniofacial bones, are formed by intramembranous ossification by which bones form directly from condensations of mesenchymal cells without a cartilage intermediate. Osteoblast differentiation occurs through a multistep molecular pathway regulated by different transcription factors and signaling proteins (Fig. 3.1). Indian hedgehog is required for endochondral but not for intramembranous ossification [16]. This factor is essential for the differentiation of mesenchymal cells into osteoblasts. Runx2 is needed in both ossification processes. Runx2-expressing cells are bipotential, which can be differentiated into either osteoblasts or chondrocytes [17]. Osterix (Osx) is an osteoblast-specific transcription factor required for bone formation [18]. Osx was first discovered as a BMP2-inducible gene in mesenchymal stem cells.

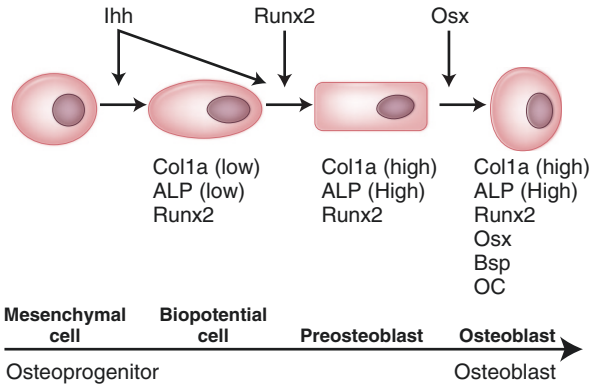

Fig. 3.1 Current model of osteoblast differentiation from stem cell. Ihh is the initiator of endochondral ossification. The *Runx2*-expressing bipotential progenitors can differentiate into either osteoblast or chondrocyte. Then cells differentiate into preosteoblasts, in which Runx2 plays an essential role. In the next step, preosteoblasts differentiate into mature osteoblasts, a process in which Osx plays a critical role. Osx is an osteoblast-specific transcription factor required for osteoblast differentiation and bone formation

Osx-null embryos have normal cartilage but show a complete absence of bone formation [18]. Wnt pathway also has an essential role in osteoblast differentiation [19–21]. Osx coordinates both osteoblast differentiation and osteoblast proliferation during bone formation. The observation that Osx inhibits Wnt pathway highlights the potential for novel feedback control mechanisms involved in bone formation [22]. Osx is also critical for the osteoblast lineage [18, 22]. Following the lineage commitment, osteoprogenitors undergo a proliferative stage. Subsequently, they exit mitosis, transit to express genes (such as ALP, bone sialoprotein, and type I collagen), and commence producing mature osteogenic extracellular matrix. Finally, they express genes involved in mineralization of extracellular matrix such as osteocalcin (*OC*). This highly regulated program of gene expression and cellular differentiation is governed by the expression and activity of different factors described above, among which Osx is responsible for the final commitment of preosteoblast differentiation into mature osteoblasts.

Osx Is Required and Specific for Bone Formation

Osx is the only bone-specific transcription factor identified so far which is required for bone formation. Osx knockout is lethal. Heterozygous *Osx* mutant mice were normal and fertile. Homozygous *Osx* mutant mice had difficulty in breathing, and died within 15 min of birth [18]. Osteoblast marker genes such as ALP and OC are undetectable in Osx-null mutant. There is no bone formation without Osx. On the other hand, it was shown that Osx was sufficient to induce the expression of osteoblast marker OC in mesenchymal stem cells in vitro [18]. Osx controls

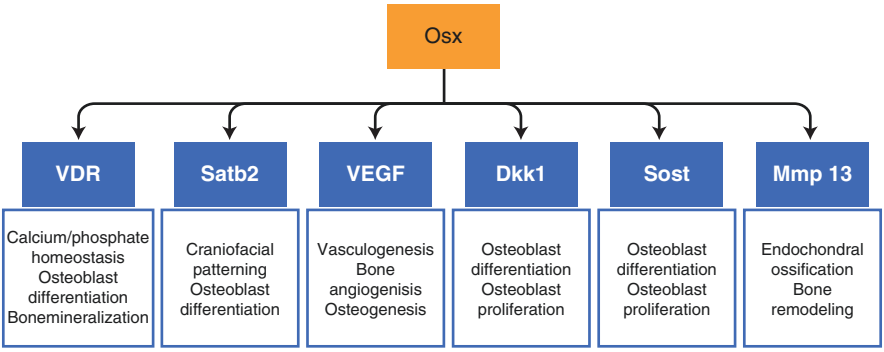

Fig. 3.2 Osx downstream target genes in osteoblasts. Downstream bone-related target genes of Osx during bone formation have been identified in our research laboratory, including Satb2, VDR, VEGF, SOST, DKK1, and MMP13. These Osx downstream targets play different important roles during bone formation, supporting the notion that Osx as an osteoblast-specific transcription factor is a master gene for osteoblast differentiation and bone formation

osteogenesis as a downstream gene of Runx2 [18]. Runx2 is required for bone formation; however, Runx2 is expressed in different cells and tissues, including osteoblasts, chondrocytes, epithelial cells, glioma cells, brain tissues, and different tumor tissues [23]. Unlike Runx2, Osx is unique and bone-specific in that it is specifically expressed in osteoblasts and at low levels in prehypertrophic chondrocytes [18]. Osx is not only critical for embryonic bone formation but also essential in postnatal bone growth and in bone homeostasis using the conditional knockout approach [24].

Despite the discovery of its significance in skeletal physiology a decade ago [18], relatively little is known about direct target genes for Osx and molecular mechanisms through which Osx controls gene transcription. Recently, our research laboratory has identified several downstream bone-related target genes of Osx during bone formation, including Satb2, VDR, VEGF, SOST, DKK1, and MMP13 [25–30]. Identification of VEGF as a downstream direct target of Osx also indicates that Osx plays an important role in coordinating osteogenesis and angiogenesis [27]. These Osx downstream targets each play important roles during bone formation (Fig. 3.2), supporting the notion that Osx, as a bone-specific transcription factor, is a master gene for osteoblast differentiation and bone formation.

Identification of Potential Chemical Leads from HTS

Identification of anabolic agents that can stimulate bone formation to treat osteoporosis has been recognized as a priority in the bone biology field. Recent genome-wide association studies have shown that *Osx* are associated with bone mineral density in both children and adults, suggesting that Osx may contribute to the cause of osteoporosis [31, 32]. Because of the tissue specificity and the critical role of Osx

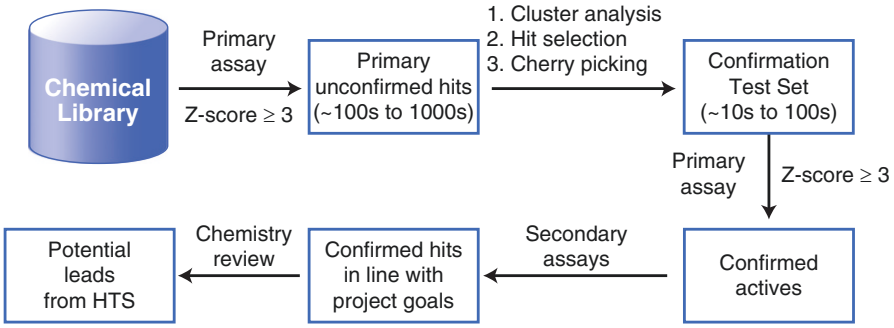

Fig. 3.3 Identification of chemical leads from high throughput screening. The following protocol will be used for assay development and HTS execution. Secondary assays will be developed in parallel to the primary assay and meet similar criteria

in bone formation, Osx can be considered an ideal novel target for the development of a therapeutic strategy to induce the anabolic pathway of bone synthesis. At present, no pharmacological approach to target Osx in osteoblasts has been identified. Focusing on novel target Osx that are responsible for driving osteoblast differentiation and bone formation increases the likelihood of discovering mechanism-based agents that are more effective and less toxic than drugs of the previous era. Therefore, HTS assay must be performed to identify compounds promoting osteoblast differentiation and bone formation through Osx. In this case, this mechanism-based approach aims to identify potential anabolic agents by targeting a bone-specific factor Osx. Protocols for assay development and HTS execution are shown in Fig. 3.3. Parameters and controls (positive, and neutral) for the proposed primary assay must be developed, refined, and validated such that it is robust (Z' values ≥ 0.45 over many assays and experimental days) [33], tolerant of effects from DMSO, free from systematic effects (e.g., plating artifacts, liquid handling errors), simple (most assays have less than three liquid additions and are endpoint assays), and efficient in use of reagents and resources. Secondary assays will be developed in parallel to the primary assay and meet similar criteria. Candidate small molecules identified by the process will be validated and characterized for their osteogenic activities using in vitro assays. The role of candidate small molecules in osteogenesis in vivo will be explored in osteoporosis animal models.

Overview of Drug Development Approval Process in the USA

How Drugs Are Developed and Approved by FDA

The FDA is an agency of the federal government's Department of Health and Human Services. Center for Drug Evaluation and Research (CDER) is the largest of the FDA's five centers in the USA. CDER is in charge of both prescription and

nonprescription or over-the-counter drugs. The CDER mission is to ensure that drugs marketed in the USA are safe and effective. CDER does not test drugs, although the Center's Office of Testing and Research does conduct limited research in the areas of drug quality, safety, and effectiveness. CDER activities include (1) reviewing drugs before marketing, (2) watching for drug problems, (3) monitoring drug information and advertising, (4) scientific research, and (5) protecting drug quality.

Companies must apply to the FDA in order to introduce a new drug into the US market. Companies have the responsibility to test the drug and submit evidence that the drug is safe and effective. A team of CDER physicians, statisticians, chemists, pharmacologists, and other scientists reviews new drug applications.

Pediatric Drug Development

The FDA Amendments Act of 2007 reauthorizes and amends the Best Pharmaceuticals for Children Act of 2002 (BPCA) and the Pediatric Research Equity Act of 2003 (PREA), both of which encourage more research in pediatric drug development. Some notable changes to BPCA and PREA are (1) authorization to establish an internal review committee (the Pediatric Review Committee will review requests for waivers and deferrals, pediatric assessments and pediatric plans prior to approval, and pediatric written requests prior to issuance); (2) clinical, clinical pharmacology, and statistical reviews are to be made public for applications submitted in response to both PREA and BPCA; and (3) adverse event reporting now affects both PREA and BPCA (review of reports has been modified to occur 1 year after labeling approval).

Introduction of Best Pharmaceuticals for Children Act

The Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) needs to oversee the activities of BPCA. The BPCA program aims to improve pediatric therapeutics through preclinical and clinical drug trials that result in drug labeling changes. Federal legislation and FDA regulations require that drugs be tested for safety and efficacy in a specific population, at a specific dosage, and for a specific time period before the drugs are finally approved for clinical use. Use of drugs without appropriate testing is considered "off-label" use.

Testing drugs in children comes with scientific, clinical, ethical, technical, and logistical challenges. Several practical challenges have discouraged drug testing in pediatric populations. These challenges include (1) lack of incentives for companies to conduct research on drugs in neonates, infants, and children; (2) lack of necessary technology to monitor patients and assay very small amounts of blood; and (3) lack of suitable infrastructure for conducting pediatric pharmacology drug trials. As a result, the majority of drugs used in children are used off-label, without

adequate understanding of appropriate dose, safety, or efficacy. This encourages pharmaceutical companies to conduct pediatric studies of on-patent drugs that are used in pediatric populations, but are not labeled for such use, by extending their market exclusivity.

BPCA Prioritization Process

The Eunice Kennedy Shriver NICHD has sought public input and obtained different collaboration within National Institutes of Health with experts in pediatrics to identify drugs in need of further study and to prioritize needs in pediatric therapeutics. Following the 2007 legislation changes, the procedure for prioritization was revised to emphasize knowledge gaps in therapeutic areas as opposed to those about specific drug products. Specifically, the legislation authorizes that the NIH, in consultation with the Commissioner of Food and Drugs as well as researchers with expertise in pediatric research, shall develop and publish a priority list of needs in pediatric therapeutics, including drugs or indications that require study. This list shall be revised every 3 years. The revised legislation also required that, in developing these priorities, the Secretary shall consider (1) therapeutic gaps in pediatrics that may include developmental pharmacology, pharmacogenetic determinants of drug response, metabolism of drugs and biologics in children, and pediatric clinical trials; (2) particular pediatric diseases, disorders, or conditions where more complex knowledge and testing of therapeutics, including drugs and biologics, may be beneficial in pediatric populations; and (3) the adequacy of necessary infrastructure to conduct pediatric pharmacological research, including research networks and trained pediatric investigators.

BPCA Clinical Studies for Pediatric Populations

The Eunice Kennedy Shriver NICHD at the National Institutes of Health and other institutes involved in the BPCA are working together with the FDA to design clinical trials for pediatric populations. The process involves creation of a Proposed Pediatric Study Request (PPSR) or Written Request (WR), the formal mechanism by which FDA notifies a drug maker that additional clinical information about a drug is needed. Once a drug company declines a WR, or if the FDA accepts a PPSR, a clinical study will be referred to the NICHD for development of a clinical trial.

Once the NICHD has initiated a funding mechanism to the most-qualified group, the final protocol for the trial is developed with input from and interaction with the appropriate institute or center (e.g., the BPCA Data Coordinating Center, the Pediatric Trials Network, the FDA, and the Institutional Review Boards). The protocol becomes the blueprint for the trial and the basis for the Investigational New Drug submission to FDA.

To ensure the participant safety, all researchers and staff involved with clinical trials must follow every federal regulation and ethical guideline in conducting the study. Frequent evaluation by those involved and by independent entities throughout the course of the trial provides added safety. Trial participant's safety is overseen during the clinical study. When the clinical study is completed, data are submitted to the FDA for evaluation by its review staff and expert advisory panels with the intent of modifying the label to improve pediatric therapeutics.

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Chapter 4
Paediatric Bone Physiology and Monitoring the Safety and Efficacy of Bone Drugs in Children

John G. Logan, J.H. Duncan Bassett, and Moira S. Cheung

Bone Physiology

To appreciate why we particularly need to monitor the effects of bone drugs in children, it is necessary to understand the molecular and cellular mechanisms underlying skeletal development and adult bone maintenance. In adults, bone size and shape remain relatively constant and bone is renewed via remodelling. In contrast, during childhood and adolescence, the size and shape of the skeleton change rapidly; bones become longer and wider, and cortical thickness and bone mass increase. Linear growth occurs at the epiphyseal growth plates and shape changes by the process of bone modelling. Approximately 90 % of bone mass is accrued during the first 18 years of life and peak bone mass is achieved by the early twenties [1]. This period of linear growth and bone mass accrual is important for long-term bone health. Disruption of this process will affect patients during childhood and may also lead to an increased risk of fracture in adulthood [2]. Thus, inappropriate use of skeletal pharmacology during childhood could leave the patient with both abnormal bone development and impaired bone strength into adulthood. This chapter focuses on the physiology of bone development and the techniques used

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to monitor the treatment of low bone mass in children. The monitoring of drugs used in the treatment of calcium and phosphate disorders is specifically covered in other chapters.

Bone Structure

Bone strength is determined by the size and geometry of the bone, its material properties and the cortical porosity. All these factors change during growth and can affect the determination of bone mineral density (BMD).

Bones initially form through either endochondral or intramembranous ossification (see Chap. 3). Within a bone there are two different types of bone tissue, cortical, or compact, bone and trabecular, or cancellous, bone. Cortical bone forms the dense outer shell of a bone and is made up of osteons; parallel cones of concentric bone matrix layers, or lamellae, with a central Haversian canal containing a neurovascular bundle [3]. The osteons are further connected by transverse perforating canals known as Volkmann's canals. Trabecular bone consists of an interconnected network of rod- or plate-like trabeculae that span the medullary cavity adding strength to bone. These also have layers of lamellae, but they are arranged longitudinally along the trabeculae and usually without any of the vascular canals found in osteons.

The geometry of bones changes throughout growth; this is explored in more detail later. The BMD of the developing skeleton increases with age due to both structural changes that result from bone modelling and increased accrual of mineral into the bone matrix. The changes in cortical porosity during growth, however, are more complicated. The Haversian canals comprise the majority of cortical pores in cortical bone; thus, cortical porosity is intrinsically linked to the osteonal development. During growth there are fewer osteons, which are larger and have canals with a large diameter. As growth finishes the number of osteons increases but they are smaller with smaller canals [4]. This results in cortical porosity initially increasing with age, peaking between 4 and 9 years of age, before gradually decreasing to adult levels. An excessively high cortical porosity alters the mechanical properties of cortical bone, as well as lowers the BMD of the cortices and results in reduced bone strength.

Bone Remodelling

Over time mechanical loading results in an accumulation of microfractures and deterioration of the structural integrity of the skeleton. The "bone remodelling cycle" is the physiological process by which damaged bone is repaired thus maintaining the biomechanical integrity of the skeleton. Discrete areas of remodelling termed "bone remodelling units", or basic multicellular units (BMUs), are formed in regions of damaged bone. Within each BMU bone resorption and formation are tightly coupled by the coordinated activity of the specialised skeletal cells, bone resorbing osteoclasts, bone forming osteoblasts and regulatory osteocytes and bone lining cells [5].

Bone remodelling begins with the recruitment of osteoclast precursors to a site of skeletal damage, and subsequently their differentiation into mature bone resorption osteoclasts. Current evidence suggests that osteocytes, buried within the mineralised bone matrix, are responsible for initiating and regulating this formation of the BMU [6]. In response to skeletal loading and microdamage osteocytes express the key osteoclast differentiation factor Rank Ligand (RANKL), thus inducing local osteoclast differentiation [7, 8]. Bone resorption is further controlled through the actions of various other cell types; most notably osteoblasts. They also produce RANKL, as well as the RANKL inhibitor osteoprotegerin (OPG), and various other modulators of osteoclast formation and activity.

Once the remodelling cycle has been initiated osteoclasts adhere tightly to the bone surface to form a "sealed zone" and become polarised. Protons and chloride ions are secreted from the ruffled border adjacent to the bone surface reducing the pH and dissolving the inorganic hydroxyapatite present and thus exposing the organic portion of the bone matrix. The organic matrix is subsequently degraded by osteoclast-derived proteases cathepsin K and matrix metalloproteinase 9 (MMP-9) [9, 10]. These enzymes digest the type I collagen that makes up 90 % of the organic bone matrix, and its degradation products are used as clinical markers of bone resorption. The C- and N-terminal cross-linked telopeptides of type I collagen (CTX, NTX), as well as hydroxyproline (HYP), pyridinoline (PYD) and deoxypyridinoline (DPD) [11] are examples of commonly used bone resorption markers.

Bone resorption ends once the osteoclasts have removed the region of bone and culminates with osteoclastic apoptosis. Subsequently, osteoblast precursors migrate to the site of resorption, in response to both growth factors released from the bone matrix by resorption and osteoclast-derived clastokines. They undergo differentiation and secrete osteoid to repair the defect. Osteoid is an unmineralised organic bone matrix containing type I collagen and multiple non-collagenous proteins. The clinical bone formation markers procollagen type I N-terminal peptide (PINP) and its carboxy-terminal variant (PICP) are generated during osteoid synthesis [11]. The bone matrix then becomes mineralised through the formation and incorporation of inorganic hydroxyapatite crystals [12]. This process is known to be facilitated through the actions of bone alkaline phosphatase (BAP) which breaks down extracellular pyrophosphate inhibitors of mineralisation [13]. Once the skeletal defect has been repaired osteoblastic bone formation ceases. The mature osteoblasts then apoptose, become embedded as osteocytes within the new bone matrix or form bone lining cells. The termination of bone formation marks the completion of the remodelling cycle.

Bone Growth and Modelling

The major difference between adult bone and paediatric or adolescent bone is that in addition to bone remodelling there are profound changes to skeletal shape and size. The mechanisms by which this occurs are collectively termed bone modelling

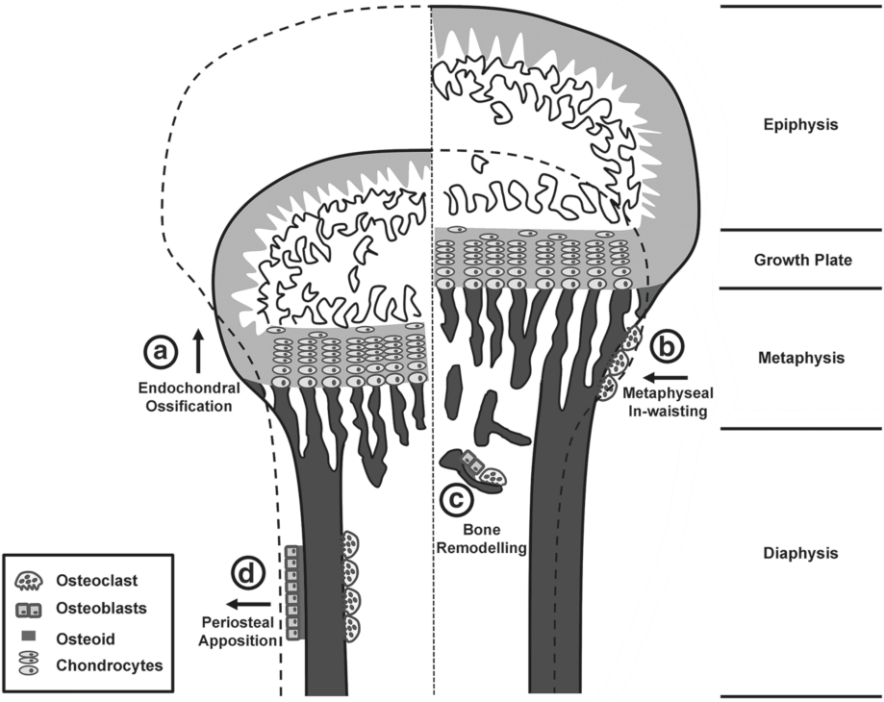

Fig. 4.1 Growth of long bones. Schematic diagram illustrating the growth of long bones: (**a**) Longitudinal growth occurs due to hypertrophic differentiation of growth plate chondrocyte. (**b**) Bone morphology is maintained by osteoclast-directed metaphyseal in-waisting. (**c**) Bone remodelling removes the mineralised cartilage scaffold and deposits new trabecular bone. (**d**) Diaphyseal bone diameter is increased by osteoblast-mediated periosteal apposition

(see Fig. 4.1). While bone remodelling maintains the bone that is already present, modelling enables bones to increase in both length and width while maintaining optimal morphology.

Long bones develop through a process of endochondral ossification [14]. Linear growth occurs at the epiphyseal growth plates that lie between the epiphyses and metaphyses at either end of long bones. The growth plate chondrocytes form highly organised columns divided into three distinct zones; the reserve zone nearest the epiphysis, the proliferative zone and the hypertrophic zone nearest the metaphysis. Reserve zone chondrocytes begin to proliferate in discrete columns extending the growth plate. Following a period of proliferation chondrocytes then undergo hypertrophic differentiation, expanding to ten times their previous volume and producing angiogenic factors. These factors lead to vascular invasion and apoptosis of the hypertrophic chondrocytes. The invading blood vessels also bring osteoclast and osteoblast precursors which convert the mineralised cartilage scaffold into new bone through remodelling [15]. At cessation of growth, chondrocyte proliferation progressively slows ultimately leading to the fusion of the metaphysis and epiphysis.

During longitudinal growth the processes of bone modelling optimise the shape of skeletal elements to their required function. In bone modelling osteoclastic bone resorption and osteoblastic bone formation are not coupled but rather actively coordinated to dynamically reshape the bone. In long bones the growth plate has the widest cross section and the diaphysis the narrowest, with the intervening tapered region termed the metaphysis. As linear growth occurs osteoclasts resorb the periosteal surface of the metaphysis (metaphyseal in-waisting) and osteoblasts lay down new bone on its endosteal surface, thus reshaping the metaphysis into the diaphysis. Importantly, anti-resorptive agents can interfere with this process, leading to club-shaped metaphysis and bone deformities known as undertubulation [16]. During growth, periosteal bone formation (periosteal apposition) also increases the diaphyseal diameter and cortical thickness is determined by the net balance of this periosteal apposition and concurrent endosteal resorption [17].

What Are the Aims of Drug Therapy?

The aim of drug therapy for osteoporosis is covered in detail in other chapters in this book, but in essence it is to enable the skeleton to achieve maximal functional capacity. Overall the intention then is to reduce the frequency of fractures, control pain and improve linear growth.

Prior to 1998 pharmacological treatment for children with fragility fractures was very limited. However, the demonstration that the anti-resorptive bisphosphonates increased BMD and reduced fracture risk in children with osteogenesis imperfecta revolutionised the field [18]. Intravenous bisphosphonates are now considered to be the mainstay of treatment for children with osteoporosis (see Chap. 5). More recently, the alternative anti-resorptive therapies have been developed and now RANKL antibody (denosumab) and cathepsin K inhibitors are also beginning to be used in children [19].

Benefits of Anti-resorptives

Anti-resorptive therapies act by impeding osteoclastic removal of bone while still allowing osteoblastic bone formation to continue. In the growing skeleton, the effect of anti-resorptive therapy on increasing bone mass is amplified and related to the rate of growth, modelling and remodelling. This leads to increased trabecular bone and cortical thickness [20]. Osteoclasts have a major role in modelling trabecular bone adjacent to the growth plate. Anti-resorptives interfere with this process and result in the retention of trabecular bone and mineralised cartilage. This increases bone mineral content (BMC) and improves mechanical strength to this region of the bone. This increase in trabecular bone is particularly beneficial in the vertebral bodies and children treated with bisphosphonates show reduced vertebral compression

fracture and improved healing. The effects of anti-resorptives on growth remain uncertain. However, improvements in height *Z*-scores in some studies suggest that treatment may also improve growth [18]. By contrast, the reduction in long bone fracture risk following anti-resorptive treatment is thought to be due to reduced endosteal resorption and thus an increased diaphyseal cortical thickness. Bisphosphonates have been used to reduce pain in fibrous dysplasia [21] and several uncontrolled trials have reported bisphosphonate-related pain relief; however, this has not yet been confirmed by randomised controlled trials.

Long-Term Adverse Effects of Anti-resorptives

During growth, treatment with anti-resorptive medication impairs bone modelling and so inhibits the normal metaphyseal periosteal resorption. This results in reduced metaphyseal in-wasting and club-shaped long bones. Anti-resorptive treatment also impairs bone remodelling resulting in altered bone structure and quality. Impaired trabecular remodelling below the growth plate results in retention of calcified cartilage [20]. Although adverse effects from retained calcified cartilage have not been directly demonstrated, it is thought to increase the brittleness and reduce the toughness of trabecular bone. Anti-resorptive treatment results in thicker and mechanically stronger cortical bone but the concomitant reduction in microdamage repair is likely to result in bone of inferior quality.

In addition to the adverse structural consequences of anti-resorptives, concerns remain regarding the retention of bisphosphonates within the skeleton after cessation of treatment. This may be clinically important as the bisphosphonate can be re-released during periods of high bone turnover such as pregnancy and lactation. This consideration however remains only a theoretical risk as no adverse events have been reported. Nevertheless, it may be prudent to use the lowest dose of bisphosphonate to achieve the desired clinical outcome. As for any therapeutic intervention, the acute and long-term benefits and risks need to be carefully considered, and continued monitoring of treatment is essential for the optimisation of therapy and safeguarding against potential adverse effects.

How Do We Monitor the Effects of Drugs on Bone Physiology?

When monitoring bone health, a number of different properties of bone and bone metabolism can be measured that reflect the state of the skeleton as a whole, or that of individual bones or bone types. There are various different techniques that can be used for this evaluation, each with benefits and limitations that must be taken into account when using them in children with bone disease. The most common techniques for monitoring bone health are discussed below.

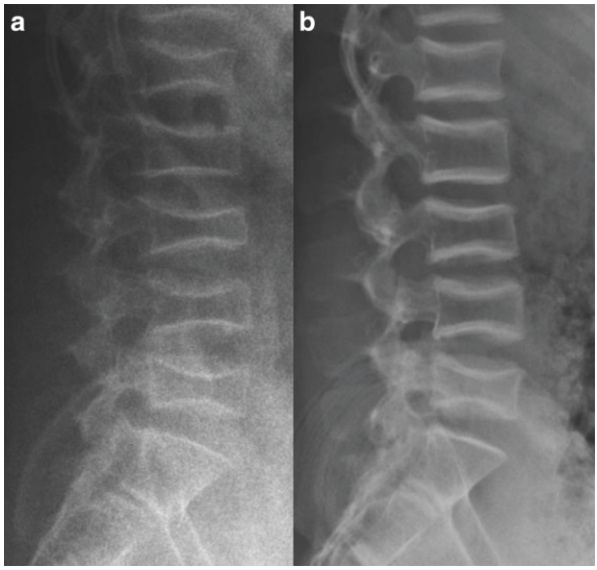

Fig. 4.2 Monitoring vertebral fracture healing during treatment using X-rays. Lateral X-rays of lumbar vertebral compression fractures in a 13-year-old boy with osteoporosis. (**a**) Prior to treatment with pamidronate. (**b**) After 2 years of treatment. Note how the shapes of the vertebrae have remodelled and healed. (Courtesy of Dr. J. Allgrove, Royal London Hospital, UK)

X-Ray Analysis

The oldest and simplest way of monitoring the skeleton in children and adolescents is to use plane X-ray. While this does not yield information regarding material properties or micro-geometry, much information can still be gained. The gross morphology, size and bone age can easily be determined and fractures and deformities readily identified. Moreover, annual X-ray of the lateral spine can be particularly helpful in monitoring vertebral compression fractures (see Fig. 4.2) since vertebral morphometry can be used to quantify the degree of compression and healing [22]. A major drawback to the use of X-ray is the relatively high radiation doses involved. These can be as high as 0.7 mSv for lumbar spine X-rays, significantly higher than other modalities [23].

Abnormal vertebral geometry is quantitated by identifying the four corners of each vertebral body and the mid-points of the end plates and then determining the distances between these six points. The anterior, posterior and mid-point heights, the lower vertebral length and the vertebral height ratios are calculated. The concavity index is determined by calculating the average ratio between the mid-point height and the posterior height for each of the first four lumbar vertebrae (L1–L4). In general, the less tall and the more concave the vertebrae the worse its vertebral shape. The monitoring of paediatric compression fractures by this method is well established and it commonly forms part of annual screening protocols [24].

Dual-Energy X-Ray Absorptiometry

Dual-energy X-ray absorptiometry (DXA) is the most widely used method for assessing bone health in children due to its availability, speed, low cost, non-invasive nature and low radiation dose, and is currently considered to be the "gold standard" for bone densitometry. Nevertheless, to prevent incorrect interpretation there are a number of important issues that must be considered when DXA is used for diagnostic or monitoring purposes. These are discussed in detail below.

A DXA system consists of a scanning X-ray source, an X-ray detector that records absorption at both high and low energy X-rays and a computer system to analyse this data. DXA analysis makes the assumption that the body is divided into two tissue compartments; bone and non-bone. The high and low energy X-rays are differentially absorbed by these two compartments allowing the conversion of the absorption data into mass values for bone and non-bone. This can be done for the whole body or for selected regions such as the lumbar spine, proximal femur or distal radius. DXA can also be utilised for the assessment of other tissues of differing densities, such as lean body mass and fat mass, thus providing important information about body composition.

DXA results are expressed in terms of either BMC or BMD. These parameters are calculated for a selected region of interest (ROI) that consists of bone tissue with the two-dimensional bone area (BA) measured in cm^2. The X-ray attenuation within this ROI is then compared to a reference standard of known mineral density, thus allowing the BMC in grams to be calculated for each pixel. The total BMC for the ROI is then calculated by summing all these pixel values. The BMD of the ROI in g/cm^2 is calculated by dividing the BMC by the bone area (BMD = BMC/BA). Importantly, DXA does not measure the true bone BMD as it is a linear absorption method and so can only provide a two-dimensional analysis of a three-dimensional structure. This means DXA quantifies the "areal" BMD or aBMD rather than the volumetric BMD or vBMD. Thus, in large bone aBMD will overestimate vBMD, while in a small bone it will underestimate it (see Fig. 4.3). In adults, bone size is constant and therefore this does not represent a major problem. However, the rapid increase in bone size in childhood means that the aBMD estimation of vBMD will progressively increase with age and this must be taken into account. Thus, DXA parameters should always be compared to normative range values for the appropriate age, rather than making direct comparisons with previous values. Since bones grow at different rates, comparisons between different ROIs are also inherently more difficult. To ensure accuracy and reproducibility of DXA measurement it is important that the position of the child and the selection of the ROI are standardised. The most commonly used ROIs include (1) total skeleton minus head, (2) lumbar spine from L1/L2 to L4 and (3) femoral neck. Total skeleton aBMD is useful for estimating overall bone health while lumbar spine and femoral neck ROIs determine aBMD at the most common fracture sites. Because these DXA parameters are compared to a normative population reference range, poor position or ROI selection can invalidate the findings (see Fig. 4.4). DXA analysis software uses algorithms to

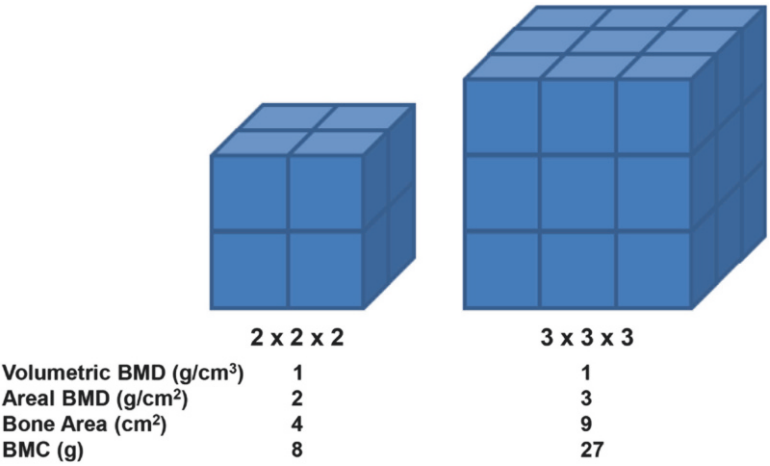

Fig. 4.3 The effect of bone size on areal BMD. A small and a large bone are schematically represented by the *small* and *large cubes*. Although both bones have identical volumetric BMD, the larger bone will have a higher areal BMD as measured by DXA

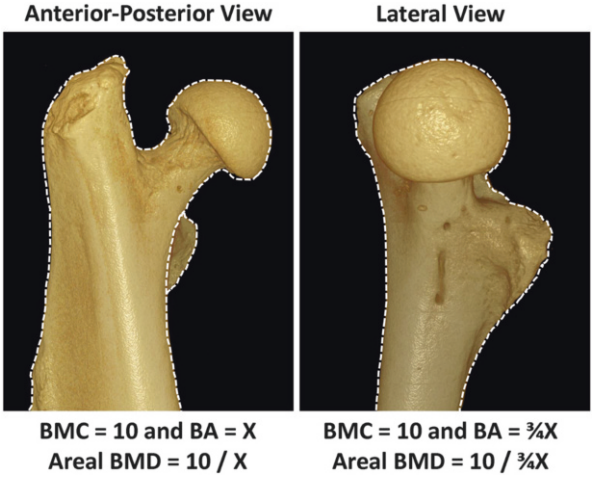

Fig. 4.4 Consistent positioning and ROI selection are required when comparing DXA scan results. The same femoral head is shown in both the lateral and AP orientations. While the volumetric BMD clearly remains the same, the change in position has altered the bone area, thus changing the areal BMD as measured by DXA

distinguish between bone and non-bone compartments when selecting the ROI. As children have a lower BMD than adults the use of an adult algorithm will result in an inaccurate ROI with the exclusion of bone with lowest density and thus an overestimation of BMD [25]. It is, therefore, important to use a modified child-specific algorithm for DXA analysis.

DXA data must be compared to a normative reference range for age before they can be interpreted. In adults the results are normally reported as a *T*-score, defined as the number of standard deviations from the population peak adult BMD. The

World Health Organisation uses this *T*-score to define osteoporosis and osteopenia ($T<-2.5$ and $T<-1$, respectively) [26] but clearly such a definition is not useful in children, as they have not yet reached peak bone mass. Instead the *Z*-score can be used, defined as the number of standard deviations from the average population BMD at the patient's age. To accurately determine the *Z*-score an appropriate reference range is therefore required. It is important to select the correct reference database and not simply to use reference data provided by the manufacturer. Previously, obtaining appropriate paediatric reference ranges was a major problem, but there is now an increasing number of databases available (Table 4.1). However, there are also many demographic factors that must be considered when selecting appropriate DXA reference data including gender, ethnicity, age, height, weight and Tanner stage. When selecting a reference range it is important to also consider the ROIs studied, ROI selection algorithms, scanner brand, scanner model and software version.

Even when an appropriate reference range has been selected, the diagnosis of childhood osteoporosis (as defined in [27]) should not be made on the basis of DXA alone, since incorrect interpretation of DXA data often results in misdiagnosis. The most frequent mistakes include the incorrect use of the *T*-score, poor ROI selection, inappropriate reference range selection and failure to account for small body size.

To improve DXA as a paediatric diagnostic tool, several methods have been proposed that adjust DXA data to take account of bone and body size. One such approach calculates bone mineral apparent density (BMAD) in the lumbar vertebrae by using the average bone width and the BA to estimate bone volume by modelling vertebrae as cubes [28] or cylinders [29]. However, individuals with abnormally narrow or wide bones will confound this adjustment, leading to an under- or over-approximation of true BMD, respectively. Furthermore, this correction must also be applied to the reference population and there are now several published BMAD reference ranges available (see Table 4.1). Recently Crabtree et al. studied the ability of unadjusted aBMD, BMAD and four other height adjustment methods to predict low trauma fractures in a paediatric population with a variety of chronic conditions [30]. They demonstrated that all height adjustment methods improved the diagnostic specificity of DXA, but that no one adjustment technique was superior. In their study, BMAD measurements from L2 to L4 were most predictive of vertebral fractures, while total body less head BMC for lean body mass adjusted for height was most predictive for long bone fractures.

Advantages of DXA

DXA is the most commonly used bone monitoring method because it is widely available, takes less than 3 minutes, is dedicated to bone analysis, has a low cost and requires exposure of less than 1 day's background radiation [23]. DXA is also more versatile than other techniques. It can be used to study the whole skeleton, or to analyse specific sites of interest, including both the long bones and vertebrae. This is of particular importance as compression fractures are common in patients with low BMD, and vertebrae are inaccessible to many other techniques.

Table 4.1 Available reference data for the analysis of paediatric bone

Year	Population (M/F)	Age range	Ethnicity	Location	Machine	Input	Output	References
DXA								
1990	70/65	1–15 years	White	France	QDR-1000	Gender, age, Ht, Wt, Tanner	LSBMD	[44]
1991	109/98	9–21 years	White	Switzerland	QDR-1000	Gender, age, Tanner	LSBMD, LSBMC, FNBMD, FNBMC	[45]
1991	84/134	1–19 years	Mixed	USA	QDR-1000	Gender, age, Tanner, Wt	LSBMD	[46]
1992	28/29	Newborn	White	France	–	GA, Ht, Wt, SA	LSBMD, LSBMC	[47]
1992	22	1–24 months	White	France	–	GA, Ht, Wt, SA	LSBMD, LSBMC	[47]
1993	86/68	5–18 years	White	Spain	–	Gender, Tanner	TBBMC	[48]
1995	137/128	4–26 years	White	Australia	Lunar DPX	Gender, age	TBBMC	[49]
1996	110/124	8–17 years	White	Canada	QDR-2000	Gender, age	LSBMC, LSBMD, FNBMC, FNBMD, TBBMD, TBBMC	[50]
1996	82/68	GA 27–42	White, Black	USA	QDR-1000	Wt	TBBMC, TBBMD, TBBA	[51]
1997	169/234	4–20 years	White	Netherlands	Lunar DPXL/PED	Gender, age, Tanner	TBBMC	[52]
1997	297/0	3–18 years	White, Black, Hispanic	USA	QDR-2000	Age, ethnicity	TBBMC	[53]
1997	0/313	3–18 years	White, Black, Hispanic	USA	QDR-2000	Age, ethnicity	TBBMC	[54]

(continued)

Table 4.1 (continued)

Year	Population (M/F)	Age range	Ethnicity	Location	Machine	Input	Output	References
1998	142/201	4–19 years	White	Denmark	QDR-1000 W	Gender, Tanner	TBBMC, TBBMD, TBBA	[55]
1999	193/230	9–25 years	White, Asian, Hispanic, Black	USA	QDR-1000 W	Gender, age, ethnicity	LSBMD, LSBMAD, HipBMD, HipBMAD, FNBMD, FNBMAD, TBBMD, BMC/Ht	[56]
2001	0/151	9–14 years	White	Netherlands	QDR-2000	Age, breast stage	LSBMC, LSBMD, FNBMC, FNBMD, FABMC, FABMD	[57]
2001	445/537	5–18 years	White, Black, Hispanic	USA	QDR-2000 W	Gender, age, disease status	TBBMC	[58]
2002	188/256	4–20 years	White	Netherlands	Lunar DPXL/PED	Gender, age, Tanner	LSBMD, LSBMAD, TBBMC, TBBMD	[59]
2002	117/139	3–18 years	White, other	USA	QDR-1000 W/ QDR-2000	Gender, age, Tanner	DFBMD	[60]
2002	107/124	5–22 years	White, other	USA	QDR-4500	Gender, age, Ht, TBBMC	TBBMC, TBBMD, TBBA	[61]
2003	210/249	3–30 years	White	Australia	Lunar DPX	Gender, age, Ht	TBBMC/LTM	[62]
2004	0/422	12–18 years	Black, non-Black	USA	QDR-4500 W	Age, Wt, ethnicity	LSBMD, LSBMAD, FNBMD, FNBMAD	[63]
2005	284/278	5–18 years	White	Poland	Lunar DPXL	Gender, age, Ht	TBBMC, TBBMD, LSBMD, LSBMC, TBBMD/LTM, LSBMD/LTM	[64]

2007	761/793	6–16 years	All ethnicities	USA	QDR-4500A, QDR-4500 W, Delphi-A	Gender, age, ethnicity	LSBMD, HipBMD, FNBMD, 1/3RadBMD, TBBMD, TBBMC, LSBMC	[65]
2007	235/200	5–18 years	White	UK	QDR Discovery	Gender, age	TBBMAD, LSBMAD, FNBMAD, LSBAforHt, LSBMCforBA, TBBAforHt, TBBMCforBA	[66]
2009	1849/4629	7–80 years	Hispanic	Mexico	DXA Lunar DPX NT	Gender, age	TBBMD, LSBMD, FNBMD, HipBMD	[67]
2009	10560/9993	8–85 years	White, Black, Hispanic	USA	QDR 4500A	Gender, age, ethnicity, Ht	TBBMC, TBBMD	[68]
2011	992/1022	5–23 years	All ethnicities	USA	QDR-4500A, QDR-4500 W, Delphi-A	Gender, age, ethnicity	TBBMD, TBBMC, LSBMD, LSBMC, HipBMD, HipBMC, FNBMC, FNBMD, 1/3RadBMD, 1/3RadBMC	[69]
2011	480/440	5–17 years	Indian	India	Lunar DPX Pro	Gender, age, Tanner	TBBMC, TBBMD, TBBA, LSBMD, LSBMAD, FNBMD, FNBMAD	[70]
2013	777/764	5–19 years	Chinese	China	Lunar Prodigy DXA	Gender, age	TBBMD, TBBMC, TBBA	[71]

(continued)

Table 4.1 (continued)

Year	Population (M/F)	Age range	Ethnicity	Location	Machine	Input	Output	References
pQCT								
2001	185/186	6–23 years	White	Germany	XCT 2000	Gender, age, Tanner	4%D.Rad—TotBMD, CortBMD, TrabBMD, TotCXSA	[72]
2001	177/185	6–23 years	White	Germany	XCT 2000	Gender, age, Tanner	65%P.Rad—CortBMC, SSI, strength modulus, polar inertia	[73]
2001	177/186	6–23 years	White	Germany	XCT 2000	Gender, age, Tanner	65%P.Rad—CortBMD, CortBMC, CortThick, CortA	[72]
2002	107/124	5–22 years		USA	XCT 2000	Gender, age	20%D.Tib—Periost circ, endost circ, cortBMD	[61]
2002	177/185	6–23 years	White	Germany	XCT 2000	Gender, age, Tanner	65%P.Rad—CortBMD, CortThick	[74]
2005	204/274	6–40 years	White	Germany	XCT 2000	Gender, age	4%D.Rad—TotBMD, TotBMC, TotCXSA, CortThick	[75]
2008	197/219	5–18 years	White	USA	–	Age, gender	4%D.Rad—TotBMD, CortBMD, TrabBMD, TotCXSA	[76]
2008	196/273	6–40 years	White	Germany	XCT 2000	Age, gender	65%P.Rad—TotBMC, TotBMD, CortBMD, TotCXSA, CortCXSA, SSI	[77]
2009	380/249	6–19 years	White	UK	XCT-2000	Gender, age, Ht	4%D.Rad—TotBMD, TrabBMD, BA, 50%D.Rad—CortArea, CortThick, CortBMC, BA	[78]

qUS								
1997	174/193	6–15 years	White	UK	Contact ultrasound bone analyser	Age, gender	Calcaneus—BUA	[79]
2000	287/309	6–20 years	White	Netherlands	SoundScan™ Compact	Age, gender, Tanner	Tibia SoS	[80]
2000	262/269	6–21 years	White	Netherlands	UBIS 3000	Age, gender, Tanner	Calcaneus—SoS, BUA (Plus SoS and BUA adjusted for heel width and foot length)	[81]
2002	678/650	3–17 years	White	Germany	DBM Sonic 1200	Age, gender, height, BMI	Finger phalanges—AD-SoS, BTT	[82]
2003	1175/0	7–80 years	White	Poland	DBM Sonic 1200	Age, Wt, Ht	Finger phalanges—AD-SoS	[83]
2003	641/586	3–16 years	White	Italy	DBM Sonic BP IGEA	Age, gender, Tanner	Finger phalanges—AD-SoS, BTT	[84]
2003	61/80	6–12 years	Turkish	Turkey	Contact ultrasound bone analyser	Gender, age	Calcaneus—BUA	[85]
2005	327/215	Newborn	Chinese	China	Sunlight Omnisense	GA, birth season, birth weight	Tibia SoS	[86]
2005	0/2850	7–77 years	White	Poland	–	Age, Wt, Ht	Finger phalanges—AD-SoS	[87]

(continued)

Table 4.1 (continued)

Year	Population (M/F)	Age range	Ethnicity	Location	Machine	Input	Output	References
2006	1513/1531	2–21 years	White	Italy	DBM Sonic BP IGEA	Age, gender, Tanner, Ht, Wt, BMI	Finger phalanges—AD-SoS, BTT	[88]
2006	290/299	3–16 years	White	Italy	DBM Sonic BP IGEA	Age, gender	Finger phalanges—AD-SoS, BTT	[89]
2007	360/366	10–21 years	Chinese	Chinese	Lunar Achilles ultrasonometer	Age, gender, Ht, Wt	Calcaneus—SoS, BUA, stiffness index (SI)	[90]
2007	1164/1016	6–13 years	Asian	Taiwan	Contact ultrasound bone analyser	Age, gender	Calcaneus—BUA	[91]
2008	101/82	Newborn	Black, White, other	USA	Sunlight Omnisense 7000	GA	Tibia SoS	[92]
2009	735/814	3–18 years	White	Greece	–	Gender, age, Tanner, physical activity	Tibia and radius SoS	[93]
2010	558/518	6–19 years	White	Sweden	Lunar Achilles model 1061	Age, gender	Calcaneus—SoS, BUA, stiffness index (SI)	[94]
2011	98/94	7–18 years	White	USA	Sahara bone sonometer	Age, gender, skeletal age	Calcaneus—SoS, BUA	[95]
2011	213/217	9–13 years	White	Portugal	Sunlight Omnisense	Age, Gender, Fat mass, physical activity	Tibia and radius—SoS	[96]
2011	417/333	6–12 years	Asian	Singapore	Contact Ultrasonic Bone Analyzer	Age, gender, ethnicity, Ht	Calcaneus—BUA	[97]

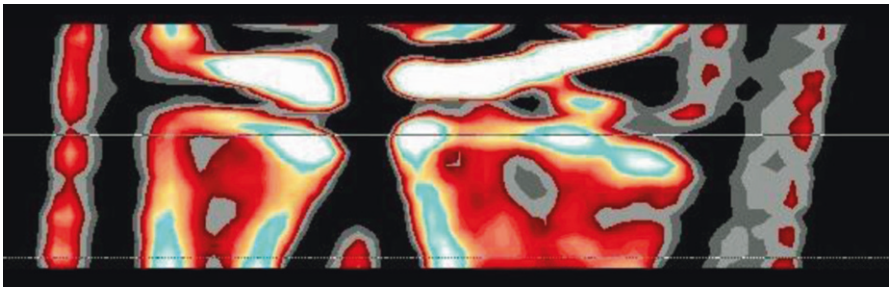

Fig. 4.5 pQCT positioning. A scout scan of a distal ulnar and radius demonstrating end plate identification and positioning

Disadvantages of DXA

The main disadvantages of DXA are its inability to discriminate between cortical and trabecular bone and its sensitivity to changes in position and bone size. These limitations are a particular problem in young children and DXA analysis is sometimes impossible in patients with severe contractures or scoliosis. Furthermore, correction of such scoliosis with spinal rods also prevents DXA analysis of the lumbar region. In such cases it is advisable to obtain baseline DXA measurements from other regions, such as proximal or distal femur, so that monitoring can continue after spinal surgery treatment. Finally children with skeletal dysplasias are particularly difficult to assess as there are currently no relevant DXA normative ranges. Furthermore, DXA cannot clearly distinguish between osteomalacia and osteoporosis as both result from insufficient bone mineral; however, the clinical history and appropriate biochemical analysis will differentiate these two conditions.

Peripheral Quantitative Computed Tomography

Peripheral quantitative computed tomography (pQCT) can be used to measure the true vBMD of the peripheral skeleton. It is a modified version of full size QCT that is specifically designed to determine vBMD in the distal forearm or tibia. A scout scan is initially performed to define the location of the bone end plate reference line (Fig. 4.5). X-ray images are then acquired from different angles at multiple sequential levels. Two-dimensional cross sections are then reconstructed and finally combined to generate a three-dimensional structure. The image consists of individual voxels each of which has a defined Hounsfield unit value (a linear scale that defines 0 as the attenuation of X-rays in water). By comparison to known standards the Hounsfield unit can be translated into BMD in g/cm^3. Cross sections are then generated for set distances, determined as a percentage of total bone length, from the reference line. A cross section adjacent to the growth plate includes cortical and

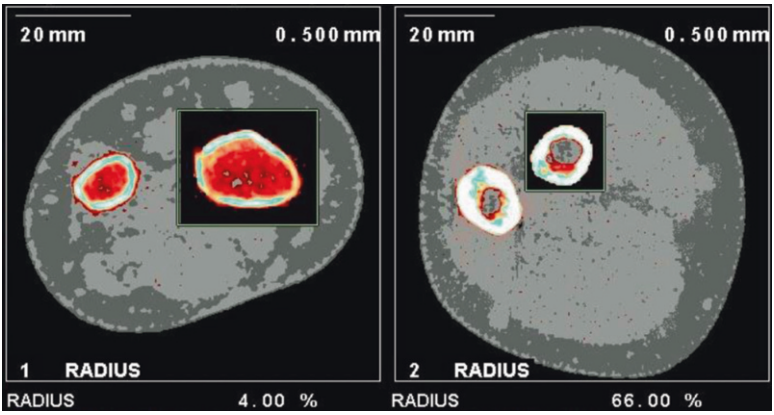

Fig. 4.6 pQCT report. pQCT images located at 4 % of the radial length (distal to proximal) and at 66 % of the radial length (distal to proximal). (Image courtesy of Novotec Medical)

trabecular bone, while a cross section in the mid-shaft includes only cortical bone (Fig. 4.6). A variety of bone parameters are calculated from this cross-sectional data, including vBMD and surrogate measures of bone strength, and these can be compared to pQCT reference ranges (see Table 4.1). Currently, however, pQCT studies lack consistency in methodology and there is limited data to determine the optimal technique for data acquisition and analysis [31]. Furthermore, comparison of upper and lower limb parameters is limited by their poor correlation resulting from the differential effects of physical loading on the developing skeleton [32].

Advantages of pQCT

The key advantage of pQCT over DXA analysis is its ability to measure true vBMD rather than aBMD. Furthermore, pQCT can differentiate between cortical and trabecular bone, thus allowing these two compartments to be analysed separately. This is important since trabecular and cortical bone can be differentially affected in different pathological conditions and pQCT has been shown to detect the early trabecular bone loss associated with high bone turnover states. In addition, pQCT can be used to determine geometric parameters and derive surrogate measures of bone strength including cross-sectional moment of inertia and the strength–strain index (SSI). The recent development of high resolution pQCT (HR-pQCT) has enabled bone micro-architectural parameters to be determined which include trabecular number, thickness and spacing and cortical porosity [33].

Disadvantages of pQCT

pQCT is currently not widely available and most pQCT has been performed in research centres. Although, whole body CT scanners, which could perform QCT of

both the axial and appendicular skeleton, are widely available they are rarely used for this purpose because the effective radiation dose is increased to 0.06–3 mSv or higher [23]. Radiation exposure is an important factor to consider in paediatric densitometry due to concerns regarding the long-term risk of radiation induced cancers. The effective radiation dose in pQCT is far lower than whole body QCT due to the distance of the ROI from radiosensitive organs. The effective radiation dose is <0.01 mSv in pQCT and 0.003 mSv in HR-pQCT [34, 35].

The major disadvantage of pQCT is that its analysis is limited to the peripheral skeleton and derived bone parameters correlated poorly with vertebral fracture risk during growth. Consequently, in children monitoring of vertebral bone requires the use of an additional imaging method. A further limitation of pQCT is its poor special resolution which leads to a "partial volume" effect. Simply, at the edge of cortical bone a number of voxels will contain both bone and soft tissue; thus, their assigned BMD will be an underestimate of the true BMD. If the resolution of the pQCT system is low, partial volume effect can cause significant problems where cortical bone is thin and algorithms to adjust for this are required [36].

pQCT analysis in children can be particularly challenging. In very young children movement between the scout scan and imaging of the selected slices will cause errors and while positioning devices have been developed to try to prevent this, movement remains a problem. Obesity can interfere with both the results and obtaining the pQCT measurements, and skeletal dysplasia or contractures can make it impossible to position a limb accurately into the pQCT device. Finally, in growing children it can be difficult to obtain pQCT measurements at the same locations in successive scans. Standard protocols attempt to adjust for this by locating cross sections at specific percentage of total bone length. However, uneven longitudinal growth means that this cannot be exact, and even a small discrepancy can result in changes in trabecular indices.

Quantitative Ultrasound

Quantitative ultrasound (QUS) is a novel non-invasive method for assessing bone status. The technique uses ultrasonic waves to interrogate peripheral bone quality [37]. Currently, the technique is not yet widely used, but its low cost, lack of radiation exposure, portability and speed have resulted in growing interest. However, uncertainties over what the QUS-derived parameters represent have limited its uptake. There are currently two different QUS methodologies; the first assesses the transmission of ultrasound waves through a peripheral bone and the second uses a pulse-echo system to measure the reflection of ultrasound waves from the surface of a peripheral bone. Transmission QUS of the calcaneus is the most commonly used method although analysis of the tibia, radius or proximal phalanges can be performed. Ultrasound probes are coupled to the foot, by use of either a water bath or coupling gel, and ultrasound vibrations applied. The shape, intensity and speed of the transmitted ultrasonic wave are determined. The velocity, measured as speed of

sound (SoS) in m/s, and the attenuation, measured as broadband ultrasound attenuation (BUA) in dB/MHz, reflect the bone density, architecture and strength. Thus, a porous or osteoporotic bone will cause the ultrasound wave to travel more slowly (lower SoS) and result in less attenuation (lower BUA). Some QUS systems also use SoS and BUA to calculate a stiffness index and the QUS index. All QUS parameters are then compared to normative data (Table 4.1).

Advantages of QUS

There are several reasons why QUS is an attractive technique for the long-term monitoring of children, the most important of which is the lack of ionising radiation exposure. In addition, QUS is easy to use, quick, portable, low cost and does not require extensive training. This makes QUS ideal for assessing bone in newborns and the very young where DXA and pQCT are impractical. Furthermore, QUS analysis provides both bone strength and quality parameters suggesting that children at risk of fracture but with normal BMD might be identified. Indeed, several studies have shown that a reduction in BUA or SoS is associated with an increased risk of fracture, but that the patients identified are not the same as those identified by DXA [38].

Disadvantages of QUS

The main disadvantage of QUS is that, while it may be a useful indicator of overall bone health, it remains unclear how BUA and SoS correlate with BMD, macro- and microstructure and stiffness. Furthermore, inconsistent probe location can easily result in errors, soft tissue thickness must be corrected for and appropriate reference ranges for age, size and Tanner stage must be used (Table 4.1).

Bone Markers

Biochemical markers of bone resorption and bone formation have been developed to assess the rate of bone turnover and the dynamics of bone metabolism. The most commonly used markers in paediatric patients are summarised in Table 4.2. Bone formation markers reflect osteoblast activity and are by-products of collagen synthesis, osteoblast enzymes or bone matrix proteins. By contrast, bone resorption markers reflect osteoclast activity and are collagen degradation products or osteoclast enzymes. Importantly, levels of many bone turnover markers are affected by both bone resorption and formation, and may also derive from non-skeletal tissues. Thus, changes in bone markers do not inevitably indicate altered bone metabolism. Bone markers may be measured in blood or urine samples with values determined

Table 4.2 Commonly used biochemical markers of bone turnover

	Acronym	Marker type
Bone resorption markers		
Hydroxyproline	HYP	Collagen degradation product
Pyridinoline	PYD	Collagen degradation product
Deoxypyridinoline	DPD	Collagen degradation product
N-terminal cross-linked telopeptide of type I collagen	NTX	Collagen degradation product
C-terminal cross-linked telopeptide of type I collagen	CTX	Collagen degradation product
MMP generated variant of C-terminal cross-linked telopeptide of type I collagen	ICTP	Collagen degradation product
Tartrate-resistant acid phosphatase type 5b	TRAcP5b	Osteoclast enzyme
Bone formation markers		
Procollagen type I N-terminal propeptide	PINP	Collagen synthesis by-product
Procollagen type I C-terminal propeptide	PICP	Collagen synthesis by-product
Osteocalcin	OC	Bone matrix protein
Bone alkaline phosphatase	BAP	Osteoblast enzyme

by enzyme-linked immunosorbent assays (ELISA), high-performance liquid chromatography (HPLC), electrophoresis or radioimmunoassays (RIA). The level of bone markers can be affected by the timing and mode of sample collection, sample handling and variation between laboratories and assay kits. Bone markers show a significant diurnal variation, particularly in urine samples, and consistent timing of sample collection is therefore essential if longitudinal comparisons are to be made [39]. In adults, 24 h urine collection can be performed but in children this is generally impractical and inaccurate so that determination of bone markers in serum samples is preferred. Furthermore, bone markers determined in urine samples must be expressed in terms of the urine creatinine level, which is subject to considerable variation with age as muscle mass increases. Finally, food intake and exercise can affect levels of some bone markers and so must also be controlled for [39].

In adults, bone markers simply reflect the rate of bone remodelling. However, in children bone markers are also affected by the rate of bone modelling and growth which results in considerable variation during childhood [40]. Bone markers are very high at birth but levels decline rapidly over the first few years of life. During prepubertal growth bone markers are relatively stable but higher than those in adults. Levels increase again during the pubertal growth spurt but then decrease to adult levels. Since the timing of pubertal growth differs between boys and girls, gender- and age-specific reference ranges are required to interpret bone markers in children [41].

Advantages of Bone Markers

The major advantage of bone markers over other bone monitoring approaches is that they can be repeated frequently without adverse consequences. Thus, bone makers

can be used to rapidly determine whether skeletal abnormalities are a consequence of high or low bone turnover and can also be used to closely monitor the effect of treatments on bone formation and resorption.

Disadvantages of Bone Markers

The use of bone markers is limited by the difficulties of sample collection, the assay variability and the lack of good age- and gender-specific reference ranges. As a consequence, in childhood, the predictive value of any individual marker is relatively poor. Thus, it is recommended that multiple markers of bone resorption and bone formation are used when monitoring bone health in children. However, such an approach may be limited by the restricted availability of these assays.

Bone markers give an overall rather than site-specific indication of formation and or resorption. This means that they can only determine whether a treatment has reduced net bone turnover and do not provide any indication of bone strength or fracture healing. Consequently, many centres do not routinely monitor bone turnover markers as they rarely change clinical management.

How Frequently Should Treatment Be Monitored?

In children treated for skeletal disorders the ideal monitoring interval will depend upon disease progression, growth velocity and type of treatment. Prior to commencing bisphosphonate treatment, children with skeletal fragility should have baseline investigations that include DXA, bone markers and skeletal surveys. If the development of a severe scoliosis is likely it is important that baseline DXA data is obtained from lumbar vertebrae and alternative sites that can be assessed after surgical intervention.

Before the next assessment of DXA BMD, sufficient time should elapse to exceed the expected inter-measurement variability. The International Society for Clinical Densitometry (ISCD) 2007 official positions state that the minimum time interval for monitoring treatment with a bone active agent or disease should be 6 months [42] but many centres only monitor BMD on an annual basis. Currently, there is no consensus on when to stop treatment. Some centres discontinue treatment when BMD approaches normal levels, while others reduce the dose when BMD becomes elevated with the aim to fully discontinue treatment when growth is complete (Fig. 4.7).

Although the regular determination of bone markers can be useful for monitoring patient compliance and in the research setting, they are rarely sufficient alone to alter management.

Spinal X-rays are frequently performed on an annual basis if there is a suspicion of a vertebral fracture, or to monitor healing of a known compression fracture. X-rays are also used to monitor healing after orthopaedic intervention as delayed osteotomy healing due to bisphosphonate therapy has been reported [43].

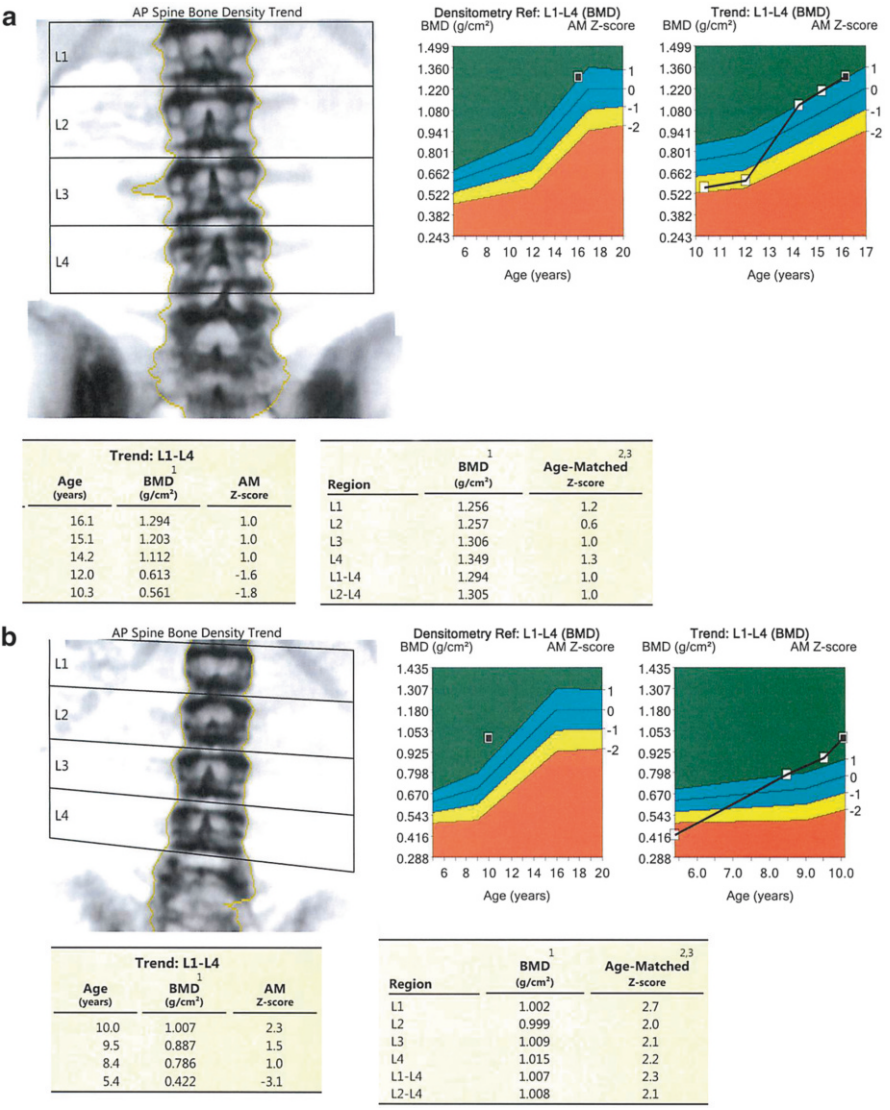

Fig. 4.7 Lumbar spine DXA reports. DXA scans of children receiving bisphosphonate treatment. (**a**) 10.3-Year-old child is commenced on bisphosphonate treatment. BMD *Z*-score initially increases gradually but then during puberty the BMD increases at a rapid rate before again slowing down and remains around +1. (**b**) 5.4-Year-old child was started on bisphosphonate treatment and did not have a repeat DXA scan until 8.5 years. Her BMD *Z*-score increased from −3.1 to +1 after which her treatment was reduced. (Courtesy of Dr. C. De Vile, Great Ormond Street Hospital, UK)

Summary

When managing children with skeletal disorders it is important to monitor the safety and efficacy of treatment. During growth the skeleton changes rapidly in size, shape and structure and to correctly interpret data from X-rays, DXA, pQCT, QUS and bone markers it is essential to understand the physiological basis of skeletal development.

Currently, the majority of treatments available for children with osteoporosis and other skeletal conditions are anti-resorptives. The most widely used method of monitoring such treatment is DXA bone densitometry, but its limitations in children are rarely fully appreciated. Other imaging modalities may provide additional information but have limitations in terms of site specificity, in the case of pQCT, or uncertainty with regard to interpretation, in the case of QUS.

Despite guidance regarding the frequency of skeletal monitoring, there is currently no consensus as to when treatment should be reduced or discontinued.

This chapter has outlined the physiology of skeletal development and described the modalities used to monitor the treatment of skeletal disorders in children. Such careful follow-up of skeletal responses to pharmacological intervention is important not only to determine the benefits of treatment but also to identify known side effects and detect long-term adverse consequences.

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Chapter 5
Bisphosphonates in Osteogenesis Imperfecta

Andrew Biggin and Craig F. Munns

Introduction

Osteogenesis imperfecta (OI) is a genetic bone fragility disorder characterised by multiple fractures, reduced bone mass, deformity and disability. Management requires a multidisciplinary approach involving paediatrician, endocrinologist (bone and mineral physician), rehabilitation specialist, orthopaedic surgeon, dentist, physiotherapist and occupational therapist. Bisphosphonate treatment is the mainstay of medical treatment in OI and has been shown to decrease bone pain, enhance well-being and improve mobility and muscle strength, in addition to reducing the incidence of fractures. This chapter summarises the historical use, safety and efficacy of bisphosphonate therapy in children and adolescents with OI.

Bisphosphonates

Bisphosphonates are synthetic analogues of naturally occurring inorganic pyrophosphates which act by inhibiting osteoclast function [1]. Their basic structure is a phosphate–carbon–phosphate bond that results in a very stable compound (Fig. 5.1). Once administered, bisphosphonates avidly bind to the hydroxyapatite crystals of bone and are quickly removed from general circulation. They therefore have a short plasma half-life but comparatively longer functional half-life as they are directly incorporated into bone mineral. As the bone is resorbed by osteoclast activity, the bisphosphonate is released and can act locally. Bisphosphonates inhibit the differentiation of osteoclast precursors and induce apoptosis of osteoclasts [2]. Newer

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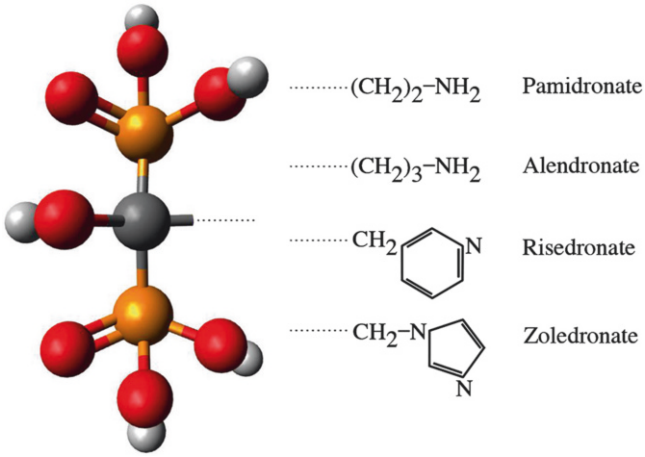

Fig. 5.1 Molecular structure of bisphosphonates. The generic bisphosphonate structure (*left*) with two phosphonate groups binding to a central carbon atom. Modern bisphosphonates have a hydroxyl group and specific nitrogen-containing side chain (*right*) attached to the central carbon atom. (*Black sphere*: central carbon atom, *orange spheres*: phosphorus, *red spheres*: oxygen, *grey spheres*: hydrogen)

bisphosphonates, such as pamidronate and zoledronate, also inhibit the mevalonate pathway of cholesterol synthesis and prevent post-translational prenylation of small guanosine triphosphate-binding proteins in osteoclasts [3].

Pamidronate and zoledronate are the most widely used intravenous bisphosphonates in children with OI, with zoledronate being about 850 times more potent [4]. Zoledronate has a high affinity for bone and has a 100-fold bone concentration compared to plasma. Adult studies have shown that the effects of zoledronate are also longstanding with only a slight decline after 6 months [5].

The use of bisphosphonates has been well established in adult patients for treatment of osteoporosis, Paget disease of bone, myeloma, hypercalcaemia and bone metastases. Its use in children is relatively more recent but bisphosphonate therapy is now the mainstay of medical treatment for osteogenesis imperfecta (OI) [6].

Osteogenesis Imperfecta

Before discussing the role of bisphosphonates in OI it is important to understand the pathogenesis and heterogeneity of this condition. OI is characterised by increased bone fragility and decreased bone mass. There is significant variability in the clinical features and severity within OI, with presentation at any age from intrauterine life to adulthood. The extreme variability in OI results in part from genetic and biochemical heterogeneity. The diagnosis can be straightforward in the more severe

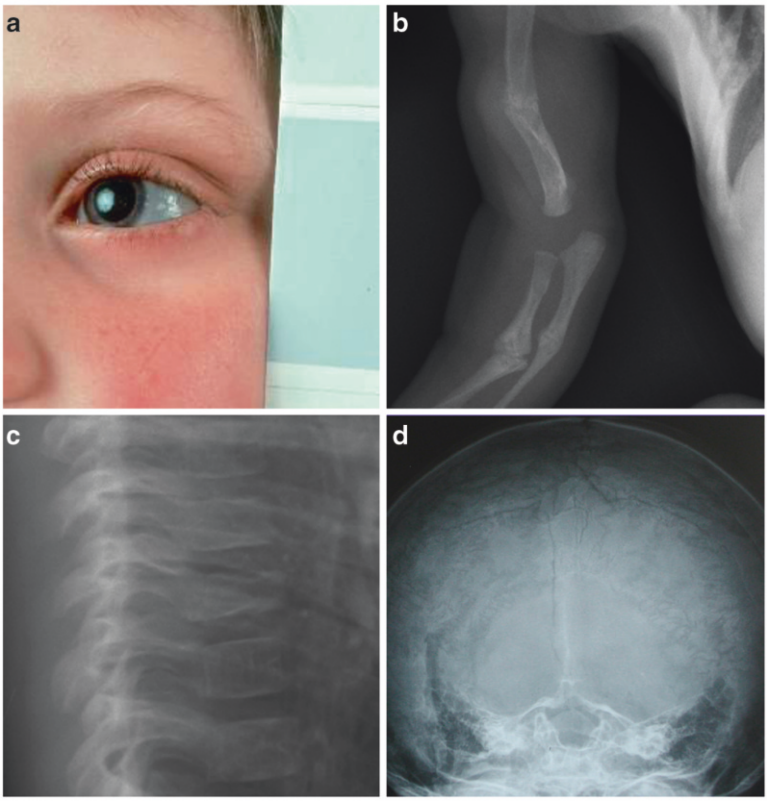

Fig. 5.2 Features of osteogenesis imperfecta. Blue sclerae (**a**), fractures ((**b**) multiple upper limb fractures, (**c**) vertebral compression fractures) and Wormian bones of the skull (**d**) are associated features of osteogenesis imperfecta

cases or when there is a positive family history and the typical features of blue sclera, fracture and Wormian bones of the skull are present (Fig. 5.2). However, a definitive diagnosis can be more difficult on clinical grounds alone in milder cases that lack these features and it is not uncommon for mildly affected adults to only be identified when their affected child presents with fractures. Other features associated with OI include joint hypermobility, hearing impairment, basilar invagination and brittle teeth (dentinogenesis imperfecta).

Various classification systems have been previously proposed to describe the heterogeneity of OI. In 1979, Sillence proposed a classification of OI types I–IV based on clinical findings with radiological subclassification [7]. Despite recent revisions of this classification to encompass the expanding molecular diagnosis, a variation of the original Sillence classification is still widely used in clinical practice (Table 5.1).

Table 5.1 Expanded Sillence classification illustrating five clinically distinct OI phenotypes

OI type	Phenotypic features	Inheritance
OI type I	Osseous fragility (variable from none through moderately severe), distinctly blue sclerae (at all ages), presenile hearing loss (or family history of hearing loss)	AD
OI type II	Perinatally lethal OI. Extremely severe osseous fragility, stillbirth or death in the newborn period. Rare long-term survival	AR and AD
OI type III	Moderately severe to severe osseous fragility, normal sclerae (blue in infancy) ± severe deformity of long bones and spine. A range of clinical and radiographic phenotypes	AR and AD
OI type IV	Osseous fragility with normal sclerae (blue in infancy) ± severe deformity of long bones and spine	AD
OI type V	Osseous fragility with normal sclerae ± severe deformity + calcification in interosseous membranes ± hyperplastic callus	AD

AD autosomal dominant, *AR* autosomal recessive

Type I OI is the most common type of OI and is distinctive because of blue sclerae and minimal bony deformity. Type I OI is classically due to reduction in the quantity of collagen type I protein due to a stop, frameshift or splice site mutation in either *COL1A1* or *COL1A2*. As this leads to a quantitative defect, the phenotype of this group tends to be mild with patients attaining normal height and having minimal functional limitations. Recurrent long-bone or vertebral fractures can however result in significant disability in some. They can present as back pain, scoliosis or be asymptomatic and during growth there is good potential to respond to treatment. The incidence of hearing impairment increases during adult life.

Type II OI is associated with perinatal lethality. These infants are the most severely affected individuals with multiple fractures and bone deformities in utero and at birth. The sclerae of these children are often deep blue or grey but can be normal. Skeletal survey reveals bones that are short and broad with very low density. Respiratory insufficiency is the leading cause of mortality due to multiple rib fractures and pulmonary insufficiency. Mutations in these babies tend to be sporadic and typically involve a glycine substitution in *COL1A1* or *COL1A2*.

Type III is the most severe form of OI in those who survive the neonatal period. They often have multiple long-bone deformities and fractures at birth, have blue or grey sclerae, severe dentinogenesis imperfecta and triangular facies. Over 50 % are wheelchair dependent at an early stage and have very short stature. These children have severe bone fragility throughout their childhood, which can in turn lead to limb and spinal deformities and result in respiratory compromise. Genetic mutations in these individuals are often due to a glycine substitution in *COL1A1* or *COL1A2* leading to a qualitative defect in the collagen protein. Mutations in other genes (see below) can lead to a very similar severe phenotype.

Type IV OI is characterised by osteoporosis leading to bone fragility without the typical features of the type I phenotype (i.e. blue sclerae and deafness). Fractures may present at any age and a majority of these patients have short stature. A small proportion experience a severe, progressive lower limb deformity rather than

recurrent fractures. Dentinogenesis imperfecta is variable but when present is associated with a greater frequency of fractures. Inheritance is autosomal dominant with mutations in *COL1A1* and *COL1A2*.

Type V OI was the first non-collagen OI type to be identified and constitutes about 4 % of the OI population. Mutations of the *IFITM5* gene have been shown to cause this autosomal dominant form of OI [8]. Classically, patients have a distinctive phenotype with moderate-to-severe bone fragility although recent family studies have shown the phenotype is variable. The hallmark of this subtype is the presence of hypertrophic callus formation and early calcification of the interosseous membrane between the bones of the forearm, which limits pronation and supination. Radial head dislocation can be identified from a young age and predates interosseous membrane calcification. Scleral colour is normal. Upon histological examination, the lamellar organisation of the bone has an irregular mesh-like appearance, clearly distinct from the normal lamellar organisation seen in type I and IV OI.

A number of other subtypes of OI have been classified based on different genetic, phenotypic or histomorphometric features. While it is important to distinguish the genetic cause for genetic counselling, the most salient clinical feature regarding the role of bisphosphonate treatment is the severity of bone fragility. Type VI OI clinically resembles other forms of moderate-to-severe OI but it has a characteristic "fish-scale" pattern of bone lamellation on bone histology. Blue sclerae and dentinogenesis imperfecta are absent in type VI OI. Mutations in *SerpinF1* have been shown to cause this subtype of OI [9] and there is evidence suggesting that response to bisphosphonate therapy, particularly gains in mobility scores and reductions in fracture incidence, is less than in other types of OI [10]. Type VII OI is a rare autosomal recessive condition that was described in the First Nations community in northern Quebec [11]. It is caused by mutations in *CRTAP* [12] and is associated with a moderate-to-severe phenotype involving fractures from birth, bluish sclerae, early lower limb deformity, coxa vara and osteopenia. Rhizomelia is a prominent clinical feature that distinguishes this form of OI.

Over 90 % of European individuals with OI have mutations in collagen type I genes (*COL1A1* or *COL1A2*) [13]. In other populations such as Southern Asian or Samoan populations, evidence suggests that mutations in other genes may be more prevalent as causes of moderate-to-severe OI.

Collagen type I has a triple helical structure consisting of two α1 and one α2 chains. For the triple helix to fold correctly, every third amino acid residue must be a glycine with the remaining amino acids rich in proline and hydroxyproline [13]. The α-chains are initially synthesised as pro-α-chains with polypeptide extensions at either end. The carboxy propeptide extension is essential for pro-α-chain association prior to triple helix assembly, which occurs in a carboxy to amino direction. Using *COL1A1* and *COL1A2* mutation analysis alone, it is difficult to make genotype–phenotype correlations in OI. In general, however, mutations resulting only in a quantitative defect in collagen type I production result in a milder phenotype than those leading to a qualitative defect [14]. A number of additional genes have been

identified that play a role in type I collagen trafficking and these have been implicated in autosomal recessive types of OI; FKBP10 [15], SerpinH1 (coding for HSP47) [16], SerpinF1 [9] and SP7/0SX (Osterix) [17]. Mutations in these genes result in moderate-to-severe OI and have subtle radiological features that may help to distinguish them from OI caused by *COL1* mutations.

Disruption of collagen results in a disorder of the mineral phase of OI bone. Human OI bone has a higher average material density than normal bone, and the murine model of moderate-to-severe OI (OIM mouse) has smaller and less well-aligned mineral crystals than the wild-type mouse [18]. It is the combination of the organic and inorganic abnormalities of OI bone that alters its biomechanical properties and makes it brittle. Histomorphometric studies of OI bone have shown a decrease in core width, cortical thickness and trabecular number and thickness [19]. Individual osteoblasts produce a reduced amount of bone in OI, but due to their increased number, the bone formation rate is increased. Osteoclastic activity is also increased so this does not lead to a net gain in bone mass. Together these findings indicate a high turnover state with minimal net gain in bone mass. The increase in bone turnover is reflected in increased serum and urinary levels of markers of bone resorption (deoxypyridinoline and N-telopeptide) and bone formation (alkaline phosphatase and osteocalcin). The reduction in core width seen on trans-iliac bone biopsies translates into thinner long bones with a reduced polar moment of inertia, further increasing the propensity to fracture.

Bisphosphonates in OI

The aim of treatment in OI is to reduce fracture frequency, maximise mobility and improve functional outcomes [20]. The use of bisphosphonates to treat OI was first described in a case report in 1987 [21]. A 12-year-old girl with OI was treated with oral pamidronate. The dosing regime comprised 250 mg daily for 2 months alternating with 2 months of abstinence for a total duration of 1 year. She showed a 33 % increase in lumbar spine bone mineral content by dual photon densitometry but still went on to sustain at least two low-trauma fractures within the following year. The first systematic assessment of bisphosphonates in OI was undertaken over a decade later when the effects of cyclic intravenous pamidronate were investigated in 30 children with severe OI [22].

Intravenous pamidronate in children with OI has been reported to decrease bone pain, enhance well-being, improve mobility and muscle strength, reduce fracture incidence, increase long bone cortical thickness, increase vertebral size with vertebral reshaping and increase bone mass and bone mineral density [22, 23]. In an attempt to prevent growth disturbance and spine and limb deformity, cyclical intravenous pamidronate has also been used in babies and infants with OI [24]. The treatment response in the younger children was more pronounced than in the older cohort and there was also an improvement in the time taken to attain motor

milestones [25]. Histomorphometry has provided valuable insight into the actions of pamidronate in children with OI [26]. The major bone effects of pamidronate were to increase cortical thickness and trabecular number. Trabecular thickness was not enhanced. Bone turnover was significantly reduced with a decrease in both bone resorption and formation below that of age-matched normal controls. There was also an increase in residual calcified cartilage within the bone. In adults with OI, pamidronate has been shown to increase spine and hip areal bone mineral density and decrease fracture rates [27]. The results in adults have not been as marked as those in children, suggesting that bisphosphonate therapy should be instigated during childhood to obtain maximal benefit.

A prospective randomised trial of 23 children with OI was carried out to assess the dosage, efficacy and safety of zoledronate compared to pamidronate [28]. This study showed a similar response in terms of improvements in serial bone density and quality of life. An increased frequency of fracture was seen in the zoledronate treatment group, which may have been due to inclusion of some children with more severe OI. Zoledronate has the benefits of being able to be administered more rapidly and of having a longer dosing interval than pamidronate, both of which may prove advantageous to patients and health care facilities.

As mentioned above, the primary aim of bisphosphonate treatment in OI is to reduce fracture frequency in order to maximise mobility. The best possible outcome is the ability to walk and bisphosphonates have revolutionised this aspect of patient management, particularly in those with moderate-to-severe disease (Figs. 5.3 and 5.4). Cyclical intravenous bisphosphonate therapy improves linear growth and bone structure via improvements in bone shape and biomechanics. Orthopaedic intervention in combination with bisphosphonate therapy has resulted in improvements in patient mobility [29].

There is growing interest in the utility of oral bisphosphonates in OI. In a placebo-controlled trial of 34 patients, olpadronate was associated with an increase in lumbar spine bone mineral density, but did not improve muscle strength, mobility, function or vertebral height [30]. A small randomised controlled study of oral risedronate showed an improvement in spine bone density and decrease in bone turnover but no change in bone biopsy or bone density data in 26 children with type I OI [31]. A large multicentre, double-blind, randomised, placebo-controlled trial of 139 patients looked at the effects of alendronate in children with moderate-to-severe OI. This study showed that while there was an improvement in bone density and decrease in bone turnover, there was no improvement in fracture rate, bone pain, vertebral height, bone histomorphometry or physical activity with treatment [32]. More recently, a large multinational placebo-controlled trial of 147 patients evaluated oral risedronate in children with milder OI. This study showed an increase in areal bone mineral density and reduction in fracture frequency [33]. The drug was generally well tolerated and the authors concluded that risedronate should be regarded as a valid treatment option for children with OI, particularly those with milder phenotypes. These data would suggest that until there is data to the contrary, oral bisphosphonates should not be used in favour of intravenous bisphosphonates

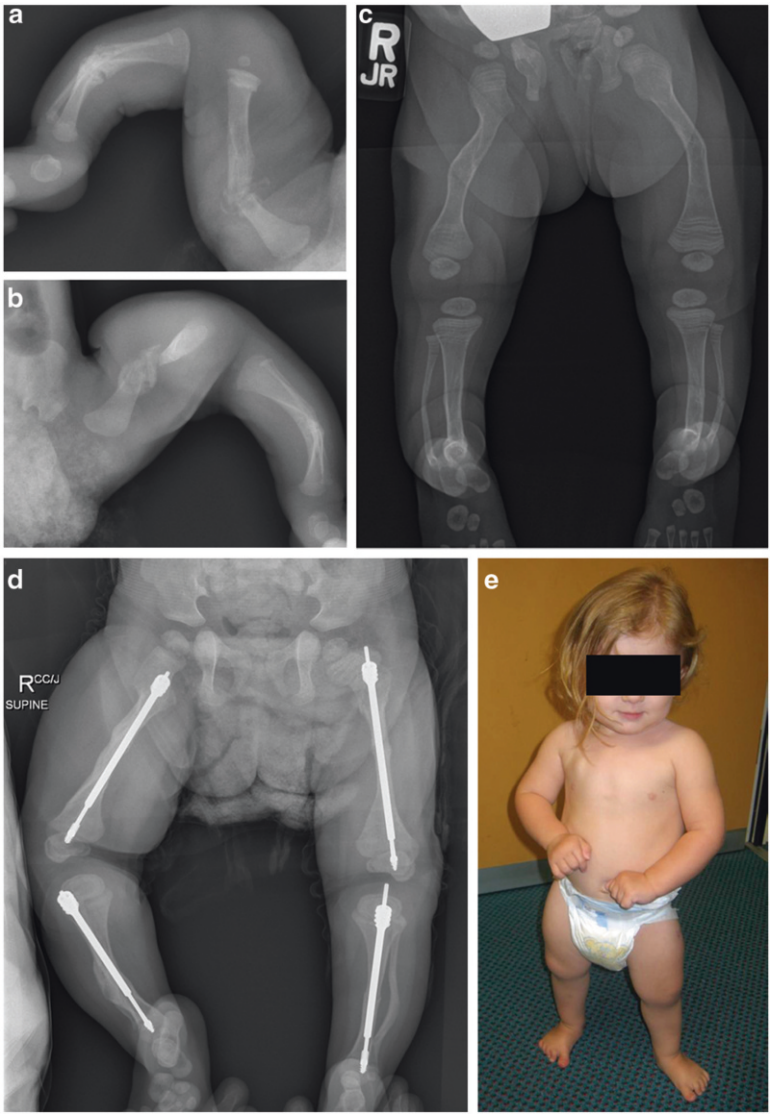

Fig. 5.3 Improvements in appendicular skeleton following bisphosphonate treatment. Multiple fractures in the neonatal period of (**a**) right leg and (**b**) left leg in a child with osteogenesis imperfecta. Cyclical intravenous bisphosphonate therapy aided fracture prevention and allowed linear growth (**c**). Note sclerotic bisphosphonate treatment lines, particularly in distal femur and proximal tibia. The bisphosphonate-treated bones were more amenable to orthopaedic intervention allowing the insertion of Fassier-Duval telescopic rods (**d**) resulting in the ability to mobilise independently (**e**)

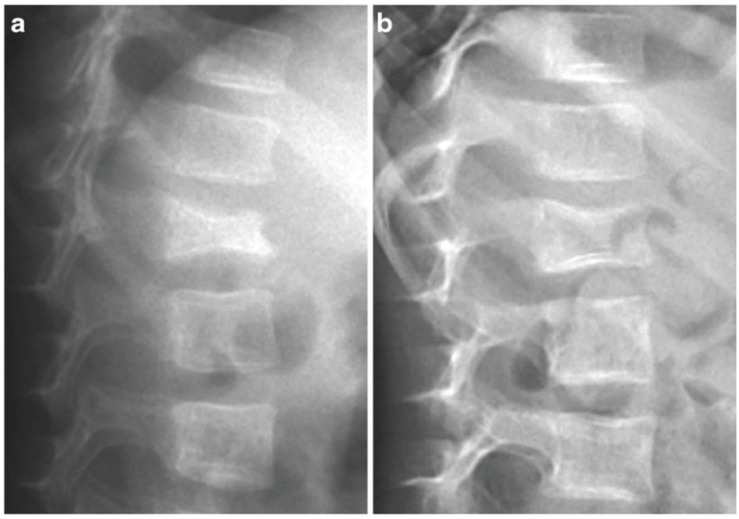

Fig. 5.4 Improvements in axial skeleton following bisphosphonate treatment. Lateral thoracolumbar spine radiograph showing vertebral crush fracture of L1 in a 3-year-old with osteogenesis imperfecta (**a**). Improvements in vertebral morphology were evident following 2 years of bisphosphonate therapy (**b**)

in the early treatment of children with moderate-to-severe OI. Oral therapy may however be of benefit as maintenance therapy in children with moderate-to-severe OI, or as initial therapy in mild OI. Once bisphosphonate therapy is discontinued entirely, there is little if any effect on the new bone produced following growth and modelling [34]. It is usually necessary to have children with OI on a maintenance dose of bisphosphonate following their acute treatment regimen. The best maintenance therapy is uncertain but is likely to be between 30 and 50 % of the acute treatment dose [35].

Side Effects and Complications

There have been numerous reports of serious adverse effects associated with bisphosphonate therapy in adults [36]. These include acute systemic inflammatory reactions, ocular complications, renal failure, nephrotic syndrome, electrolyte abnormalities and osteonecrosis of the jaw. By far the most frequent adverse effect seen in children is an acute-phase reaction following the first dose. This typically includes fever, nausea, diarrhoea, malaise and muscle and bone pain. These symptoms begin 24–48 h following the initiation of treatment and rarely recur with subsequent doses [37]. This appears to be mediated by T-cell release of interferon gamma and tumour

necrosis factor [38]. These side effects can be minimised by the administration of acetaminophen (paracetamol) or the anti-inflammatory medication Ibuprofen [39]. Bisphosphonates lower serum calcium concentrations and this is most marked following the first infusion cycle [40]. In vitamin D-replete individuals receiving the recommended calcium intake, the hypocalcaemia is self-remitting.

Infants with severe OI and pre-existing respiratory compromise have been reported to experience an acute respiratory distress associated with the first pamidronate infusion [41]. The aetiology remains unclear, but may relate to cytokine release and/or haemodynamic compromise from fluid administration during the first infusion. This highlights the fragile state of young children with severe OI and the need for close monitoring during treatment.

Animal studies have shown that high-dose bisphosphonates can suppress growth and concerns have been raised of this possibility in children. Counter to these concerns, pamidronate has been shown to significantly improve the growth of children and adolescents with moderate-to-severe OI compared to historical controls over a 4-year treatment period by preventing limb and spine deformity [42]. In the same report, rapid weight gain was noted in a number of children with severe OI. The aetiology of the weight gain remains unclear, but excessive weight could have a detrimental effect upon function and increase fracture risk.

Pamidronate suppresses bone turnover in children with OI to well below that of normal-aged matched controls [26]. At high doses, pamidronate can interfere with bone modelling and result in undertubularisation of long bones. In the growing skeleton, a reduction in bone remodelling results in the accumulation of mineralised cartilage within the bone, which contributes to the increase in bone density seen with pamidronate treatment [43]. Further, acute reductions in remodelling and the persistence of calcified cartilage in bone account for the characteristic sclerotic metaphyseal lines seen on long-bone radiographs of children receiving pamidronate therapy [44] (Fig. 5.3). Pamidronate therapy has also been associated with delayed healing of osteotomy sites after intramedullary rodding procedures [45] but multivariate analysis did not show a significant delay in healing after fractures. However, when children with OI sustain a fracture and are due a scheduled bisphosphonate dose, the general pragmatic approach is to delay treatment until there is radiological evidence of callus formation.

Osteonecrosis of the jaw has emerged as a major issue in adult patients treated with high-dose or potent bisphosphonates. It is most simply described as non-healing, painful jaw wounds following dental extraction or other dental procedures. At the present time the risk from normal exfoliation of deciduous teeth in children is not quantified but presumed to be extremely small. There has been no report of children treated with bisphosphonates long term developing osteonecrosis of jaw. A study of 64 young people with OI treated with bisphosphonates for up to 12 years revealed no instance of jaw osteonecrosis [46]. Current clinical practice varies but a pragmatic approach is for children to undergo dental review prior to staring bisphosphonate treatment and have necessary reparative work undertaken. This is then followed by annual review by a paediatric dentist. There

are no data to guide recommendations on timing of dental extractions once bisphosphonate treatment has commenced. A reasonable approach may be to preserve teeth where possible, but extraction is required to undertake this at least 3 months from the last bisphosphonate infusion and to wait for gingival healing before the next dose is given.

Bisphosphonates are contraindicated during pregnancy, and all females of reproductive age should have a negative pregnancy test before each bisphosphonate treatment cycle or before commencing oral bisphosphonates. Because bisphosphonates persist in mineralised bone for many years, concern has also been expressed that bisphosphonates administered before conception could be released from the maternal skeleton during the pregnancy and effect the foetus [47]. Reports have been published of two women with OI who became pregnant after 5 years of pamidronate therapy. No pamidronate was administered following conception. Both pregnancies went to term and there were no maternal complications noted. It could not be excluded, however, that the adverse events noted in the babies, hypocalcaemia and talipes equinovarus, were related to maternal pamidronate therapy [48]. Clearly, further systematic follow-up of pregnancy outcome in this cohort is required and females should be counselled about the uncertainty surrounding this aspect of bisphosphonate treatment.

Summary

Over the last 20 years, the use of bisphosphonates has proven to be very effective in the treatment of OI. It has led to a reduction in fracture rate, pain and disability in children with this disorder. Through a multidisciplinary approach and the appropriate use of bisphosphonate therapy, the quality of life for children with OI and their families has been significantly improved. Bisphosphonates have potential side effects and their use should remain limited to children with skeletal fragility. Treatment needs to be monitored closely by institutions experienced in the care of children with OI and the use of bisphosphonates. Despite the widespread use of bisphosphonates in the management of patients with OI, there remain a number of unanswered questions. Key areas requiring further elucidation include the long-term effects of treatment, the optimal treatment regimen to maximise benefit and minimise potential long-term side effects, the use of newer bisphosphonate preparations and the outcome following cessation of therapy. These issues can only be addressed through the continued systematic evaluation of patients receiving bisphosphonate therapy and ability to obtain long-term follow-up data on these individuals (Box 5.1).

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Box 5.1 Summary of Bisphosphonates in Osteogenesis Imperfecta

1. Cyclic intravenous bisphosphonates, particularly pamidronate, have good short-term safety and efficacy in children and adolescents with moderate-to-severe osteogenesis imperfecta.
2. Bisphosphonate therapy should be offered to children with moderate-to-severe OI as defined by: two or more long-bone fractures per year, and/or vertebral crush fractures, and/or long-bone deformities, and/or children with OI type III.
3. In severe cases, treatment can be started during infancy, but these children need to be monitored very closely, especially during the first infusion cycle.
4. Treatment continues to be effective in older teenagers and the upper age limit of responsiveness still remains to be defined.
5. Several dosage regimens appear to be effective:
   (a) Low dose frequent administration—pamidronate 0.5–1 mg/kg/month [49].
   (b) High dose infrequent administration—pamidronate 9 mg/kg/year, with a dose and treatment interval that vary with age [40]. Zoledronate 0.05 mg/kg 6-monthly.
6. All children treated with a bisphosphonate should be part of a comprehensive surveillance programme, preferably in the context of a clinical trial. This will enable the short- and long-term safety and efficacy of these compounds can be adequately evaluated.
7. After completion of the acute treatment phase, most children will require ongoing maintenance therapy.

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Chapter 6
Use of Bisphosphonates in Genetic Diseases Other than Osteogenesis Imperfecta

Maria Luisa Bianchi

Introduction

This chapter focuses on the bone complications—i.e., low bone mineral density (BMD) and fragility fractures—of genetic diseases other than osteogenesis imperfecta (OI), and on their treatment with bisphosphonates (BPs). What we currently know about the efficacy of BPs in these diseases is essentially based on case reports on single patients or small patient samples. Studies with BPs on sufficiently large populations or placebo-controlled studies are very few, since most genetic diseases are quite rare and their bone complications have only recently aroused interest.

In the genetic diseases characterized by low bone mass and fractures, treatment with BPs is justified by their efficacy in increasing bone density and reducing fracture risk, well demonstrated in postmenopausal osteoporosis and also in osteogenesis imperfecta. In other genetic diseases, characterized by abnormal formation of bone, the use of BPs is justified as an attempt to prevent calcification through their physiochemical action on the calcification process (inhibition of formation and delay of aggregation of calcium phosphate crystals) [1].

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It is recommended that in all cases of primary or secondary osteoporosis[1] in children and adolescents, whether of genetic origin or not, BP treatment should be decided only with the advice of a pediatric bone expert. Adequate calcium intake and vitamin D levels, and, whenever possible, regular physical activity are always recommended as a basic intervention, before and during BP treatment.

The first section of this chapter is dedicated to the rare genetic diseases, for which little data is available, and the second to the more prevalent genetic diseases.

Rare Genetic Diseases

Bruck Syndrome (OMIM 259450)

Inheritance: Autosomal recessive.

Genetic defect:
1. Mutations in *PLOD2* gene (probably located in the chromosome 17p12 region) resulting in deficiency of a bone-specific telopeptidyl lysyl hydroxylase that catalyzes the formation of cross-links in the telopeptide region of type I collagen in bone (but not ligaments or cartilage). The deficiency leads to aberrant cross-linking due to under-hydroxylation of the lysine residues [2].
2. Mutations in *FKBP10* gene, encoding FKBP65, an extracellular matrix binding protein, whose mutations affect type I procollagen production [3].

Prevalence: Very rare, only few cases reported worldwide.

Bruck syndrome (BS) is a rare, autosomal recessive disorder, with only few cases known.

Like OI (see Chap. 5), it is characterized by deformity of the spine and extremities, low bone mass, bone fragility fractures, wormian bones, and blue or white sclerae [2–4]. The main differences are the presence of congenital joint contractures—a prominent finding in BS—and the absence of the typical alterations in type I collagen that characterize OI [5].

[1] According to the Pediatric Position Development Conference (PPDC) 2013 of the International Society of Clinical Densitometry (ISCD), the diagnosis of osteoporosis in children and adolescents should not be made on the basis of densitometric criteria alone. Osteoporosis can be diagnosed in the presence of one or more vertebral compression (crush) fractures, not attributable to local disease or high-energy trauma. In the absence of such fractures, a diagnosis of osteoporosis requires the presence of both a BMD Z-score ≤ -2.0 and a clinically significant fracture history (defined as "two or more long bone fractures by age 10 years" or "three or more long bone fractures at any age up to 19 years"). The ISCD PPDC 2013 document can be read at: http://www.iscd.org/2013-iscd-official-positions-pediatric/.

In this disease, the rationale for using BPs is much the same as for OI: low bone mass and fragility. BPs seem to improve bone quality and strength, with fewer fractures, and to reduce bone pain.

The few published case reports have shown a positive effect of BPs. Shaheen et al. used a parenteral BP in two brothers with BS, and observed a reduction of fractures [3]. Cyclic pamidronate was successfully used by Andiran et al. to treat a boy with multiple fractures: an increase in BMD, a reduction of pain, and fewer fractures (only two during 2 years of treatment) were observed [6].

Osteoporosis-Pseudoglioma Syndrome (OMIM 259770)

Inheritance:	Autosomal recessive.
Genetic defect:	Mutations (likely loss-of-function) in the *LRP5* gene in osteoblasts. The *LRP5* gene (probably located in the chromosome 11q13.4 region) encodes the low-density lipoprotein receptor-related protein 5 (LRP5), whose defects impair the Wnt and Norrin signal transduction [7–9]. At least 12 homozygous and 15 heterozygous mutations have been described [8–12].
Prevalence:	1:2,000,000 [9].

The osteoporosis-pseudoglioma syndrome (OPPG) is a rare autosomal recessive disease [9]. It has some of the characteristics of moderate-to-severe OI—such as reduced bone mass, short stature, and skeletal deformity—but a major difference is the presence of congenital or infancy-onset blindness, due to the presence of a pseudoglioma. Obligate heterozygotes may have a slightly reduced BMD but do not have ocular pathology.

The affected children develop osteoporosis with vertebral and limb fractures that may cause severe pain. The rationale for BP use in OPPG is the same as for Bruck syndrome (see above) and OI. Positive effects have been observed in a few case reports. Three children (aged 9–11 years) with vertebral fractures were treated with pamidronate or clodronate for 2 years and had less pain, improved mobility, greater vertebral body size, no new fractures, and normal growth and puberty, leading to the conclusion that BPs are justified in OPPG in the presence of symptomatic vertebral fractures [12]. A 21-year-old woman treated with pamidronate had less bone pain, improved mobility, and increased BMD at lumbar spine (LS) and femoral neck (FN) [13]. Different BPs (oral risedronate, intravenous pamidronate, oral alendronate) were used in four children (aged 2–8 years) for 1.5–6.5 years: the highest increase in BMD *Z*-score was observed with alendronate (1 mg/kg/day) [14]. Barros et al. treated two brothers with pamidronate for 3 years and observed an increase in BMD and a decrease in fracture rate [15]. Three Turkish children, in whom three novel *LRP5* mutations were discovered, were treated with BPs for 3.5–7 years and showed improved BMD *Z*-scores at lumbar spine, reduced bone pain, and better quality of life [16].

Homocystinuria (OMIM 236200)

Inheritance: Autosomal recessive.

Genetic defect: Most commonly, mutations in the cystathionine beta-synthetase (CBS) gene, encoding a key enzyme of the trans-sulfuration pathway (conversion of methionine to cysteine).

Prevalence: 1:344,000.

Homocystinuria is an autosomal recessive connective tissue disease, due to defects in methionine metabolism, resulting in elevated plasma levels and urinary excretion of homocysteine. The disease is characterized by mental retardation, ectopia lentis, marfanoid habitus, early-onset thrombotic vascular disease, and osteoporosis. Homocysteine reacts with many biological substances, including proteins. In particular, it may damage the glycoprotein fibrillin-1, a major component of elastin (found in arteries, cartilage, skin, the suspensory ligament of the lens, and bone). This explains the many similarities of homocystinuria with Marfan syndrome (MFS).

Homocysteine-induced bone damage (due to alterations of the growth plate during endochondral ossification) has been investigated in cellular and animal studies, but systematic studies on bone density and fractures in patients affected by homocystinuria are lacking.

Only a few case reports mention the problem of low BMD in this disease, and BP treatment has been reported in only one case, a 22-year-old woman with multiple vertebral fractures and reduced BMD (measured by dual-energy X-ray absorptiometry, DXA), who received zoledronic acid by i.v. infusion once every 12 months. No adverse effects were reported but the final results have not yet been published [17]. Like most diseases with increased bone fragility, homocystinuria may benefit from BP treatment in the presence of reduced BMD and increased fracture risk, also considering that the standard treatment with betaine has not shown positive effects on bone [18].

Fibrodysplasia Ossificans Progressiva (OMIM 135100)

Inheritance: Autosomal dominant in hereditary cases; most cases due to spontaneous new mutations.

Genetic defect: Mutations of the *ACVR1* gene. ACVR1/ALK2 (activin A type I receptor/activin-like kinase 2) is a type I receptor for bone morphogenetic proteins (BMPs). An identical heterozygous substitution of a single nucleotide (G→A) has been demonstrated in all individuals with the classic presentation of fibrodysplasia ossificans progressiva (FOP). The diagnosis can be confirmed by DNA testing (determination of the DNA sequence of the *ACVR1* gene).

Prevalence: 1:2,000,000. Fewer than ten families with inheritance of FOP known worldwide [19].

FOP (formerly called myositis ossificans) is a rare but extremely disabling genetic disease of the skeletal system, characterized by the formation of extraskeletal (heterotopic) ossification within connective tissues (skeletal muscles, ligaments, tendons). The involved tissues are destroyed and replaced by bone through a process of endochondral ossification, leading to progressive restriction of mobility, particularly of the upper limbs. Heterotopic ossification can also be induced by trauma, including surgical attempts to remove the newly formed bone. The affected children appear normal at birth except for some typical malformations, such as short great toes, hallux valgus, short thumbs, and hypoplasia of digital phalanges. Skeletal malformations may also develop during embryonic development [19–24]. The diagnosis can be made even before radiographic evidence of heterotopic ossification, on the basis of rapidly appearing, painful soft tissue inflammatory swellings (flare-ups) during the first years of life, associated with deformity of the great toes [25]. The diagnosis can be confirmed by sequencing the *ACVR1* gene.

FOP was the first disease in which a BP (etidronate) was therapeutically used in humans [26]. The rationale was based on the physiochemical properties of BPs as inhibitors of calcification and bone formation [1, 27]. Over some years, a few other publications reported positive results with BPs (mainly etidronate) [28, 29], but there are no recent publications.

Fibrous Dysplasia (OMIM 174800)

Inheritance:	None.
Genetic defect:	Activating mutations of the GNAS gene (in chromosome 20q13), with increased expression or function of the alpha subunit of the stimulatory G protein (G_s-alpha) of adenylyl cyclase. The mutations occur in a postzygotic phase, resulting in mosaicism. The affected cells suffer from excessive production of cAMP.
Prevalence:	?

Fibrous dysplasia (FD) is today the preferred name for a disease also known as osteitis fibrosa, polyostotic fibrous dysplasia, and McCune–Albright syndrome. FD is a complex syndrome, characterized by single or multiple skeletal alterations (commonly but superficially described as an overgrowth of fibrous tissue in bone, hence the name “osteitis fibrosa”), often but not invariably associated with various endocrinopathies and skin pigmentation.

In FD, bone growth and modeling are abnormal, due to localized formation of excess bone tissue, and bone remodeling is abnormally high. The result is an architecturally deficient bone, in which the organization of cortical bone, trabecular bone, and marrow space is lost, with bone deformities and fractures. In particular, in the dysplastic lesions, bone trabeculae are thinner and more numerous than normal [30].

FD develops during bone growth, and an earlier presentation usually means a more widespread disease. Thus, FD more often appears as the polyostotic form in infants, and as the monostotic form in adolescents. The ratio of polyostotic to monostotic disease is about 1:10.

The craniofacial, axial, or appendicular skeleton may be variably involved, and the disease severity depends on the number and extension of bone lesions. After puberty, new major skeletal lesions do not usually appear, but the existing lesions may still evolve. Monostotic lesions may be asymptomatic, and the diagnosis is usually made after a fragility fracture, or because of deformity or persistent bone pain. The polyostotic forms are diagnosed on the basis of a combination of pain, fracture, and deformity. In some cases there are extraskeletal complications, such as precocious puberty in females, that may appear even before any apparent skeletal involvement. Bone cysts are very common, but are different from those observed in hyperparathyroidism (brown tumors).

In FD, the high rate of bone remodeling and the production of low-quality, fragile bone are the rationale for BP use, considering its inhibitory action on osteoclasts and bone turnover.

Many patients with FD have been treated with BPs (mainly i.v. pamidronate, but also oral alendronate or risedronate), usually in combination with calcium and vitamin D supplementation, often with positive results [31]. However, Plotkins et al. reported that in seven patients treated with pamidronate for 2.2 years on average, the dysplastic bone lesions were not significantly improved [32].

We treated two cases of polyostotic cystic fibrous dysplasia (unpublished data). The first was a 15-year-old girl sent to our attention, after her 13th fracture, by an orthopedist who had followed her for 10 years. The diagnosis had been made at 4 years of age, after a fracture of the left foot and X-ray evidence of cysts in the left leg. After a second fracture of left femur, histological examination confirmed the diagnosis. The disease was locally destructive and painful, and led to 11 further left leg fractures for minor trauma, or even spontaneous, in the following years. The clinical and radiological evolution was continuous, until cysts were present in the whole limb. BMD was always within normal range. We decided to start treatment with pamidronate (0.8 mg/kg i.v., once a month, then 30 mg every 2 months). The girl was successfully treated for 4 years, with resolution of bone pain, no new fractures, and stabilization of the cystic lesions at MRI (Figs. 6.1 and 6.2). Our second case was a 23-year-old young lady affected by polyostotic cystic fibrous dysplasia localized in the frontal-parietal area of the skull. She suffered from frequent headaches and presented with moderate left exophthalm and a wide area of softened or destroyed cystic cranial bone, where pulsating endocranial structures could be felt. We started i.v. neridronate, 50 mg every month at first, then every 2 months. After 4 years of treatment, she had no more headaches, the exophthalm was significantly improved, and MRI demonstrated reduction of the cystic bone area.

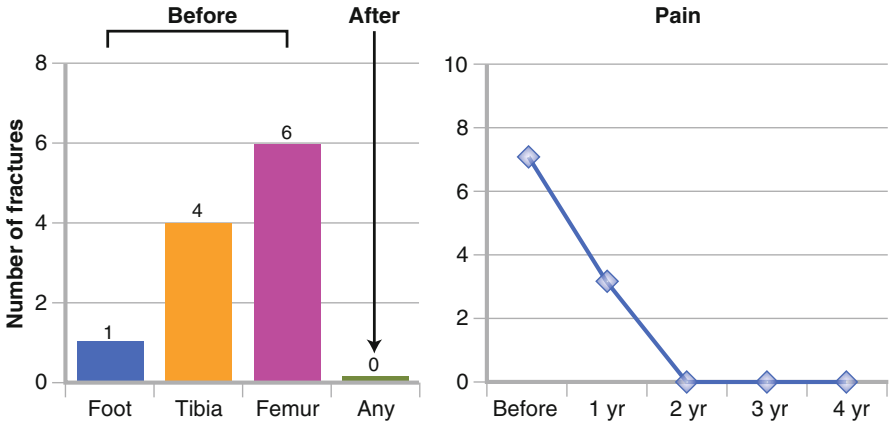

Fig. 6.1 A 15-year-old girl affected by polyostotic cystic fibrous dysplasia interesting the whole left lower limb, with a history of multiple fractures. Effect of pamidronate therapy on number of fractures and bone pain

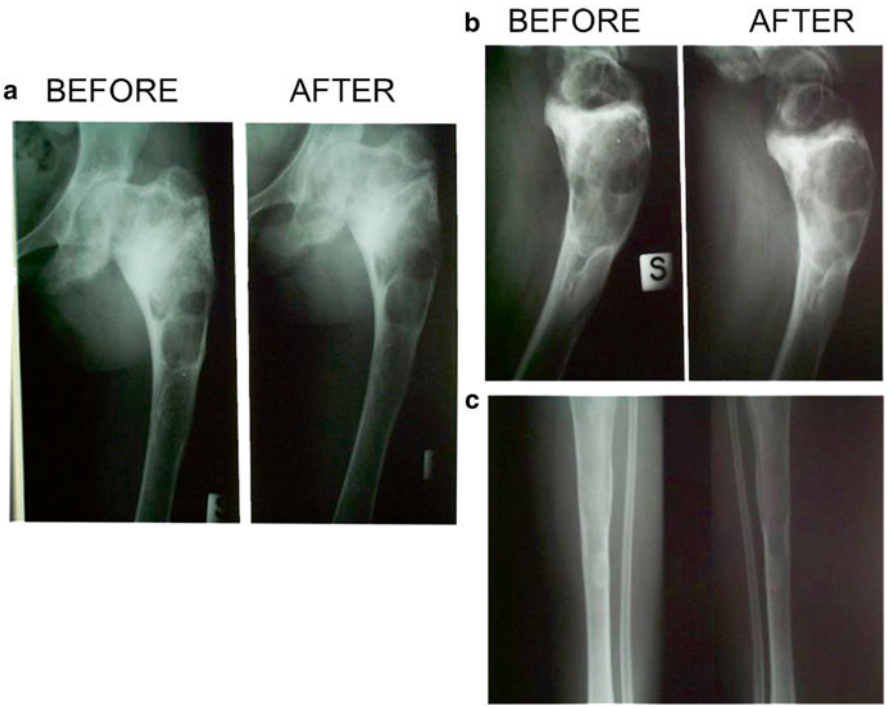

Fig. 6.2 (**a–c**) MRI images of the left lower limb in the same patient before and after pamidronate, showing stabilization of the cystic lesions

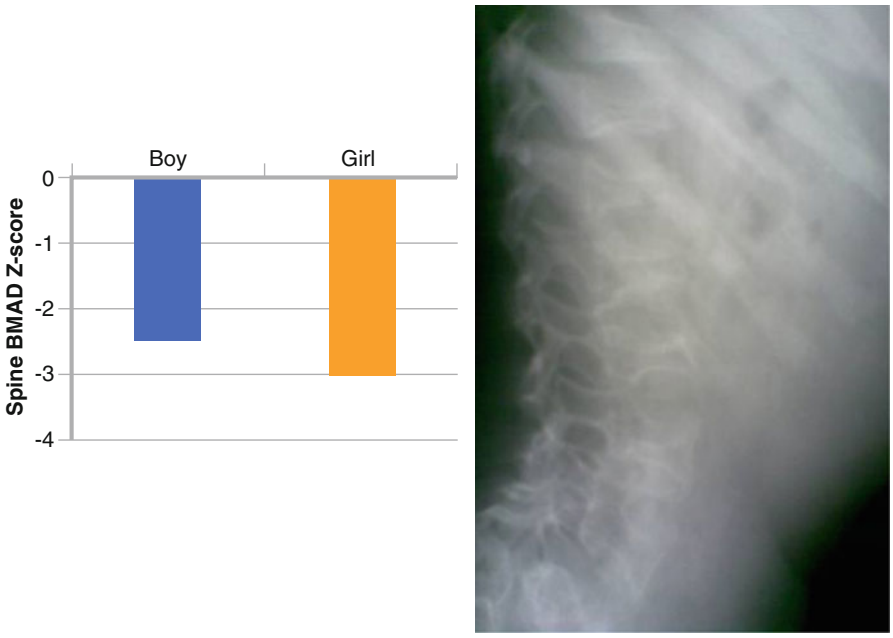

Fig. 6.3 Lumbar spine BMAD *Z*-scores in boy and girl affected by unbalanced translocation t(9;14) (*left*). Multiple vertebral fractures in the girl before pamidronate therapy (*right*)

Trisomy 9p (OMIN 190685)

Inheritance: In some cases, due to balanced chromosomal rearrangement in one of the parents; in others, arising from de novo errors in early embryonic development.
Genetic defect: Duplication of the short arm of chromosome 9.
Prevalence: 200 cases reported worldwide.

Trisomy 9p is a rare chromosomal abnormality, first described by Rethoré in 1970 [33]. Unbalanced translocation t(9;14) (i.e., trisomy of the short arm of chromosome 9 and monosomy of the short arm of chromosome 14) is a rarer variant. These anomalies are compatible with long survival. The clinical manifestations are very variable: mental retardation, short height, prominent or bulbous nose, downturned corners of the mouth, hypertelorism, strabismus, and foot and hand anomalies are most often described [33–36]. Only delayed bone maturation has been reported [35], but not low BMD and fragility fractures.

We personally observed both low BMD and fragility fractures (osteoporosis according to the ISCD PPDC 2013 definition) in two Italian children with unbalanced translocation t(9;14), referred to our institute (Fig. 6.3). The first was a 12-year-old boy who had sustained two subsequent fractures after minimal trauma, and the second was a 6-year-old girl with multiple, apparently atraumatic, vertebral

fractures. In both cases, DXA revealed very low lumbar spine BMAD *Z*-scores (−2.5 and −3.0, respectively),[2] and we decided to start treatment with i.v. pamidronate (0.5 mg/kg, for 3 consecutive days every 3 months). After, respectively, 8 months (boy) and 2 years (girl) of treatment, both had a significant BMAD increase (+8.9 %, boy; +11.4 %, girl) and, above all, no more fractures. As often happens in the growing age, the girl also showed a good recovery in height of the crushed vertebral bodies (unpublished data).

More Frequent Diseases

Ehlers–Danlos Syndrome (OMIN 13000–13050)

Inheritance: Autosomal dominant; autosomal recessive; due to spontaneous new mutations.

Genetic defect: Various defects in extracellular matrix proteins. Gene mutations described for *ADAMTS-2*, *COL1A1*, *COL1A2*, *COL3A1*, *COL5A1*, *COL5A2*, *PLOD1*, and *TNX-B*.

Prevalence: 1:5,000.

Ehlers–Danlos syndrome (EDS) refers to at least nine different genetic disorders of connective tissues, caused by defects in one of the genes encoding extracellular matrix proteins (including collagens and small leucine-rich proteoglycans like decorin). The main clinical features are skin hyperextensibility, joint hypermobility, and tissue fragility of the skin, ligaments, blood vessels, and internal organs.

Yen et al. measured BMD in 11 patients with EDS (children, adolescents, and young adults) and observed osteoporosis in all of them [37]. Dolan et al. reported an increased fracture rate in EDS patients [38]. These findings suggest reduced bone strength, but further studies on larger patient samples affected by the various EDS forms are needed to assess the actual impact of this disease on bone. There are no published data on BP use in EDS. However, if the presence of reduced BMD and increased fracture rate are confirmed, treatment with BPs might improve the condition and should be investigated.

[2] DXA measures an "areal" BMD (aBMD, i.e., BMC in g/cm^2 of bone projection area) and not the true "volumetric" BMD (vBMD, i.e., BMC in g/cm^3 of bone volume). For mathematical reasons, if two bones of equal vBMD (g/cm^3) are analyzed with DXA, the smaller bone will have a lower aBMD than the larger bone; that is, DXA overestimates aBMD as bone size increases. For this reason, aBMD is unsuitable for the study of growing subjects, and a surrogate of vBMD, called "bone mineral apparent density" (BMAD, g/cm^3), and its *Z*-score are preferred. The BMAD is calculated by assuming that the vertebral body is a cube or a cylinder, whose volume can be easily calculated from the projection height and width.

Marfan Syndrome (OMIN 154700)

Inheritance: Autosomal dominant.
Genetic defect: Mutations in *FBN1* gene, one of the two genes encoding fibrillin-1 (the main structural component of elastin-associated cross-links). Rare mutations in *COL1A2* gene. Rare mutations in *TGFBR2* gene, encoding transforming growth factor beta (TGF-beta) receptor 2.
Prevalence: 1:5,000.

MFS is a relatively common autosomal dominant genetic disease, variably affecting the skeleton, eye, and cardiovascular system. The most characteristic clinical feature is long, thin, hyperextensible fingers (arachnodactyly). The most common gene mutations (*FBN1*) lead to an increased activation of TGF-beta, which stimulates osteoblast proliferation and differentiation, influencing bone mass and the properties of bone matrix [39]. Some studies evaluated BMD in adults and children affected by MSF: the findings are inconsistent for children, reporting reduced as well as normal BMD, while both reduced BMD and increased fracture risk are observed in adults [40–42]. These features may constitute the rationale for BP treatment, at least in selected cases. Currently, there is only one published study that used alendronate in an animal model, Fbn1 (mgR/mgR) mice with severe MFS and osteopenia [43]. These mice have normal osteoblast differentiation and bone formation, but excessive osteoblast-stimulated pre-osteoclast differentiation and increased osteoclastogenesis, leading to osteopenia. Alendronate treatment improved bone quality by reducing osteoclast activity, but had no effect on aneurysm progression.

Duchenne Muscular Dystrophy (OMIN 310200)

Inheritance: X-linked recessive.
Genetic defect: Mutation in the dystrophin gene (at locus Xp21, in the short arm of chromosome X).
Prevalence: 1:3,500 male births.

Duchenne muscular dystrophy (DMD) is the most frequent muscular disease affecting children. In most cases, proximal muscle weakness begins before 3 years of age, then patients become progressively unable to walk. Notwithstanding substantial therapeutic advances, essentially due to early treatment with glucocorticosteroids (GCs), most patients still die in early adulthood.

The presence of low bone mass, mainly due to both physical inactivity and GC treatment, has been frequently reported in DMD. Fractures are a common complication and a major factor in the precocious loss of independent ambulation [44]. The few published studies on bone density in DMD concluded that BMC and BMD were lower than normal at different skeletal sites [45–51], particularly at lower limbs [47, 49]. In a retrospective study on 143 DMD boys, the GC-treated subjects had 2.6 times more long-bone fractures than the untreated;

and vertebral fractures occurred in 32 % of GC-treated patients, versus none in the untreated [45]. In a study on 33 boys with DMD, treated with GCs for 100 months, 24 sustained vertebral fractures [46]. In 46 boys with DMD, treated with deflazacort for 4 years, 26 suffered 37 fractures (14 vertebral) and significant BMD decrease was observed [47]. A recent study on 25 boys (mean age 7.4 years), however, did not show any detrimental effects of GCs (given for 30 months) on lumbar vertebrae [48].

The use of BPs in children affected by DMD is justified by the increased fracture rate due to low BMD (cytokine-induced increased osteoclastogenesis and disuse osteopenia) and long-term GC treatment, a well-known cause of osteoporosis. Different BPs (pamidronate, alendronate, risedronate) have been used in DMD boys on long-term GC treatment. The few published studies consistently report increases in BMD and fewer fractures. In 23 deflazacort-treated boys, with only a slight decrease of the BMD *Z*-score and no fractures, alendronate treatment plus calcium and vitamin D supplements for 2 years improved total body and lumbar spine BMD *Z*-scores [52]. In three patients with reduced BMD *Z*-score, fractures, and generalized bone pain, oral alendronate (10 mg/day, for 14–25 months) was well tolerated, without adverse effects, and led to increased lumbar spine BMD, reduction of bone pain, and no incident fractures [53]. A recent retrospective study on a cohort of 44 Canadian patients, treated with GCs (prednisone or deflazacort) at a single center, even reported that BP treatment was associated with a significant improvement in survival rate, compared with treatment with steroids alone. This interesting finding should however be confirmed by larger studies [54]. We have been using BPs (i.v. pamidronate or oral alendronate) in children and adolescents with DMD and osteoporosis (i.e., BMD *Z*-score less than or equal to −2 and a history of fragility fractures) in the last 4 years, and observed improvements in BMD and fewer fractures (Fig. 6.4) (unpublished data).

Cystic Fibrosis (OMIN 219700)

Inheritance:	Autosomal recessive.
Genetic defect:	Mutations in a gene (*CFTR*) encoding the cystic fibrosis transmembrane conductance regulator (CFTR) protein.
Prevalence:	1:2,000 newborns in Europe and 1:3,000 white newborns in the USA.

Cystic fibrosis (CF) is among the most frequent genetic diseases. It is caused by mutations in the *CFTR* gene, encoding a chloride channel that modulates the transport of salt and water across the membranes of epithelial cells. The genetic defect leads to alteration in the quantity, viscosity, and/or salt concentration of the secretions of exocrine glands, with severe pulmonary, pancreatic, hepatic, and gastrointestinal complications and premature death. The most severe clinical manifestations, including osteoporosis, and the highest mortality are seen in the presence of delta-F508 mutations.

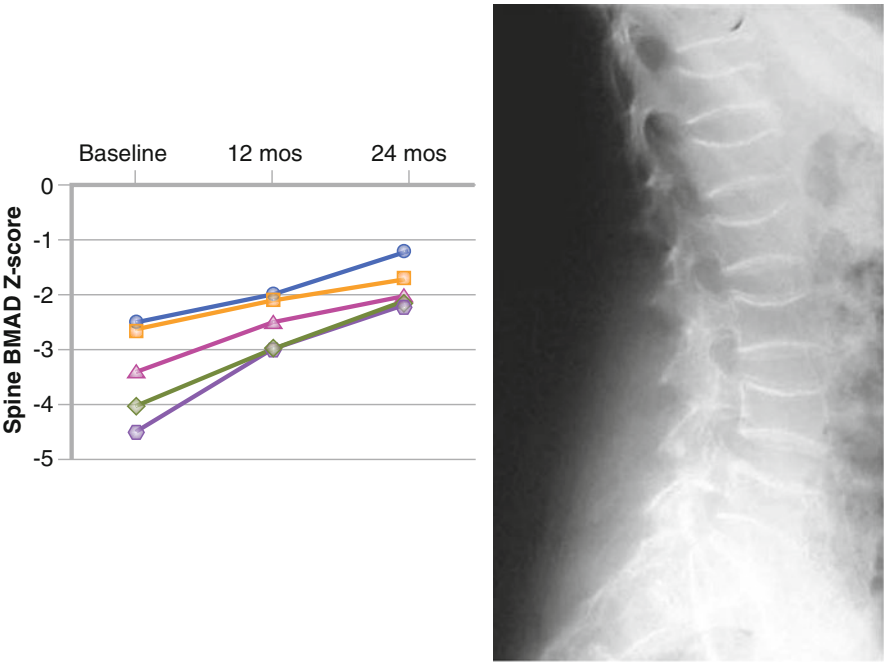

Fig. 6.4 Vertebral fractures in a child affected by Duchenne muscular dystrophy, on long-term glucocorticosteroid treatment (*right*). Effect of i.v. pamidronate or oral alendronate in five children/adolescents affected by DMD, with low bone density. Spine BMAD increased +22 % on average after 24 months of therapy (*left*)

Reduced bone mass in CF seems due to both inadequate calcium deposition and excessive bone resorption. Reduction of fracture risk is extremely important, because all fractures lead to reduced mobility and a higher risk of pulmonary infections, and rib and vertebral fractures limit the possibility of respiratory physical treatment and lung transplantation. BPs have been successfully used in adult CF patients in the presence of fragility fractures or significant BMD reduction, while waiting for solid transplants, or when long-term treatment with systemic GCs was being started [55–57]. In children and adolescents they have been rarely used, mainly after vertebral fractures. We recently published the results of a 12-month randomized controlled trial with oral alendronate versus placebo, showing that alendronate is able to significantly improve BMD in young patients with CF [58]. Two recent articles [59, 60], the first a Cochrane review, evaluated the effects of BPs in adult patients with CF and concluded that they are superior to placebo in improving BMD and reducing fracture risk, but may have adverse effects such as bone pain with i.v. administration. The recent European CF guidelines [61] suggest that BP treatment can be considered for CF children, after failure of optimal conservative treatment for BMD, in the presence of total body or lumbar spine BMD *Z*-score less than or equal to −2 and a history of

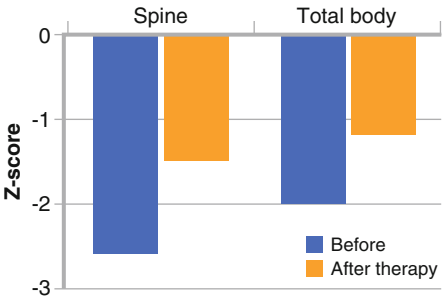

Fig. 6.5 Mean lumbar spine BMAD *Z*-score and total body less head BMD *Z*-score before and after oral alendronate therapy in six children affected by cystic fibrosis, with low bone density and fractures

low trauma fractures. In particular, BP treatment should be considered for CF children with low BMD who are taking continuous systemic GCs, or have undergone solid organ transplantation or are waiting for it. Figure 6.5 shows the response to alendronate treatment in six children affected by cystic fibrosis, and Fig. 6.6 shows the recovery of vertebral fractures in one of these patients (unpublished data).

Gaucher Disease (OMIN 23100: 23080–23090)

Inheritance:	Autosomal recessive.
Genetic defect:	Mutations in the *GBA* gene (1q21) encoding lysosomal acid beta-glucosidase (glucocerebrosidase).
Prevalence:	1:50,000 (type I; most common among Ashkenazi Jews); 1:100,000 (type II); 1:100,000 (type III).

Gaucher disease is the most common lysosomal storage disease. It is characterized by lysosomal accumulation of a sphingolipid (glucosylceramide) in the macrophages of liver, spleen, bone marrow, lung, and other organs. The lipid-repleted cells (Gaucher cells) cause tissue damage, fibrosis, and increased release of cytokines. Three clinical types, of different severity, are recognized: non-neuropathic (type I); acute infantile neuropathic (type II); and chronic neuropathic (type III). Types II and III are characterized by extensive brain infiltration and lead to premature death, respectively, in infancy/childhood or adolescence/early adulthood. Since 1991, a corrective treatment for Gaucher disease type I (enzyme replacement therapy, ERT) is available and has become the standard of care.

Skeletal involvement is common, and bone architecture, bone strength, BMD, and bone metabolism are all affected. Bone pain, deformities, osteonecrosis, and pathological fractures are the main clinical features [62, 63]. ERT, good nutrition, and judicious exercise are the first-line measures to help achieving a satisfactory peak of bone mass in young patients with Gaucher disease. ERT restores bone growth in children, reduces the frequency of avascular necrosis and nonspecific bone pain [64], and according to recent evidence may also help improving BMD [65]. Several characteristics of the bone derangements observed in Gaucher disease, including bone pain, may justify a trial with BPs, and they have sometimes been

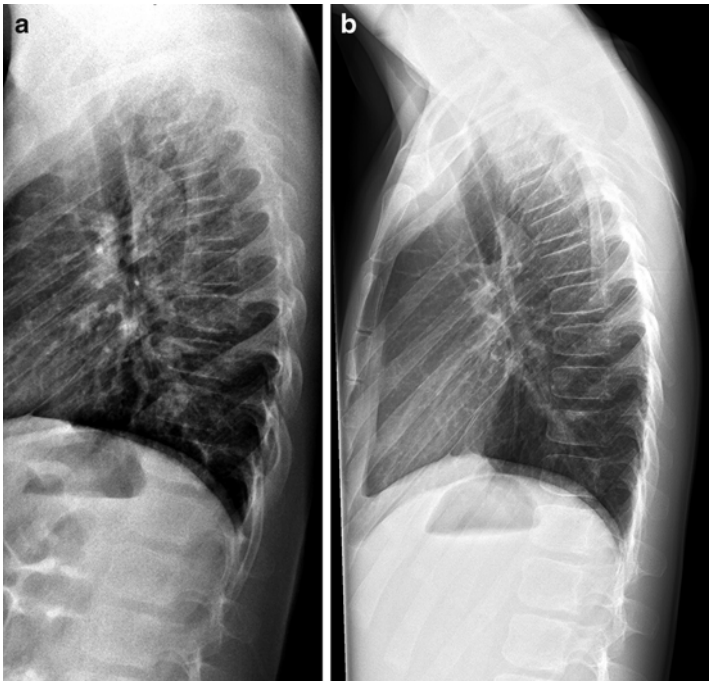

Fig. 6.6 Vertebral fractures in a 11-year-old boy with cystic fibrosis and vertebral fractures, showing improvement of vertebral wedging after only 1 year of pamidronate (PAM) treatment. (**a**) *Before PAM treatment* reveals a reduction in height of vertebrae D5–D9, with initial anterior wedging of D5-D6-D7-D8. (**b**) *After 12-month PAM treatment* shows an improvement in the height reduction of all vertebrae, with less anterior wedging of D5-D6-D7 and no more wedging of D8

used, although few data are available [66]. Oral BPs rapidly increase BMD in adult patients treated with enzyme infusions [67], but their effect on bone strength and fracture risk is unknown. In children, parenteral administration of BPs (e.g., pamidronate) has been reported to alleviate bone pain [68–72]. Overall, the current evidence is insufficient to recommend BPs as a standard of care, except in children or young adults with vertebral collapses or recurrent pathological fractures due to severe osteoporosis.

Other Genetic Diseases

In several genetic diseases with inborn errors of metabolism (glycogen storage diseases (GSD), Niemann–Pick disease, mucopolysaccaridoses), progressive reduction of BMD, osteoporosis, and increased risk of fractures have been described. In

particular, muscle weakness, loss of muscle function, and reduced mobility could be major factors of bone impairment in GSD type II (Pompe's disease) [73–75].

In at least some of these diseases, despite the lack of published data, treatment with BPs might be considered in the presence of severe bone loss and high fracture risk. We treated a 7-year-old male child (affected by Niemann–Pick disease type A/B) with a history of multiple fractures: i.m. clodronate (25 mg every 10 days for 2 years 5 months) led to BMD increase and no more fractures (unpublished data).

Looking Ahead

Most genetic diseases are so rare that a single medical center, even at a national level, will never be able to recruit sufficiently large patient samples to perform randomized controlled trials of BPs for their bone complications. A way to overcome this substantial difficulty would be the creation of an international rare disease databank to permit the accumulation of a sufficient number of cases to attempt multicenter trials.

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Chapter 7
Bisphosphonates in Pediatric Burn Injury

Gordon L. Klein

Background: The Pathophysiology of Post-Burn Bone Loss in Children

Use of bisphosphonates in the prevention of post-burn bone loss in children represents a unique situation in which the novel off-label use of a drug has been studied in children before being studied in adults. The reason for this is entirely circumstantial in that the mechanisms of bone loss following burn injury were being studied in a children's hospital setting.

While discussed elsewhere in this book, mechanisms of secondary bone loss following burn injury are diverse and constitute at least three distinct entities. First, the damage done to the body's main barrier to infection, the skin, allows entry of a wide variety of organisms into the body through the burn wound. Furthermore, by a mechanism not as yet clarified, gut permeability increases allowing for translocation of intestinal bacteria into the blood. This invasion of the body from both without and within triggers a *systemic inflammatory response*. The immediate consequence of this inflammatory response is a markedly increased production of cytokines by the inflammatory cells. In particular there is a threefold rise in circulating interleukin (IL)-1β and a hundredfold rise in circulating IL-6 [1].

These cytokines in particular are known to stimulate osteoblast production of the ligand for the *r*eceptor *a*ctivator of the *n*uclear transcription factor κB, otherwise known as RANK Ligand or RANKL. RANKL has been shown to stimulate the differentiation of bone marrow stem cells into osteoclasts [2] as opposed to white blood cells, thereby increasing osteoclastic bone resorption.

Also, within 24 h of the burn injury itself, by uncertain mechanisms and possibly in conjunction with the systemic inflammatory response, a *stress response* is elicited

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within the body. This stress response entails the increased endogenous production of catecholamines and glucocorticoids. In the case of the former, catechols represent the end product of sympathetic nervous system activation, and the sympathetic nervous system, through β-adrenergic receptors on the osteoblast, is postulated to increase osteoblastic production of RANKL and therefore increases bone resorption [3]. Endogenous glucocorticoids, as measured by urine free cortisol, are elevated 3- to 8-fold post-burn depending on whether 50 or 125 μg/24 h is taken as the upper limit of normal for urinary excretion of free cortisol [1, 4]. The endogenous glucocorticoids appear to have a biphasic effect on bone. Initially, while the osteoblasts are viable they stimulate osteoblast production of RANKL and increase osteoclastic bone resorption. It is currently not known whether the inflammatory and glucocorticoid effects on RANKL production by the osteoblasts are additive. However, by approximately 2 weeks post-burn osteoblasts disappear from the bone surface [1, 4] and tetracycline labeling demonstrates both the absence of the double label and shortening of the length of surface uptake [1, 4, 5]. Moreover, glucocorticoid receptors in bone show a trend toward reduction when analyzed by RT-PCR and culture of marrow stromal cells from burned children demonstrates significantly reduced markers of osteoblast differentiation, including alkaline phosphatase, cbfa1, otherwise known as runx2, and BMP-2 [4]. All of these findings are hallmarks of glucocorticoid-induced toxicity to bone.

The role of *immobilization* in the pathogenesis of bone loss is a bit more controversial. After burn injury patients are restricted in movement not only by pain but also by necessity to allow operative skin grafts to take in situ. Therefore, especially in the early weeks post-burn patients are intermittently immobilized. In studies of a rat model of burn injury and immobilization by Baer et al. [6] immobilization led to increased bone turnover and burn injury to reduced biomarkers of bone formation and decreased bending strength. The most severely affected rats were those which were both immobilized and subject to burn injury. Thus it is entirely possible that children who are victims of severe burn injury are subject to the synergistic effects of burn injury, i.e., inflammation and stress, and the effects of early immobilization. What remains puzzling, however, is that the effects of immobilization are purported to be mediated by the sympathetic nervous system [3, 6]. If this is so, then much like the catecholamines driven by the stress response sympathetic nervous system stimulation should affect the osteoblastic β-adrenergic receptor. This signaling pathway poses two problems. The first is that due to the effects of endogenous glucocorticoid production as part of the stress response, osteoblasts become apoptotic by approximately 2 weeks post-burn [4]. Osteoblast apoptosis then should limit the duration of sympathetic influence on bone unless other as yet undescribed pathways also exist. Further, in a pilot study the β blocker propranolol when administered to burned children from time of hospital admission failed to improve bone mineral content by 6 months post-burn as compared to placebo controls [7].

There is preliminary evidence that bone resorption begins within the first week following burn injury in that Leblebici and colleagues [8] found elevated urinary pyridinoline excretion in adults who suffered a burn injury of at least 25 % of their body surface area. Urinary pyridinoline is a biomarker of type I collagen cross-link breakdown as would uniquely occur with bone resorption.

Efforts to Treat the Bone Loss

At first, the anabolic agents recombinant human growth hormone and oxandrolone were studied in an attempt to treat the bone loss and to restore bone density to normal [9]. These efforts are described elsewhere in this book.

The off-label use of bisphosphonates, especially limited to one or at most two doses, had not been previously attempted in children. The only clear-cut successful use of bisphosphonates prior to the work with burns patients has been for the symptomatic treatment of children with osteogenesis imperfecta, and this experience is also described elsewhere in this volume.

Rationale for Clinical Trials of Bisphosphonates

With regard to the prospective use of bisphosphonates in children following burn injury several questions needed to be addressed. The first question involved the route of administration, oral versus intravenous. Given that little work had been done on intestinal absorption following burn injury we elected to use the intravenous route to ensure delivery of the drug. The next question was timing. Should the drug be given acutely following the burn injury or should it be given after the patient was stabilized? The answer was not clear. If it is assumed that bone resorption begins within 24 h of the injury then clearly the earlier the drug could be given the less bone the patient might lose. It was arbitrarily decided to administer a dose within the first 10 days following the burn injury after the initial determination of bone mineral content of the total body and bone mineral content and density of the lumbar spine.

With acute administration came the question of whether giving a bisphosphonate would block bone calcium release and lead to profound hypocalcemia. Inasmuch as burned children become hypocalcemic and hypoparathyroid [10] most likely secondary to cytokine-mediated up-regulation of the parathyroid calcium-sensing receptor [11] this concern was disturbing. There was no experience available to guide us in the resolution of this issue, so it was decided arbitrarily to closely monitor ionized calcium concentrations in the blood during the infusion of bisphosphonate. Furthermore, we would monitor the amounts of calcium required to be given intravenously in order to maintain blood ionized calcium concentrations in the normal range throughout the period of hospitalization in both the group receiving a bisphosphonate and the group receiving a saline placebo.

The final concern was growth. Given that children burned over 40 % of their body surface area have been shown to have retarded growth velocity most pronounced in the first post-burn year [12] might it be possible that the bisphosphonate will further impair bone growth by migrating to the growth plate and preventing osteoclasts from resorbing the cartilage that needed to be replaced by bone in order for bone growth to occur? Different bisphosphonates have different affinities for bone matrix.

The more tightly bound the drug to the bone matrix the less likely it will be to migrate to the growth plate. Thus, for example, in the case of oral bisphosphonates alendronate is more tightly bound to the matrix than is risedronate [13]. Therefore, risedronate would be more likely to migrate to the growth plate than alendronate. With regard to bisphosphonates for intravenous administration pamidronate did not affect bone growth in children with osteogenesis imperfecta and therefore we assumed its affinity for bone matrix was sufficiently strong so as it would not migrate to the growth plate. Based on the overall positive experience of the patients with osteogenesis imperfecta we selected pamidronate to use in this study of acute bisphosphonate administration to children who suffered severe burn injuries.

The Clinical Trials Themselves

The initial design of the study was that of a double-blind randomized controlled trial of administration of pamidronate at a dose of 1.5 mg/kg intravenously in 1 L of a 5 % dextrose and water solution over 12 h or of an equivalent volume of saline placebo. This calculated dose regimen was extrapolated downward from the 60–90 mg doses given to adult women with body weights ranging from 40 to 60 kg. It was mandatory that the first dose be administered within 10 days of the burn injury and following an initial dual energy X-ray absorptiometry (DXA) scan of the total body and lumbar spine with a second and final dose given 1 week after the first. The administration of the second dose did not always take place for a variety of clinical reasons, so the subjects in the experimental arm of the study received no more than two doses and no less than a single dose. Subjects were unblinded 6 months after the burn injury and preliminary results were evaluated [14]. Afterward the unblinded subjects were studied on follow-up visits to the outpatient clinic at 6-month intervals till they reached 2 years post-burn [15].

Parameters followed included total body bone mineral content, lumbar spine bone mineral content, and bone mineral density, as well as bone mineral density *Z* scores for age and sex at the conclusion of the study 2 years post-burn. In addition we evaluated blood ionized calcium concentrations and amounts of calcium administered intravenously that were necessary to maintain normal blood levels of ionized calcium. Intraoperative iliac crest bone biopsies with double tetracycline labeling were performed at 2 weeks and approximately 1 year post-burn. The study results were striking in terms of both which outcome parameters were affected and which were not.

In the blinded part of the evaluation by 2 months post-burn, or the approximate time of hospital discharge following treatment for the acute burn injury, total body bone mineral content did not differ significantly between pamidronate ($n = 18$) and placebo ($n = 15$) groups [15], although there was a trend toward increasing total body bone mineral content in the pamidronate group and a downward trend in the placebo group. These trends became significant at 6 months post-burn (Fig. 7.1) when total body bone mineral content demonstrated a 5 % increase from baseline in the pamidronate group ($n = 14$) and a 3 % reduction from baseline in the placebo group ($n = 11$).

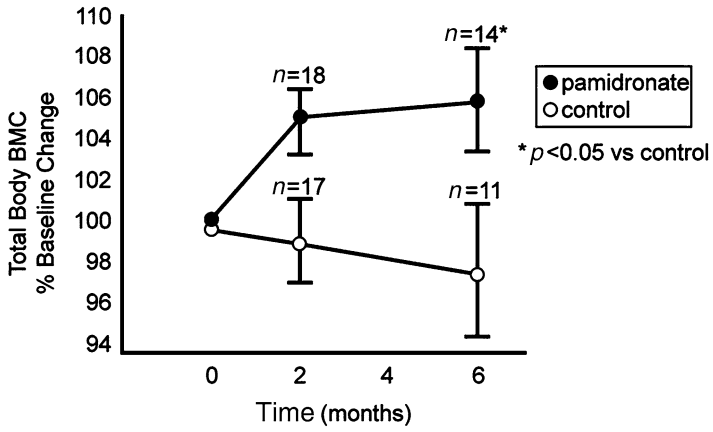

Fig. 7.1 Changes in total body bone mineral content (BMC) at discharge (2 months) and at 6 months post-burn, expressed as a percentage of baseline (admission) values. Data are shown as mean and standard error. Statistical significance versus control is designated by an asterisk. (Reproduced from Klein et al. [14] by permission of Springer Science + Business Media.)

Of interest is that this pattern of change was more striking in the lumbar spine, where bone mineral content at discharge increased by around 5 % in the pamidronate group ($n = 17$) and decreased by 7 % in the placebo group ($n = 12$), a difference that was statistically significant at hospital discharge. By 6 months post-burn lumbar spine bone mineral content rose by 10 % from baseline admission levels in the pamidronate group ($n = 7$) while stabilizing at 7 % below admission baseline in the placebo group ($n = 6$), also being significantly different from the pamidronate group (see Fig. 7.2).

Bone resorption at 2 weeks post-burn did not appear to differ between pamidronate and placebo groups either by measure of free deoxypyridinoline biomarkers in the urine or by quantitative iliac crest resorptive surface. This would ordinarily be surprising in light of the potential mechanisms of bone loss discussed earlier in the chapter. It is possible, however, that the evaluation methods were not ideal. In the case of the free deoxypyridinoline, it is not as sensitive to changes in resorption as total deoxypyridinoline, the assay for which was not available at the time this study was carried out. Also, the iliac crest consists of both cortical and trabecular bone, and inasmuch as cortical bone changes relatively little following burn injury compared to trabecular bone, it is possible that any increase in resorptive surface of the trabecular component may have been offset by the stability of the larger cortical component.

One other consideration regarding bone resorption must also be addressed here. That is that urine calcium excretion remained elevated in both placebo and pamidronate groups. Although the quantity of calcium excreted in the urine over 24 h was about 25 % lower in the pamidronate group at about 2 weeks post-burn the difference from the placebo group was not statistically significant. Could the increased urinary calcium excretion in both groups indicate increased resorption?

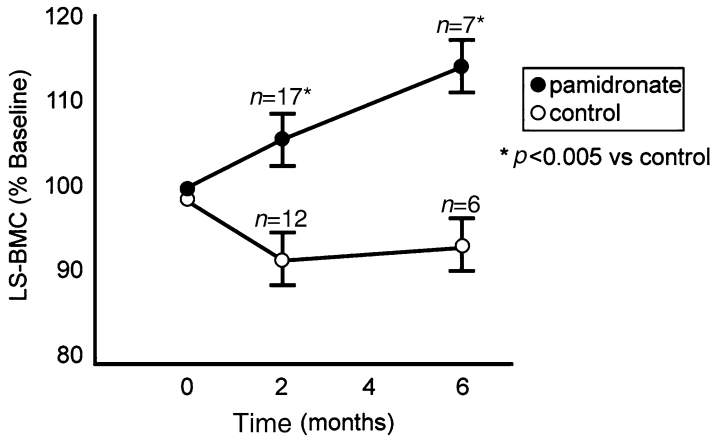

Fig. 7.2 Changes in lumbar spine (LS) bone mineral content (BMC) at discharge (2 months) and at 6 months post-burn, expressed as a percentage of baseline (admission) values. Data are shown as mean and standard error. Statistical significance versus control is designated by an asterisk. (Reproduced from Klein et al. [14] by permission of Springer Science + Business Media.)

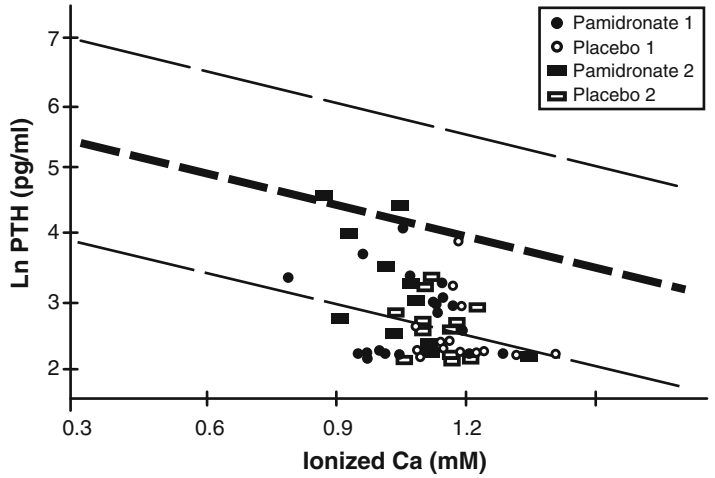

Fig. 7.3 Nomogram depicting the mean and 99 % confidence interval of the natural logarithmic (ln) PTH response to blood ionized calcium levels (expressed as mM). The majority of PTH values remain below the 99 % confidence interval for ionized calcium regardless of administration of placebo or pamidronate. The designation 1 refers to baseline values and the designation 2 refers to hospital discharge. (Reproduced from Klein et al. [14] by permission of Springer Science + Business Media.)

While it is tempting to think so interpretation of the hypercalciuria is confounded by the inappropriately low levels of parathyroid hormone (PTH) in the blood in response to the low circulating ionized calcium concentrations (Fig. 7.3). This hypocalcemic hypoparathyroidism, which has previously been noted [10], is likely due to

the inflammatory cytokine-mediated up-regulation of the calcium-sensing receptor of the parathyroid gland, which has been documented to occur in a sheep model of burn injury [11]. The consequence of this up-regulation is that a lower, often abnormally low, level of circulating calcium is sufficient to suppress PTH secretion by the parathyroid glands and the resultant hypoparathyroidism is permissive of urinary calcium excretion. Therefore, at the present time it is unclear if the hypercalciuria reflects bone resorption or simply the oversupply of calcium given intravenously in an attempt to correct the hypocalcemia. What was shown in this present study, however, is that the amount of calcium administered to patients receiving pamidronate and those receiving placebo was not significantly different in the attempt to achieve normal circulating levels of ionized calcium. Therefore, by blocking bone resorption pamidronate is not exacerbating existing hypocalcemia and its lack of effect in reducing hypercalciuria underscores the likelihood that the source of the urinary calcium excretion is the parenterally administered calcium. One additional consideration is the timing of the urine collection. Inasmuch as patients underwent the urine collection by 10 days post-burn it is possible that at the time of collection active resorption was slowing down due to onset of osteoblast apoptosis. In this case the urinary calcium from the placebo group might have been higher if it had been quantitated earlier post-burn and the differences in urinary calcium excretion between the two groups might then have reached statistical significance.

Now that we have addressed though not solved the bone resorption issues raised by this study, we must address the finding that pamidronate failed to prevent the loss of osteoblasts as seen on histomorphometry of the specimens taken from the iliac crest. Given this observation how do we explain the continued gain in bone mineral content over 6 months' time in the burned children receiving pamidronate, especially in the lumbar spine region?

Studies have shown that bisphosphonates have antiapoptotic properties [16]. While this may be the case the failure to preserve osteoblasts at the bone surface at 2 weeks post-burn must be addressed. One possibility that was not examined by this study is that the antiapoptotic properties become manifest at a later time than the antiresorptive properties. This may be plausible due to initially large quantities of endogenous glucocorticoids produced by the adrenal glands in response to the stress of the burn injury [1, 4]. As these levels drop off it is possible that the antiapoptotic activity of pamidronate is unmasked. This explanation may have been better addressed by examining bone biopsies between 2 weeks and the 1 year post-burn follow-up specimens or by systematically and sequentially examining biomarkers of bone formation. These data were not available in the study discussed here. Another possible explanation would be that the antiapoptotic properties of pamidronate are more clearly discernible in purely trabecular bone, such as in lumbar spine. That possibility also needs to be pursued.

While it would appear that pamidronate acts only on bone and on no other aspect of the abnormal calcium homeostasis brought about by burn injury, data on muscle mass, which were available from total body DXA studies, were not included in the data analysis or the publication. Given the present degree of interest in the interaction between muscle and bone this oversight requires attention. While it is clear that

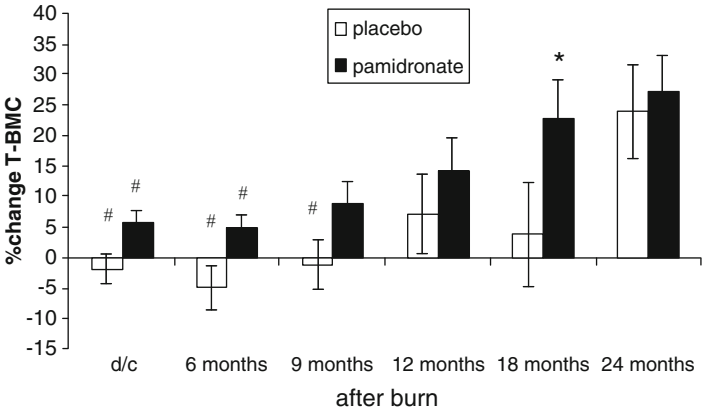

Fig. 7.4 Percent change in total bone mineral content from baseline to 24 months after burn (d/c: hospital discharge). Values are means and standard error of the means. Significant differences between placebo and pamidronate are designated by *asterisk* with $p<0.05$. Significant time effects within a group when compared to changes at 24 months are designated by *hash*, with $p<0.05$. (Reproduced from Przkora et al. [15] with permission of Elsevier.)

during this 6-month time period the catabolic response and likely the heightened production of endogenous glucocorticoids as part of the stress response produce negative nitrogen balance and muscle wasting [17] it is not certain whether the anti-resorptive activity of the bisphosphonates and the positive effect they have on bone mineral content by whatever mechanism could attenuate the loss of lean body mass. If not, then bisphosphonates can be directly targeted to reduce bone loss without any likelihood of affecting any other adaptive response. If there were to be an attenuation of muscle wasting concomitant with improvement in bone mineral content then perhaps bisphosphonates could play an adjunctive role in the limiting of post-burn catabolic response as well.

We will next examine the longer-term effects of the acute administration of pamidronate up to 2 years following the burn injury. Subjects were unblended after 6 months but new subjects had also been added to both groups in randomized fashion to provide a total of 57 subjects, 32 having received pamidronate and 25 having received saline placebo. Enrolled children were followed every 6 months with DXA studies. After 2 years attrition resulted in having a complete set of values in only 21 subjects, 8 of whom received pamidronate and 13 of whom received saline placebo. There appeared to be no attribution of dropout to any side effects of pamidronate.

The results demonstrated that total body bone mineral content as a percentage change from acute admission values exhibited a steady increase over the 6-month intervals until reaching 2 years post-burn in those who were given pamidronate. In contrast those subjects who had received placebo remained significantly behind in the total body accretion of bone mineral content until 18 months post-burn, at which point the percentage change from admission rapidly rose to equal the percentage change from admission in the experimental group (see Fig. 7.4).

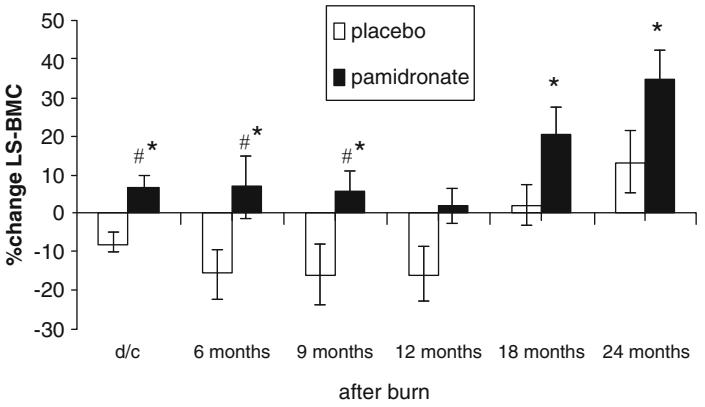

Fig. 7.5 Percent change in lumbar spine bone mineral content (d/c: hospital discharge). Values are presented as means and standard error of the means. Significant differences between placebo and pamidronate are designated by *asterisk* with $p<0.05$. Significant time effect within each group when compared to changes at 24 months is designated by *hash*, with $p<0.005$. (Reproduced from Przkora et al. [15] with permission of Elsevier.)

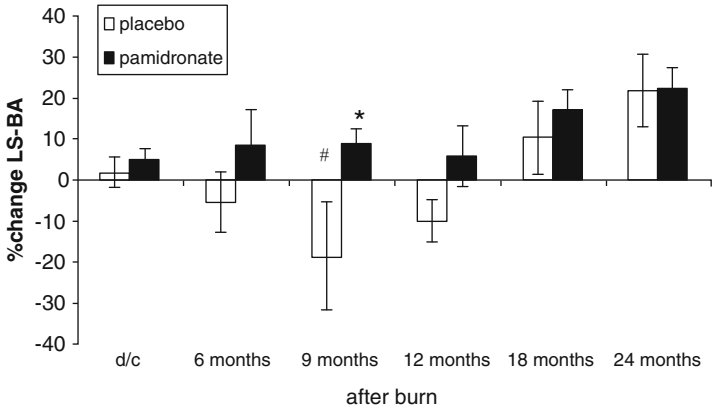

Fig. 7.6 Percent change in lumbar spine bone area from baseline to 24 months after injury (d/c: hospital discharge). Values are presented as means and standard error of the means. Significant differences between placebo and pamidronate are marked with *asterisk* with $p<0.05$. Significant time effect within each group when compared to changes at 24 months is designated by *hash*, with $p<0.05$. (Reproduced from Przkora et al. [15] with permission of Elsevier.)

As was seen at 6 months post-burn in the acute study [14], the beneficial effect of pamidronate was more pronounced in the trabecular bone of the lumbar spine. In the lumbar spine the significant difference between pamidronate and control groups in bone mineral content but not bone area persisted for the entire 2-year follow-up period (see Figs. 7.5 and 7.6). With lumbar spine bone mineral density the significant difference in percentage change persisted throughout the first 12 months

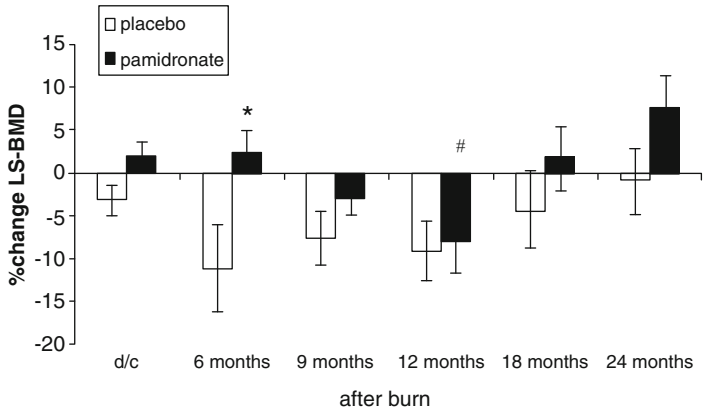

Fig. 7.7 Percent change in lumbar spine bone mineral density when measured by dual energy X-ray absorptiometry (d/c: hospital discharge). Values are means and standard errors of the means. Significant difference between placebo and pamidronate is designated by *asterisk*, with $p<0.05$. Significant time effect within each group when compared to changes at 24 months is designated by *hash*, with $p<0.05$. (Reproduced from Przkora et al. [15] with permission of Elsevier.)

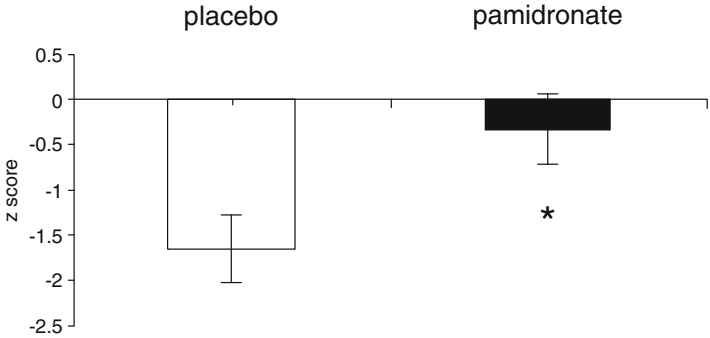

Fig. 7.8 Lumbar spine bone mineral density *Z* scores at 2 years after burn. Note *Z* scores represent the number of standard deviations from normal, healthy age- and sex-matched children. Values are displayed as means and standard deviation of the means. Significant difference between pamidronate and placebo is designated by *asterisk*, with $p<0.05$. (Reproduced from Prkzora et al. [15] with permission of Elsevier.)

post-burn and while statistical significance was not observed between the two groups at 18 and 24 months post-burn the differences trended in the same way as during the first year and the failure to demonstrate statistically significant differences between pamidronate and placebo groups at these time points may be attributable to the reduced number of subjects in each group and the large variability in percentage change from admission during that second year post-burn (see Fig. 7.7). More to the point, however, the significant difference in lumbar spine bone mineral density *Z* scores between the pamidronate and placebo subjects at 2 years post-burn (see Fig. 7.8) speaks to the persistence of the beneficial effect of acute pamidronate

administration for at least 2 years on the bone mineral content and bone mineral density of burned children.

Finally, this study examined changes in bone histomorphometry in both pamidronate and placebo groups of subjects from 2 weeks post-hospital admission for acute severe burn injury to 12 months following the burn injury. We measured static parameters of bone turnover, including bone area, osteoid area, surface, and width, all measures of matrix calcification, and eroded surface, a measure of resorption. We also measured the dynamic parameters of bone formation, such as mineral apposition rate and bone formation rate based on double tetracycline labeling.

What was a clear-cut outcome of this part of the study was that by 12 months post-burn bone remodeling resumed in the 16 patients, 8 who received pamidronate and 8 who received placebo, who took up at least a single tetracycline label (see Fig. 7.9). There were no significant differences in either static or dynamic parameters of bone histomorphometry mainly because the range of normal values in pediatric patients is so widely variable. Furthermore, a variety of factors may have contributed to the histological picture seen in these patients.

Among the variables that may have affected the acute bone histomorphometry are the precise timing of bone resorption and the shutdown of bone formation in individual subjects, the quantity of endogenous glucocorticoid production, and the intensity and duration of the inflammatory response. It is unclear how any of these factors might have influenced the resumption of bone remodeling at 1 year post-burn, nor do we know with certainty whether the rate of bone remodeling is uniformly normal by that time as too few specimens have been quantitated.

Summary of Findings

In summary, the timely intravenous administration of one or at most two doses of pamidronate actually prevented acute post-burn bone loss and permitted serial increases in both cortical and trabecular bone mineral content as well as in trabecular bone mineral density. These results are consistent with acute resorption being the chief mechanism of post-burn bone loss for reasons previously discussed and they also suggest that bisphosphonates given intravenously have not only an antiresorptive effect but also an osteoprotective effect, whether it be by an antiapoptotic mechanism or by some other as yet unidentified mode of action.

While there are no data currently available as to whether bisphosphonates exert any secondary effects on muscle catabolism in the setting of acute burn injury, or whether they exert any effects on any other aspects of calcium homeostasis, it must be assumed for the present that the changes observed following bisphosphonate administration are bone-specific. These findings are summarized by the diagram shown as Fig. 7.10.

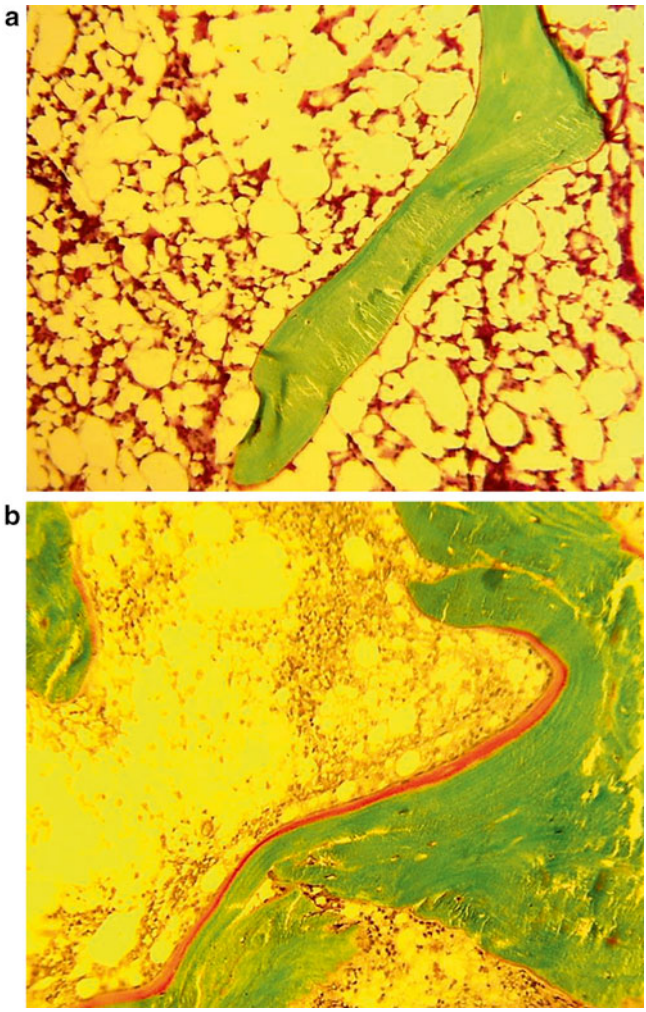

Fig. 7.9 (**a**) A Goldner trichrome stain of the iliac crest at 100× magnification. The sample was taken at approximately 2 weeks post-burn. Note the absence of a significant osteoid seam. (**b**) Iliac crest bone biopsy from the same patient at 12 months following burn injury. Note the presence of a long, smooth lamellar seam lined with active osteoblasts. These photomicrographs were provided courtesy of Susan M. Ott MD, Department of Medicine, University of Washington School of Medicine, Seattle, WA. (Reproduced from Przkora et al. [15] with permission of Elsevier.)

Potential Applications and Implications

The manner in which the bisphosphonate administration was shown to be effective in preventing bone loss, i.e., the acute one-time, or at most two-time, intravenous dosing, may offer prospects for success in managing other types of acute bone loss that do not result from chronic conditions in pediatrics.

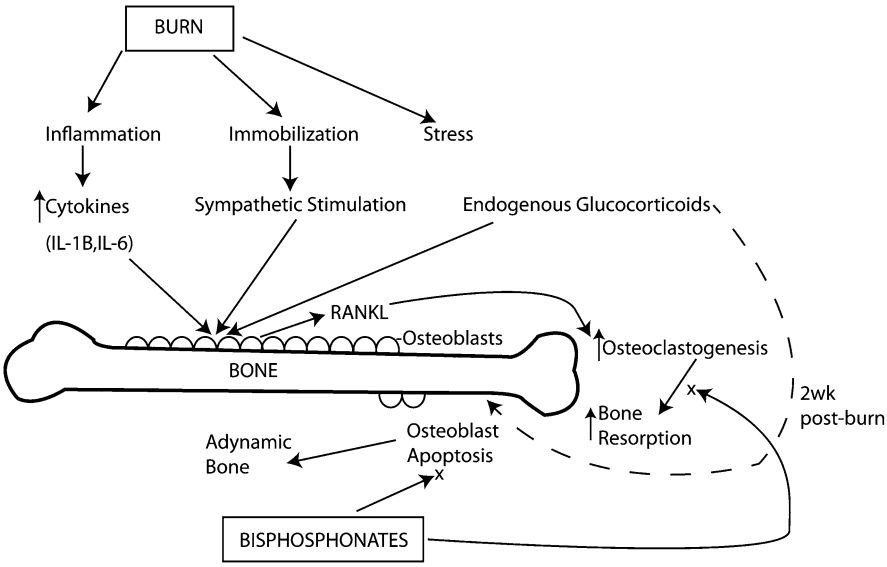

Fig. 7.10 A schematic diagram illustrating the presumptive effects of bisphosphonates on both post-burn bone resorption and bone formation

Consideration might be given for bisphosphonate use in clinical trials for conditions in which bone loss occurs as a consequence of an isolated event, for which a single dose of bisphosphonate might prevent bone loss from ever occurring. Thus conditions that might be appropriately considered for acute use of bisphosphonate as prevention might be the initiation of glucocorticoid therapy or pre-transplantation of solid organs, such as kidney, heart, or liver. In both settings glucocorticoid administration may play a role in bone loss along with the unspecified roles of other immunosuppressives given concomitantly. In fact successful bisphosphonate treatment for glucocorticoid-associated bone loss has already been employed in adults and now has approval from the United States Food and Drug Administration (FDA) as an indicated therapeutic use.

Despite receiving FDA approval for use in adults, no such clinical trials have been undertaken in children even though the incidence of pediatric vertebral fracture is 6 % in children with rheumatologic conditions treated with glucocorticoids [18]. Furthermore, a single oral or intravenously administered dose of alendronate was shown by Nakhia et al. [19] to have a bioavailability that was similar to children receiving this treatment for osteogenesis imperfecta and the single dose received by the subjects was reported to be generally well tolerated.

With regard to post-transplant bone loss, a study by Mitterbauer and colleagues [20] entailing a meta-analysis of five randomized controlled trials of bisphosphonates in 180 subjects found that bone loss at the lumbar spine was significantly attenuated and bone loss at the femoral neck demonstrated a trend toward becoming significantly lower in those subjects receiving bisphosphonates. More recently

Torregrossa et al. [21] showed that a single dose of 30 mg pamidronate given intravenously at the time of renal transplantation and repeated 3 months later significantly reduced lumbar spine bone loss and normalized biomarkers of bone formation compared to controls. The authors found no difference between the groups in terms of fracture incidence. However, this conclusion was drawn because no subject enrolled in the studies experienced a fracture during the relatively short period of follow-up. The data for brief acute use of bisphosphonates in renal transplantation patients thus appear promising, but no studies have been published regarding pediatric patients who have undergone solid organ transplantation. On the basis of the findings reported in pediatric burns patients perhaps the same type of study should be considered in the former category.

Single-dose treatment with a bisphosphonate has the theoretical advantage of limiting toxicity given the infrequency of administration. However, the implication of the successful use of limited-dose therapy with bisphosphonates for pediatric burn victims is that other conditions which involve the inflammatory or stress responses, or perhaps immobilization as well, may also render patients susceptible to subclinical bone loss by means of these same operative nonspecific adaptive responses.

Therefore, it would be instructive to consider designing studies in which bisphosphonates are used as a treatment adjunct in conditions such as pediatric rheumatoid arthritis, inflammatory bowel disease, and various connective tissue disorders, such as lupus, in which not only may there be an acute inflammatory response but also acute exacerbations may be treated with glucocorticoids. Lack of randomized clinical trials involving large numbers of subjects is the chief impediment to obtaining the data needed to ultimately evaluate the safety and efficacy of this class of drugs in children [22]. Until the data available become adequate for such an evaluation, the debate over the use of this class of drugs in children will continue.

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Chapter 8
Growth Hormone and Bone

Daisuke Harada and Yoshiki Seino

Physiology of Growth Hormone

Regulatory Mechanism of GH Secretion

Daily rhythmical growth hormone (GH) secretion controls its serum concentration. A total of 70 % of daily GH secretion occurs with the first episode of slow-wave sleep [1]. GH secretion is also affected by serum glucose density, amino acids, free fatty acids, drugs, and GH itself. These various conditions stimulate the hypothalamus and regulate secretion of three hypothalamic hormones, growth hormone releasing hormone (GHRH), ghrelin, and somatostatin. These signals eventually control GH secretion from GH-producing cells, expressing the specific receptors for them, in the pituitary gland [2] (Fig. 8.1).

GHRH selectively induces GH secretion from the pituitary gland through the GHRH receptor [3, 4]. Somatostatin suppresses GH pulse amplitude and frequency, and inhibits central GHRH release via direct synaptic connections with hypothalamic neurons, but does not affect GH biosynthesis [2]. Hypothalamic GHRH and somatostatin are secreted in independent waves and interact to generate pulsatile GH release together with additional GH secretagogues (GHS). Ghrelin binds to the GHS receptor to induce hypothalamic GHRH and pituitary GH [5, 6]. The greatest amount of ghrelin is secreted from gastric cells rather than from the hypothalamus. Plasma ghrelin concentrations increase when fasting, and decrease after food intake [7].

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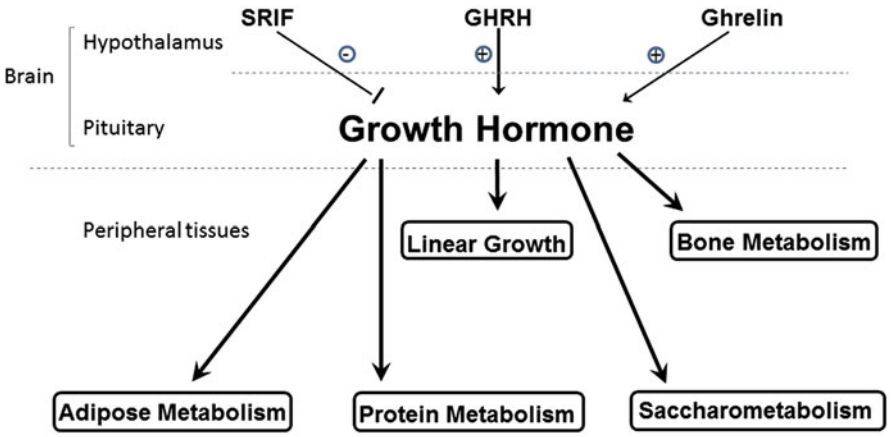

Fig. 8.1 Stimulation and action of intrinsic GH. GH is secreted in the pituitary gland and stimulated by hypothalamic hormones, such as GHRH and ghrelin, and is suppressed by somatostatin. GH acts on many peripheral tissues and plays a role in linear growth, bone metabolism, adipose metabolism, protein metabolism, and saccarometabolism

Mechanism of GH Action

GH stimulates linear body growth through differentiation and proliferation of the cells in the growth plate in children. GH also acts on many peripheral tissues other than the growth plate, and plays important roles in homeostasis, such as glycemic effects, hydration, protein anabolism, and lipid degradation [8] (Fig. 8.1).

At least part of the growth effect by GH is through endocrine, autocrine, and paracrine mechanisms of insulin-like growth factor I (IGF-I). GH action in body growth may be explained through three pathways involving IGF-I. In one pathway, GH acts through GH receptor (GHR) expression in hepatocytes and generation of IGF-I [9]. Consequently, serum IGF-I levels increase and IGF-I acts on peripheral tissues as a hormone by an endocrine mechanism. In a second pathway, GH acts on peripheral tissues, not the liver, promoting IGF-I generation, and this IGF-I affects local tissues by an autocrine/paracrine system [10]. Expression of GHR, IGF-I, and IGF-I receptor has been detected in chondrocytes, osteoblasts, osteoclasts, myocytes, and adipocytes. In a third pathway, GH affects peripheral tissues directly. For Laron syndrome in which there is deficiency of GHR, extrinsic IGF-I administration does not have a sufficient effect on growth in spite of its biological activities, such as improvement of hyperglycemia [11]. This phenomenon implies a direct action of GH.

GH Treatment

History

In the 1950s, human GH was first used to stimulate linear growth in a child with hypopituitarism [12]. At that time, GH was extracted and purified from the pituitary grand. Because the supply of the extracted GH was limited, GH treatment was restricted to children with the most severe and unequivocal GH deficiency (GHD). Delays in diagnosis and treatment, interruptions in treatments, and dosage restrictions were common during this time. Consequently, while GH accelerated growth of these individuals, adult height was usually less than average [13–15].

In 1985, Creutzfeldt–Jakob disease (CJD) was recognized in patients who had received GH. Distribution of pituitary-derived GH was stopped. Subsequently, in the United States, CJD was diagnosed in seven recipients of GH [16, 17]. Fortunately, 192- and 191-amino-acid biosynthetic GHs were approved in 1985 and 1987, respectively. The production of GH by biological systems transplanted with the *GH* gene yields a virtually unlimited supply of GH.

Biosynthetic GH treatment eliminated the risk of CJD and offered children with severe GHD an opportunity for optimal treatment. Children with milder forms of inadequate GH secretion, previously excluded from receiving GH, could become treated. In addition, metabolic effects of GH, apart from linear growth promotion, are now being studied extensively, leading to new indications for GH treatment [18].

Approved Disorders and the Efficacy of GH Treatment

Approved disorders for GH treatment have been expanding in the world in spite of its high cost, with expectations of promoting linear growth. Currently, these growth disorders are GHD, short children with small for gestational age (SGA), Turner syndrome (TS), chronic renal insufficiency (CRI), Prader–Willi syndrome (PWS), short stature homeobox (SHOX) haploinsufficiency, achondroplasia (ACH), hypochondroplasia (HCH), Noonan syndrome (NS), and idiopathic short stature (ISS) (Table 8.1). The type of approved disorders, criteria of diagnosis, and treatment dose vary and depend on the country.

GH treatment was started primarily for classical GHD patients to promote linear growth. Untreated patients with GHD have profound short stature, averaging nearly −5 standard deviation (SD) [19–21]. In many countries, pediatric endocrinologists have developed guidelines for diagnosis, criteria for starting treatment, treatment regimens, criteria for continuing treatment, and criteria for finishing treatment. GH treatment in GHD patients gradually improved their adult height SD score by approximately −1.3 SD, although most patients failed to reach their genetic target heights [22, 23].

Table 8.1 Approved diseases for GH in various countries as of 2013

	GHD	Adult GHD	SGA	TS	CRI	PWS	SHOX haploinsufficiency	ACH	HCH	NS	ISS
USA	○	○	○	○	-	○	○	-	-	○	○
UK	○	○	○	○	○	-	○	-	-	-	-
France	○	○	○	○	○	○	○	-	-	-	-
Germany	○	○	○	○	○	○	○	-	-	-	-
Sweden	○	○	○	○	○	○	-	-	-	-	-
Japan	○	○	○	○	○	○	-	○	○	-	-
Taiwan	○	○	○	○	-	-	-	-	-	-	-
Australia	○	○	-	○	○	-	-	-	-	-	-

○: Approved, -: not approved; *USA* United States of America, *UK* United Kingdom of Great Britain, *GHD* growth hormone deficiency, *TS* Turner syndrome, *CRI* chronic renal insufficiency, *SGA* small for gestational age, *PWS* Prader–Willi syndrome, *ACH* achondroplasia, *HCH* hypochondroplasia, *NS* Noonan syndrome, *ISS* idiopathic short stature

SGA is a term used to describe a neonate's birth size based upon appropriate auxological standards for healthy infants. Approximately 86 % of SGA children achieve a length within the normal range by 12 months [24, 25]. Catch-up growth in the normal range is virtually always complete by 2 years of age [26]. Overall, 8–14 % of SGA infants become short in stature with an adult height of approximately 1 SD [27, 28]. SGA children achieve a final height within the normal height range after 7.8 years of GH treatment [29]. The effects of GH extend beyond linear growth and potentially include important effects on body composition, muscle mass and function, bone mass, metabolism, behavior, and cognitive function, and even quality of life, IQ, and bone mineral content [30, 31].

TS is characterized by short stature, cubitus valgus, webbing of the neck, and sexual infantilism [32]. Over 95 % of TS patients eventually fall below the −2 SD, and their adult height is typically approximately 20 cm below the mean for females of their respective ethnic group. GH treatment in TS patients improves their final height to 8.5 cm above the mean projected adult height and there is a mean height gain due to GH of +7.2 cm [33, 34].

Growth failure is still a major obstacle to successful rehabilitation of children with CRI. The mean height SD score at the start of renal replacement therapy is approximately −2, indicating that half of the patients have a short stature [35, 36]. Similarly, the mean final height SD score of CRI patients is reported to be significantly reduced and varies between −1.4 in girls and −2.2 in boys in various reports [37, 38]. GH treatment for short stature in CRI became available approximately 20 years ago [39]. The final height of CRI patients after extended GH treatment appears to be an average of 1.0–1.5 SD [40, 41].

PWS is a neurogenetic disorder characterized by mental and physical abnormalities. The mean adult height achieved by men and women with PWS is 155–162 and 148–150 cm, respectively [42, 43]. The GH-deficient state commonly associated with PWS, as evidenced by reduced GH secretion, low serum IGF-I levels, and clinical features typical of GHD, has provided a rationale for trials assessing the

efficacy of GH treatment. However, currently, the duration of treatment is limited. Longitudinal growth has been shown to increase by GH treatment in PWS [44, 45]. Some reports have shown that growth continues to improve by GH treatment in PWS, with the result that the target height SD score can be reached [46].

NS is a genetic syndrome with many features similar to TS, and is characterized by pulmonary valvular stenosis, visual problems, clotting disorders, and short stature [47]. Although approximately half of NS patients will reach an adult height within 2 SD of the population mean, the mean adult height of NS is approximately 162.5 cm and 153 cm for males and females, respectively [48]. GH treatment in NS improves the final height SD score to 1.7 [49]. Additionally, pretreatment baseline cortical bone mineral density (BMD) is reported to be in the low–normal range and it increases over 2 years of GH therapy [50]. In the majority of reports, GH treatment induced catch-up growth in most of the NS patients. First data on long-term outcome demonstrate an effect comparable with or even better than that in TS.

ISS is a purely descriptive term that refers to a child, adolescent, or adult with a height below the age reference for population and sex, in whom, with current diagnostic tools, no etiological diagnosis is made [51]. The mean final height is similar to the mean predicted height in ISS. There is a large interindividual variation that is primarily correlated with the initial height SD score and bone age delay at start of GH treatment [52]. GH for ISS in a supraphysiological dosage increases the final height by approximately 7 cm, but for the individual child, the height gain is difficult to predict.

Side Effects

Recombinant biosynthetic GH preparations are highly purified and free of contaminants. The possibility of viral transmission through GH has been virtually eliminated. Antigenicity of GH preparations is also low, although GH antibodies can be detected in 10–30 % of treated children [53]. With rare exceptions (less than 0.1 %), these antibodies do not impede effects of GH.

Laboratory indications of hypothyroidism can be found in as many as 25 % of GHD children treated with GH [54]. GHD patients, who display subnormal nocturnal thyroid-stimulating hormone surges, signifying preexisting central hypothyroidism, are more likely to display subnormal T4 and free T4 levels during GH therapy [55]. However, most studies have indicated that children with normal thyroid function before treatment do not develop significant perturbations in thyroid hormone metabolism during GH therapy.

Administration of unphysiological high concentrations of GH may lead to defects in glucose metabolism [56]. When intrinsic GH secretion is increased, as in sleep, oral or intravenous glucose tolerance tests show a defect in glucose metabolism [57]. This defect of glucose metabolism lasts even after finishing GH treatment and normalization of serum GH concentrations.

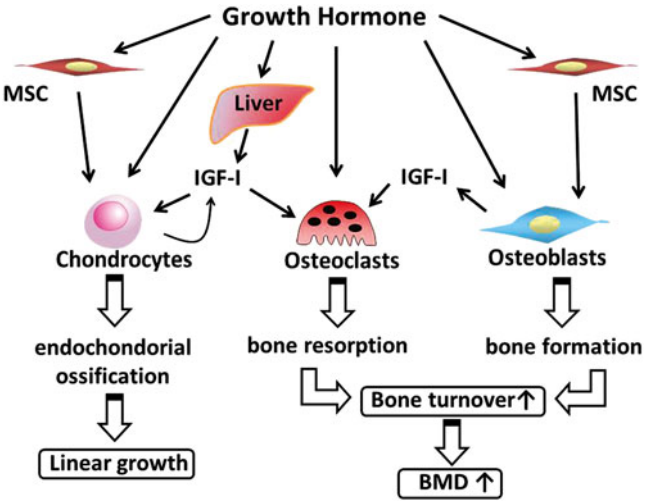

Fig. 8.2 Schematic representation of bone metabolism by GH. GH stimulates differentiation and proliferation of chondrocytes directly and through IGF-I synthesis. This endochondral ossification leads to linear growth. GH also stimulates differentiation and proliferation in osteoblasts and osteoclasts. Consequently, GH affects bone metabolism and, consequently, linear growth and bone mineral density

Edema and sodium retention rarely occur early in the course of GH therapy, which is attributable to an anti-natriuretic effect on the renal tubules of GH and/or IGF-I. Minor elevations in plasma renin activity and aldosterone observed in the first 3 days of treatment resolve within 1 or 2 weeks [58]. Occasionally, fluid shifts within the central nervous system are sufficient to cause benign intracranial hypertension, with symptoms of headache, visual loss, vomiting, and papilledema. Direct fluid-retaining properties of GH and/or action of locally produced IGF-I on cerebrospinal fluid production are speculated to be causative. Cessation of GH therapy reverses symptoms in spite of continued GH treatment [59]. Resumption of GH treatment has been successfully accomplished with re-initiation at a lower dosage and a gradual return to the initial dosage. Performing a fundoscopic examination is recommended in all patients before initiation of GH therapy and periodically thereafter [60].

Growth Hormone and Bone

Effect of GH on Bone and Cartilage Metabolism

GH acts directly on the perichondrial layer in the growth plate of growing bones, and promotes proliferation and differentiation of pre-chondrocytes, as well as promotes IGF-I synthesis (Fig. 8.2). Pre-chondrocytes proliferate and differentiate to

chondrocytes in the proliferative zone of growth plates by acquiring the ability for reaction to IGF-I and for generation of IGF-I [61].

Osteoblasts express GHR and IGF-I receptor, and have the ability of generating IGF-I [62]. Therefore, GH promotes synthesis of IGF-I in osteoblasts, and IGF-I acts on these cells through the autocrine/paracrine system. In osteoblastic culture, IGF-I stimulates differentiation of osteoblasts to osteocytes by promoting proliferation of osteoblasts, expression of type I collagen, and activation of alkaline phosphatase, and by suppressing expression of matrix metalloproteinase 1 [63]. However, it is still unclear whether GH action on osteoblasts occurs through IGF-I or there is a direct pathway.

GH action is also detected in osteoclasts. Precursors of osteoclasts express GHR, and GH promotes their differentiation to osteoclasts. Factors promoting osteoclast differentiation are generated by GH-stimulated osteoblasts and bone marrow cells [64]. These findings show that GH promotes bone resorption through its direct effect on bone marrow cells or through osteoblasts. In fact, when GH is administered in pediatric patients, bone resorption markers are elevated before growth is detected and bone formation markers are elevated.

GH promotes bone turnover, including bone generation and bone resorption; GH consequently promotes longitudinal bone growth while maintaining BMD suitable for increasing quantities of bone (Fig. 8.2). Since GH also increases mass and strength of the skeletal muscles, mechanical stress may be another factor for GH effect on increasing BMD [65]. Although the effect of GH on BMD is still controversial in certain conditions such as burn injury, BMD is indeed correlated with nocturnal GH secretion in young healthy men and acromegaly [66, 67]. Moreover lumbar BMD is reduced in pediatric GHD patients, and GH treatment increases BMD in GHD and other diseases [68–71].

For the considerable variability in response to GH treatment, several prediction models that attempt to estimate the growth response to GH treatment have been developed [72–75]. As a result, in growing children, markers of bone metabolism reflect skeletal growth and development. For example, urinary deoxypyridinoline and serum pyridinoline, bone resorption markers, are strongly related to height velocity. These results imply that bone metabolism and linear growth are closely related to each other.

Approved GH Treatment in Skeletal Dysplasia

Skeletal dysplasia is a heterogeneous group of diseases affecting the skeleton. The estimated incidence is 30–45 in every 100,000 newborns. The final height differs substantially between the various disorders, but is often in the range of 110–130 cm [76]. Currently, although a remarkably short stature has been detected in various skeletal dysplasias, only three skeletal dysplasias have been approved for GH treatment: ACH, HCH and SHOX haploinsufficiency [77] (Table 8.1).

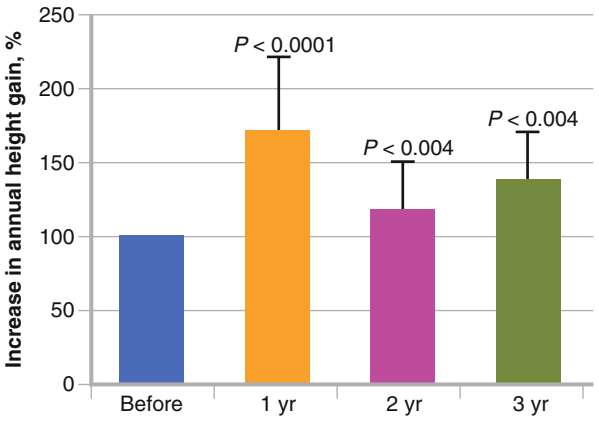

Fig. 8.3 Short-term effect of GH treatment in achondroplasia (ACH). The graph shows the percentage increase in height in ACH patients. Growth velocity is increased when GH treatment is started and is maintained at a higher level than that before GH treatment during 3 years [83]

ACH is the most common type of rhizomelic short-limb dwarfism caused by activating point mutations in the fibroblast growth factor receptor 3 (*FGFR3*) gene [78, 79]. The incidence of ACH is estimated as 1 in 25,000 live births. The average adult height of ACH is approximately 132 cm (−6.8 SD) for males and 124 cm (−6.4 SD) for females [80]. FGFR3 is expressed in the growth plate, and its activation suppresses IGF-I expression and cell proliferation, and promotes apoptosis of chondrocytes. GH administration increases *IGF-I* expression in chondrocytic cell lines expressing mutated *FGFR3* and prevents these cells from apoptosis [81]. This could explain one of the mechanisms by which GH therapy improves disturbed bone growth in ACH.

GH treatment in ACH has been approved only in Japan, since 1997. As a short-term effect, GH administration increases height velocity from (mean ± SD) 3.8 ± 0.9 to 6.6 ± 1.6 cm/year in patients with ACH for at least 6 months [81]. In longer-term studies, GH treatment in ACH patients promotes their height velocity in the first treatment year and promotes their linear growth, with a gain of 1–1.5 SD over 3–6 years, although height velocity is low after the second year of treatment (Fig. 8.3) [82–84]. More than 15 years have passed since approval, but reports on the long-term effect of GH on ACH regarding the prognosis of height and bone mineral metabolism have still not been published.

HCH is also mainly caused by mutations of the *FGFR3* gene and is characterized by short stature and abnormal body proportions, although not as severe as in ACH. The final height in HCH is compromised and in the range of 132–147 cm [85, 86]. GH treatment for HCH has been approved only in Japan at the same time as ACH in 1997. Several reports have shown that the median height SD score is approximately −3.2 SD at the start of GH therapy for HCH and it improves plus 1 SD after 2–5 years of GH treatment [87, 88]. Because some HCH patients have no mutation in the *FGFR3* gene, but characteristic facial features, bone deformities, and disproportionate short stature are observed, there are still some doubts as to the certainty

of the diagnosis in some of the patients diagnosed with HCH. Therefore, clinical studies of GH treatment, including genetic background data, are required.

Dyschondrosteosis, or Leri–Weill syndrome, is a mesomelic skeletal disorder caused by a deletion or mutation in the *SHOX* gene [89]. In dyschondrosteosis, there are abnormal proportions due to short legs, and the adult height in these individuals is variable, but in most patients it is reduced. However, a reduction in height appears to be sex-specific, with a greater loss of height in females compared with males [90]. Isolated SHOX haploinsufficiency is observed in 56–100 % of patients with Leri–Weill dyschondrosteosis and in 1–14 % of ISS [91]. Short stature observed in patients with TS is partially explained by haploinsufficiency of the *SHOX* gene [92]. Because GH treatment in TS improves the final height SD score, GH treatment in patients with SHOX haploinsufficiency has been approved in some countries. Prepubertal children with isolated SHOX defects treated with GH during 2 years present with a similar growth response to that of TS patients [93] and reach their final height with a height SD score gain of 1.1 ± 0.7 after 4.7 years [94]. The gain in the height SD score during the first year of GH therapy for patients with SHOX haploinsufficiency shows an increase of 0.7 SD [95]. The sitting height ratio SD score does not change during 1 year of GH treatment in patients with SHOX haploinsufficiency. Adult height in GH treatment for dyschondrosteosis has not been published yet.

Challenging Trials of GH Treatment

GH treatment has been attempted in many diseases with short stature, such as Down syndrome, Cornelia de Lange syndrome, Kabuki syndrome, Fanconi anemia, Rubinstein–Taybi syndrome, Klippel–Feil syndrome, Diamond–Blackfan anemia, and skeletal dysplasia. We discuss below regarding GH treatment in skeletal dysplasia, focusing on GH and bone, such as osteogenesis imperfecta (OI) and X-linked hypophosphatemic (XLH) rickets. Because the final height of each disorder has not been determined yet, further evidence of GH treatment in all challenging disorders needs to be gathered.

Osteogenesis Imperfecta

OI is an autosomal dominant disorder caused by dysfunction of type I collagen proteins. OI is characterized by congenital-decreased BMD, bone fragility, short stature, blue sclerae, progressive bone deformities, and dentinogenesis imperfecta [96, 97]. Clinical severity varies widely from lethal to mild with non-deformity. Recently, OI patients were classified into eight types according to their severity [98, 99].

The most popular internal treatment of OI is bisphosphonates suppressing bone resorption. Bisphosphonates in OI children increase BMD and result in dramatically decreased bone fractures [97]. Because growth deficiency is constantly present in severe OI and common in mild to moderate forms of OI, GH could be used in OI for stimulating bone metabolism or for increasing linear growth [100, 101].

Although there are few reports of GH treatment in patients with OI, GH action positively affects bone growth and bone turnover by stimulating osteoblasts, collagen synthesis, and longitudinal bone growth [102, 103]. A recent study also suggested that combined bisphosphonate and GH treatment in OI patients for 1 year positively increases BMD and growth velocity, and does not affect the peripheral fracture rate [104]. Although GH treatment in OI has not been approved yet, GH may be expected to improve symptoms of OI patients.

X-Linked Hypophosphatemic Rickets

XLH rickets is the most common form of hereditary rickets and is characterized by short stature, rickets, osteomalacia, and hypophosphatemia [105]. XLH rickets is due to mutations in the phosphate-regulating gene with homologies to endopeptidases on the X chromosome (*PHEX*), which encodes a membrane-bound endopeptidase expressed in mineralizing tissues (i.e., bone and teeth) [106]. Although the precise function of PHEX protein still remains to be determined, inactivation of PHEX reduces serum phosphate levels by suppressing proximal tubular phosphate reabsorption and intestinal phosphate absorption though synthesis of fibroblast growth factor 23 [107]. This has been shown to be causative for renal phosphate wasting and diminished 1α-hydroxylation of 25(OH) vitamin D [108, 109].

Combined treatment with oral phosphates and activated vitamin D (calcitriol) has been shown to improve growth and skeletal abnormalities in XLH rickets [110–112]. Even with optimal medical treatment, many XLH rickets patients do not demonstrate catch-up growth to achieve normal stature [113–115]. Mean adult height in cohorts of treated XLH rickets patients ranges from −2.8 to −1.7 SD [116].

IGF-I increases *Phex* expression in bone and sodium-dependent phosphate cotransporter mRNA expression in the kidney, and increases circulating phosphate concentration through these two mechanisms [117]. Previous studies have shown that administration of GH increases renal tubular phosphate reabsorption and serum concentrations of $1,25(OH)_2D$, suggesting that GH is involved in phosphate homeostasis and in renal 1α-hydroxylation of vitamin D through IGF-I [118, 119]. Although some studies have shown that GH treatment increases the growth rate of XLH rickets patients, some studies have suggested that GH might make deformities of XLH rickets worse. The effect of GH in XLH rickets is still controversial [118–122]. Further investigation may be able to add GH treatment to the standard choice in the future.

Other Skeletal Dysplasias

Metaphyseal chondrodysplasia (MCD) Schmid type is caused by inactive mutations in the *type X collagen* gene and treatment-free adult height is approximately 130–160 cm [123]. One-year GH treatment in patients with MCD Schmid type was reported to improve height from −3.2 SD to −2.7 SD [124]. No final heights from GH treatment in patients with MCD Schmid type are available.

MCD McKusick type, also known as cartilage-hair hypoplasia (CHH), is characterized by short-limbed short stature, hypoplastic hair, defective immunity, and diminished erythrocyte generation [125]. CHH is caused by several mutations in the *RNA component of mitochondrial RNA processing endoribonuclease* gene [126]. Adult height of CHH patients is reported as approximately 131.1 cm in males and 122.5 cm in females [127]. GH treatment increased height of a CHH patient from −4.2 to −2.1 SD together with limb lengthening [128], but another report showed no benefit of the treatment [129].

Spondyloepiphyseal dysplasia is caused by the mutation or deletion of the type II collagen (*COL2A1*) gene and is characterized by severe short stature and a markedly short trunk, with a final height of 100–125 cm [130]. GH treatment in 17 patients with spondyloepiphyseal dysplasia did not result in a significant increase in the height SD score during the first year of treatment, although some patients did appear to benefit [123]. However, the sitting height SD score was improved from 2.7 to 1.8. No adult heights from GH treatment in patients with spondyloepiphyseal dysplasia are available.

Pseudoachondroplasia is caused by mutation in the cartilage oligomeric matrix protein (*COMP*) gene and is characterized by severe short stature with a waddling gait, deformity of the legs, short fingers, loose joints, and ligamentous laxity [131]. The final height is approximately 80–130 cm. Only one report described that GH treatment was given to four patients with pseudoachondroplasia, and that GH did not increase annual height gain in the first year of the treatment [124]. The increase in height of seven patients with pseudoachondroplasia was not significant, with a median gain of 0.2 SD [123]. No adult heights in patients with pseudoachondroplasia who had GH treatment have been reported.

Conclusion

Physiological GH secreted by the pituitary gland and extrinsic recombinant GH have the ability to promote bone elongation and linear growth of children, as well as regulate bone mineral metabolism with accelerating bone turnover. Currently, GH treatment has been established for many disorders. However, there are many types of diseases with short stature, and treatment of these patients is still limited. Although more evidence is required, GH has the possibility to be useful for other skeletal dysplasias, including metabolic bone diseases, because of its potential capacity for regulating bone mineral metabolism.

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Chapter 9
Growth Hormone and Oxandrolone in Burned Children

Nigel Tapiwa Mabvuure, Alexis N. Thomas, and Linda E. Sousse

Introduction

The persistent catabolic and hypermetabolic stress responses from thermal injury lead to several adverse outcomes in pediatric patients. These include an increase in resting energy expenditure, a negative muscle protein balance, and bone wasting. These secondary consequences result in stunted overall growth and weight, although it is important to note that we have yet to find evidence to support a direct link between bone loss and growth impairment.

Animal models were used to initially explore the mechanisms of post-burn bone loss. In Edelman et al.'s mouse model of burn injury, burned mice had significantly lower total femur dry and ash weights 10 days after burn injury compared with sham and control mice [1]. Total calcium, as well as the percentage of fluorochrome-labeled bone surfaces (i.e., those that are actively bone-forming) and bone formation rates, was also significantly lower in burned mice. In a follow-up study, Miller et al. investigated the effects of a 20 % total body surface area (TBSA) burn on bone mass, structure, elongation, and dynamics of C3H/HeN and Balb/c mice [2]. There was significantly less bone volume and total bone mass in the burned models of both strains compared to their respective controls. The burned mice exhibited increased

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osteoclast counts and significant growth velocity impairment. Miller's results showed that within 10 days of thermal injury, a marked increase in bone resorption and a decrease in bone formation led to the reduced total bone mass.

Human studies have corroborated the findings from these animal studies. A retrospective analysis by Rutan et al. of 80 burned children (>40 % TBSA burn, ages 2–18 years) found that both height and weight velocities were reduced up to 3 years post-burn [3]. Growth arrest occurred despite the patients receiving adequate nutritional support and performing rehabilitative exercises such as long bone stresses, cardiovascular exercises on the elliptical, and treadmill and weight-bearing exercises such as leg extensions and curls. An earlier study by Mooney et al. demonstrated that even localized flame burns to the hands may result in premature metacarpal and/or phalangeal epiphyseal fusions, causing arrested bone growth [4].

Post-burn bone disease may result in bone loss with bone mineral content (BMC) reductions and growth velocity impairment [2]. The problems of bone disease are compounded in burned patients by (1) the use of albumin, (2) the risk of aluminum exposure from use of antacids, (3) partial immobilization, and (4) hypercortisolaemia from possible suppression of intestinal calcium absorption [2, 5]. Several strategies have been employed to investigate the mechanisms underlying burn-related bone disease. Bone biopsies of burned patients show a disproportionate reduction in bone formation, leading to a loss in bone mineral density (BMD) [5]. The effects of burns on BMD have been demonstrated by assessing the lumbar BMDs and clinical outcomes (such as fracture incidence) of 68 burned children (15–36 % TBSA burn [$n=16$] and >40 % TBSA burn [$n=52$]) [6]. Children with larger burns had significantly lower BMDs than those with moderately sized burns and experienced more fractures within the 5-year follow-up period. The observed reduced serum level of osteocalcin (a marker of bone formation) and increased serum levels of type I collagen telopeptide and urinary calcium (both markers of bone resorption) suggest that bone formation is reduced while resorption is increased (although no precise pathophysiological link between the two mechanisms has yet been elucidated). Histomorphometric investigation by Klein et al. in 1995 corroborated these findings by revealing a lack of bone-forming osteoblasts at the osteoid seam in bone biopsies of burned children [7].

However, there remains some controversy—some studies have found no statistically significant decrease of osteocalcin levels in patients with burns covering >25 % TBSA up to 4 weeks post-burn [8], so it may be that bone formation may only be affected by extremely large burns. In smaller burns, a statistically significant increase in deoxypyridinoline levels (a marker for bone resorption), supporting previous findings was noted. This suggests that increased resorption may be a more important mechanism than decreased bone formation in short-term post-burn osteopenia. Hypercalciuria, bone marrow suppression, reduced skeletal loading, and magnesium depletion may also contribute to post-burn osteopenia [5]. Post-burn osteopenia may increase the risk of early-onset osteoporosis [6], so it is crucial that remedial strategies are instituted in the early treatment pathway.

A multifaceted and targeted therapeutic approach is needed in burns bone management [9]. Exogenous recombinant human growth hormone (rhGH) and oxandrolone are anabolic drugs that have been shown to modulate the hypercatabolic stress response to burns. These drugs also improve immune function and, thus, wound healing. This chapter focuses on their effects on bone health following thermal injuries.

Rationale for Exogenous Augmentation of Growth Hormone Concentrations

Growth hormone (GH), or somatotropin, is a 91-amino acid, single-chain polypeptide hormone secreted from the anterior pituitary. The hypothalamus regulates GH levels by altering the concentrations of GH-releasing hormone and GH-inhibiting hormone (somatostatin) (see Fig. 9.1) [10]. The anabolic properties of GH are particularly important during prepubescent linear growth. Reduced levels of GH during pediatric development result in growth impairment and short stature.

Growth hormone, initially from cadaveric pituitaries, was first used clinically in 1958 to treat disorders of reduced stature, but it was not until the development of rhGH in 1985 that its use beyond growth hormone replacement therapies became a possibility. In severe burns, rhGH, injected subcutaneously daily, acts to improve wound healing rates [11], muscle protein kinetics [12], and overall mortality [13], with a good safety profile in a pediatric population [11].

The physiology of GH is described in detail elsewhere in this book. Briefly, several mechanisms may explain the effects of GH on bone turnover and linear bone growth but this remains an area that is not yet fully understood [14]. Although GH improves both bone growth and bone remodeling, the mechanistic link between these two processes is yet to be proven. One potential mechanism begins with the activation of chondrocyte GH receptors, which activate the MAPK/ERK pathway and stimulate proliferation [14]. Another potential mechanism focuses on growth hormone's activation of the JAK-STAT signaling pathway in the liver, which results in the production of insulin-like growth factor-1 (IGF-1). IGF-1 circulates as part of a complex formed by one molecule of IGF-1, IGF-binding protein-3 (IGFBP-3), IGF-binding protein-5 (IGFBP-5), and an acid-labile subunit [14]. In excess, IGFBP-3 and -5 inhibit IGF-1 action [14]. IGF-1 has anabolic effects in a wide range of organs including in bone, where it stimulates osteoblasts and growth plate chondrocytes [15], promoting bone growth [16].

Several animal studies have demonstrated the anabolic effects of GH on bone. In one study, infusion of [14]C-labeled-GH into hypophysectomized male rats resulted in dose-related increases in the width of the tibial epiphyseal cartilage [17]. Jeschke et al. also used animal models to define the relationship between rhGH, hepatic IGF-1 expression, and subsequent serum levels of IGF-1 [18]. Sprague–Dawley rats received a 60 % TBSA third-degree scald burn and were randomized to receive either rhGH (2.5 mg/kg/day by intramuscular injection) or saline (control). Serum

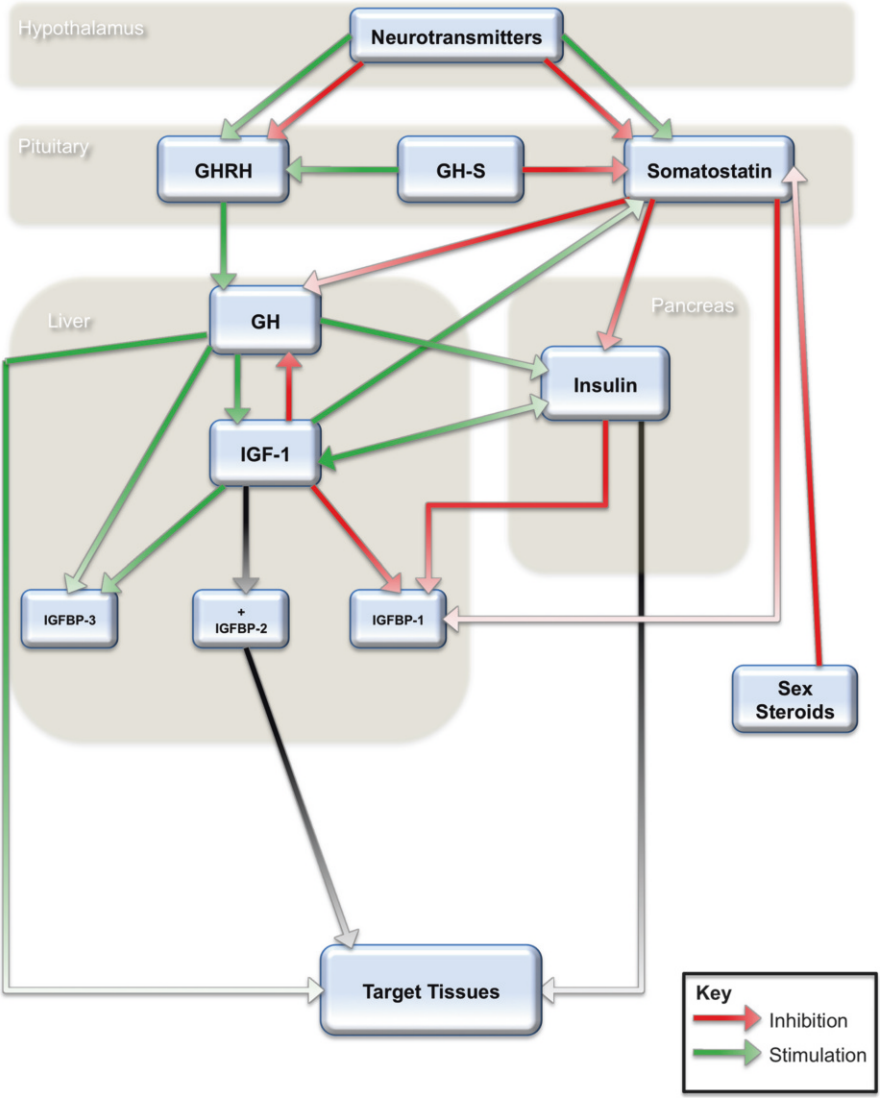

Fig. 9.1 This figure illustrates the interlinking cascade of the growth hormone system throughout the body. *GH* growth hormone, *GHBP* GH binding protein, *GH-S* GH secretagogues, *IGF-1* insulin-like growth factor-1, *IGFBPs* IGF-binding proteins. *Arrows* indicate direction and mechanism of action, either stimulatory or inhibitory. Adapted by permission from BMJ Publishing Group Limited. [Molecular Pathology, Z Laron, 54, 311–316, ©2001]

IGF-1, hepatic IGF-1 mRNA, and IGF-1 protein expression were significantly increased in rhGH-treated rats, whereas levels decreased in untreated rats. This, coupled with the observation that IGF-1 increased both liver and muscle protein concentrations, led to the conclusion that rhGH modulated post-burn hypermetabolism through an IGF-1-mediated mechanism. The observation that the decreased

IGF-1 mRNA levels in bone from hypophysectomized rats are restored following GH replacement supports this proposed IGF-1-mediated mechanism [19]. Several other studies of hypophysectomized rats have also demonstrated the positive effect of GH on bone formation and mass [20, 21].

Clinical Trials: Growth Hormone

Several studies in humans have also demonstrated the effects of GH on bone (Table 9.1). In a study examining the effects of rhGH on circulating levels of several catabolic hormones, Fleming et al. in 1992 demonstrated that 0.2 mg/kg/day of rhGH given to six children with large burns (>40 % TBSA) significantly elevated IGF-1 levels [22]. The hypothesis that rhGH improves the impaired bone turnover state associated with severe burns was derived from these observations and from several studies including those in which rhGH was administered to children with GH deficiency [23]. These studies demonstrated that rhGH increases the levels of IGF-1 [24], BMD [25], osteocalcin [24–26], and type I procollagen propeptide (PICP) [25].

In 1995, Klein et al. studied bone histomorphometry and biochemistry following large severe burns (>42 % TBSA) in 24 pediatric patients. Five of these patients received 0.2 mg/kg/day of subcutaneous rhGH to improve wound healing during their in-patient treatment period [7]. No histomorphometric or biochemical differences were seen between the treatment and control groups. However, this study's small sample size and lack of randomization limit its applicability at a pediatric population level. Therefore, better designed prospective studies with larger sample sizes were performed to further investigate the effect of GH on bone.

In 1998, Klein et al. performed the first study testing the hypothesis that rhGH improves bone turnover in burned children [23]. In this randomized double-blind controlled trial, 19 children with large burns (>40 % TBSA) were treated with either subcutaneous rhGH (0.2 mg/kg/day, $n=10$) or saline ($n=9$) from admission to wound healing completion. Blood levels of IGF-1, IGFBP-3, IGFBP-4, IGFBP-5, osteocalcin, and serum procollagen type I C-terminal peptide (PICP) were measured on admission and on completion of wound healing. These measures were then correlated with lumbar BMD. On completion of wound healing, IGF-1 and IGFBP-3 levels were significantly higher in the rhGH-treated group. IGFBP-4 (an inhibitor of the anabolic effects of GH on bone and other tissues) was raised on admission and continued to increase beyond the normal range, regardless of rhGH administration. Serum concentrations of IGFBP-5 (a binding protein that may link IGF-1 to bone), osteocalcin, and PICP did not differ significantly between the study groups. These findings suggest that short-term acute treatment with rhGH does not increase bone formation, but this may have been secondary to the short study treatment period. There is also a possibility that increased keratinocyte production of IGFBP-4 during wound healing may have inhibited IGF-1. These findings necessitated larger-scale studies with longer rhGH treatment periods.

Table 9.1 Summary of clinical trials of growth hormone use for burned children

Authors, year, journal	Aim of study	Study type	Outcomes	Main findings	Conclusions
Klein et al., 1995, *Bone* [7].	Investigate acute histomorphometric and biochemical response of bone to severe burn injury in 24 severely burned children (>40 % TBSA)	Five participants were treated with GH (0.2 mg/kg/day) throughout admission to accelerate wound healing	Markers of bone formation (PICP) and resorption (urinary type I collagen crosslinks, pyridinoline, deoxypyridinoline and calcium) were measured. Iliac crest bone biopsies were also taken	No difference in markers of bone turnover (formation and resorption) or histopathological appearance was present between those receiving and not receiving GH	
Klein et al., 1998, *J Clin Endocrinol Metab* [23]	Investigate the short-term effects of rhGH on bone formation in severely burned children (>40 % TBSA)	Nineteen children [ages 5.36 ± 3.6 years (SD)] were randomized to receive either rhGH (0.2 mg/kg/day) sc ($n = 10$)or placebo ($n = 9$) from within 72 h of admission until wound healing was considered 95 % complete	Serum IGF-1; IGFBP-3, -4, and -5; osteocalcin; and PICP concentrations were measured on admission and end of treatment. Lumbar BMD was measured at the end of treatment	At wound healing: IGF-1 and IGFBP-3 levels were significantly higher in the rhGH group; BMD, osteocalcin, and PICP levels did not differ at any time	Short-term rhGH does not increase bone formation or bone in burned children
Hart et al., 2001, *Ann Surg* [27]	Investigate whether long-term low dose rhGH attenuated post-burn bone loss in severely burned children (>40 % TBSA)	Double-blinded RCT: patients were randomized to receive either rhGH (0.05 mg/kg/day) ($n = 19$) or saline ($n = 21$) for a year post-burn	Height, LBM, BMC, IGF-1, and IGFBP-4 and -5 were determined at discharge and 6, 9, and 12 months after injury	IGF-1 and IGFBP-4 and -5 levels were not different between groups ($p > 0.05$). Osteocalcin remained low despite rhGH. BMC and gains in height were significantly ($p > 0.05$) greater in the rhGH group at 12 months	Long-term rhGH abates bone loss and improved linear growth but did not increase bone formation

Przkora et al., 2006, *Ann Surg* [28]	Investigate the efficacy of rhGH in severely burned children (>40 % TBSA) up to 2 years post-burn: the immediate 12 months post-burn on rhGH and the following 12 months after drug discontinuation	Double-blinded RCT: patients were randomized to receive either rhGH (0.05 mg/kg/day) ($n=19$) or placebo ($n=25$) for a year post-burn	Height, LBM, BMC, IGF-1, IGFBP-3, and osteocalcin were measured at discharge and 6, 12, 18, and 24 months after injury	The rhGH had significantly greater ($p<0.05$) height gains, and BMC from 12 to 24 months. IGF-1 levels were higher ($p<0.05$) in the rhGH from 9 to 18 months after injury and osteocalcin was only significantly greater 18 months after injury	The significant increases in height and BMC following rhGH treatment persist up to a year after treatment is discontinued
Branski et al., 2009, *Ann Surg* [29]	Summarize one institution's experience treating children with large burns (>40 % TBSA) with three doses of rhGH up to 2 years post-burn: the immediate 12 months post-burn on rhGH and the following 12 months after drug discontinuation	Double-blinded RCT: patients received either placebo ($n=94$) or long-term rhGH at 0.05, 0.1, or 0.2 mg/kg/day ($n=101$)	Height, LBM, BMC, IGF-1, IGFBP-3, and osteocalcin were measured at discharge and 6, 12, 18, and 24 months after injury	Overall, rhGH patients gained significantly more height from 9 to 24 months (0.1 mg/kg/day rhGH resulted in the most sustained growth) Overall, rhGH increased IGF-1 at 6, 9, and 12 months post-burn (0.05 rhGH mg/kg/day did not increase IGF-1) Osteocalcin levels did not differ ($p>0.05$) Only 0.05 mg/kg/day rhGH significantly increased BMC (12–24 months post-burn)	The effects of rhGH may be dose-dependent. Use of 0.2 mg/kg rhGH in the acute phase following thermal injury, and 0.1 mg/kg rhGH for at least 12 months immediately following injury was recommended

NB: Only bone-relevant outcomes are included

TBSA total burn surface area, *BMC* bone mineral content, *LBM* lean body mass, *rhGH* recombinant human growth hormone, *GH* growth hormone, *IGF-1* insulin-like growth factor-1, *IGFBP* insulin-like growth factor binding protein, *sc* subcutaneously, *PICP* procollagen type I C-terminal peptide

Hart et al. randomized 72 severely burned children (>40 % TBSA) to receive either rhGH or saline in their double-blinded study [27]. Treatment with rhGH (0.05 mg/kg) or control was continued for 1 year post-burn. Only 40 children completed the study: 19 within the treatment and 21 in the control groups, respectively. Height, lean body mass (LBM), BMC, and serum levels of GH, IGF-1, and IGFBP-4 and -5, among other markers, were determined at discharge, 6, 9, and 12 months post-burn. Children treated with rhGH demonstrated a significant increase in height compared to controls at 12 months (1.4 ± 1.5 cm versus 7.9 ± 2.1 cm) ($p < 0.05$). Both groups showed bone loss for the first 6 months. However, the control group demonstrated no further change in BMC, whereas the rhGH-treated children continued to gain BMC. The difference between the BMC of both groups neared significance at 6 months ($p = 0.06$) and reached significance at the 12-month time point ($p < 0.05$). Unlike Klein et al.'s findings [23], IGF-1, IGFBP-4, and IGFBP-5 levels did not differ between groups. However, there was concordance with their finding that IGFBP-4 levels remained persistently elevated throughout the trial. Osteocalcin levels remained low despite rhGH therapy, signifying low or no bone formation. Hart's study demonstrated that low dose rhGH for 1 year post-burn increased linear growth, LBM, and BMC. Although no rise in IGF-1 was shown, this finding is not enough to disprove the suggested IGF-1-mediated mechanism of GH [27]. LBM increased approximately 3 months before BMC—these results do not exclude the possibility that BMC rises were stimulated by skeletal loading. Although a limitation of this study was the large drop-out rate, the numbers of children completing the trial were similar for both groups, reducing bias [27].

A criticism of the studies by Klein, Hart, and their respective teams [23, 27] is that they neglected to assess whether GH resulted in functional improvements. Concerns were also raised about the possibility of the suppression of endogenous GH production secondary to extended rhGH treatment, resulting in a rebound phenomenon upon cessation of treatment. Przkora et al. addressed these concerns in a study to investigate functional improvements up to 2 years post-burn [28]. In this double-blinded study, children were randomized to receive daily doses of either rhGH (0.05 mg/kg) ($n = 19$) or control ($n = 25$) for 12 months from discharge. Follow-up was performed at 6, 12, 18, and 24 months post-burn. The following bone-related measurements were taken at each follow-up appointment: child height, LBM, BMC, GH, IGFBP-3, IGF-1, and osteocalcin (among other serum markers). Strength testing of children aged ≥7 years was also conducted on their dominant leg extensors. The percentage change in height from baseline was significantly higher in the rhGH-treated group from 12 months up to 24 months post-burn. Likewise, BMC in rhGH-treated patients continued to improve with a steeper gradient than in controls and was significantly greater from 12 to 24 months post-burn. Strength was significantly greater in the treated group at 12 months, but this effect did not persist after drug discontinuation. Although GH, IGF-1, and IGFBP-3 were all significantly increased in the treated group during the first 12 months, only IGF-1 was persistently elevated after the discontinuation of treatment (via mechanisms not yet elucidated). Osteocalcin was elevated 18 months post-burn in the treated group. No adverse effects were recorded. Up to 1 year after the discontinuation of rhGH treatment, no rebound phenomenon was observed.

Przkora's study provided the first data on the functional improvements from rhGH treatment, including improved muscle strength and cardiac function. The rises in IGF-1 and IGFBP-3 following rhGH treatment are supportive of the hypothesis that GH acts through the IGF-1 complex. However, several study limitations were raised. There was no "healthy" control group with which expected development in the absence of morbidity could be compared. This study, like that by Hart et al., also suffered from a high drop-out rate. A possible explanation for this is that 70 % of the children treated at Shriners Hospital for Children (Galveston, USA), where both studies were performed, came from Mexico and this patient population does not tend to remain in the USA for the extended time period of the trial.

The positive effects of GH on bone health were further confirmed in a 2009 study by Branski et al. [29]. Burned children (>40 % TBSA) were randomized to receive either subcutaneous placebo ($n=94$) or rhGH in doses of 0.05, 0.1, or 0.2 mg/kg ($n=101$) for up to 12 months post-burn. The same bone-related markers described above were measured at discharge, 6, 9, 12, 18, and 24 months post-burn. The heights of patients receiving rhGH were significantly greater than controls from 9 to 24 months and approached normalcy (50th percentile) from 12 to 24 months. rhGH doses of 0.1 mg/kg led to the most sustained height gains whereas 0.2 mg/kg did not lead to any significant height improvement. As might be expected, 0.2 mg/kg doses led to the greatest increases in LBM, although this finding was only significantly better than the control group within the period of active treatment. Overall, BMC values did not differ significantly between the groups. Surprisingly, only the lower dose of 0.05 mg/kg led to significant differences in BMC in the two groups (12–24 months). Serum IGF-1 levels were only significantly higher in the entire rhGH group from 6 to 12 months post-burn. Only rhGH doses of 0.2 mg/kg resulted in significantly raised osteocalcin levels. Hence, no significant differences in osteocalcin levels were observed between the controls and the entire rhGH group. Two cases of hypercalcemia following 0.2 mg/kg doses of rhGH and one case of hyperglycaemia were the only adverse effects recorded. These results suggest that some effects of GH may be dose-related. For example, the decrease in BMC with 0.2 mg/kg/day rhGH was thought to be due to sustained suppression of parathyroid hormone and high bone turnover (as indicated by a raised osteocalcin) in this group. The authors therefore suggested using 0.2 mg/kg rhGH in the acute phase post-burn to maximize gains in LBM and 0.1 mg/kg rhGH for at least 1 year post-burn to maximize gains in other bone health indices.

Summary of GH Studies

These randomized placebo-controlled clinical trials provide high level evidence (level 1 on the American Society of Plastic Surgeons Evidence Rating Scale for Therapeutic Studies) [30] of the efficacy and safety of rhGH in improving bone health. Although this chapter has focused solely on bone health indices, GH has myriad other benefits to burned patients. These include improved cardiac function,

improved muscle protein kinetics, maintained muscular growth, improved wound healing, and improved resting energy expenditure, denoting a less heightened metabolic state [31]. Considering the wide range of possible side effects of GH in children [32], only minor, easily correctible adverse affects were seen. Despite the apparent positive safety profile of rhGH, there remain concerns following recent trials in adults which demonstrated significantly higher mortality rates (up to 44 %) compared with controls when administered to adult ICU patients [33]. In addition, GH is administered subcutaneously, potentially reducing compliance. These issues, combined with rhGH treatment costs of approximately $18,000 per patient in the USA, have made alternative anabolic agents desirable.

Rationale for Exogenous Augmentation of Androgen Concentrations

Testosterone levels fall below baseline from approximately 60 days post-burn up to 3 years, suggesting the need for an anabolic replacement and/or augmentation [34, 35]. Animal models have supported the rationale for androgen supplementation. In 2000, Erben et al. showed that androgen-deficient orchidectomized rats suffered substantial global loss of trabecular bone and sustained increases in bone turnover [36]. Other studies have corroborated these results. Short-term androgen deficiency caused significant increases in markers of bone turnover in aged male rats [37]. Several androgens including testosterone and 5 alpha-dihydrotestosterone effectively prevented this rise in bone turnover.

In severely burned male patients (>70 % TBSA), the supplementation of testosterone has been shown to attenuate muscle catabolism and improve protein synthesis and LBM [38]. However, testosterone may cause significant side effects such as virilization and hepatotoxicity. Testosterone also causes premature epiphyseal maturation [39]. It may therefore be inappropriate in burned children, who may already be predisposed to premature epiphyseal maturation [4]. Alternatives may also be necessary in women to avoid virilization.

The non-aromatizable androgen, oxandrolone, is a synthetic orally active testosterone derivative. Oxandrolone has lower hepatotoxicity and only 5 % of the virilizing activity of testosterone. It is less androgenous than testosterone, but has been implicated in causing premature puberty in some case studies [40]. Oxandrolone is therefore favored for use in treating women and prepubescent boys. In skeletal muscle, oxandrolone binds to intracellular androgen receptors (ARs). The androgen receptor–oxandrolone complex then moves to the cell nucleus and binds to DNA, stimulating protein synthesis and anabolism.

The hypothesis that oxandrolone positively improves bone health is derived from both basic science and clinical studies. In 2007, Bi et al. investigated whether oxandrolone increased osteoblastic production of type I collagen and whether this action occurs via oxandrolone transactivating the androgen receptor (AR), a mediator of androgen activity [41]. Thirty micrograms per milliliter oxandrolone was sufficient

to transactivate the AR, as shown by increased fluorescence in the nuclei of oxandrolone-treated osteoblasts. Control cultures did not fluoresce. Fifteen micrograms per milliliter and 30 μg/mL oxandrolone-treated osteoblasts expressed significantly more type I collagen than controls, as confirmed by immunohistochemistry and immunoquantitative assays. Oxandrolone also increased expression of alkaline phosphatase (ALP), a marker of osteoblast activity, in a dose-dependent manner. Levels of osteocalcin, a marker of bone turnover, mirrored ALP and were significantly greater in 10, 15, and 30 μg/mL oxandrolone-treated cultures as compared with controls. These results suggest that oxandrolone may act directly on the osteoblast in addition to effects that result in increasing skeletal loading [41]. Similar results to this in vitro study were observed in murine models [42]. Although serum levels of ALP rose following oxandrolone administration, this also occurs 3–6 months following an increase in LBM, so the in vivo applicability of Bi et al.'s findings remains unclear [43].

Oxandrolone is the only androgenic steroid that is FDA approved to maintain body weight in catabolic states, including AIDS, major surgery, infections, malnutrition, and neuromuscular diseases. Oxandrolone's androgenic properties have also been harnessed since the 1970s to stimulate growth in children with constitutional short stature or other causes such as Turner's syndrome [44–46]. Some studies have actually found oxandrolone to be more efficacious than rhGH in children with constitutional growth delay [47]. In burned children, several studies (discussed below) have demonstrated that oxandrolone attenuates hypercatabolism and increases LBM, BMC, strength, and BMD [43, 48–50].

Clinical Trials: Oxandrolone

Several studies have investigated the effects of oxandrolone on BMC in burned children (Table 9.2). The first was a single center study by Murphy et al. in 2004 [43]. A double-blinded randomized controlled trial (RCT) was performed to investigate the effect of twice-daily oral oxandrolone (0.1 mg/kg) in pediatric patients (ages 0.5–18 years). Eighty-four children in total were randomized into demographically matched treatment and control arms ($n=42$ per group). Oxandrolone treatment began at discharge and continued for 1 year. Baseline bone measurements included LBM, BMC, and BMD via dual energy X-ray absorptiometry (DEXA). Serum osteocalcin levels were also measured. Measurements were repeated at 6, 9, and 12 months after the burn and the experience of side effects was explored via patient questionnaires.

Oxandrolone significantly increased LBM in burned children whereas the control group lost an additional 4.5 % of their LBM in their 6-month post-burn period. The treatment group consistently showed significant rises in LBM at every follow-up assessment. A significant 15 % improvement in BMC was observed in the oxandrolone group compared with a smaller 4 % rise in the control group. The majority of bone deposition occurred between 9 and 12 months. A steeper rise in BMC was

Table 9.2 Summary of clinical trials of oxandrolone use for burned children

Author, year, journal	Aim of study	Study type	Outcomes	Main findings	Conclusions
Murphy et al., 2004 [43]	To investigate whether long-term oxandrolone promotes BMC accretion in severely burned children (>40 % TBSA)	Double-blinded RCT: patients received either twice daily oral oxandrolone (0.1 mg/kg) ($n=42$) or placebo ($n=42$) from discharge until 1 year after injury	Osteocalcin, LBM, BMC, and bone mineral density (BMD) were measured at discharge, and at 6, 9, and 12 months after the injury	BMC was significantly higher in the oxandrolone group after 12 months ($p<0.05$) LBM was significantly greater in the oxandrolone group from 6 to 12 months post-burn ($p<0.05$) The oxandrolone group had significantly better age- and gender-matched z-scores throughout ($p<0.05$)	A 1-year course of oxandrolone safely improves LBM, BMC, and BMD
Przkora et al., 2005, *Ann Surg*. [48]	Investigate the efficacy of oxandrolone in severely burned children (>40 % TBSA) up to 2 years post-burn: the immediate 12 months post-burn on rhGH and the following 12 months after drug discontinuation	Double-blinded RCT: patients were randomized to receive either oxandrolone (0.1 mg/kg/day) ($n=30$) or placebo ($n=31$) for a year post-burn	Height, LBM, BMC, IGF-1, IGFBP-3, and osteocalcin were measured at discharge and 6, 12, 18, and 24 months after injury	From 18 to 24 months, oxandrolone-treated children were more likely to be in the >25th percentile/total for height ($p<0.05$) LBM and BMC were significantly higher in the oxandrolone group only at 12 months IGF-1 was significantly higher in the oxandrolone group from 12 to 18 months	Some effects attributable to oxandrolone such as gains in height persist after drug discontinuation

Porro et al., 2011, *J Am Coll Surg*. [50]	Investigate the efficacy of oxandrolone in severely burned children (>30 % TBSA) up to 5 years post-burn: the immediate 12 months post-burn on rhGH and the following 4 years after drug discontinuation	Double-blinded RCT: patients were randomized to receive either oxandrolone (0.1 mg/kg/day) ($n=70$) or placebo ($n=152$) for a year post-burn	Height, LBM, BMC, BMD, IGF-1, IGFBP-3, and osteocalcin were measured at discharge and 6, 12, 18, and 24 months after injury. Measurements were also taken annually thereafter	Children aged 7–18 years had a significantly greater change in height percentile up to 4 years post-burn ($p<0.05$) Only oxandrolone-treated children aged 7–18 had significantly greater BMC than controls (2–5 years post-burn) ($p<0.05$) BMD and LBM differences were not significant ($p>0.05$) IGF-1 was significantly higher in the oxandrolone from discharge to 2 years post-burn ($p<0.05$) Three female patients suffered reversible clitoral hood edema	Oxandrolone safely improves bone health indices up to 5 years after initial injury

NB: Only bone-relevant outcomes are included

TBSA total burn surface area, *BMC* bone mineral content, *LBM* lean body mass, *rhGH* recombinant human growth hormone, *GH* growth hormone, *IGF-1* insulin-like growth factor-1, *IGFBP* insulin-like growth factor binding protein, *sc* subcutaneously, *PICP* procollagen type I C-terminal peptide

seen in the oxandrolone group. From 6 months onwards, the treated group had significantly higher levels of ALP compared to controls, which continued up to the 12-month point. With regard to adverse events, clitoromegaly was seen in two subjects, although the authors hypothesized that post-burn edema was a more likely explanation since the clitoral hypertrophy resolved after drug discontinuation.

Murphy's study provided the first evidence that oxandrolone could improve bone formation in severely burned children. However, since BMC improvements occurred 6 months after LBM rises, these data did not explain the exact mechanism by which oxandrolone promotes bone formation. At least two possibilities exist: oxandrolone either directly stimulates bone-forming osteoblasts or first increases LBM, causing increased skeletal loading and, hence, increased BMC [41]. Although the rise in BMC likely leads to reduced fracture risk, longer follow-up was required to investigate this further.

A study by Przkora et al. investigated the effects of oxandrolone in severely burned pediatric patients up to 2 years after the burn [48]. The 2-year period comprised of twice-daily oral oxandrolone (0.1 mg/kg) for the 12 months immediately post-burn followed by a further 12 months of follow-up after the drug was discontinued. Sixty-one pediatric patients with burns covering more than 40 % of their TBSA were included and randomized to oxandrolone ($n=30$) or placebo ($n=31$). At 18 and 24 months, there were significantly more patients from the oxandrolone group with heights above the 25th percentile. Contrary to the finding by Murphy et al. [43] LBM rise did not precede BMC rise. Both variables were significantly better in the oxandrolone group at 12 months. However, there was no evidence of premature epiphyseal plate closure which has been demonstrated in other studies [4]. Three patients with perineal burns suffered clitoral hood edema, which, as in the previous study, resolved after drug discontinuation.

One of the main findings of Przkora et al.'s study in relation to bone health was that although the positive effects of oxandrolone on LBM and BMC were only seen during the treatment period, height and weight percentiles were significantly improved even after the discontinuation of treatment. Another important suggestion was that since oxandrolone did not cause premature epiphyseal fusion, height improvements were likely to be permanent. However, the authors conceded that larger series were needed to study the effects of diet and exercise, to assess whether the frequency, duration, and intensity of growth spurts were affected by oxandrolone treatment. Continuation of oxandrolone for at least 2 years was deemed necessary in order to evaluate its effect on functionality, rather than weight and height gains alone, along with other clinical outcomes such as bone fracture risk.

A further study was therefore undertaken to investigate outcomes of oxandrolone treatment at 5 years post-burn. Burned pediatric patients ($n=222$, ages 0.5–18 years, >30 % TBSA burn) were randomized to receive either oxandrolone ($n=70$) or placebo ($n=152$). Oxandrolone was given at 0.1 mg/kg twice daily for 12 months after the burn. Measurements of osteocalcin, LBM, BMC, and BMD were made at discharge, 6, 9, 12, 18, and 24 months after burn, then annually up to 5 years post-burn. Bone age was calculated at each time point using radiographs of patients' hands and knees. Height velocities were not significantly different between the groups at

3 years post-burn, although significance was seen at 2 years post-burn. Patients receiving oxandrolone had positive change in height percentiles whereas controls had negative changes. These differences were only significant up to 2 years post-burn. Growth arrest was significantly more likely to occur in the control group. Oxandrolone was most beneficial to patients who were aged between 7 and 18 years at the time of injury. These children had a significantly greater gain in BMC than age-matched controls from 2 to 5 years post-burn. The differences in LBM approached but did not reach significance ($p = 0.06$) at any time point. No virilization was observed and bone ages did not differ significantly between the groups. As in the previous studies, the patients who suffered clitoral edema ($n = 3$) had full reversal of the hypertrophy within 3 months of discontinuing treatment.

These results support the previous finding of sustained improvements in growth 2 years post-burn in children receiving oxandrolone for 12 months immediately after the burn. In the 7–18 age groups, gains in BMC persisted up to 5 years post-burn. However, this study was not powered to determine eventual adult heights of these children. Side effects were minimal and easily reversible.

Summary and Potential Implication of Findings

In addition to attenuating hypermetabolism, oxandrolone and growth hormone have long-term bone-protective properties in pediatric burn patients. These drugs are relatively safe to administer to burned children for periods of up to a year following thermal injury. These drugs are therefore useful additions to consider in multifaceted burn management and rehabilitation programs. However, several questions remain unanswered and require consideration before universal adoption of these treatments.

Several drugs, apart from oxandrolone and GH, have been shown to reduce the metabolic sequelae of burn injury including the beta-blocker propranolol, metformin, insulin, and incretin analogues [31]. It is likely that optimum treatment regimens will integrate concurrent use of two or more of these drugs. It is therefore important to further delineate the specific roles each drug would have in such a pharmaceutical cocktail. Comparative studies have not yet been performed to ascertain the most efficacious protocol. For example, oxandrolone has been administered as an adjunct to GH in other conditions. It is thought to reduce the waning effect often seen during sole GH therapy [51, 52], as well as increasing the sensitivity of somatotrophs to GH [53]. This has not yet been investigated in burn cohorts. Studies comparing the effects of sole GH and sole oxandrolone therapy or combined GH and oxandrolone may define additive beneficial effects but may also identify unfavorable effects.

It is important to note that these drugs comprise only one aspect of a multifaceted approach to burn management. As large burn injuries present long-term biological, psychological, and social challenges to patients, it is important to also investigate the long-term biopsychosocial functional recovery of these patients. Despite these

limitations, the discussed studies provide the first data on pediatric outcomes up to 5 years post-burn. Further studies, particularly on long-term quality of life, will provide additional variables to factor into cost–benefit analyses.

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Chapter 10
Pediatric Bone Drugs: Calcium and Vitamin D

Melissa S. Putman and Catherine M. Gordon

Introduction

Calcium and vitamin D play an important and interconnected role in bone health across the age spectrum. Vitamin D is a prohormone that exerts critical effects on calcium homeostasis via the actions of its metabolite, calcitriol [1, 2]. As is illustrated by cases of severe calcium and/or vitamin D deficiency resulting in rickets and osteomalacia, these nutrients are essential for the normal growth and maintenance of the skeleton. Childhood and adolescence are a particularly important time for the acquisition of bone mass that occurs during periods of rapid growth [3, 4]. The effects of calcium and vitamin D intake on the improvement of pediatric skeletal health, as well as the optimal method of calcium and vitamin D administration, have received increasing attention over the past decade. However, studies focusing on short- and long-term outcomes are currently limited in this population. As a result, controversies remain regarding the use of calcium and vitamin D as pediatric bone drugs.

This chapter will review the dietary and supplemental sources, metabolism, and physiology of calcium and vitamin D, along with the effects of these nutrients on the skeleton. Focusing in particular on pediatric studies, we will discuss the medical literature that has shaped our understanding of calcium and vitamin D requirements across infancy, childhood, and adolescence. Current recommendations regarding the supplementation of calcium and vitamin D in the healthy pediatric population,

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as well as those young patients who are at risk for vitamin D deficiency and/or low bone density, will be reviewed, along with approaches to the treatment of rickets and vitamin D deficiency.

Calcium Metabolism and Physiology

Calcium is required for multiple cellular and tissue functions, exhibited in a variety of different mechanisms. Complexed with phosphate, calcium forms hydroxyapatite, the primary mineral in bone that provides strength and hardness to the skeleton. Ninety-nine percent of the body's calcium is contained in bone, which serves as the primary reservoir for this mineral. Only a small fraction of the body's calcium circulates in plasma, where it is available for function as an intracellular messenger involved in vascular reactivity, nerve conduction, muscle contraction, and hormone secretion and action [5]. Tight control of blood calcium is required to maintain these adequate cellular functions, representing a highly regulated balance of dietary intake, intestinal absorption, bone formation and resorption, and urinary excretion. Parathyroid hormone (PTH) and vitamin D, through its activated form calcitriol, are the primary regulators of this critical calcium balance [6–8].

Calcium is absorbed through the intestinal mucosa through a combination of active and passive transport. Active transport is under the control of calcitriol, which acts on vitamin D receptors (VDRs) in intestinal cells, primarily in the duodenum [9, 10]. Passive transport occurs throughout the intestine across electrochemical gradients between the lumen and serosa [11]. Because calcium is absorbed passively down a gradient, this process typically occurs under conditions of higher calcium intake, and active transport increases during times of low-to-moderate calcium intake in response to increased calcitriol levels. Selective calcium channels of the transient receptor potential (TRP) family of membrane proteins, particularly TRPV6, are expressed in intestinal epithelial cells and play an important role in calcium absorption [12, 13]. In general, approximately 25 % of ingested calcium is absorbed, and this amount can vary based on multiple factors including calcium intake, vitamin D status, and age [14–16]. For example, when calcium intake is low, the fraction of calcium absorbed rises, mediated by increased active transport, to improve calcium availability. In addition, age has a significant effect on intestinal calcium absorption, and the fraction of calcium absorbed is highest in infants and adolescents and declines with age in adulthood [16–19].

Calcium homeostasis is also maintained by the kidney and skeleton. Approximately 98 % of filtered calcium is reabsorbed by passive or active processes in the kidney throughout the proximal tubule, loop of Henle, distal tubule, and collecting ducts, mediated by a number of mechanisms including PTH, the calcium sensing receptor, and calcitriol. Epithelial TRPV5 channels, similar in structure to intestinal TRPV6 proteins, are involved in this renal calcium reabsorption [20, 21]. The skeleton, as the primary storage reservoir for calcium and phosphate,

also plays a critical role in maintaining normal serum calcium concentrations. The balance of bone formation and bone resorption is mediated by complex interactions from multiple hormonal controls and cellular actions that will be reviewed in further detail below.

Vitamin D Metabolism and Physiology

Vitamin D exists in two forms, vitamin D2 (ergocalciferol) and vitamin D3 (cholecalciferol), whose chemical structure varies based on differences in side chain structure (Fig. 10.1). Vitamin D2 is primarily plant-derived (from yeast), whereas vitamin D3 is synthesized in the skin and is present in animal-based foods such as oil-rich fish. The primary source of vitamin D in humans has historically been production of vitamin D3 in the skin from UV exposure. Both forms of vitamin D are used in food fortification and are available in a supplement form. Food sources such as milk and orange juice are fortified with approximately 400 IU (international unit)/L of vitamin D3, and other foods such as some bread products, cereals, yogurt, and cheeses can also be vitamin D fortified (Table 10.1) [22].

Both vitamin D2 and vitamin D3 are converted in the liver to 25-hydroxyvitamin D [25(OH)D], also known as calcidiol, which is the primary storage form and primary metabolite of vitamin D that circulates in human blood. Because of its long half-life and direct relationship to vitamin D exposure and supply, 25(OH)D is considered to be the best indicator of total body vitamin D status [22–24]. The metabolite, 25(OH)D, is converted to the activated form of vitamin D, 1,25-dihydroxyvitamin D [1,25(OH)2D or calcitriol], by 1-alpha-hydroxylase (CYP27B1) in the kidney [1].

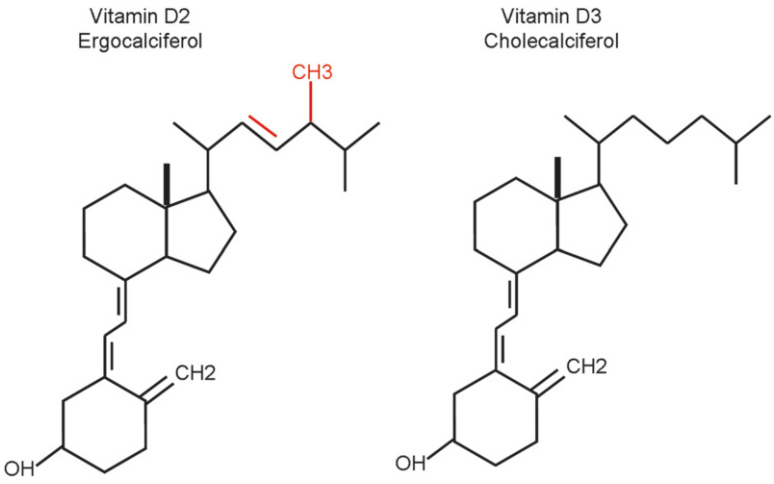

Fig. 10.1 Molecular structure of D2 and D3

Table 10.1 Food sources of vitamin D and calcium

Dietary sources of calcium and vitamin D	Calcium content	Vitamin D content
Breast milk	250 mg/L	16 IU/L
Standard infant formula (8 oz)	130 mg	100 IU
Fortified milk (8 oz)	300 mg	100 IU
Fortified almond or soy milk (8 oz)	300 mg	100 IU
Cheese (1 oz)	200–300 mg	30 IU
Yogurt (6 oz)	250 mg	200 IU
Ice cream (4 oz)	100 mg	–
Fortified tofu (4 oz)	435 mg	50 IU
Fortified juice (8 oz)	300 mg	100 IU
Fortified cereal (1 serving)	1,000 mg	400 IU
Egg (1)	43 mg	20–40 IU
Almonds (24 nuts)	70 mg	–
Dark leafy vegetables (4 oz cooked)	50–135 mg	–
Beans (4 oz)	60–80 mg	–
Canned fish with bones (salmon, mackerel, sardine, tuna) 3.5 oz	70–100 mg	230–600 IU
Fresh salmon, wild (3.5 oz)	–	600–1,000 IU
Fresh salmon, farmed (3.5 oz)	–	100–250 IU

Calcitriol synthesis comprises a tightly regulated process controlled primarily by PTH and fibroblast growth factor 23 (FGF23). PTH acts to increase calcitriol levels by up-regulating 1-alpha-hydroxylase, whereas FGF23 down-regulates this enzyme's activity and subsequently decreases circulating calcitriol levels. In an important feedback loop, calcitriol can then suppress PTH secretion via action on VDRs in parathyroid cells [25]. In addition, calcitriol functions to regulate itself by ensuring a net positive calcium balance through increased calcium availability, which subsequently decreases the stimulus for PTH secretion. The metabolism of vitamin D is illustrated in Fig. 10.2.

The primary role of calcitriol is to maintain calcium and phosphate homeostasis. Through interaction with the VDR in the nuclei of target cells, calcitriol acts to increase the availability of calcium in the blood by three primary mechanisms: (1) stimulation of intestinal calcium absorption through active transport processes (2), mobilization of calcium from bone (with PTH), and (3) increased renal distal tubule calcium reabsorption. Calcitriol also affects phosphate metabolism, though this mechanism of action is less clear. Calcitriol stimulates increased intestinal phosphate absorption along with calcium and also induces FGF23 synthesis by osteocytes, thus exerting both positive and negative effects on phosphate levels.

As detailed above, active calcium absorption in the intestine is mediated by calcitriol action on VDRs among intestinal cells. In adults, studies have suggested that intestinal calcium absorption varies by serum 25(OH)D level, ranging from 10 to 15 % without vitamin D present, to 30–40 % in vitamin D sufficient states [26, 27]. During puberty, an increase in calcitriol production allows for increased intestinal

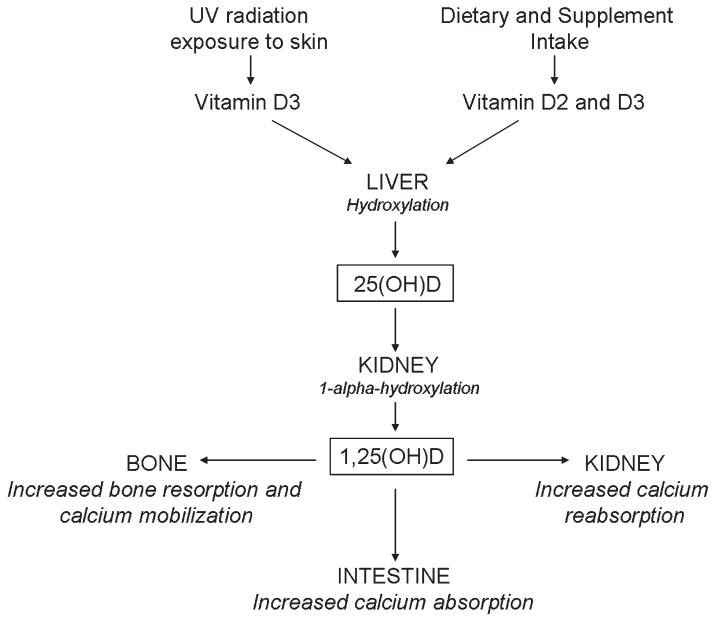

Fig. 10.2 The metabolism of vitamin D and its major effects on calcium regulation

calcium absorption to ensure adequate calcium availability during peak bone accrual that occurs during this period of rapid growth [28, 29].

Calcitriol also acts in concert with PTH to mobilize calcium from bone by stimulating bone resorption via inducing the expression of the receptor activator of nuclear factor kappa-B (RANK) ligand, which induces osteoclast differentiation and activation [30]. There may also be a possible direct anti-resorptive effect of calcitriol on bone [31]. At the same time, calcitriol positively affects bone mineralization by ensuring the availability of calcium and phosphate through the above-mentioned intestinal and renal effects on the absorption of these nutrients.

Effects of Calcium and Vitamin D on the Skeleton

Recently, calcium and vitamin D have received significant attention regarding their role in pediatric and adult bone health. The amount of vitamin D and/or calcium supplementation required for optimal bone accrual and maintenance, as well as for the prevention of bone loss across the age spectrum, is controversial. In addition, the optimal 25(OH)D concentration to ensure skeletal health, including at what level bone turnover markers, PTH, and gut calcium absorption are optimized, is under debate.

Despite these uncertainties, it is well known that calcium and vitamin D are important to maintain skeletal integrity. This point is perhaps best illustrated by the physiological changes noted within the setting of calcium and/or vitamin D deficiency. When a negative calcium balance occurs, PTH secretion is stimulated, leading to increased calcitriol production, which in turn stimulates intestinal and renal calcium absorption. Increased bone resorption from enhanced osteoclastogenesis may also ensue. This secondary hyperparathyroidism maintains normal blood calcium concentrations, but does so at the expense of calcium loss from bone. Thus, vitamin D and/or calcium deficiency can cause secondary osteoporosis, and in more severe cases, deficiency can result in inadequate mineralization of osteoid, leading to rickets in children and osteomalacia in adults.

Rickets and Osteomalacia

Calcipenic rickets occurs in the setting of insufficient calcium substrate to accommodate the needs of the growing pediatric skeleton. This condition is marked by defective mineralization of cartilage in the epiphyseal growth plates and failure of normal mineralization of newly formed osteoid. Classic features of rickets include widening of the long bone epiphyses, progressive bowing of the limbs (genu varus or valgus), craniotabes, delayed closure of fontanelles, parietal and frontal bossing, rachitic rosary from enlargement of the costochondral junctions of the ribs, hypoplasia of dental enamel, muscle weakness, and bone pain. In severe cases, hypocalcemia can result in seizures, particularly in infants less than a year of age [32].

Nutritional rickets can be due to inadequate calcium and/or phosphorus, insufficient vitamin D (from diet and/or sun exposure), or a combination of both. Low calcium intake of less than 200–300 mg/day has been associated with an increased risk of rickets, although the exact amount of calcium intake required to prevent rickets is unclear [33]. Similarly, the degree of vitamin D deficiency associated with the development of nutritional rickets is not well defined. However, in a recent review, a serum 25(OH)D concentration below 11 ng/mL (27.5 nmol/L) was found to be consistently associated with rickets, although the level above which rickets did not occur could not be determined [34]. Concurrent low calcium intake may also increase the level of vitamin D required for rickets prevention.

Nutritional rickets is a worldwide problem, most common in underdeveloped nations, but also occurring in industrialized countries. Although rickets may be less prevalent in the United States, one review noted at least 166 case reports of nutritional rickets identified between 1986 and 2003 [35], and the incidence appears to have risen over the past decade [36]. Patients at risk for rickets include infants who are solely breast-fed (due to low amounts of vitamin D present within breast milk), children with dark skin pigmentation, premature infants, and malnourished children. In addition, children with chronic medical disorders may be at high risk for rickets, particularly those with gastrointestinal malabsorption, pancreatic insufficiency, renal insufficiency, liver disorders, significant burn injury, and receiving

medications that alter vitamin D metabolism (i.e., anticonvulsant and antiretroviral medications) [32]. In children who have sustained burn injuries, cutaneous vitamin D synthesis is not normal even a year after the burn. Interestingly, unburned adjacent skin has similarly decreased conversion of 7-dehydrocholesterol to 1,25-dihydroxyvitamin D3 [37]. Moreover, the vitamin D deficiency after the burn injuries is progressive, with low 25(OH)D levels noted 2 years postburn, while 1,25(OH)2D levels are normal [38].

Underlying genetic abnormalities can also result in the development of rickets, either by affecting the production of calcitriol or by inducing resistance to the actions of vitamin D. For example, vitamin D-dependent rickets type 1 is an autosomal recessive disorder that causes abnormally low activity of 1-alpha-hydroxylase leading to low calcitriol levels. This results in the classic skeletal manifestations of rickets and can cause significant hypocalcemia, typically presenting in the first year of life. Treatment includes calcitriol administration along with adequate calcium intake [39, 40]. Hereditary vitamin D-resistant rickets, formerly known as vitamin D-dependent rickets type 2, is an autosomal recessive condition that causes calcitriol resistance due to a mutation in the VDR gene. Patients typically present in the first several years of life with skeletal findings, including rachitic changes and often alopecia [41, 42]. In these patients, increased calcium supplementation or intravenous calcium infusions may be effective in treating rickets [43–46]. In the setting of end-organ resistance to vitamin D, the fact that calcium alone can treat this condition illustrates the important point that one of the principal roles of vitamin D is related to its effects on calcium availability, and that calcium (along with phosphorus) may be more critical than vitamin D in regard to effects on bone.

After closure of the growth plates, severe calcium and/or vitamin D deficiency can result in defective mineralization referred to as osteomalacia. Histologically, the resulting bone is marked by an abundance of undermineralized osteoid. This is not an uncommon finding in the general adult population and contributes to increased bone fragility [47–50]. Like rickets, the specific serum 25(OH)D concentration that leads to the development of osteomalacia is unknown, although a recent study of postmortem bone biopsies and blood 25(OH)D levels among 675 adults, aged 20–100 years, suggested that osteomalacia is most likely to occur at a 25(OH)D level below 20 ng/mL and does not occur at levels above 30 ng/mL [51].

Treatment approaches for rickets and osteomalacia with calcium and vitamin D will be reviewed later in this chapter.

Calcium and Pediatric Bone Health

Calcium Requirements in the Pediatric Population

Bone accretion occurring in growing infants, children, and adolescents leads to varying calcium requirements across the age spectrum. Infancy in particular is a time of high calcium requirement, typically met with a combination of high

intestinal calcium absorption and high calcium content in breast milk and/or formula. Premature infants require even higher amounts of calcium due to the high rate of growth and bone mineralization that occurs in the third trimester, and these infants typically require fortified formula with a dose of calcium of 80–120 mg/kg/day in addition to that received in milk, for a total of 200 mg/kg/day [52]. If this calcium need is not met, these infants are at risk for "osteopenia of prematurity" (previously known as "rickets of prematurity"). In term infants, total body calcium mass at birth is estimated at 30 g, increasing to approximately 80 g by 12 months of age [53, 54]. To account for this gain, calcium accretion rate approximates 100 mg/day during the first year of life [55], which is readily available in breast milk and infant formulas.

Calcium absorption in infants is initially primarily passive [56, 57] and then gradually involves more active transport [58]. Breast-fed infants have the highest calcium absorption rate at approximately 60 %, and this rate is somewhat lower in formula-fed infants at 30–40 %, compensated by the higher calcium content in formula compared to breast milk [19, 55, 59, 60]. According to the recent guidelines of the Institute of Medicine (IOM), the recommended daily allowance (RDA) for infants 0–6 months is 200 mg/day, and increases to 260 mg/day from 6 to 12 months as solid foods are introduced [61].

Bone growth and calcium accrual continue into childhood and reach a peak during adolescence. In children, average calcium accretion rate is estimated at approximately 140 mg/day, increasing to 140–160 mg/day as puberty approaches [55, 62, 63]. The pubertal growth spurt leads to increases in bone mass and size, such that up to 40 % of bone mass is acquired in a 3- to 5-year period [3, 64]. The average calcium accretion during this period varies by gender ranges from 90 to 200 mg/day [65] and can peak at 300–400 mg/day [66]. At the completion of puberty, a small amount of bone accretion may occur after the age of 20 years, although at a much lower rate [67], and peak bone mass is typically reached by age 30 years [68]. To accommodate this increased calcium requirement during adolescence, intestinal calcium absorption rises as puberty progresses, from approximately 28 % during childhood to 34 % during adolescence, and back down to 25 % thereafter [18]. This increased absorption is primarily mediated through rise in circulating PTH concentrations, leading to higher calcitriol levels [29]. In order to meet these calcium needs, the IOM has set the RDA for calcium as 700 mg/day in ages 1–3 years, 1,000 mg/day in 4–8 years, and 1,300 mg/day in 9–18 years [61].

Pediatric Studies Evaluating the Effects of Calcium on Bone Health

Although it is clear that insufficient calcium intake is harmful to skeletal health, whether calcium intake above this amount, particularly via calcium supplementation, is beneficial is less apparent. A majority of studies assessing calcium intake and bone health have been performed in adults, particularly focusing on postmenopausal

women and patients with osteoporosis. These studies have confirmed the importance of adequate calcium intake in maintaining bone health in adults and have driven recommendations for daily calcium requirements. Although less well represented than in the adult literature, multiple studies evaluating calcium intake and bone health in pediatrics have been performed, providing important insights into our understanding of this complex issue.

As detailed above, calcium requirements in infants are primarily met in breast milk and formula, allowing for bone accrual to occur. Whether additional calcium during this period improves long-term bone health is unclear. In one study, premature infants receiving formula with 700 mg/L of calcium had greater bone mineral content (BMC) at a corrected gestational age of 3 and 9 months compared to those receiving formula with 350 mg/L [69]. However, other studies suggest that the effects of higher calcium intake on bone mass accrual in infancy may be transient [70, 71], and further studies are required.

More data are available assessing calcium interventions in childhood and adolescence. Multiple randomized controlled trials have been performed in this population, and the majority have suggested neutral or modest improvement in bone outcomes. Several studies have suggested that bone mineral density (BMD) and bone accretion are improved with calcium intake that is increased above a baseline of 900 mg/day [72–78]. However, other studies suggest that this may only be a transient effect [79–81]. For example, in a trial of adolescents in the United States ages 15–18 years, improvement in hip and forearm BMD was noted after calcium supplementation [78]. A 3-year randomized controlled trial evaluated the effect of 1,000 mg of calcium supplementation compared to placebo in twins, revealing increased BMD in prepubertal supplemented subjects, although not in pubertal or postpubertal subjects [72]. A meta-analysis of 19 trials involving 2,859 subjects treated with calcium supplementation at a dose of 300–1,200 mg daily showed a 1.7 % increase in upper limb BMD compared to placebo, without an effect on other sites [82, 83]. Overall, these studies suggest that increased calcium intake and supplementation may increase skeletal size and mineralization, although it is unclear whether the effect is sustained.

The effect of calcium supplementation on fractures in the pediatric population is also unclear. In the above-mentioned meta-analysis, there was uncertain benefit on fracture rate [82, 83]. Epidemiologic studies suggest that there is an inverse association between calcium intake and childhood fracture rate [84–88]. For example, one case–control study in 100 Caucasian girls aged 3–15 years with distal forearm fractures and 100 controls without fractures found that older girls with fractures reported lower calcium intake than controls [85]. Another study suggested a decreased odds ratio for fracture in children, mean age 14 years, with higher calcium intake [88].

The effect of calcium supplementation in childhood and adolescence on the future development of low bone density and osteoporosis in adulthood also remains to be elucidated. Although intuitively it would be reasonable to hypothesize that any intervention that could optimize the achievement of peak bone mass might improve bone health into adulthood, this has not been proven to date in prospective long-term interventional studies. In observational and retrospective studies, there may be

a suggestion of support for this hypothesis. For example, BMD measured by pQCT of the dominant radius in postmenopausal women correlated with retrospective recall of dietary history of calcium intake in childhood [89]. Given the limits of this type of data, the question remains as to the long-term efficacy of calcium supplementation on future osteoporosis risk.

Vitamin D and Bone Health in the Pediatric Population

Defining Vitamin D Deficiency and Sufficiency

The level of 25(OH)D required for the optimization and maintenance of bone health is controversial, and recommendations from different academic and professional medical societies vary. Many of the studies used in the determination of definitions for vitamin D deficiency and sufficiency have primarily involved adults, and data in the pediatric population are limited. In assessing optimal vitamin D status, studies have focused on determination of the PTH plateau (i.e., the 25(OH)D level at which PTH levels stabilize), calcium absorption studies, bone turnover markers, BMD measures, and fracture outcomes. Because many of these factors are affected by multiple confounders, such as concurrent calcium intake, age, and sunlight exposure, it has been difficult to establish consensus on this issue.

In adults, several studies have evaluated the association between serum 25(OH)D and PTH concentrations to determine whether there is a minimum 25(OH)D level that elicits a rise in PTH, as well as the maximum level of 25(OH)D at which PTH no longer declines. This is referred to as the PTH plateau. Initial influential studies in adults suggested that a PTH plateau occurs at a 25(OH)D level of 30 ng/mL (75 nmol/L) [90, 91]. However, this has been called into question, and more recent data suggest that there may not be an absolute threshold of 25(OH)D at which the PTH plateau occurs, but rather this may range from a 25(OH)D level as low as 12 ng/mL (30 nmol/L) to over 50 ng/mL (125 nmol/L) depending on multiple other confounding factors [92]. In the pediatric population, fewer studies have evaluated this question, and a clear point of PTH inflection based on 25(OH)D level has not yet been identified in children and adolescents [93].

Calcium absorption studies have also been used as a consideration for determining the ideal serum concentration of 25(OH)D that maximizes intestinal absorption of calcium, as this has important implications on calcium availability for bone health. In adults, a threshold of 32 ng/mL (80 nmol/L) was suggested as the level at which maximal efficacy in intestinal calcium absorption is reached [27]; however, other studies in adults have suggested that calcium absorption is not necessarily optimized at levels above 30 ng/mL [17, 94, 95]. Studies addressing this question in children are fewer. In one dual-labeled calcium absorption study in 251 children and adolescents ages 5–17 years, higher 25(OH)D level was not found to affect total or fractional calcium absorption in school-age children, and there was a modest effect

of higher 25(OH)D on calcium absorption in early pubertal subjects [96]. In this study, highest calcium absorption was noted at the range of 11–20 ng/mL. Other pediatric studies have also suggested that calcium absorption may not necessarily be increased with the achievement of a higher 25(OH)D level [97, 98].

Adult studies have also illustrated that bone turnover markers are affected by circulating 25(OH)D level, such that higher 25(OH)D levels were associated with lower bone turnover markers such as serum osteocalcin and C-telopeptides, and urinary N-telopeptides [99]. In children, cross-sectional studies have similarly indicated that an inverse association exists between bone turnover markers and serum 25(OH)D, with lower markers of bone resorption with an improved vitamin D status [100–102]. In clinical trials of vitamin D supplementation in children and adolescents, effects on markers of bone turnover have been varied, with some reporting a decline in bone resorption markers [103], an increase in bone formation markers [104], or no change [105, 106].

Studies assessing associations between serum 25(OH)D concentration and both BMD and fracture risk have primarily focused on adults, with a particular emphasis on postmenopausal women because this population is at highest risk for osteoporosis and fracture. Although out of the scope of this chapter, observational data have supported the association of higher 25(OH)D with improved BMD and reduced fracture risk, although randomized controlled trials have yielded more variable results [34, 107]. Clinical studies assessing these outcomes in children, as well as how this affects future adult BMD and fracture risk, will be reviewed in more detail below.

Based on the above data regarding vitamin D and skeletal outcomes, the Institute of Medicine 2010 Report redefined vitamin D sufficiency in the healthy pediatric and adult population as a 25(OH)D level above 20 ng/mL and vitamin D insufficiency as less than 20 ng/mL [61], a level that is lower than many experts have recommended [22, 108, 109]. The IOM Committee interpreted available data as showing that higher serum 25(OH)D levels were not consistently associated with greater benefit, and possible U-shaped associations were seen with some outcomes suggesting that both high and low levels of 25(OH)D may be associated with greater risk of negative skeletal outcomes [61]. In contrast, The Endocrine Society published a Clinical Practice Guideline aimed at pediatric and adult individuals at risk for vitamin D deficiency or low bone density, defining vitamin D sufficiency in these patients as a 25(OH)D level above 30 ng/mL, vitamin D insufficiency as a level 20–30 ng/mL, and vitamin D deficiency as a level less than 20 ng/mL [110]. Future studies will be required to determine the long-term effects of these recommendations, particularly in children as they progress to adulthood.

Studies Evaluating Vitamin D and Bone Health in the Pediatric Population

In the following section, the important observational studies, randomized control trials, meta-analyses, and reviews involving infants, school-age children, and adolescents will be reviewed.

Infants

During development, fetal 25(OH)D concentrations are dependent on transplacental passage of vitamin D from the mother; therefore, 25(OH)D levels in neonates are directly related to mother's vitamin D status. Apart from the previously presented data on rickets, the effects of vitamin D on bone health in infants and long-term repercussions are largely unknown. Studies in a relatively small number of infants have shown inconsistent results regarding associations between serum 25(OH)D levels and BMC in this age group [111–116]. Although some case–control studies suggest a direct association between BMC and 25(OH)D [113, 114], clinical trials have not confirmed this finding. In a randomized controlled trial of 18 breast-fed infants testing 400 IU as a supplementation dose, the treated infants had higher 25(OH)D levels and a transient increase in BMC at the radius at 12 weeks, although BMC changes did not persist at 26 weeks [111]. In a similar study in 46 infants, no difference in BMC was noted at 6 months [115]. Recently, a double-blind randomized clinical trial of 132 1-month old breast-fed infants receiving vitamin D supplementation at doses of 400, 800, 1,200, or 1,600 IU found that 97 % of all infants achieved a 25(OH)D level of 20 ng/mL after 3 and 12 months of supplementation regardless of dose [116]. There were no differences in growth or BMC between groups at 12 months. Only the dose of 1,600 IU daily led to 25(OH)D levels above 30 ng/mL at 3 months in 97.5 % of subjects; however, supplementation with this dose resulted in 25(OH)D levels that exceeded the healthy population target of 50 ng/mL, raising the possibility that this dose may lead to vitamin D toxicity in this population [116].

Long-term effects of vitamin D supplementation are also unclear. In one retrospective cohort study of 106 girls, higher BMD was noted at age 7–9 years in subjects who had received vitamin D supplementation during the first year of life [117] Similarly, whether low vitamin D levels are associated with increased fracture risk in infancy or in future childhood or adulthood has yet to be established. For example, a cross-sectional study of 380 healthy infants and toddlers found that 12 % had 25(OH)D <20 ng/mL, and of these 33 % had demineralization on wrist and knee radiographs [118]. In those 40 infants with 25(OH)D levels less than 20 ng/mL, none sustained a fracture after 2 years of follow-up [119]. Further studies evaluating the effects of vitamin D supplementation in infancy on bone health, fracture risk, and future peak bone mass are required.

Children and Adolescents

Observational studies in children and adolescents have suggested a direct association between serum 25(OH)D levels and BMC or BMD [100, 101, 120–122]. One of the largest cross-sectional studies in this population evaluating 576 12-year-olds and 489 15-year-olds showed that girls (although not boys) with higher 25(OH)D levels had significantly greater forearm BMD [101]. Vitamin D intake in

adolescence has also been associated with higher adult BMD in males (although not females) [122]. 25(OH)D levels were directly associated with cortical BMD at the radius and tibia in another study of 10- to 12-year-old girls [100]. In the recent AHRQ review published in 2009, experts determined that there was fair evidence from observational studies in older children and adolescents to support a direct association between serum 25(OH)D levels and baseline BMD, as well as a change in BMD or BMC [107].

However, randomized control trials in this population have shown inconsistent results of vitamin D supplementation on bone density across different skeletal sites, ages, and gender [107]. For example, a trial of 26 healthy girls did not find an effect of supplementation with 400 IU vs. 800 IU daily of vitamin D for 1 year on BMC compared to placebo [123]. However, a larger study of 179 girls ages 10–17 years randomized to weekly oral vitamin D doses of 1,400 or 14,000 IU or placebo found that bone area and total hip BMC increased significantly after 1 year in the high dose group compared with the placebo and lower dose groups [124]. In a meta-analysis of available pediatric randomized clinical trials encompassing a total of 541 subjects receiving vitamin D supplementation and 343 controls [125], vitamin D supplementation did not have a statistically significant effect on total body BMC or hip or forearm BMD, although a slight effect (nonsignificant trend) was noted at the lumbar spine. However, in subjects with a low serum vitamin D (less than 14 ng/mL or 35 nmol/L), total body BMC and lumbar spine BMD had a significantly greater increase from baseline, by 2.6 % and 1.7 %, respectively, in supplemented subjects compared to placebo. Authors concluded that vitamin D supplementation was unlikely to be beneficial in pediatric subjects with normal vitamin D levels, but may result in clinically useful improvements in BMD in those with vitamin D deficiency [125]. Given the variability in these prior findings, further studies are needed. These conflicting results may also be due to varying corrections for bone size, or the lack thereof. Dual X-ray absorptiometry (DXA) measures in children can be confounded by short stature. Therefore, height will be an important variable for which the BMD *Z*-score should be adjusted in future clinical trials. In addition, studies utilizing quantitative computed tomography to evaluate three-dimensional bone geometry and strength may be helpful to evaluate the effect of vitamin D on growing bones.

The effect of vitamin D levels and supplementation on fracture risk in childhood and later in adulthood is also unclear. In one observational study, there was 50 % reduced risk of stress fracture in preadolescent and adolescent girls with higher vitamin D intake compared to those with lower intake [126]. However, these results have yet to be replicated in prospective trials of vitamin D supplementation.

In conclusion, a direct association between 25(OH)D and BMD or BMC in children and adolescents has been suggested in observational studies, but randomized clinical trials have not yet confirmed that vitamin D supplementation improves bone health in healthy children and adolescents. Further studies are needed to delineate the short- and long-term effects of vitamin D on future BMD and fracture risk in adulthood.

Calcium as a Pediatric Bone Drug

As detailed in Table 10.2, the IOM has developed recommendations for calcium intake in infants, children, and adolescents based on available calcium balance studies, with the tolerable upper limit of calcium set at 3,000 mg/day [61]. Although these recommendations are believed to account for the calcium requirements of the growing skeleton, it has yet to be confirmed that these recommendations will have long-term effects on BMD in children. However, these recommendations appear to be safe [127], and it is known that extremely low intakes (e.g., less than 600 mg/day) may put children at risk for inadequate calcium retention [128], suggesting that adequate intake (AI) at these levels could prevent inadequate mineralization.

Calcium within food is primarily found in dairy products such as milk, cheese, and yogurt, as well as some vegetables, legumes, fish, and grains. Foods such as orange juice and cereal may also be fortified with calcium (Table 10.1). Recent studies have suggested that the actual amount of dietary calcium intake in children, and particularly in adolescents, is likely significantly lower than the recommended amounts. In adolescents, calcium intake likely ranges between approximately 500–1,000 mg/day [18, 29, 129, 130], rather than the RDA of 1,300 mg/day. Calcium supplements are also an option for ensuring adequate calcium intake and optimizing bone health; however, calcium supplementation also is associated with both benefits and potential side effects.

Oral calcium supplements are primarily available as calcium carbonate and calcium citrate, and less commonly calcium glubionate. Intravenous forms, such as calcium gluconate and calcium chloride, may be used for the treatment of severe hypocalcemia or for stabilization of the myocardium during emergency situations. Calcium availability varies based on the form of calcium administered (Table 10.3), and oral amounts above 500 mg should be administered in divided doses due to limitations on the amount of calcium that can be absorbed at a given time [131]. The most common form of calcium supplement, calcium carbonate, contains

Table 10.2 2010 Institute of Medicine report on dietary reference intakes for calcium and vitamin D

IOM recommendations for calcium and vitamin D intake by age [61]				
	Vitamin D		Calcium	
	RDA	UL	RDA	UL
Infants				
0–6 months	400 IU[a]	1,000 IU	200 mg[a]	1,000 mg
6–12 months	400 IU[a]	1,500 IU	260 mg[a]	1,500 mg
Children				
1–3 years	600 IU	2,500 IU	700 mg	2,500 mg
4–8 years	600 IU	3,000 IU	1,000 mg	2,500 mg
Adolescents				
9–13 years	600 IU	4,000 IU	1,300 mg	3,000 mg
14–18 years	600 IU	4,000 IU	1,300 mg	3,000 mg

[a]Adequate intake (AI) was provided in these age groups due to insufficient evidence for the development of a recommended daily allowance (RDA)

Table 10.3 Types of calcium supplements and elemental calcium content

Type of calcium	Route of administration	Elemental calcium per 1 g calcium salt (mg)
Calcium carbonate	Oral (tablet)	400
Calcium citrate	Oral (tablet)	211
Calcium glubionate	Oral (liquid)	66
Calcium gluconate	IV	93
Calcium chloride	IV	273

approximately 40 % elemental calcium. Because acid is required for absorption, it is best taken with food and is less well absorbed with medications that reduce stomach acid (e.g., proton pump inhibitor or H_2 blocker medications). Calcium citrate has a lower elemental calcium content of approximately 21 %, but this form may be better absorbed than calcium carbonate [131] and is less dependent on an acidic gastric environment, making it a better choice in patients on acid blockade [132–134]. Calcium glubionate has low elemental calcium content but is also available in a liquid form. Head-to-head trials comparing different forms of calcium supplements on bone outcomes are lacking, particularly in pediatrics.

Risks of Calcium Supplementation

Calcium supplementation may also be associated with risks, particularly when total calcium intake is excessive. These risks include hypercalcemia, which can lead to anorexia, weight loss, polyuria and dehydration, constipation, and renal insufficiency. Milk alkali syndrome is a condition marked by excess intake of calcium leading to hypercalcemia, metabolic alkalosis, and renal failure, typically seen at levels of intake greater than 3,000 mg/day [135–137]. Hypercalciuria can also develop with calcium supplementation, considered to be a urinary calcium level of above 250 mg/day in women, above 275–300 mg/day in men, and above 4 mg/kg/day in children over age 2 years. Hypercalciuria can predispose to nephrocalcinosis and nephrolithiasis. Although the development of kidney stones is often multifactorial related to different dietary and non-dietary factors, there may be an increased risk of nephrolithiasis with calcium supplementation in adults. For example, in the Women's Health Initiative, where 36,000 postmenopausal women were randomized to calcium and vitamin D supplementation (1,000 mg/day and 400 IU/day, respectively) or placebo, the supplemented group had an increased risk of kidney stones (HR 1.17) [138]. Both supplemented and placebo groups in this study were noted to have a high baseline dietary intake of calcium of 1,100 mg/day, which may have contributed to this finding. Studies in children are limited, but two studies suggested that calcium supplementation does not significantly affect urinary calcium excretion at daily intakes (diet plus supplement) of 1,800 mg/day [139] or 1,560–1,740 mg/day [140]. In addition, because IV calcium gluconate formulations contain aluminum, prolonged administration of IV calcium gluconate may lead to aluminum toxicity, including osteomalacia and bone pain [141].

Recent studies in adults have also raised the question as to whether calcium supplementation may predispose to vascular and soft tissue calcification, potentially leading to increase in cardiovascular disease and higher mortality. For example, a recent meta-analysis evaluating 15 randomized controlled trials of calcium supplementation involving 20,000 adult participants suggested an increased risk of myocardial infarction with this intervention [142]. Although these data are not available in the pediatric population, this does raise concern regarding the advisability of routine calcium supplementation, and optimizing dietary calcium may be a safer approach than supplements.

Vitamin D as a Pediatric Bone Drug

As previously reviewed, the level of serum 25(OH)D at which bone health is optimized in the pediatric population remains controversial, and various professional societies have differed regarding the 25(OH)D level that represents sufficiency. Depending on the definition used, low vitamin D concentrations are common among individuals across the United States. Data from the 2001–2006 National Health and Nutrition Examination Survey showed that 18 % of children ages 1–11 years had a 25(OH)D level less than 20 ng/mL, and 1 % had a level less than 10 ng/mL, although this varied by age, gender, and race [143]. Reasons for vitamin D deficiency most commonly include inadequate intake or sun exposure, and other potential causes may be inadequate absorption due to underlying gastrointestinal issues or malabsorption, accelerated metabolism (i.e., from antiepilectic drugs or certain HIV medications), impaired liver hydroxylation due to hepatic disease, impaired 1-alpha-hydroxyaltion due to renal disease, or excess adipose tissue leading to vitamin D sequestration in obese patients.

Vitamin D supplements are administered orally in the form of vitamin D2 and vitamin D3. These supplements can be taken on an empty stomach and are available in a liquid form or in pill or capsule form. In the United States, vitamin D is not currently available in intravenous or intramuscular formulations. In patients dependent on intravenous parental nutrition, these preparations typically contain multivitamins that include 400 IU/L of vitamin D, although this may not be adequate to prevent vitamin D deficiency in these patients [144].

Vitamin D2 vs. Vitamin D3

Although vitamin D2 and vitamin D3 are often considered bioequivalent and interchangeable, recent questions have been raised as to whether differences in efficacy may exist. As previously mentioned, these two forms of vitamin D differ based on side change structure (Fig. 10.1), but available evidence suggests that the steps involved in their metabolism and action are the same [1], including hydroxylation

[145, 146], binding to vitamin D binding protein [147], and binding of their activated forms to VDR [148].

In adults, some clinical studies suggest equal potency between vitamin D2 and vitamin D3 [149, 150] whereas others have suggested that vitamin D3 may be more effective [151–153]. A recent meta-analysis of seven trials involving supplementation with vitamin D2 vs. D3 found a significantly greater overall change in 25(OH)D levels with vitamin D3 over vitamin D2; however, the studies included in this meta-analysis utilized a variety of supplementation regimens ranging from a single very high dose of vitamin D to daily supplementation with lower doses [154]. When studies evaluating only daily supplementation regimens were evaluated, no significant difference between D2 and D3 was noted, suggesting that this difference in efficacy may only be present at very high doses of vitamin D.

Pediatric studies evaluating vitamin D2 and D3 regimens have been fewer and have also come to varying conclusions regarding the efficacy of these formulations. In 40 otherwise healthy infants and toddlers with serum 25(OH)D levels below 20 ng/mL, three different supplementation regimens (vitamin D2 50,000 IU weekly, vitamin D2 2,000 IU daily, or vitamin D3 2,000 IU daily) resulted in a similar tripling of serum 25(OH)D over a 6-week period, without significant differences between regimens [155]. In contrast, in a study of children and adolescents ages 5–20 years with underlying inflammatory bowel disease, the authors found that a regimen of vitamin D3 2,000 IU daily and vitamin D2 50,000 IU weekly was superior to vitamin D2 2,000 IU daily in raising 25(OH)D [156]. Whether compliance differences were at play, especially among the adolescent subjects, is unclear.

Given the current available data, vitamin D3 may potentially be more effective in raising serum 25(OH)D in adults. However, no difference in efficacy between the two forms used in daily supplementation has been confirmed, and the effects of these different formulations in children remain unclear. In addition, whether vitamin D2 or vitamin D3 has differential effects on bone health and fracture risk in children and adults requires study. Provision of vitamin D and optimal compliance with whichever regimen that is prescribed appear to be the most critical issues.

Recommendations for Vitamin D Supplementation in Healthy Infants, Children, and Adolescents

The dose of supplemental vitamin D that should be recommended in the pediatric population has been debated, and recommendations have changed over the past several years. In the most recent Institute of Medicine Report in 2010, the vitamin D supplementation dose recommended for infants 0–12 months was 400 IU; the dose for children and adolescents was 600 IU daily [61]. Prior to this Report, the IOM had previously recommended 200 IU daily in all pediatric age groups [157]. In contrast, the American Academy of Pediatrics previously recommended 200 IU daily for children and adolescents [158], which was increased to 400 IU daily in 2008 [159]. Based on the variability among these recommendations, it is often up to the individual clinician and the specific patient to decide what should be administered.

In healthy adults, studies have suggested that each 100 IU of vitamin D will increase serum 25(OH)D by approximately 1 ng/mL [150, 160]. Some pediatric studies have shown similar effects on serum 25(OH)D with vitamin D supplementation [124, 155]. For example, two clinical trials in healthy children and adolescents compared vitamin D administered weekly at doses of 1,400 IU (equivalent to 200 IU daily), 14,000 IU (equivalent to 2,000 IU daily), or placebo over 1 year [124, 161]. Both of these studies showed a significant difference in mean change in serum 25(OH)D levels between treatment groups, with an increase in serum 25(OH)D over 12 months of 3–4 ng/mL with low dose supplementation (similar to placebo) and 21–24 ng/mL with the higher dose. These studies also suggest that at least 2,000 IU daily may be required to maintain 25(OH)D levels above 30 ng/mL in healthy adolescents [161]. In contrast, in a clinical trial of healthy adolescents with baseline vitamin D sufficiency (serum 25(OH)D above 20 ng/mL), supplementation with doses of 200 IU daily and 1,000 IU daily over 11 weeks during winter maintained stable 25(OH)D levels without a difference between treatment groups [106]. Differences in study outcomes are likely related to multiple factors including study design, subject age, baseline 25(OH)D level, and confounders such as sunlight exposure, race, and compliance. Further studies are needed to identify the specific dose of vitamin D required for both the healthy pediatric population.

Recommendations for Vitamin D Supplementation in At-Risk Pediatric Populations

Some pediatric patient populations may be at higher risk for compromised bone density and vitamin D deficiency and may, therefore, require different vitamin D supplementation doses to maintain optimal serum 25(OH)D concentrations than those recommended by the IOM, which was intended to address the needs of 97.5 % of the healthy population. Table 10.4 includes conditions that may warrant special attention to vitamin D levels and supplementation. Patients with underlying gastrointestinal diseases, malabsorption, malnutrition, metabolic bone diseases, inflammatory disorders, endocrinopathies, renal diseases, and significant burn injury, and those treated with medications detrimental to bone are at risk for not attaining peak bone mass and may benefit from higher vitamin D doses and target serum 25(OH) D levels to optimize their bone health,

To address the care of these “at-risk” patients, The Endocrine Society developed a Clinical Practice Guideline in 2011 [110]. Among these patients, a serum 25(OH) D level of less than 20 ng/mL is consistent with vitamin D deficiency, a level between 20 and 29 ng/mL with vitamin D insufficiency, and above 30 ng/mL with vitamin D sufficiency. These definitions are in agreement with other professional societies, including the International Osteoporosis Foundation [162] and the National Osteoporosis Foundation [163]. To achieve sufficient 25(OH)D levels, the Endocrine Society Guideline-recommended doses of supplemental vitamin D were 400–1,000 IU daily in infants 0–12 months and 600–1,000 IU daily in children and

Table 10.4 At-risk pediatric populations

Pediatric patient populations at risk for vitamin D deficiency and low bone density	
Solely breast-fed and/or premature infants	Liver failure
Individuals with dark skin pigmentation	Chronic kidney disease
Metabolic bone diseases	Endocrinopathies
Idiopathic juvenile osteoporosis	Hyperthyroidism
Primary hyperparathyroidism	Hypogonadism
Hereditary forms of rickets	Diabetes mellitus
Osteogenesis imperfecta	Growth hormone deficiency
Connective tissue disorders	Solid organ or bone marrow transplant recipients
Inflammatory diseases	Medication use
Rheumatoid arthritis	Glucocorticoids
Systemic lupus erythematosis	GnRH agonists
Inflammatory bowel disease	Aromatase inhibitors
Gastrointestinal malabsorption	Antiepileptic drugs
Celiac disease	Immunosuppressants
Short gut syndrome	Chemotherapeutic agents
Malnutrition	Depot medroxyprogesterone acetate
Anorexia nervosa	
Burn injury	

adolescents, though authors acknowledge that higher doses may be required in order to maintain 25(OH)D levels in the sufficient range [110]. Whether these recommendations will result in long-term bone health improvements in these at-risk patients requires further study.

Treatment Regimens for Rickets and Vitamin D Deficiency

Treatment of pediatric patients with rickets and vitamin D deficiency requires higher doses of vitamin D than standard supplementation along with concurrent close attention to adequate calcium intake in diet or supplement form. For infants and young children diagnosed with vitamin D deficiency rickets, recommendations for treatment regimens vary but typically consist of at least 6 weeks of vitamin D2 or D3 1,000–2,000 IU daily in infants 0–12 months old, and 2,000 IU daily in children over 12 months old. Alternatively, vitamin D2 50,000 IU weekly for 6 weeks can be prescribed [110, 155, 164]. Other regimens have also been studied, including observed administration of a single dose of 150,000–600,000 IU orally (also known as "stoss therapy"), particularly when compliance or follow-up is uncertain; however, concerns about possible hypercalcemia limit this option [165, 166]. Higher doses, sometimes 2–3 times more than standard recommendations, may be required if the patient has underlying obesity, malabsorption, or increased vitamin D metabolism [110]. To avoid the complication of hypocalcemia from "hungry bone syndrome" as vitamin D deficiency is corrected and calcium is mobilized back into bone, patients should

also be prescribed concurrent calcium supplementation, typically at a dose of elemental calcium 30–75 mg/kg/day in three divided doses. If a patient presents with hypocalcemic seizure, then intravenous calcium gluconate 10–20 mg/kg/dose run over 5–10 min may be required for rapid correction of hypocalcemia.

After initiation of therapy, serum studies should be monitored at least monthly in patients with rickets, including PTH, calcium, phosphorus, alkaline phosphatase, 25(OH)D, and a spot urine calcium/creatinine ratio; the latter should be repeated with a 24-h urine collection if the spot sample is abnormal. These results can determine if the intervention is effective, as well as guide the duration of treatment. In most patients, biochemical and radiologic improvement can be appreciated after 3 months of treatment, and the patient can be changed to maintenance vitamin D supplementation once labs have normalized and healing is noted on X-ray. In patients with vitamin D deficiency without rickets, documentation of normalization of 25(OH)D after treatment can be helpful to guide subsequent management. Maintenance vitamin D supplementation is required after completion of high dose therapy to prevent recurrence of vitamin D deficiency.

Of note, rickets can also be seen in cases of severe dietary calcium deficiency even in the face of normal serum 25(OH) levels, and in these cases, calcium supplementation is the primary treatment. In addition, rickets due to genetic resistance to vitamin D (VDDR1 and hereditary vitamin D resistance rickets) requires a different treatment approach, consisting of calcium and activated vitamin D (calcitriol), although the detailed approach to this clinical scenario is out of the scope of the present chapter.

Vitamin D Toxicity

Vitamin D intoxication can result in hypercalcemia, leading to clinical effects previously discussed including altered mental status, anorexia, dehydration, constipation, hypercalciuria, and renal insufficiency. The precise level of serum 25(OH)D that is associated with toxicity is not well established in children or in adults, and studies suggest a wide range of serum 25(OH) levels that have been reported to cause hypercalcemia, with most cases occurring at levels above 100–140 ng/mL [167–170]. This variability is likely due to the fact that multiple other factors affect calcium levels in addition to serum 25(OH)D, especially concurrent dietary calcium intake, renal function, and so forth.

Interestingly, some studies in adults have suggested a possible U-shaped curve signifying an increased risk of mortality occurring at low levels of 25(OH)D, as well as potentially at higher levels [171–173]. In addition, there has been a suggestion in the adult literature that single large doses of vitamin D may also be deleterious. For example, in a randomized trial of 2,300 elderly subjects greater than 70 years old, subjects receiving vitamin D supplementation with 500,000 IU annually for 3 years had a higher fall and fracture rate compared to placebo. This finding was particularly apparent during the first 3 months after vitamin D was administered, a

time when serum vitamin D would be the highest [174]. Data in children are limited, but have not suggested the presence of this U-shaped curve.

Based on the available, albeit limited, data on vitamin D intoxication and the suggestion of potential deleterious effects of higher 25(OH)D levels, the IOM has recommended that upper limits of vitamin D supplementation be set at 1,000 IU daily in infants 0–6 months, 1,500 IU daily in infants 6–12 months, 2500 IU daily in toddlers 1–3 years, 3,000 IU daily in children 4–8 years, and 4,000 IU daily in older children and adolescents [61]. The Endocrine Society recommendations for safe upper level of intake were higher, consisting of 2,000 IU daily in infants under 12 months and 4,000 IU daily in ages 1–18 years [110]. As illustrated by the recently published clinical trial of vitamin D supplementation in infants by Gallo et al. [116], it would be prudent to monitor 25(OH)D levels and routinely assess for potential signs or symptoms of vitamin D toxicity in pediatric patients in whom higher 25(OH)D levels are targeted and higher doses of vitamin D are recommended. Supplemental doses below these suggested upper limits appear safe based on available data.

Conclusion

In conclusion, calcium and vitamin D play an important and interrelated role in maintaining and optimizing pediatric bone health. Although controversies exist regarding the optimal level of serum 25(OH)D and dose of vitamin D supplementation, it is clear that deficiency in this vitamin as well as insufficient calcium intake is harmful for the growing skeleton, as best illustrated by cases of nutritional rickets. Studies evaluating calcium and vitamin D supplementation on bone health in pediatric populations and the long-term effects on adult skeletal health are currently limited but suggest that both are important in optimizing peak bone mass, particularly in at-risk populations. Although calcium and vitamin D supplementation may not be sufficient to prevent compromised bone health in patients at risk for low bone density complicating underlying medical conditions, ensuring adequate calcium intake and maintaining higher serum 25(OH)D levels (e.g., 30 ng/mL) is a reasonable approach to optimizing the care of these patients. Future studies on this important area of pediatric care are awaited with great anticipation.

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Chapter 11
Pediatric Maxillofacial Conditions and Drugs

Srinivas M. Susarla, Christina M. Jacobsen, and Shelly Abramowicz

Introduction

There are several systemic conditions involving bone and/or bone metabolism in craniofacial skeleton (maxilla, mandible, midfacial, orbital, and frontal bones). Primary bone pathology in these locations is unique because it is often asymptomatic. It is usually incidentally discovered during routine dental or maxillofacial imaging or as part of ongoing treatment or routine care. Many craniofacial bone disorders involve areas that cannot be easily examined and undergo significant natural change (e.g., eruption and exfoliation of teeth, growth of facial bones, and associated soft tissue changes). When the condition appears prior to completion of facial growth, intervention may impede facial skeletal development. Clinicians must consider the risks and benefits of intervention, and affects of treatment on skeletal growth and development, and determine length and method of surveillance.

Patients with maxillofacial bony lesions typically have diagnostic plain films (panoramic radiographs, skull films, or cephalograms) and/or maxillofacial computed tomography scans (CT; cuts $\leq$3mm thickness, with the field extending from the supraorbital rims to the hyoid bone). These are used for diagnosis, characterization, extent of involvement, three-dimensional views for operative planning, and

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follow-up. Patients with vascular malformations are typically imaged with magnetic resonance imaging (MRI) with gadolinium contrast and plain films and/or CT if there is skeletal involvement.

In this chapter, we will focus on management of craniofacial syndromes with bony involvement and vascular malformations. While the management of these patients can be complex, there are general principles to guide treatment.

Craniofacial Syndromes

Giant Cell Tumors and Associated Syndromes

Giant Cell Tumor

Giant cell tumor (GCT) is thought to be mesenchymal in origin, though the cell type of origin has not been identified [1–3]. GCTs can be categorized as inflammatory, osteoclastic, or vascular in nature, as they possess clinical and histological characteristics that are consistent with each of these classifications. GCTs occur over a wide range of ages (2–80 years), with most patients under 30 years of age [4]. They are more common in females and are more often seen in the mandible than the maxilla [1–4]. The anterior portions of the jaws are most commonly involved, with large mandibular lesions often seen crossing the midline [4].

Clinically, the majority of lesions are asymptomatic; they are detected on routine radiographs for dental or orthodontic purposes. Physical examination may be notable for painless expansion of the bony cortices sometimes leading to facial asymmetry. Less commonly, patients present with pain, paresthesia, or mucosal ulcerations. Radiographically, the lesions appear as uni- or multiloculated radiolucencies that may be associated with resorption of tooth roots, displacement of teeth, cortical thinning, or perforation (Fig. 11.1). There is a large variability in size (<1 to >10 cm). Smaller lesions may be confused with periapical pathology, whereas larger lesions must be distinguished from odontogenic/non-odontogenic neoplasms.

Based on biologic behavior, GCTs are classified into two groups, as originally described by Chuong and Kaban in 1985 [3]. Aggressive lesions are ≥5 cm in size, display recurrence after treatment, or have three of the following characteristics: root resorption, tooth displacement, cortical bone thinning, or cortical perforation. The more common non-aggressive lesions lack these criteria and are typically asymptomatic. There is some evidence to suggest that the difference in biologic behavior may be related to increased levels of angiogenic factors in aggressive lesions [5, 6].

Treatment protocols are based on biologic behavior. Non-aggressive lesions predictably respond to enucleation/curettage. Aggressive lesions are treated with en bloc resection to achieve histologically clear margins (1 cm), or with nerve/tooth-sparing enucleation with adjuvant anti-angiogenic therapy (such as interferon-alpha,

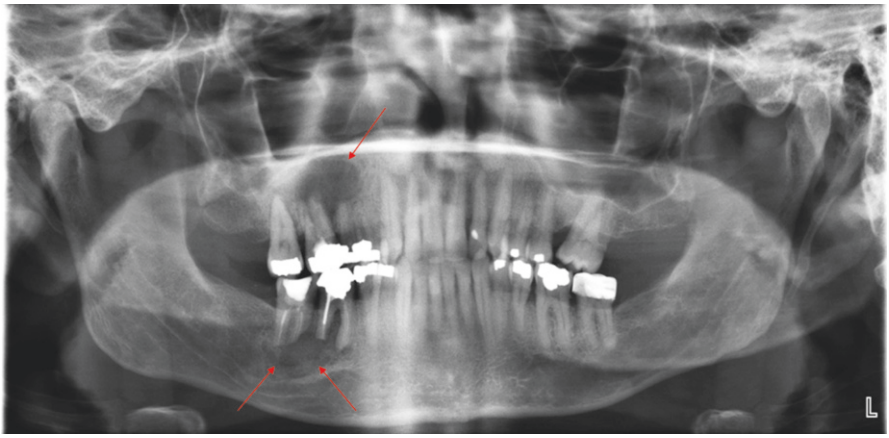

Fig. 11.1 Panoramic radiograph of a 31-year-old male patient with giant cell tumors of the right maxilla and mandible (*arrows*). The lesions are associated with root resorption of the adjacent teeth

IFN) [7–9]. Patients who have difficulty with interferon therapy (lethargy, malaise, fevers, etc.) can be successfully managed with bisphosphonate therapy. However, bisphosphonates should be used with caution if dental extractions are anticipated, due to the potential risk of osteonecrosis [1, 2].

Cherubism

Cherubism is a rare autosomal dominant condition resulting from mutation in the SH3BP2 gene (4p16.3) [10–18]. Cherubism is characterized by bilateral, symmetric GCTs in the facial bones most commonly in the mandible. Maxillary lesions and extragnathic involvement (ribs, humerus) have been reported [10, 15–17]. The condition is typically diagnosed around ages 7–10 with initial presentation around 2–5 years of age [10]. Replacement of mandibular bone with fibrous tissue gives rise to the classic appearance of "cherubic" faces, originally described by Dr. William A. Jones in 1933 [11, 12]. Involvement of the bones of the orbit can result in an upward gaze ("eyes towards heaven"). The presence of these lesions may be associated with premature loss of primary teeth and delayed eruption of permanent teeth. Extensive bony involvement can result in vision and hearing changes. In most cases, the condition becomes quiescent during late adolescence, with the facial features of most adult patients approaching normalcy by age 40 [13, 14].

Radiographically, the lesions appear as bilateral symmetric multilocular radiolucencies with associated expansion of the bony cortices (Fig. 11.2). Treatment consists of observation. Once the lesions become quiescent, treatment is aimed at recontouring excessive fibrous tissue to reestablish normal bony contours. Early, aggressive intervention has been associated with regrowth and worsening

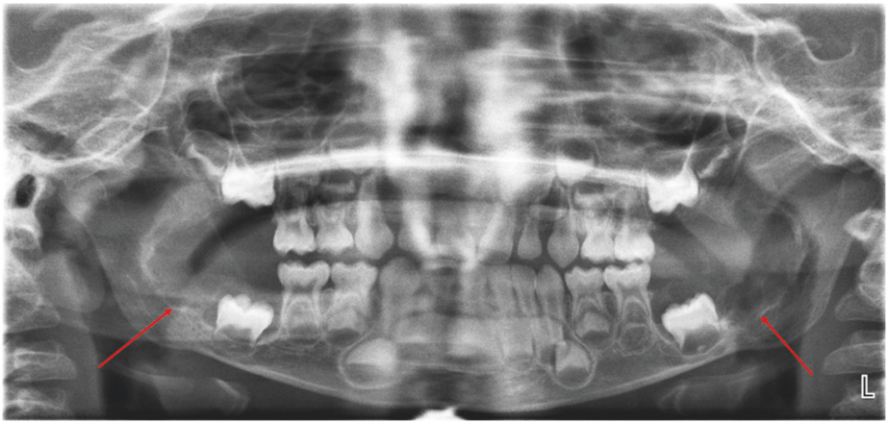

Fig. 11.2 Panoramic radiograph of a 4-year-old male with cherubism. Bilateral multilocular radiolucencies are seen in the posterior mandible (*arrows*)

deformity. Radiation therapy is contraindicated due to reports of sarcomatous degeneration [10]. During active clinical growth, annual plain film imaging is recommended. Following established quiescence, plain film imaging is recommended every 3–5 years [10, 14].

Noonan/Multiple Giant Cell Lesion Syndrome

Noonan syndrome was first described in 1963 by Dr. Jaqueline Noonan at the University of Iowa. Patients with the classic phenotype have short stature, hypertelorism, posteriorly angulated and low-set ears, congenital cardiac defects (most commonly pulmonic valve stenosis), bleeding diatheses (von Willebrand disease, factor deficiencies, thrombocytopenia), developmental delay, and cryptorchidism in males [19, 20]. In 1991, Cohen and Gorlin identified 15 cases of patients with Noonan syndrome and giant cell lesions of the jaws [21]. They coined the term Noonan/multiple giant cell lesion syndrome (NMGCLS) and considered it to be a distinct entity from Noonan syndrome and cherubism.

More recently, mutations in the SOS1 and PTPN11 genes have been identified in patients with Noonan syndrome, allowing for a molecular distinction between Noonan syndrome and cherubism [22, 23]. Molecular analyses have provided evidence to suggest that NMGCLS is a variant along the spectrum of Noonan syndrome [24, 25].

It is important for the clinician to distinguish between NMGCLS and cherubism. Clinical behavior of the associated GCTs, and thus, treatment, can be dramatically different. In NMGCLS, GCTs can demonstrate aggressive behavior and lead to significant damage to surrounding tissues [10, 26]. Genetic testing becomes particularly important in cases where there is bilateral, symmetric jaw involvement, as it may be difficult to distinguish cherubism from NMGCLS clinically or radiographically (Fig. 11.3).

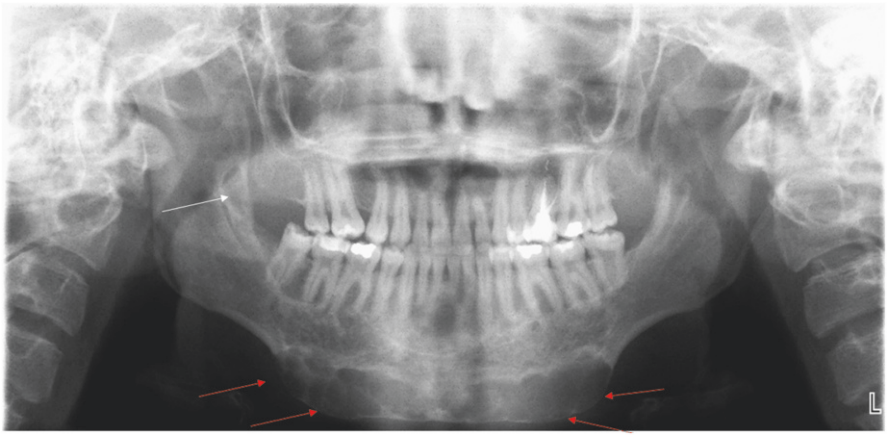

Fig. 11.3 Panoramic radiograph of a 19-year-old female with Noonan multiple giant cell lesion syndrome. An expansile, multilocular radiolucency is seen in the anterior mandible (*red arrows*). A smaller lesion is evident in the right mandibular ascending ramus (*white arrow*) (Radiograph courtesy of Leonard B. Kaban, DMD, MD)

Differentiation between the GCTs in NMGCLS and cherubism has important consequences for management. Typically, GCTs in Noonan syndrome behave more aggressively and usually require surgical intervention with adjuvant treatment [10]. Thus, it is imperative that the practitioner utilize clinical exam and diagnostic testing to differentiate between the different entities.

Fibrous Dysplasia

In fibrous dysplasia (FD), fibrous tissue and woven bone replace normal bone and marrow [27, 28]. FD is caused by somatic activating mutations in the a-subunit of the G_s protein of GNAS gene [29, 30]. Monostotic FD is more common; polyostotic FD may include McCune–Albright syndrome (polyostotic fibrous dysplasia, café-au-lait macules, precocious puberty, endocrinopathy). Patients are typically between ages 5 and 20 [27]. Clinically, patients may be asymptomatic or present with a painless swelling. Growth of the lesions tends to correlate with somatic growth and hormonal changes, with the fastest rates observed during childhood and adolescence. Quiescence typically occurs by age 25. Recrudescent growth may be seen during periods of hormonal changes (e.g., pregnancy, use of oral contraceptives). Major growth changes outside of these events are unusual and should alert the clinician to pursue additional workup.

In the maxillofacial skeleton, the most common sites of involvement are the maxilla and the mandible. In the maxilla, involvement of the orbital floor may produce paresthesia or proptosis [27]. Mandibular FD is most common at the angle; it must be distinguished from masseter hypertrophy, primary jaw tumors, or

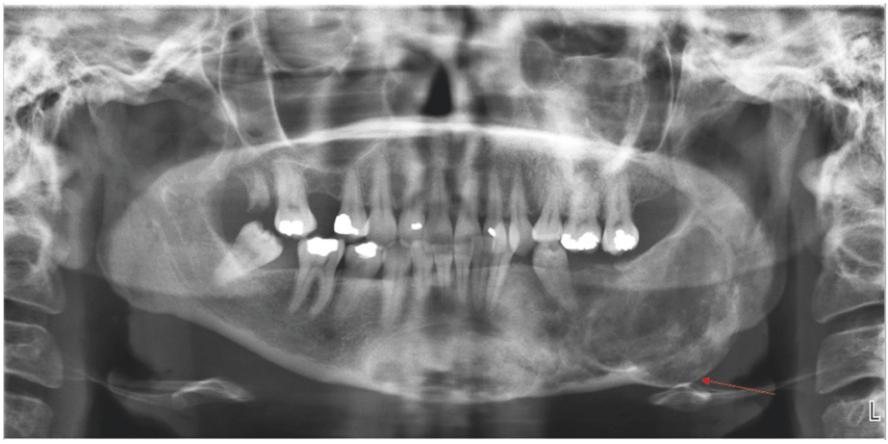

Fig. 11.4 Panoramic radiograph of a 44-year-old female with fibrous dysplasia of the mandible. Multilocular, mixed lesions are seen in the left mandibular body and mandibular symphysis, with associated cortical expansion (*arrows*) (Radiograph courtesy of Leonard B. Kaban, DMD, MD)

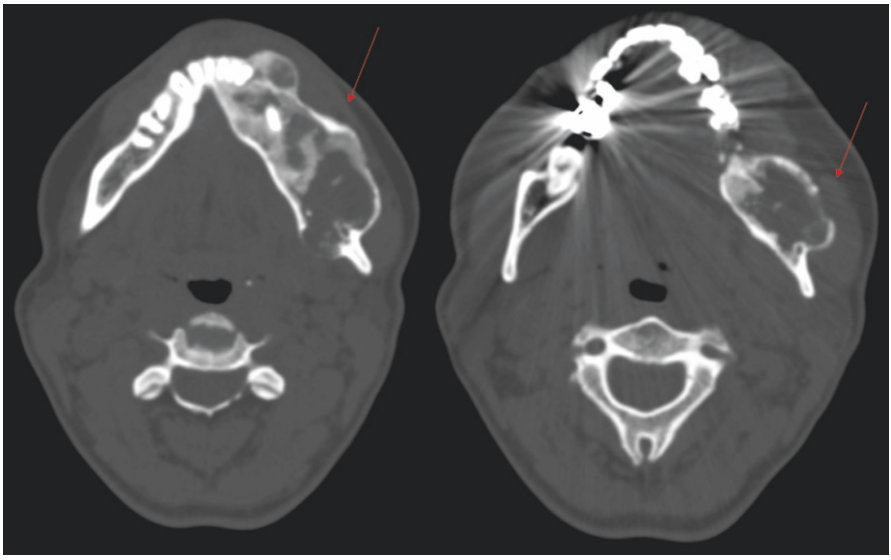

Fig. 11.5 Maxillofacial axial computed tomography of the patient from Fig. 11.4. Notice classic "ground glass" appearance and cortical expansion (*arrows*)

osteomyelitis. Radiographically, FD appears as multilocular radiolucencies or mixed radiolucent–radiopaque appearance (Fig. 11.4). On CT, FD has characteristic "ground glass" appearance (Fig. 11.5) [28]. Delayed dental eruption and splaying of the roots in the affected regions are frequently seen (Fig. 11.6). Serum levels of calcium, phosphate, and alkaline phosphatase are typically normal for monostotic

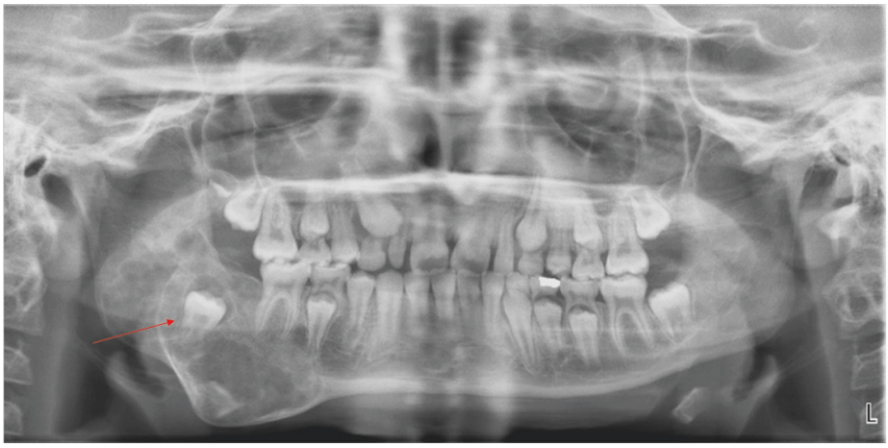

Fig. 11.6 Panoramic radiograph of an 11-year-old female with fibrous dysplasia of the right mandible. There is a large, expansile, multilocular radiolucency in the right posterior mandible, with displacement of the right mandibular third molar (*arrow*) (Radiograph courtesy of Leonard B. Kaban, DMD, MD)

fibrous dysplasia. In patients with McCune–Albright syndrome, disease activity can be monitored with serum alkaline phosphatase levels [31].

Management of FD is twofold [8]. First, biopsy is done to confirm the diagnosis. Asymptomatic, quiescent, or slow growing FD can be enucleated, observed, or contoured. Patients with significant asymmetry, paresthesia, and/or proptosis are considered to have aggressive/rapidly growing FD. Management consists of symptomatic debulking/contour resection [27]. Among patients with quiescent disease, rapid growth, new onset of pain, paresthesia, and/or functional deficit (e.g., hearing or vision changes, trismus, nasal obstruction) can indicate disease reactivation and surgical intervention should be considered. Among adult patients, contouring can be performed after 1 year of documented clinical stability. Similar to cherubism, sarcomatous change has been reported following radiotherapy; such treatment should be avoided [2, 10].

Vascular Malformations

Vascular malformations occur as a result of aberrant morphogenesis of blood vessels or lymphatic structures [32–35]. Vascular malformations are present at birth but may not become clinically apparent until late infancy or childhood [33, 34]. Unlike vascular tumors, vascular malformations grow proportionately with the patient and do not regress. They may increase in size at any time including somatic growth, trauma, infection, or hormonal changes such as puberty and pregnancy [32–36].

Vascular malformations are categorized according to the vessel type involved (capillary, lymphatic, venous, arterial) and the rate of flow (slow or fast) [32–35]. While most are composed of a single-type of vascular entity, combined malformations have been described such as capillary-lymphatic, capillary-venous, lymphatico-venous, and capillary lymphatico-venous [34–36]. The biology of vascular malformations has been studied extensively. Distinction between a vascular malformation and a vascular tumor must be made. Vascular malformations enlarge by dilatation of abnormal vasculature, whereas vascular tumors demonstrate proliferative growth. As such, vascular malformations are typically not treated by resection, with the exception of some low-flow venous malformations (VMs) of the jaws.

The clinical presentations of vascular malformations vary according to the type of vessel involved. Capillary malformations (CMs) change from pink, with a smooth surface in infancy, darkening through childhood, and appearing purple, with a tessellated surface in older patients. Venous malformations (VMs) are typically soft, easily compressible, bluish-hued lesions that may have associated palpable masses (phleboliths). They may be present on the skin or mucosa, or may span multiple tissue planes. Their size typically changes with maneuvers that increase venous pressure (dependency or Valsalva) [35]. Lymphatic malformations (LMs) are typically colorless while lymphatic-venous malformations are blue-purple. LMs can expand secondary to intralesional bleeding, bacterial infection, or during a period of lymphatic "stress" (e.g., upper respiratory infections). LMs with large cystic cavities (historically referred to as "cystic hygromas") can be identified with transillumination. Both lymphatic and capillary-lymphatic malformations have irregular surfaces [34, 35].

Arteriovenous malformations (AVMs) are high flow lesions and are typically warm and tender to palpation. In cases where there is shunting, pulsatile flow and a palpable bruit may be present. Intraoral AVMs cause gingival hypertrophy, mucosal staining, and intraoral gingival bleeding. Teeth in the vicinity may be periodontally compromised and may demonstrate gross mobility [36–38].

In approximately 30 % of cases, vascular malformations are associated with skeletal changes such as difference in morphology or bone density adjacent to the malformation, or a frank intraosseous malformation [33–36]. The most common associated skeletal anomaly is overgrowth or expansion of bone deep to a slow-flow malformation [33–36]. The evolution of these changes has been difficult to elucidate, due to a paucity of radiographs taken at birth. However, it has been reported that approximately 80 % of patients with LM demonstrate evidence of altered skeletal growth by 10 years of age [35, 36].

Management of vascular malformations depends upon the vessel type and rate of flow. CMs typically do not require special precautions; tooth extractions, orthodontic treatment, and osteotomies can all be safely performed. LMs of the maxillofacial region typically involve the floor of mouth, mandible, and submandibular/cervical tissues. As such, feeding difficulties and airway obstruction become sources of considerable morbidity. These patients are typically managed with serial-staged excisions. Orthognathic surgery can take place once skeletal growth is completed.

Patients with venous or lymphatic-venous malformations who have dental or dentoskeletal deformities but no concomitant intraosseous involvement can have orthodontic treatment and orthognathic surgery without fear of excessive bleeding. Intraosseous involvement (i.e., microshunting) is associated with a higher risk for major hemorrhage, either from the malformation or from local or systemic coagulopathy [35]. In the latter situations, stasis and turbulence within the malformation lead to localized and, occasionally, disseminated intravascular coagulation. Prothrombin and partial thromboplastin times may be normal, but fibrin split products may be elevated and fibrinogen and platelet levels are reduced [35, 36]. Surgical intervention can take place once the coagulopathy is corrected; in the interim, patients are typically systemically anti-coagulated [35]. In cases of large venous malformations, direct injection of 100 % ethanol to sclerose the area has been demonstrated to be effective [35]. Smaller malformations may be sclerosed with injection of 1 % sodium tetradecyl sulfate [35, 36]. Tender phleboliths may be effectively treated with aspirin (325 mg daily) for an indefinite course [35].

High flow lesions, such as AVMs, are currently managed by staged procedures. The first stage involves occlusion of the nidus by arterial embolization. Proximal embolization and ligation are contraindicated due to the rapid formation of collateral flow. In the second stage (24–72 h post-embolization), surgical resection is completed, with the goal of complete resection. Unfortunately, AVMs are typically not well localized and the recurrence rates may be high (approximately 40 %) [39]. In recurrent cases, reoperation is not always feasible and repeat embolization is palliative [35, 39].

Osteogenesis Imperfecta

Osteogenesis imperfecta (OI) refers to a group of heritable disorders related to defects in collagen maturation. Both autosomal dominant and recessive patterns have been described. Most cases are associated with mutations in two genes involved in the formation of type I collagen: COL1A1 (Chromosome17) and COL1A2 (Chromosome 7) [40]. Due to the ubiquitous nature of type I collagen as a constituent of multiple types of connective tissues (bone, dentin, ligament, skin, etc.), the clinical manifestations vary. Abnormalities in collagen formation result in diffuse osteoporosis, bone with thin cortices, and aberrant callus formation with injury. In addition, affected individuals may have blue sclera, hearing loss, joint hyperextensibility, and alterations in tooth structure.

There is no definitive treatment for OI. Patients with OI are prone to fractures and aberrant healing and management can be complicated. Severe attrition of tooth structure and premature tooth loss are common. Surgical rehabilitation with osseointegrated implants is challenging due to the poor quality of bone. Among patients with skeletal malocclusion, orthognathic surgery can be performed, albeit with judicious planning [41–43].

Systemic Conditions Affecting Maxillofacial Structures

Patients with significant hematologic, renal, oncologic, or immunosuppressive conditions can be challenged with maxillofacial problems ranging from dental caries to osteonecrosis. These patients, while complicated from a medical treatment standpoint, do not necessarily require complex regimens for management of dental and maxillofacial conditions. In general, a patient should be medically optimized to the extent possible prior to elective surgical interventions. In situations where the risks of surgical interventions (e.g. sepsis, endocarditis, risks from anesthesia) outweigh the benefits, improvement of overall health and non-invasive intervention to improve oral health are performed. Fortunately, common surgical procedures in and around the oral cavity carry low attendant risks and, in most instances, can be carried out with minimal modifications to patient treatment protocols.

Treatment

Pharmacological therapies for conditions involving the pediatric maxillofacial skeleton are limited. No drugs are currently approved for the prevention of low bone density and possible pathologic fractures in children. However, therapies are currently in use off-label in clinical practice.

Vitamin D and Calcium Supplementation

Vitamin D deficiency is extremely common in the pediatric population [44, 45]. Although the level of vitamin D needed for bone health remains somewhat controversial, it has been shown that levels of 25-hydroxy vitamin D below 20 ng/mL increase bone turnover and the risk of low bone density [46]. In patients with conditions causing low bone density such as OI, supplementation to ensure a higher level of 25-hydroxy vitamin D of 30 ng/mL may be appropriate [47]. Evidence of the benefits of higher levels of supplementation on organ systems besides the skeleton is mixed [48]. There are few risks to vitamin D supplementation such as hypervitaminosis D and resulting hypercalcemia. Although rare, they have been reported at doses needed to maintain the above levels, indicating the need for monitoring of patients on supplementation [49].

Calcium supplementation has been shown to increase bone density in children with low dietary intake [50]. In general, children require 1,000–1,500 mg of elemental calcium a day. Patients with low bone density and high bone turnover may have increased requirements [51]. Patients not receiving adequate dietary calcium should receive supplementation. However, if vitamin D levels and dietary calcium

are adequate, supplementation is not required. The risks of calcium supplementation are also relatively low. Studies in adult patients disagree whether calcium supplementation may increase the risk of nephrocalcinosis and cardiovascular calcifications [48, 50, 52, 53].

Bisphosphonates

Bisphosphonates decrease bone turnover by preventing osteoclasts from breaking down bone, increase bone density, and reduce risk of pathologic fracture. They increase bone mass but not bone quality in high bone turnover conditions such as OI [54–57]. In FD, they reduce bone pain and decrease the risk of pathologic fractures, but do not affect the natural history of the disease [58].

Although they have not been approved in children, in most instances, bisphosphonates are used off-label as treatment for systemic pediatric conditions known to cause decreased bone density such as cerebral palsy, inflammatory conditions requiring long-term steroid treatment, and oncologic disorders. Most protocols originate from initial studies done in children with OI [54, 55]. Oral bisphosphonates are less effective in children and have been shown to increase bone density but not reduce fracture risk in patients with OI [56].

Pediatric patients are generally not treated with bisphosphonates regardless of diagnosis until they have experienced at least one pathologic fracture and are at risk for more, with the exception of FD patients treated for pain relief. Standard dosing is 1 mg/kg of pamidronate up to a maximum of 60 mg/kg every 6 weeks to 2 months. Patients with severe bony disease may experience fever, body aches, and bone pain with infusions, especially with the first administration. All patients receiving bisphosphonates are at high risk for hypocalcemia due to inactivation of the osteoclasts and resulting decrease in calcium release from the skeleton [59]. Vitamin D is critical to maintain adequate calcium absorption from the diet and patients should be documented to have a 25-hydroxy vitamin D level of at least 20 ng/mL prior to therapy. Patients also must maintain adequate calcium intake before and after the infusions either through dietary sources or by supplementation. Some studies suggest that prior or concurrent bisphosphonate therapy adversely affects bone healing, although other studies report no clinically significant effects when bisphosphonates are given before or soon after a fracture [60–62].

Among patients exposed to bisphosphonates, a small number will develop bisphosphonate-related osteonecrosis of the jaws (BRONJ). Most cases reported in literature were in adult patients with oncologic disorders treated with bisphosphonates who subsequently underwent dental procedures or had local trauma to the jaw. While the etiology is unknown, an anti-angiogenic effect of the medication has been proposed [63]. The estimated frequency of BRONJ ranges from 0.0004 to 0.06 % for oral bisphosphonates. Estimates for those exposed to parentral bisphosphonates are lacking, but are higher than for oral bisphosphonates [43–46]. The risk for

BRONJ is higher among patients receiving parenteral bisphosphonates, patients on oral bisphosphonates for >3 years, or those who have been exposed for <3 years but received concomitant steroids [44].

Though expected to parallel the effects seen in adults, there is a paucity of literature describing the risk for BRONJ in children and adolescents. There are two European studies of 102 and 64 children and adolescents treated with bisphosphonates which did not reveal any cases [64, 65]. A study in Canada specifically looked at 15 pediatric patients with OI who were treated with bisphosphonates, mostly IV pamidronate, and underwent dental procedures including tooth extraction but did not report any cases of ONJ [66]. As such, clinicians should use experience and clinical judgment regarding the necessity of dental or oral surgical interventions. Discussion with parent, patient, and pediatric dental practitioners prior to initiation of treatment should take place. Significant efforts should be made to treat decayed, infected, or impacted teeth, and other maxillofacial pathology. Among children who require treatment with bisphosphonates, initiation of treatment should be delayed, where feasible, until dental evaluation and any necessary dental and/or oral surgical treatment is completed. A drug holiday of at least 3 months prior to oral surgical intervention is currently recommended [67, 68]. Among some patients, given the long half-life of intravenous bisphosphonates, cessation is not practical [68]. It is possible that BRONJ may not become evident until skeletal maturity is reached, but further research is necessary to explore this area.

New Therapies: Denosumab and Recombinant Parathyroid Hormone

In the past several years, two new therapies became available for the treatment of skeletal disorders. Denosumab is a humanized monoclonal antibody to the NF-kB ligand (RANKL), a cell surface protein involved in osteoclastogenesis. Similar to bisphosphonates, it reduces bone turnover by preventing osteoclast activity although through a different mechanism. It has not been widely used “off-label” in the pediatric population since the relatively recent FDA approval for use in adult patients. There are reports in literature of successful pain management in patients with FD as well as the treatment of hypercalcemia following a stem cell transplant for osteopetrosis. However, the FD patients developed secondary hyperparathyroidism during treatment and severe hypercalcemia after the discontinuation of therapy requiring hospitalization and IV bisphosphonate therapy [69, 70].

Teriparatide is a truncated version of parathyroid hormone (PTH) given by subcutaneous injection to increase bone density in adult patients. Teriparatide is different than other mentioned therapies in that it has an anabolic effect on bone, increasing bone formation instead of preventing bone turnover. However, animal studies showed an increase in bone tumors in juvenile rats. Thus, it is not used in the pediatric population [71].

Conclusion

The management of systemic conditions of bone metabolism affecting the maxillofacial skeleton is complex and requires collaboration from multiple specialists. Craniofacial pathology may be asymptomatic and diagnosed incidentally by imaging. Surgical management is appropriate for some conditions and medical management with pharmacologic intervention necessary for others. Treatment must consider risks and benefits of the interventions on the immature maxillofacial skeleton undergoing rapid growth.

New therapies for disorders of bone metabolism are currently in clinical trials, including new anabolic therapies that act on osteoblasts to increase bone production. For example, therapies targeting Sclerostin, an endogenous inhibitor of a cell signaling pathway known to increase bone density, are currently being studied [72]. Although the side effects of these therapies in children are unknown, they may affect future treatment regimens with ultimate improvements in patient outcomes.

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Chapter 12
Newer Adult Bone Drugs

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Introduction

In this chapter we review new concepts in pharmacologic therapy for osteoporosis in adults. We cover denosumab, the most recent addition to the antiresorptive category of medications; innovations in osteoanabolic and combination therapy; and newer therapeutic classes that show promise in clinical trials. A summary is provided in Table 12.1. We will not discuss FDA-approved therapies that have been available for more than 5 years or agents that have not yet been studied in humans or that are no longer under active investigation.

Antiresorptive Therapy

Bisphosphonates are the mainstay of osteoporotic therapy. The only recent innovation in this class is the approval of a delayed-release form of risedronate. All oral bisphosphonates require patients to take the drug in the fasting state with plain water and to wait approximately 30–60 min before eating, drinking, or taking other medications. These stipulations help to ensure maximal absorption of the bisphosphonates which are at best poorly absorbed [1]. Less than 1 % of the administered oral bisphosphonate dose is absorbed under these optimized conditions. Some individuals cannot tolerate this approach because they are unable to take drugs while fasting. Other individuals report upper gastrointestinal intolerance under these conditions. To deal with these concerns, a weekly 35 mg delayed-release form of risedronate

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Table 12.1 Novel drugs in the treatment of osteoporosis

Class	Drug	Route of administration	Timing	Mechanism of action
Antiresorptive	Denosumab[a]	Subcutaneously	Twice yearly	Decreases bone resorption by inhibiting RANKL
Osteoanabolic	PTH(1-34) (teriparatide)[b]	Subcutaneously, transdermal, chip	Daily, weekly	Increase bone turnover, with the increase in bone formation preceding and exceeding bone resorption, characterizing the "anabolic window"
	PTH(1-84)[c]	Subcutaneously	Daily, weekly	
	PTHrP(1-36)	Subcutaneously	Daily	
	PTHrP analog	Subcutaneously	Daily	
Cathepsin K inhibitors	ONO-5334	Oral	Daily	Inhibit cathepsin K, a key enzyme for bone collagen breakdown. The effect on osteoclast function is limited so that osteoclasts continue to positively signal osteoblasts. As a result, bone resorption is reduced without suppressing bone formation to an appreciable degree
	Odanacatib	Oral	Weekly	
Antisclerostin antibodies	Romosozumab	Intravenous and subcutaneously	Not defined yet	Inhibition of sclerostin subsequently prevents the inhibition of Wnt/β-catenin signaling. The release of inhibition in this pathway increases bone formation and reduces bone resorption
	Blosozumab	Subcutaneously	Not defined yet	
Nitric oxide	Isosorbide mononitrate	Oral	Daily	Direct effect on osteoclasts and osteoblasts, leading to a decrease in bone resorption and an increase in bone formation
	Nitroglycerin	Transdermal	Daily	

[a]Denosumab is approved by the FDA for the following indications: treatment of postmenopausal women with osteoporosis who are at high risk for fracture; treatment to increase bone mass in men with osteoporosis at high risk for fracture; treatment to increase bone mass in women at high risk for fracture receiving adjuvant aromatase inhibitor therapy for breast cancer; and treatment to increase bone mass in men at high risk for fracture receiving androgen deprivation therapy for nonmetastatic prostate cancer. In Europe, denosumab is approved for the treatment of osteoporosis in postmenopausal women at increased risk for fracture and for bone loss due to hormone ablation in men with prostate cancer

[b]PTH(1-34) by subcutaneous injection daily is approved worldwide for advanced osteoporosis in men and women at high risk of fracture as well as glucocorticoid-induced osteoporosis; PTH(1-34) by subcutaneous injection weekly is approved for use in Japan

[c]PTH(1-84) by subcutaneous injection daily is approved for use in Europe for postmenopausal osteoporosis

was developed to be taken postprandially, after breakfast. The delayed-release formulation was demonstrated to be effective compared to immediate-release risedronate in a non-inferiority trial in which both groups showed similar gains in bone mineral density (BMD) at the lumbar spine and hip [2]. As with other oral bisphosphonate preparations, patients are advised to remain upright for 30 min after taking the medication to ensure successful transit of the medication.

The most recent addition to the antiresorptive class of therapeutics for osteoporosis, denosumab, is based on a mechanism that is completely different from the bisphosphonates. Receptor activator of nuclear factor-kB ligand (RANKL) is a key mediator of osteoclast formation, activity, and survival [3]. Denosumab, a fully human monoclonal antibody, binds with high affinity and specificity to RANKL and, thus, prevents its access to mature and developing osteoclasts. As a result, osteoclast-mediated bone resorption is profoundly affected. By reducing bone turnover, denosumab increases BMD [4–6]. At vertebral, hip, and other nonvertebral sites [7, 8], denosumab reduces fracture incidence, as compared to placebo controls.

The FREEDOM trial was a 3-year international, randomized, placebo-controlled trial that enrolled 7,868 postmenopausal women with osteoporosis. Subjects were randomized to receive either 60 mg of denosumab or placebo subcutaneously (SC) every 6 months [7]. Compared to placebo, subjects taking denosumab had a significant reduction in the risk of new radiographic vertebral fractures by 68 % (relative risk (RR), 0.32; 95 % confidence interval (CI), 0.26–0.41; $p<0.001$), hip fracture by 40 % (RR, 0.60; 95 % CI, 0.37–0.97; $p=0.04$), and all nonvertebral fractures by 20 % (RR, 0.80; 95 % CI, 0.67–0.95; $p=0.01$). By 3 years, patients in the denosumab group had a 9.2 % increase in lumbar spine BMD and a 6 % increase at the total hip. Although infections are a theoretical concern [9, 10] because RANKL is expressed in lymphocytes, the rate of serious infections was not higher with denosumab treatment. However, cellulitis and eczema were seen with greater frequency in those who took denosumab.

The gains in BMD were progressive. In the phase III trials, a linear increase was seen at all sites over the entire 3-year period [9]. Gains for as long as 8 years have been reported in the phase II trial [10]. In a subset of 99 subjects from the FREEDOM trial, quantitative computed tomography of the spine and hip was performed to estimate bone strength using finite element modeling [11]. Hip and spine strength increased for the denosumab group compared with the placebo group by 14.3 % ($p<0.0001$) and 22.4 % ($p<0.0001$), respectively, at 36 months.

Denosumab treatment for 36 months has also been shown to reduce the incidence of new vertebral fractures in men receiving androgen deprivation therapy for nonmetastatic prostate cancer (RR, 0.38; 95 % CI, 0.19–0.78; $p=0.006$). Rates of adverse events and overall incidence of infections were similar for both the treatment and placebo groups [8].

A phase III trial compared efficacy of denosumab vs. alendronate on BMD and bone turnover markers [12]. A total of 1,189 postmenopausal women with low bone mass were randomized to receive either denosumab 60 mg every 6 months or alendronate 70 mg weekly. At 12 months, subjects in the denosumab group had a greater increase in BMD at the lumbar spine, total hip, femoral neck, and 1/3 radius.

Denosumab reduced the bone formation marker procollagen type I intact N-terminal propeptide (P1NP) to a greater extent than alendronate, but the median reduction in the bone resorption marker C-terminal telopeptide (CTX) was similar for both groups. There was no difference in the overall incidence of adverse events between the two treatment groups.

The reversibility of the antiresorptive effects of denosumab was shown in a 2-year extension study of a randomized, blinded, placebo-controlled, dose-ranging trial in which patients were randomly allocated to further treatment with active drug or placebo [13]. Despite a marked suppression during the treatment period with denosumab, bone turnover markers rapidly increased after the drug was discontinued, with the most pronounced changes within the first 6 months off-therapy. The increase in bone turnover markers temporarily exceeded the control, pretreatment values. This "overshoot" was associated with a reduction in BMD. Bone turnover markers and BMD returned to values near baseline after 24 months off-treatment. Subjects in whom treatment was discontinued for 12 months before denosumab was reintroduced for an additional 12 months showed increments in BMD to an extent similar to that observed during the first period of denosumab treatment.

Denosumab is approved by the FDA for the following indications: treatment of postmenopausal women with osteoporosis who are at high risk for fracture; treatment to increase bone mass in men with osteoporosis at high risk for fracture; treatment to increase bone mass in women at high risk for fracture receiving adjuvant aromatase inhibitor therapy for breast cancer; and treatment to increase bone mass in men at high risk for fracture receiving androgen deprivation therapy for nonmetastatic prostate cancer. Denosumab is also approved to prevent skeletal-related events in patients with bone metastasis from solid tumors. In Europe, denosumab is approved for the treatment of osteoporosis in postmenopausal women at increased risk for fracture and for bone loss due to hormone ablation in men with prostate cancer.

Osteoanabolic Therapy

Parathyroid hormone (1-84) [PTH(1-84)] and its fully active, foreshortened variant, PTH(1-34) (teriparatide), represent the only currently available osteoanabolic therapies for osteoporosis. Teriparatide was demonstrated to be effective in the pivotal clinical trial conducted by Neer et al. [14]. It is indicated for the treatment of men and women with advanced osteoporosis at high risk for fracture. It is also approved for the treatment of glucocorticoid-induced osteoporosis. PTH(1-84) is approved in Europe, but not in the United States, for the treatment of postmenopausal osteoporosis. Both teriparatide and PTH(1-84) are administered by subcutaneous injection daily for up to a 2-year course. Teriparatide carries an FDA-instructed "black box" warning because of toxicity noted in rats, osteosarcoma [15]. PTH(1-84) also causes osteosarcoma in rats. Concerns that this rat toxicity might also be seen in human subjects have not been substantiated. Reviews of this subject have established that

after 10 years of teriparatide use and 6 years of PTH(1-84) use amounting to a cumulative experience of over one million subjects and several million patient-years, the incidence of osteosarcoma is not greater than what would be expected in the general population not exposed to these PTH formulations [16, 17].

In contrast to antiresorptive therapies, osteoanabolic agents directly stimulate bone formation. PTH therapy increases BMD in the lumbar spine, a site comprising primarily cancellous bone. Increases in the hip region are more modest and PTH therapy may actually be associated with a reduction in BMD at the distal 1/3 radius, a cortical site. PTH therapy results in an initial rapid increase in bone formation markers subsequently followed by an increase in bone resorption markers. These changes in markers of bone formation are accompanied by histomorphometric observations that confirm an effect of PTH to increase processes associated with bone formation without any early evidence for bone resorption. This effect is reminiscent of bone metabolism in growing children in whom bone *modeling* is the dominant process. Thereafter, teriparatide leads to an increase in bone resorption giving rise to the more typical characteristics of bone metabolism in adults, namely bone *remodeling*. The "anabolic window" describes the period of time when PTH stimulates bone formation directly, before bone remodeling is stimulated [18]. Even after bone turnover is stimulated, there is more ongoing bone formation than bone resorption, thus maintaining the anabolic window at least for a finite period of time.

Different Timing and Delivery Systems of Teriparatide

Weekly Administration of PTH(1-84) or Teriparatide

A randomized, double-blind, placebo-controlled trial [19] examined the use of weekly teriparatide 200 IU (56.5 μg) in healthy Japanese men and postmenopausal women (65–95 years) with 1–5 prevalent vertebral fractures and low lumbar spine BMD (<80 % of young adult mean) at any site. Subjects were randomly assigned to receive a 72-week course of weekly teriparatide injection ($n=286$; 13 men) or placebo ($n=286$; 10 men). Compared to placebo, treatment with teriparatide increased BMD at the lumbar spine (0.3 % vs. 6.7 %), total hip (0.1 % vs. 3.1 %), and femoral neck (−0.5 % vs. 1.8 %), and reduced the relative risk of vertebral fracture by 80 % (14.5 % vs. 3.1 %) ($p<0.01$ for all). In a subset of these patients [20], a single dose of teriparatide led to a transient decrease in bone formation markers with a subsequent increase over baseline levels up to 72 h later, lasting more than 7 days after administration. Markers of bone resorption transiently increased after administration and then decreased to below baseline levels from 24 h until the next injection. Computed tomographic imaging studies of a subgroup of these patients [21] showed that teriparatide treatment increased cortical thickness and area in the femoral neck, inter-trochanter, and shaft, while tending to decrease cortical perimeter and cortical volumetric BMD in the inter-trochanter (but not the femoral neck or shaft), compared to placebo. Weekly teriparatide also improved the biomechanical properties of section modulus and buckling ratio.

After the completion of the original weekly teriparatide trial [19], 465 subjects were enrolled in a follow-up study in which patients were treated for 1 year with bisphosphonates or other therapeutic regimens at the discretion of their physicians [22]. Among the 447 subjects who completed the study, 205 were in the post-teriparatide group and 242 in the post-placebo group. Approximately 45 % of subjects in the post-teriparatide group and 54 % in the post-placebo group were treated with bisphosphonates. The other regimens included selective estrogen receptor modulators, calcitonin, alfacalcidol, or no osteoporosis drugs. New vertebral fracture occurred in 3.4 % of subjects in the post-teriparatide group and 13.7 % in the post-placebo group (RR, 0.23; 95 % CI, 0.10–0.52; $p<0.05$).

Outcomes from the original weekly teriparatide trial are comparable to those obtained with daily teriparatide [14], with the exception that daily teriparatide decreased cortical BMD of the femoral neck. Weekly teriparatide is now approved for use in Japan [23].

Weekly PTH(1-84) administration has also been studied [24]. A double-blind, placebo-controlled trial randomized 50 postmenopausal women 45–70 years of age with low BMD at the femoral neck to receive PTH(1-84) 100 μg or placebo daily for 1 month, followed by weekly injections of PTH or placebo for 11 months. At 1 year, lumbar spine BMD increased by 2.1 % in PTH-treated women, significantly greater than placebo ($p=0.03$), although there were no significant differences at the hip [25].

Transdermal Teriparatide

In phase I trials, a transdermal teriparatide delivery system was shown to deliver PTH(1-34) with a rapid time to maximal concentration, comparable area under the curve, and shorter half-life than the subcutaneous route [26]. A subsequent 6-month, randomized, placebo-controlled, positive control, multidose phase II trial compared a daily transdermal microneedle teriparatide patch with a placebo patch and subcutaneous teriparatide 20 μg injection in 165 postmenopausal women [27]. Bone turnover markers (P1NP and CTX) increased in all treatment groups in a dose-dependent manner compared to placebo. At 6 months, lumbar spine BMD increased by 3.0 %, 3.5 %, and 5.0 % in the 20-, 30-, and 40-μg teriparatide patch groups, respectively, and by 3.6 % in the subcutaneous teriparatide group ($p<0.001$ vs. placebo (–0.3 %) for all). The 40 μg teriparatide patch increased total hip BMD compared to both placebo patch and subcutaneous teriparatide injection ($p<0.05$). The 40 μg dose is entering into phase III trials [28].

Delivery of Teriparatide by Chip Technology

A novel approach to deliver teriparatide through a wirelessly controlled implantable microchip was recently described [29]. The microchip-based devices, containing discrete doses of lyophilized teriparatide, were implanted in the subcutaneous tissue of the abdomen of eight osteoporotic postmenopausal women for 4 months and

wirelessly adjusted to release doses once daily for up to 3 weeks. A computer-based programmer communicated wirelessly with the implant to program the dosing schedule and to receive implant status verifying proper operation. For comparison, each study subject subsequently received subcutaneous injections of teriparatide in escalating doses. The device produced similar pharmacokinetics of teriparatide to standard daily subcutaneous injections with a lower coefficient of variation in seven of the eight study subjects. This novel approach to deliver teriparatide increased the bone formation marker P1NP, confirming that the device was clinically effective. There were no toxic or adverse events due to the device or to the drug, and patients stated that the device did not compromise quality of life. This new cutting edge technology has promise.

PTH-Related Peptides (PTHrP(1-36); PTHrP Analog)

PTH-related peptide (PTHrP) was first brought to medical attention as a cause of humoral hypercalcemia of malignancy. It was subsequently demonstrated to have key physiological actions in the skeleton as well as other systems [30]. Despite the fact that both PTH and PTHrP act at a common receptor, the proteins are products of different genes. While there is limited overall sequence homology between the two peptides, the N-terminal regions are intensely homologous. This N-terminal sequence homology helps to explain the shared interaction with a common PTH–PTHrP receptor. In the normal state, PTHrP has evolved to regulate local tissue functions, as opposed to the systemic hormonal role of PTH [31]. Similar to PTH in primary hyperparathyroidism, PTHrP is catabolic to bone when administered continuously, and therefore intermittent administration has been studied. Intermittent administration of PTHrP increases bone mass in rodents with varying potency in relationship to PTH [32].

Initial short-term studies with PTHrP(1-36) by Horwitz et al. [33, 34] showed early effects that appeared to favor a rather exclusive stimulation of bone formation. Results from a phase II study [35] in 105 postmenopausal women aged 45–75 years ($n = 35$ in each group) are now available. Subjects were randomized to daily treatment with PTHrP(1-36) 400 μg, PTHrP(1-36) 600 μg, or PTH(1-34) 20 μg for 3 months. The primary outcome measures were bone turnover markers, with secondary outcome measures of BMD and safety. PTH(1-34) and PTHrP(1-36) stimulated bone formation early (day 15), although by day 90 PTH(1-34) increased bone formation markers two to fourfold greater than PTH(1-36) at the 600 or 400 μg doses, respectively ($p < 0.05$). As expected, the increase in bone resorption occurred later (day 60 for PTH(1-34) and day 90 for both PTH(1-36) groups), and was not as dynamic. The increase in bone resorption at day 90 was threefold greater for the PTH(1-34) arm than either of the PTH(1-36) dosage groups ($p < 0.05$), which were not different from each other. At 3 months, PTH(1-34) and PTHrP(1-36) at both doses significantly increased BMD by about 2 % at the lumbar spine. There were small but significant increases in hip BMD in the PTHrP(1-36) groups but not in

the PTH(1-34) group. There were no significant differences in BMD at the forearm. Adverse effects were similar between the PTH(1-34) and PTHrP(1-36) groups, with the exception of more frequent episodes of mild hypercalcemia in the PTHrP(1-36) group.

A PTHrP analog is being developed by Radius Health, Inc. [36]. The first 22 residues are identical to PTHrP but thereafter, from amino acids 22 to 34, the molecule contains several replacement residues designed to optimize its osteoanabolic potential. The results of a multicenter, randomized, double-blind, placebo-controlled phase II investigation of safety and effects on BMD and bone markers were recently presented [36]. Postmenopausal women with osteoporosis, aged 55–85 years, were randomized to placebo, BA058 20, 40, or 80 μg, or teriparatide 20 μg for 24 weeks. After completion of the 24-week study, subjects were then eligible to participate in a 24-week extension study. One hundred and eighty-four of 221 patients completed 6 months of treatment. The mean percent change in lumbar spine BMD at 24 weeks was 1.6 % with placebo, 2.9 %, 5.2 %, and 6.7 % with BA058 20 μg, 40 μg, and 80 μg, respectively, and 5.5 % with teriparatide ($p < 0.001$ compared to placebo for BA058 40 and 80 μg and teriparatide). Further dose-dependent increases in lumbar spine BMD were noted during the extension ($n = 55$), with a mean percent change at 48 weeks of 0.7 % with placebo, 5.1 %, 9.8 %, and 12.9 % with BA058 20 μg, 40 μg, and 80 μg, respectively, and 8.6 % with teriparatide. The investigators also found dose-dependent increases in total hip BMD. At 24 weeks, changes were noted in serum and urine bone turnover markers from baseline ($p \leq 0.05$) for BA058 40 and 80 μg and for teriparatide. BA058 was well tolerated, with an adverse event profile comparable with blinded placebo. BA058 is the focus of a phase III placebo-controlled, 18-month international study in postmenopausal women [24]. The primary endpoint is the incidence of new vertebral fractures at a dose of 80 μg in comparison to placebo. A transdermal microneedle technology is also under development for BA058 and in phase I and II trials (www.radiuspharm.com).

Combination Osteoanabolic and Antiresorptive Therapy

In theory, the combination of an antiresorptive and osteoanabolic agent offers the potential for increased efficacy over monotherapy with either drug class given their differing mechanisms of action. If bone resorption is inhibited by an antiresorptive while bone formation is stimulated by an osteoanabolic agent, combination therapy might give better results than therapy with either agent alone. Despite the intuitive attraction of this reasoning, important data to the contrary have been provided by Black et al. [25] and Finkelstein et al. [37]. These two groups independently conducted trials using a form of PTH alone, alendronate alone, or the combination of the PTH formulation and alendronate. Black et al. studied postmenopausal women treated with 100 μg of PTH(1-84) and Finkelstein et al. studied men given 40 μg of teriparatide. PTH monotherapy was associated with greater densitometric gains

than with combination therapy or alendronate alone at the lumbar spine with both dual energy X-ray absorptiometry (DXA) and quantitative computed tomography. Combination therapy was not different from alendronate alone. Bone turnover markers followed the expected course for monotherapy with anabolic or antiresorptive agents. For combination therapy, however, bone markers followed the course of alendronate, not PTH, with reductions in bone formation and bone resorption markers. The findings from these two trials suggest that the impaired response to combination therapy was due to the dominating effects of alendronate on bone remodeling dynamics when both drugs are used in combination.

The results of these combination therapy studies with alendronate led to the concept that an antiresorptive agent that did not impair the anabolic actions of PTH to increase bone formation while mitigating its effects on bone resorption may be a more effective approach to combination therapy. Deal et al. [38] studied the effects of raloxifene, a less potent antiresorptive agent than the bisphosphonates, in combination with teriparatide over 6 months. Their results support the idea that combination therapy might be advantageous with a mild antiresorptive drug but are not conclusive because of the short duration of the study. As a further test of this hypothesis, Walker et al. [39] investigated the combination of teriparatide and risedronate, a bisphosphonate with less potent effects on bone turnover than alendronate or zoledronic acid. Men with low BMD were randomized to receive risedronate 35 mg weekly plus daily injected placebo, teriparatide 20 μg subcutaneously daily plus weekly oral placebo, or risedronate plus teriparatide (combination) for 18 months. At study conclusion, all three treatment arms significantly increased lumbar spine BMD, the primary endpoint, but there were no between-group differences. In contrast, at the total hip, BMD increased to a greater extent in the combination group (3.86 ± 9.2 %) vs. teriparatide (0.29 ± 8.0 %) or risedronate alone (0.82 ± 8.0 %; $p < 0.05$ for both). Bone turnover markers in the combination group paralleled the teriparatide alone arm, supporting the idea that an antiresorptive that does not have a profound effect on bone turnover might permit salutary effects of combination therapy with an osteoanabolic agent. The results of this proof-of-concept study are favorable but require further investigation.

Cosman et al. [40] studied the use of a single dose of zoledronic acid in combination with daily teriparatide. This approach was based upon animal studies of Gasser et al. [41] in which a single dose of zoledronic acid led to greater improvements in BMD in rats treated simultaneously with teriparatide than those treated with bisphosphonate therapy alone. With the combination of zoledronic acid and teriparatide, BMD increased after 6 months at the spine and hip to a greater degree than in either monotherapy arm. With combination therapy, there was a rapid, but only transient, reduction in bone turnover markers. At the lumbar spine, there were significantly greater changes after 6 months with combination therapy but by the end of the study at 12 months, there were no differences between combination therapy and teriparatide alone (7.3 % vs. 7.3 %; $p = \text{NS}$). At the hip, similar results were observed with the combination therapy arm showing a greater enhancement of BMD than zoledronic acid alone, but by the 12-month endpoint of the study,

differences were no longer appreciated (2.3 % vs. 2.2 %; p=NS). However, if one considered the lumbar spine and hip sites together as a composite endpoint, only combination therapy provided improvement in BMD that was greater than either zoledronic acid or teriparatide alone.

The first section of this chapter is devoted to denosumab, a potent inhibitor of RANKL. Several interesting properties have surfaced over continued investigation of this therapy. The distal 1/3 radius, a cortical site, increases and remains above baseline for 8 years. The second observation is that PTH levels rise after denosumab administration by approximately twofold and remain above baseline for about 3 of the 6-month interval between doses [5, 42, 43]. To relate these observations to each other requires appreciation of the fact that PTH requires RANKL for its catabolic actions [44–46]. As a RANKL inhibitor, denosumab blocks this catabolic pathway. The increase in PTH associated with denosumab, therefore, may preferentially exploit the anabolic *Wnt* signaling pathway [47, 48]. The increase in cortical bone density (distal 1/3 radius) is compatible with this hypothesis. Seeman et al. [49] have provided additional evidence for this view when they showed that the higher PTH in connection with denosumab is associated with lower cortical porosity. In the control arm of this study, alendronate administration was associated with increases in PTH but there was a positive relationship with higher PTH levels associated with greater porosity. These observations led to the hypothesis that denosumab and teriparatide in combination may be more beneficial than the combination of teriparatide with other antiresorptives. The Denosumab and Teriparatide Administration (DATA) Study [50] has in fact shown a densitometric benefit to combination therapy in postmenopausal women. At 12 months, lumbar spine BMD increased more in the denosumab and teriparatide combination group (n=30; 9.1 %) than the teriparatide alone group (n=31; 6.2 %, p=0.014) or denosumab alone (n=33; 5.5 %, p=0.0005). Femoral neck BMD also increased to a greater extent in the combination group (4.2 %) than the teriparatide alone (0.8 %, p=0.0007) or denosumab alone arms (2.1 %, p=0.0238), with similar findings at the total hip site. There was an increase of 2.6 % in BMD at the distal 1/3 radius in the combination therapy arm and 1.7 % in the denosumab alone arm without between-group differences, although both groups differed when compared to teriparatide alone, which demonstrated a 1.8 % decline in BMD. As expected, teriparatide alone increased bone turnover markers and denosumab alone decreased bone turnover markers. While the combination therapy group decreased the bone resorption marker CTX to a similar extent as the denosumab alone group, bone formation decreased more gradually and to a lesser extent.

It should be noted that these and other combination studies have not been designed with fracture outcome as a definitive endpoint. These trials have only targeted surrogate endpoints such as BMD and bone turnover markers, and have also been shorter than typical definitive fracture trials. One must therefore exercise caution in the interpretation of these data. In addition, the use of two pharmacologic therapies simultaneously carries with it the potential for more adverse events than the use of a single therapy as well as added expense.

Newer Therapies

Cathepsin K Inhibitors

Cathepsin K, a member of the papain family of cysteine proteases, is highly expressed by activated osteoclasts, and promotes degradation of type I collagen (Fig. 12.1) [51]. It plays an important role in the process of bone resorption through its actions to remove collagen, an initial step in the creation of the bone remodeling unit [51]. Clues to the therapeutic potential of inhibiting the actions of cathepsin K came from studies of a human genetic disorder, pycnodysostosis, a disorder of excess bone due to an inactivating point mutation in the gene encoding cathepsin K [52, 53]. Several oral compounds have been designed to inhibit cathepsin K [54]. Preclinical studies of cathepsin K inhibitors noted that the drug reduces bone resorption without suppressing bone formation to an appreciable degree [55–57]. Different from the bisphosphonates, cathepsin K inhibitors appear to permit certain functions of the osteoclast such as signaling to the osteoblast while preventing its classical actions to excavate bone.

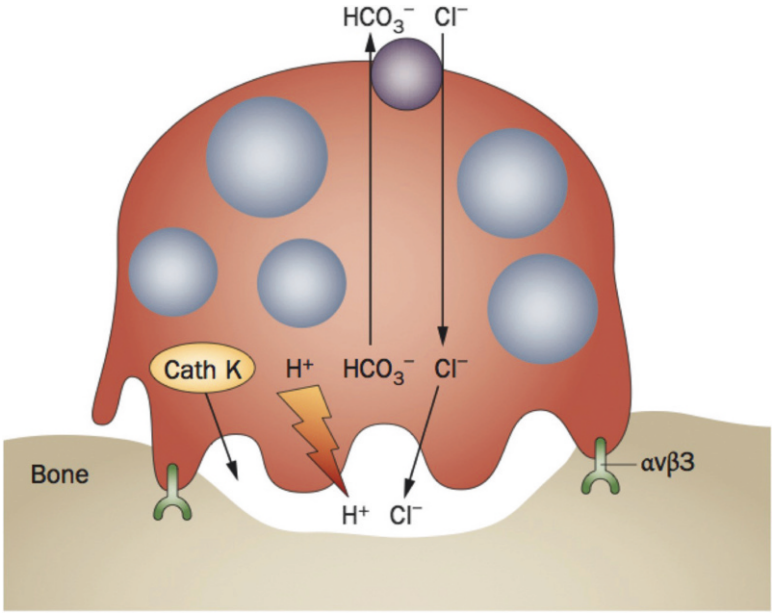

Fig. 12.1 Cartoon depicting osteoclast-mediated bone resorption. Hydrochloric acid and proteases, including cathepsin K (Cath K), are secreted into the sealing zone created by the adhesion of the osteoclast to bone through integrin αvβ3. The organic collagen matrix, 90 % of which is composed of type I collagen, is subsequently degraded. Reprinted by permission from Macmillan Publishers Ltd: Costa AG, Cusano NE, Silva BC, Cremers S, Bilezikian JP. Cathepsin K: its skeletal actions and role as a therapeutic target in osteoporosis. Nat Rev Rheumatol. 2011;7(8): 447-456

Several molecules in this new class are noteworthy. They are all under investigation at this time. The cathepsin K inhibitors, ONO-5334 and odanacatib, have shown encouraging results in clinical trials so far. ONO-5334 is a hydrazine-based cathepsin K inhibitor developed in Japan. A double-blind, placebo-controlled, single-dose escalation study enrolled 52 healthy postmenopausal women to evaluate safety, tolerability, pharmacokinetics, and pharmacodynamics of ONO-5334. ONO-5334 was administered at 3, 10, 30, 100, 300, or 600 mg to patients and compared to a placebo control. ONO-5334 was well tolerated at all doses. Suppression of bone resorption markers was observed for at least 24 h with doses of 100 mg and above vs. placebo. Food ingestion did not interfere with total drug exposure, although the time to reach peak maximal concentration was delayed by about 30 min [58]. In another early study, little effect was noted on bone formation markers while bone resorption markers were suppressed by once and twice daily administration of ONO-5334 at doses of 10–600 mg/day for up to 28 days [58].

A phase II randomized, placebo- and active-controlled parallel group study evaluated the efficacy, safety, and tolerability of ONO-5334 in 285 Japanese postmenopausal women with osteoporosis (55–75 years old). There were four study arms: daily 100 or 300 mg, twice daily 50 mg or placebo; the active comparator group received 70 mg of weekly alendronate [59]. Treatment with ONO-5334 suppressed bone resorption markers (serum and urinary CTX and urinary N-terminal telopeptide (NTX)), but had a much smaller effect on bone formation markers bone-specific alkaline phosphatase (BSAP) and P1NP. Dose-related increases in BMD were noted with the 300 mg dose, showing gains that were similar to alendronate. Increments in femoral neck and total hip BMD were similar at the 50 and 300 mg dose regimens [59, 60]. Serious adverse events were reported in 11.1 % of patients on study drug, as compared to a 7 % incidence in the placebo and alendronate groups. The most frequent adverse events reported by patients treated with the active drug were hypertension and dyspepsia. There was no imbalance among the ONO-5334 arms and the placebo and alendronate arms in dermatologic reactions, an area of potential concern because cathepsin K is found in dermal fibroblasts [61].

Odanacatib (MK-0822) is another cathepsin K inhibitor in development at this time. Similar to ONO-5334, odanacatib inhibits bone formation markers to a much smaller extent than its effects on bone resorption markers. A randomized, multicenter, placebo-controlled trial enrolled 399 postmenopausal women with low BMD (T-score $\leq$–2.0 and $\geq$3.5 at the lumbar spine, femoral neck, total hip, or trochanter) [62]. Designed to be a 12-month study with a 12-month extension phase, patients received one of the four odanacatib weekly regimens (3, 10, 25, and 50 mg) or placebo. By 12 months, lumbar spine and proximal femoral BMD increased in a dose-dependent manner, except at the lowest odanacatib dose. Further BMD increments were noted after 24 months. Bone resorption markers were suppressed in a dose-dependent manner, and remained below baseline levels at months 12 and 24. Despite an initial decline, bone formation markers gradually increased after 6 months to levels similar to controls, except at the 50 mg dose in which P1NP levels remained lower than controls through the 2-year study. At the lowest dose of

odanacatib (3 mg) bone resorption and formation markers increased. At all doses, odanacatib was well tolerated and adverse events were similar among the groups [62]. This 2-year study was extended for a third year in a subset of patients maintaining the double-blind, placebo design. Patients were re-randomized to odanacatib 50 mg weekly or placebo, such that three groups were created with the 2-year:1-year extension periods following this sequence for the three groups: ODN/ODN; PLB/PLB; ODN/PLB [63]. Further gains in BMD (lumbar spine: +2.3 %; femoral neck: +1.6 %) were noted in the ODN/ODN group, with a cumulative densitometric gain of 7.9 % at the lumbar spine, 5.8 % at the total hip, and 5.0 % at the femoral neck. There was an initial decline of bone formation markers (P1NP and BSAP) but after reaching a nadir, P1NP levels returned to baseline and BSAP levels rose thereafter to above baseline values by the end of year 3. Bone resorption markers CTX and urinary deoxypyridinoline showed a transient suppression, but returned to baseline levels, whereas urinary NTX showed a cumulative 50 % decline in the ODN/ODN group. Tartrate-resistant acid phosphatase *5b*, a marker of osteoclastic number, increased above baseline similarly in the ODN/ODN and placebo groups, which confirms preclinical studies that have shown either no change or an increase in osteoclast numbers with odanacatib [51, 64].

In the ODN/PLB group, the switch to placebo after 2 years of active drug was followed by significant bone loss, particularly during the first 6 months after the study drug was discontinued. After 12 months without further odanacatib treatment, femoral neck BMD still remained slightly above baseline (+2.3 %) but BMD at the lumbar spine, total hip, and trochanter returned to baseline levels. A rapid increase in urinary NTX and serum CTX was seen after odanacatib was discontinued, reaching levels that were above baseline 12 months after stopping odanacatib. Bone formation markers increased during the first 6 months off-treatment, followed by a return to baseline levels. The rapid reversibility observed upon discontinuation of odanacatib is similar to that seen with most other antiresorptive agents, such as estrogens, selective estrogen receptor modulators, and denosumab [13, 51, 65, 66]. Odanacatib was well tolerated. More uncomplicated urinary tract infections were seen in the odanacatib group compared to placebo (12 vs. 3), but all other adverse events, including dermatologic ones, were similar between the two groups [63, 67].

In a phase III trial, 214 postmenopausal women with low BMD were randomized to receive weekly oral odanacatib 50 mg or placebo for 2 years [68]. Women receiving active drug showed significantly greater gains in BMD at lumbar spine, femoral neck, total hip, and trochanter sites as early as 1 year of treatment, and treatment difference at 2 years were 5.4 %, 3.8 %, 3.3 %, and 5.5 %, respectively. By computed tomography, trabecular volumetric BMD increased with odanacatib at the spine, hip, and hip subregions; integral volumetric BMD at the hip and hip subregions at 2 years. The treatment difference between odanacatib and placebo for volumetric trabecular BMD at spine was 11.5 % ($p<0.001$). Finite element-estimated strength also increased at both the hip and spine with odanacatib [68]. Other phase III trials are currently ongoing.

Sclerostin

The canonical Wnt/β-catenin pathway has profound effects upon osteoblast proliferation, differentiation, and survival (Fig. 12.2) [69]. When enhanced, Wnt signaling promotes bone formation [70]. Components of the Wnt/β-catenin pathway are potential targets for osteoanabolic treatment. Sclerostin and DKK-1, two negative regulators of this pathway, are the subjects of great interest in this regard

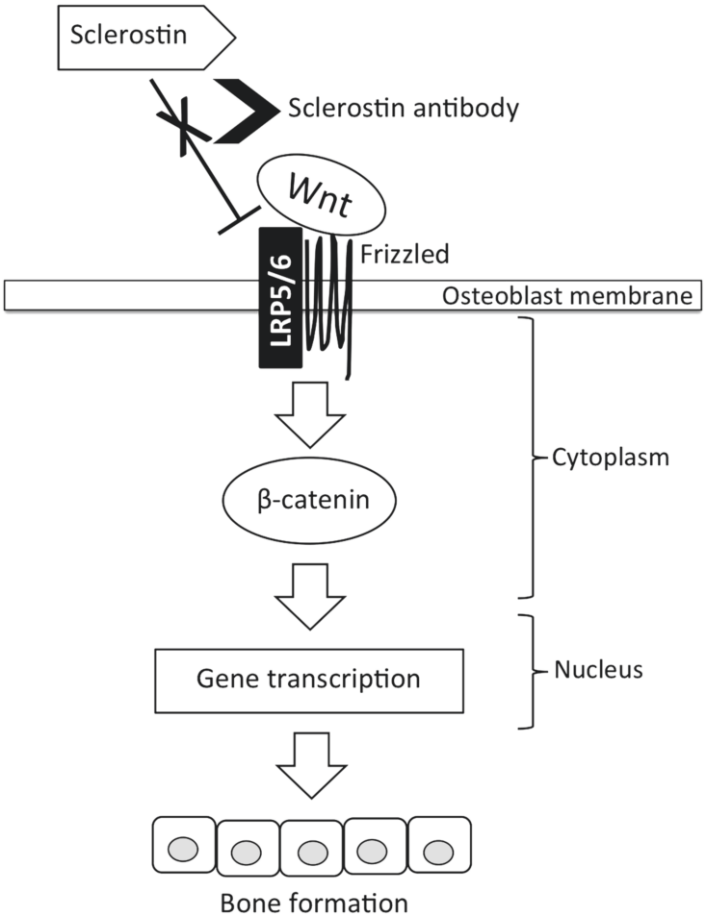

Fig. 12.2 Schematic depiction of sclerostin and the canonical Wnt signaling pathway. Wnt binds to the osteoblast receptors LRP5/6 and Frizzled, leading to stabilization of cytoplasmic β-catenin and subsequent translocation into the nucleus. In the nucleus, genes that regulate bone formation are activated. Sclerostin inhibits Wnt binding and reduces this anabolic pathway. Inhibition of sclerostin with antisclerostin antibodies alleviates this inhibition. Adapted by permission from: Lewiecki EM. Sclerostin: a novel target for intervention in the treatment of osteoporosis. Discov Med. 2011;12(65):263-73

[71]. Drug development has concentrated so far on the development of human antibodies capable of inactivating sclerostin [72, 73].

Preclinical studies have shown beneficial effects of sclerostin antibodies on bone formation in several different animal models of bone loss [74–77]. Ovariectomized rats treated for 5 weeks with murine sclerostin antibodies had higher BMD than controls, in addition to increased trabecular thickness (+57 %). Trabecular volumetric BMD of the distal femur recovered to levels similar to sham controls. Trabecular bone volume at the proximal tibia was restored, and bone strength was enhanced in the animals treated with sclerostin antibody as compared to controls [74]. The anabolic effects of sclerostin antibodies were also noted in rats, which were previously or concurrently treated with bisphosphonates [78]. Aged male rats demonstrated positive results with the sclerostin antibodies with dose-related increments in BMD, bone microarchitecture, and bone strength [77]. Adolescent female cynomolgus monkeys that received monthly administration of sclerostin antibodies showed a general increase in BMD associated with increments in bone formation in a dose-dependent manner. A reduction in bone resorption was seen at the 10 mg/kg dose [73].

Other studies using sclerostin antibodies have focused on animal models of secondary causes of osteoporosis such as glucocorticoid-induced bone loss, ulcerative colitis, and immobilization [75, 76, 79]. Treatment with sclerostin antibodies countered the deleterious effects of dexamethasone on bone mass, although deleterious effects on linear growth in young mice were not prevented [75]. The use of sclerostin antibodies in a mouse model of ulcerative colitis did not influence the inflammatory features of the disease; however, bone loss was prevented when given prophylactically [79]. Sclerostin antibodies were shown to be effective in increasing bone formation and decreasing bone resorption when given to hind limb-immobilized rats [76]. In two other experimental models, sclerostin antibodies improved fracture healing through increased callus size at the fracture site, increased bone mass and bone strength not only at the site of the fracture but also in nonfractured bone, and by enhancing the fracture healing process overall [80, 81].

These preclinical studies in rats and monkeys have been followed by studies in human subjects. Early clinical trial results with romosozumab (AMG-785) are promising [82]. In the phase I study, a total of 72 healthy patients (16 men, 56 women) aged 45–59 years were randomized to receive different dosing regimens of the drug SC (0.1, 0.3, 1, 3, 5, and 10 mg/kg) or IV (1 mg and 5 mg/kg). Romosozumab reached peak concentration within 1 week of subcutaneous administration, presenting a biphasic decline thereafter [82]. Bone turnover markers changed in a dose-dependent manner, with remarkable increments in bone formation (with 10 mg/kg SC, P1NP: +184 %, BSAP: +126 %, OCN: 179 %; and with 5 mg/kg IV, P1NP: +167 %, BSAP: +125 %, OCN: +143 %) and substantial reduction of bone resorption marker CTX (10 mg/kg SC: −54 % and 5 mg/kg IV: −49 %). After 3 months, BMD had increased in a dose-dependent manner at nearly all doses at the lumbar spine (10 mg/kg SC: +5.3 %; 5 mg/kg IV: +5.2 %) and at the total hip (10 mg/kg SC: +2.8 % and 5 mg/kg IV: +1.1 %) [82]. Adverse events were not different between romosozumab and placebo. The most common adverse events included

injection site bleeding, erythema, headache, constipation, back pain, arthralgia, and dizziness. Overall, there was no apparent relationship between adverse events and dose or route of administration of study drug, except for the injection-related adverse events such as injection site erythema and minor bleeding. Antibodies against the drug developed in 6 out of the 54 subjects, and neutralizing antibodies were positive in two subjects on the highest doses [82].

Preliminary results of a phase II randomized, placebo-controlled trial evaluated safety, efficacy, and tolerability of romosozumab and tested different subcutaneous monthly doses (70, 140, 210 mg) and every 3-month doses (140, 210 mg) compared to placebo and to open-label active comparators: teriparatide 20 mg SC daily and oral alendronate 70 mg once weekly [83]. After 12 months, all regimens of romosozumab were associated with increments in lumbar spine, total hip, and femoral neck BMD ($p < 0.005$) when compared to placebo. The largest BMD gains were obtained with the 210 mg monthly dose (11.3 % at lumbar spine and 4.1 % at total hip). The BMD increase was significantly less with alendronate and teriparatide ($p < 0.0001$). Markers of bone formation (P1NP) increased whereas CTX was reduced when compared to baseline by 1 week. Overall, adverse events were balanced between romosozumab groups and placebo; however, mild injection site reactions were higher with romosozumab (12 %) vs. placebo (4 %) [83].

Other studies are being conducted [84, 85], including a phase II multicenter, double-blind, randomized alendronate-controlled study in postmenopausal women with osteoporosis. The primary endpoints are the incidence of clinical and new nonvertebral fractures and the study is designed to last 24 months [49].

Blosozumab, another antisclerostin antibody, is also under investigation [86, 87]. A randomized, parallel-design, double-blind, placebo-controlled study was designed to study the effects of different doses of blosozumab in postmenopausal women with low BMD. There were five subcutaneous regimens: 270 mg every 12 weeks, 180 mg every 4 weeks, 180 mg every 2 weeks, 210 mg every 2 weeks, and placebo. The primary endpoint was the densitometric response at the lumbar spine at 1 year. A total of 154 postmenopausal women were enrolled with a mean baseline age of 65 years and an average lumbar spine *T*-score of −2.8. The percent change from baseline for these five groups are noted here: +6.7 %; +8.4 %; +14.9 %; +17.8 %; and −1.5 % for placebo. Adverse events did not differ across treatment groups. Mild-to-moderate injection site reactions were observed in those receiving blosozumab.

Nitric Oxide

Nitric oxide, a short-lived free radical implicated in several physiological processes, has been shown to promote bone formation and to reduce bone resorption by direct beneficial effects on osteoblast/osteoclast function and number [88, 89]. In population-based case–control studies, moderate doses of nitrates have been associated with increased BMD and a reduction in hip and overall fracture risk [90, 91]. However, while intermediate exposure to organic nitrates appears to be beneficial to

bone, higher doses and/or continuous nitrate exposure might be detrimental to the skeleton [89, 91–93].

Randomized clinical trials in postmenopausal women have shown that nitric oxide donors, such as nitroglycerin or isosorbide mononitrate, have positive effects on BMD, bone size, and bone remodeling. In healthy postmenopausal women, 5 or 20 mg/day of isosorbide mononitrate for 12 weeks increased the bone formation marker BSAP and decreased the bone resorption marker NTX [94]. Nitroglycerin ointment at a dose of 15 mg/day for 24 months led to a significant increase in BMD at the lumbar spine (+6.7 %), total hip (+6.2 %), and femoral neck (+7 %) as compared to placebo [95]. Peripheral qualitative computed tomography studies of the radius and tibia in these subjects also showed increased volumetric trabecular and cortical BMD, cortical thickness, and periosteal circumference with a consequent increase in bone strength. Compared with placebo, nitroglycerin-treated patients displayed uncoupling of bone formation and resorption, with a 34.8 % increase in BSAP and 54 % decrease in NTX [95]. Additionally, a randomized trial involving osteoporotic postmenopausal women taking either isosorbide mononitrate 20 mg daily or alendronate 70 mg weekly for 12 months showed a comparable effect between the two treatments, with a 10.8 % and 12.1 % increase in lumbar spine BMD, respectively [96].

In contrast, the Nitroglycerin as an Option: Value in Early Bone Loss (NOVEL) study, a randomized, double-blind, placebo-controlled trial, failed to demonstrate a positive effect on BMD in women treated with nitroglycerin ointment at 22.5 mg/day as compared to placebo-treated subjects [97].

Conclusions

We have reviewed new developments in the established therapeutic categories and new dosing regimens and formulations of osteoanabolic therapy with PTH, PTHrP, and their analogs. We have also presented information about new molecules with therapeutic potential currently under active investigation, including cathepsin K inhibitors, antisclerostin antibody, and nitric oxide. The advances summarized in this chapter suggest that further important developments in this field will be appreciated over the next decade.

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Chapter 13
Conclusion: Whither (or Wither?) the Pharmacology of Pediatric Bone?

Gordon L. Klein

You have now read a summary of the availability and use of drugs to prevent or to treat bone loss in children. When you compare the pediatric chapters with current progress in adult pharmacology it is clear that we in pediatrics have a long way to go to reach the level of sophistication at which internists select and use these drugs to benefit their patients. So, how do we get to that level? Clearly a wealth of information is still required and in order to obtain it we pediatricians need to change our mind-set.

To illustrate the dilemma we face I will describe what I call "The Coke Bottle Paradigm." Many years ago when I lived in Peru I recall becoming very thirsty for a Coca Cola one hot day. I walked into the corner bodega and asked the owner if I could buy a bottle of Coke. He said, "certainly, just bring in an empty one first." As this was my first time attempting to buy a Coke I asked him how I would procure an empty bottle, in answer to which he simply shrugged his shoulders. The analogy to pediatric drug testing is clear. We are reluctant to test a drug in children unless we have some assurances that it is safe to do so, but we cannot be certain that a drug is safe for children unless we test it in children. Furthermore, we in pediatrics tend to interpret the first part of the Hippocratic Oath: "First do no harm" to mean "do nothing." In other words, if testing a drug involves a risk of harm do not test. I would ask those who feel this way whether we do not violate this Oath by consequently withholding from children the potential benefits a drug may have to offer. All potential studies must balance potential risks against potential benefits in order to provide Institutional Review Boards with sufficient data to make an informed decision regarding approval of a drug study.

In the chapter on Drug Discovery (Chap. 3) you can find a description of the Best Pharmaceuticals for Children Act passed by the United States Congress and designed to identify candidate drugs for testing in children under the sponsorship

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of the Eunice Kennedy Shriver National Institute for Child Health and Human Development. This mechanism covers drugs that are FDA approved for a specific purpose in adults and proposes that they be tested for those approved indications in children. These candidate drugs would not have been tested in children by the pharmaceutical companies that developed them. As a participant in this process for the past 3 years I have witnessed the identification of many candidate drugs but only a relative few can afford to be tested by cooperating medical centers. Where does this leave the bulk of the drugs? Whose responsibility is it to test them in children?

I would argue that it is our collective responsibility to undertake the testing of new drugs that offer the most potential benefit for children we care for. These studies should be carefully designed with the most current pharmacologic and pharmacokinetic information available. Study subjects should be carefully screened to minimize the possibility of side effects. Similarly, statistical methods should be well thought out to minimize the chances of inconclusiveness. Once safety and efficacy have been suggested by a pilot study, a large multicenter study should be undertaken to confirm the findings. For patients suffering from rare genetic disorders, specific international registries should be established to identify them and to facilitate their inclusion in multicenter international studies.

To conclude, we cannot leave the testing of drugs with potential benefits to others. If the patients we care for suffer from conditions which can potentially be helped by experimental drugs, we must find the resources and the funding to undertake these studies, and any information, however minimal, can be a step toward the comprehensive evaluation of these drugs.

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