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Benjamin Z. Leder
Marc N. Wein *Editors*

Osteoporosis

Pathophysiology and Clinical Management

Third Edition

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Preface

The second edition of *Osteoporosis: Pathophysiology and Clinical Management* was edited by Robert Adler. It was a successful compilation that combined key topics in basic science bone biology with clinical discussions regarding osteoporosis diagnosis and management. In this third edition, we have tried to keep to this same strategy for most topics in the exciting and ever-growing literature of osteoporosis. Some new chapters have been added to reflect the new insights and controversies in this rapidly evolving field. Chapters on basic and clinical aspects of potent new therapeutics (denosumab, romosozumab, and PTH analogs) have been added. We are grateful to Drs. Lewiecki, Tabacco, Bilezikian, Baron, and Gori for their contributions on these important new therapies.

Recently, the topic of safety of osteoporosis therapeutics has garnered considerable attention among physicians, patients, and the lay press. Therefore, we are pleased to add a new chapter on safety considerations for osteoporosis therapies by Drs. Lianne Tile and Angela Cheung. Despite the considerable benefit of our current osteoporosis therapeutics, exactly how these agents should be used in combination and over time remains to be defined. As such, we have included a new chapter on combination and sequential use of therapeutics that highlights very important new studies on this topic.

Finally, recent years have witnessed an explosion in knowledge regarding the basic mechanisms controlling how bone cells function in health and disease. As such, we have added new chapters on osteoblast, osteoclast, and osteocyte function and are pleased to include a new chapter that details recent advances in the genetics of bone density, fracture risk, and response to osteoporosis therapies. In addition, we would like to highlight the recent advances in structural biology, reviewed by Dr. Thomas Gardella, that have revolutionized the field of parathyroid hormone receptor signaling. We hope that this textbook will represent a valuable resource for a wide variety of skeletal biology researchers, clinical trainees, and clinicians.

We need to thank all the contributors for producing quality work. In an era when time is precious and all of us are stretched, writing a chapter is not usually high on the priority list. Therefore, the tremendous work of the contributors to this volume, all recognized experts in their fields, is greatly appreciated.

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Abbreviations

aBMD	Areal BMD
ADT	Androgen deprivation therapy
AFM	Atomic force microscopy
AIS	Androgen insensitivity syndrome
ALPL	Alkaline phosphatase
AP-1	Activator protein 1
AR	Androgen receptor
ATF4	Activating transcription factor 4
BMD	Bone mineral density (by dual X-ray absorptiometry)
BMDD	Bone mineralization density distribution
BP	Bisphosphonate
BSU	Bone structural unit
BV/TV	Bone volume fraction (bone volume/total volume)
BW	Body weight
Ca	Calcium
COL2A1	Collagen 2A1
CT	Computed tomography
CTX	c-terminal telopeptide
Cx43	Connexin 43
DHEA	Dehydroepiandrosterone
DHT	Dihydrotestosterone
DKK1	Dickkopf WNT signaling pathway inhibitor 1
DM	Diabetes mellitus
DMP1	Dentin matrix protein 1
DXA	Dual-energy X-ray absorptiometry
E2	Estradiol
ECR5	Evolutionarily conserved region 5
ERα	Estrogen receptor α
ESSA	Exercise and Sports Science Australia
FE	Finite element
FEA	Finite element analysis
FGF23	Fibroblast growth factor 23
GC	Glucocorticoids
GR	Glucocorticoid receptor
HA	Hydroxyapatite
HAL	Hip axis length
HPP	Hypophosphatasia

HR-pQCT	High resolution peripheral quantitative computed tomography
IGF	Insulin-like growth factor
IGFBP	Insulin-like growth factor binding protein
IL	Interleukin
LIFMOR	Lifting intervention for muscle and osteoporosis rehabilitation
LRP	Frizzled receptors and low-density lipoprotein receptor-related proteins
LS	Lumbar spine
μCT	Micro computed tomography
MAR	Mineral apposition rate
MEF2C	Myocyte enhancer factor 2
MEPE	Matrix extracellular phosphoglycoprotein
MM	Multiple myeloma
MMPs	Matrix metalloproteinases
MORE	Multiple Outcomes of Raloxifene Evaluation trial
MRI	Magnetic resonance imaging
MSCs	Mesenchymal stem cells
NIH	National Institutes of Health
OCs	Oral contraceptives
OI	Osteogenic index
OPG	Osteoprotegerin
P1NP	Procollagen type I propeptide
PAQ	Physical activity questionnaire
PCOS	Polycystic ovarian syndrome
PHEX	Phosphate-regulating neutral endopeptidase, X-linked
Pi	Phosphate ions
PI	Proteasome inhibitors
PMMA	Polymethylmethacrylate
pmOP	Postmenopausal osteoporosis
PP	Pyrophosphate
PPARγ	Peroxisome proliferator-activated receptor γ
pQCT	Peripheral quantitative computed tomography
PTH	Parathyroid hormone
PTH1R	Parathyroid hormone receptor 1
PTHrP	Parathyroid hormone-related protein
QCT	Quantitative computed tomography
RANKL	Receptor activator of nuclear factor kappa B ligand
RBPJK	Recombination signal-binding protein 1 for j-kappa
RCT	Randomized controlled trial
ROS	Reactive oxygen species
RUNX2	Runt-related transcription factor 2
SARM	Selective androgen receptor modulator
SAXS	Small angle X-ray scattering
SD	Standard deviations
SERM	Selective estrogen receptor modulators
SHBG	Sex hormone-binding globulin
SIBLING	Small integrin-binding ligand, N-linked glycoprotein
SR-μCT	Synchrotron radiation micro computed tomography

SrR	Strontium ranelate
T	Testosterone
TBS	Trabecular bone score
TEM	Transmission electron microscopy
TGF	Transforming growth factor
TMD	Tissue mineral density
TNFα	Tumor necrosis factor α
TNSALP	Tissue nonspecific alkaline phosphatase enzyme
vBMD	Volumetric BMD
VERT-NA	Vertebral Efficacy with Risedronate Therapy, North American trial
WAT	White adipose tissue
WAXS	Wide angle X-ray scattering
WBV	Whole-body vibration
Wnts	Wingless-MMTV integration site family members
XLH	X-linked hypophosphatemic rickets

Basic Aspects of Osteoblast Function

1

Christina Vrahnas and Natalie A. Sims

Key Points

- The osteoblast lineage includes pluripotent precursors, preosteoblasts, osteoblasts, osteocytes, and bone lining cells.
- Osteoblasts are the cells responsible for formation of the collagen-rich bone matrix (osteoid) which becomes mineralized by the deposition and accumulation of mineral crystals.
- Mineralization of the bone matrix is regulated by proteins produced by the osteoblast lineage including alkaline phosphatase and non-collagenous proteins in the bone matrix.
- Osteoblast lineage cells also control the differentiation of osteoclasts through their production of receptor activator of NF-KB ligand (RANKL), macrophage colony-stimulating factor (M-CSF), and osteoprotegerin (OPG).
- Bone lining cells have the potential to be a source of osteoblast precursors.

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Introduction to the Osteoblast Lineage: Multiple Stage-Specific Functions

Osteoblasts are specialized mesenchymal-derived cells that produce and deposit the collagenous bone matrix and regulate the mineralization of that matrix by their production of additional non-collagenous proteins. The osteoblast lineage includes not only these bone-forming osteoblasts but also their pluripotent and lineage-committed precursors, bone lining cells, and matrix-embedded osteocytes (Fig. 1.1). Each of these stages of the osteoblast lineage has distinct functions, morphologies, particular locations relative to the bone surface, and increasingly well-defined markers of differentiation (noted on Fig. 1.1 and discussed below).

The different stages of osteoblast differentiation allow these cells to perform three major functions that determine skeletal structure (noted on Fig. 1.1 and discussed below): (1) production of bone matrix (osteoid), (2) regulation of osteoid mineralization by production of non-collagenous proteins, and (3) support of osteoclast formation. In addition, osteoblast lineage cells produce paracrine factors, such as IL-6 family cytokines, parathyroid hormone-related protein (PTHrP), and contact-dependent molecules such as EphrinB2, that regulate their own differentiation and activity [1–3]. Osteoblasts have also been suggested to act as "reversal" cells, allowing communication

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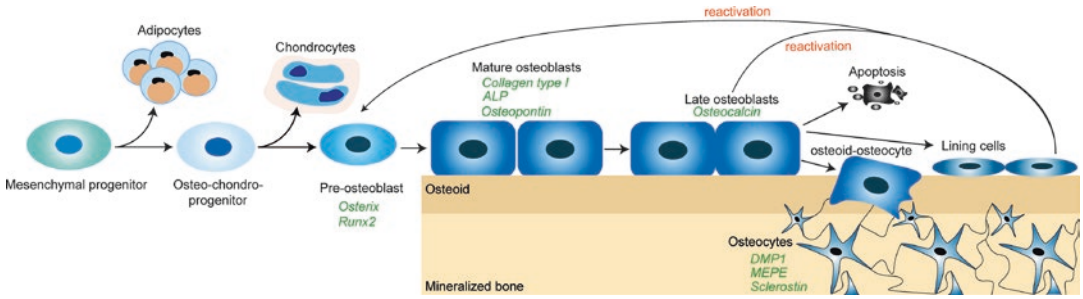

Fig. 1.1 Stages of the osteoblast lineage. The osteoblast lineage arises from pluripotent mesenchymal progenitors, capable of differentiating into adipocytes or into chondrocytes or osteoblasts. Commitment to the osteoblast lineage is determined by expression of transcription factors including Runx2 and osterix. Once osteoblasts become mature, they deposit collagen type I-rich matrix (osteoid) as a template for bioapatite mineral deposition and express alkaline phosphatase (ALP), osteopontin, and osteocalcin, proteins that regulate bone mineralization. Osteoblasts then undergo one of three fates: (1) apoptosis, (2) remain on the bone surface as bone lining cells, or (3) become embedded within their collagenous bone matrix as "osteoid-osteocytes," which then become terminally differentiated osteocytes. Osteocytes also regulate the mineralization of the bone matrix through their production of DMP-1, MEPE, and sclerostin. Bone lining cells appear to be capable of reactivation to become active osteoblasts or osteoblast precursors

between osteoclasts and osteoblasts, during the bone remodeling process [4]. The functions of the osteoblast lineage are not limited to the control of bone structure. They also regulate the hematopoietic stem cell niche [5, 6], contribute to hematopoietic malignancies [7], and to B cell homeostasis [8]. The osteoblast lineage also has endocrine functions in phosphate homeostasis [9] and glucose metabolism [10]. This chapter will focus on describing the stages of osteoblast differentiation and the functions of the lineage that regulate bone structure and bone matrix composition.

Osteoblast Differentiation and the Stages of the Osteoblast Lineage

Osteoblast Precursors

The osteoblast lineage arises from pluripotent mesenchymal progenitors. In vitro, these cells can be induced to differentiate into other mesenchymal origin cells such as chondrocytes, adipocytes, myoblasts, or fibroblasts [11] (Fig. 1.1). In vivo, bone marrow-derived mesenchymal progenitors have a more restricted future, being capable of differentiating into chondrocytes, osteoblasts, and adipocytes [12]. The location of these cells in the marrow has been refined by cell lineage-tracing studies (using genetically altered mice with fluorescent tags that are retained throughout differentiation) to be in close association with vascular structures [13]. This provided support for much earlier studies proposing that the pericyte, a cell found wrapped around endothelial cells, can behave as an osteoblast progenitor [14, 15]. Pericytes in different tissues appear to behave in an organ-specific manner, dictated by their anatomy and position; only bone marrow-residing pericytes appear capable of becoming osteoblasts [12]. This illustrates the importance of the microenvironment in determining differentiation. For more details, the reader is directed to a recent focused review on the identity of osteoblast progenitor populations [16].

The source of osteoblast progenitors is not restricted to the bone marrow pericytes. During embryonic bone development, perichondrial cells were identified as precursors giving rise to osteoblasts on trabecular bone [17]. This has been confirmed by lineage-tracing studies, which also identified these precursors as entering the marrow space with invading blood vessels and thereby contributing to both bone development and fracture healing [18]. Similar observations have been made that differentiated hypertrophic

chondrocytes at the growth plate can "transdifferentiate" into osteoblasts during development and fracture healing [19], again confirming much earlier in vitro work [20]. Lineage tracing studies have also suggested that bone lining cells [21] and recently embedded osteocytes [22] can act as osteoblast precursors, although the latter remain highly controversial. It is likely that lining cells are already committed to the lineage, rather than having the potential to differentiate in chondrocytes or adipocytes. This suggests that there are multiple sources of osteoblast progenitors in vivo, with differentiation that is both context- and location-specific.

Commitment of precursors to the osteoblast lineage is controlled by the expression of a range of transcription factors. Absolutely essential for the commitment to the preosteoblast stage are runt-related transcription factor 2 (Runx2) and osterix [23, 24]. Other transcription factors including activating transcription factor 4 (ATF4) [25], activator protein 1 (AP-1) [26], and CCAAT/enhancer-binding proteins β and δ (C/EBPβ and C/EBPδ) [27] promote the transition to matrix-producing osteoblasts.

Since osteoblasts and adipocytes are derived from common precursors, many of these transcription factors also inhibit mesenchymal progenitor commitment to adipogenesis [26, 28]. Alternatively, transcription factors such as peroxisome proliferation-activated receptor γ (PPARγ) [29] and CCAAT/enhancer-binding protein α (C/EBPα) [30] promote differentiation into adipocytes. This inverse relationship between osteoblast and adipocyte differentiation was first observed in cell culture [31]. This has also been described in vivo in genetically altered mouse models, either where high osteoblast numbers are associated with low marrow adipocyte volume [26] or where low osteoblast numbers are associated with high marrow adipocyte volume [32–34]. Similar reciprocal regulation has been made in animal models of ovariectomy-induced bone loss [35]. There are exceptions to this, such as the C3H/HeJ mouse strain which has high bone mass [36] and high marrow adiposity [37]. Reciprocal regulation of osteoblasts and adipocytes has also been observed clinically: increased marrow adiposity is associated with age-related osteoporosis [38]. Understanding the relationships between osteoblast and adipocyte commitment remains an area of active research, since it may allow the development of additional treatments to increase bone mass.

The osteoblast precursor can also give rise to chondrocytes; this is important in the context of developmental and pediatric bone growth, and fracture healing, and may be of relevance for methods to repair joint cartilage. The osteoblast commitment transcription factors Runx2 and osterix not only promote osteoblast commitment but also stimulate the final stage of chondrocyte differentiation prior to vascular invasion in endochondral ossification [39–41]. Reciprocal regulation of chondrogenesis versus osteoblastogenesis from the same common precursor has also been suggested [42], as described above for adipocytes, but mechanisms controlling this have not yet been identified.

Matrix-Forming Osteoblasts

Mature matrix-forming osteoblasts are characterized by a cuboidal morphology and are located in groups with extensive cell-cell contact [43–45]. Osteoblasts are also located in close apposition to the bone surface; this indicates that as they differentiate to this stage, these cells must migrate, probably in groups to the bone surface, likely in response to coupling factors produced by osteoclasts or other cells within the basic multicellular unit [46–48]. There are two exceptions to this. During skeletal development, osteoblasts can form bone de novo (without a surface to work on), and during endochondral ossification, calcified cartilage serves as a template on which osteoblasts deposit bone.

At the electron microscope level, matrix-forming osteoblasts exhibit abundant endoplasmic reticulum, in line with their major function as factories for production of type I collagen, the main component of the osteoid matrix (see below). Matrix-producing osteoblasts also express a range of non-collagenous proteins. These include proteins involved in regulating the

incorporation of mineral into the osteoid matrix (alkaline phosphatase [49], osteocalcin [50], and osteopontin [50]) and receptors that regulate their response to factors influencing their further differentiation and function, such as receptors for IL-6 family cytokines [33, 51] or the receptor used by parathyroid hormone (PTH) and PTH-related protein (PTHrP), PTH1R [52]. The mechanisms of matrix production and mineralization will be discussed below.

When their production of osteoid matrix is complete, mature osteoblasts undergo one of three fates: [1] remain on the surface of bone as less metabolically active bone lining cells, [2] die by apoptosis, or [3] become entrapped within the osteoid matrix and, as the osteoid is mineralized, further differentiate to become osteocytes (Fig. 1.1).

Osteocytes

Osteocytes are embedded within the bone matrix during the process of bone formation, and through their extensive dendritic processes and their fluid-filled network of communicating channels, they sense and respond to mechanical strain and microdamage to bone. They are the most abundant cells in bone by far, forming a highly complex cellular communication network through the bone matrix with a total of ~3.7 trillion connections throughout the adult skeleton [53].

How osteoblasts become embedded into the bone matrix remains unknown. The manner in which osteoblasts become osteocytes has been described as "encased," "buried," and "merged" into the matrix suggesting that the manner of transformation may depend on the type of bone formed [54]. It is possible that the type of bone being made (woven vs lamellar) or mode of ossification as well as location (periosteal/endocortical/trabecular) can determine how an osteoblast becomes embedded into the matrix. There are no specific signals made by the osteoblast that have been found to directly control this process. When an osteoblast transitions into the recently secreted matrix (osteoid) to become an osteocyte (Fig. 1.1) they are termed "osteoid-osteocytes" [55]. The most striking difference between osteoblasts and osteoid-osteocytes is the morphological change that occurs during this transition. The cuboidal morphology of the osteoblast changes into a less cuboidal cell which eventually transforms into a smaller cell body with many dendritic cellular projections characteristic of osteocytes. Upon mineralization of the osteoid, the ultrastructure of the osteocyte changes in line with its reduced protein-production capacity, including reduced endoplasmic reticulum and Golgi apparatus [56].

Differentiated osteocytes reside within lacunae in the bone matrix and form an extensive intercellular network throughout the bone matrix and regulate both bone formation and resorption. Cell contact is a notable feature of this network [53], as is the ability of these cells to sense and respond to mechanical load and microdamage [57]. In addition to controlling osteoblast activity on the bone surface by the release of local factors such as sclerostin [58], and oncostatin M [33], osteocytes regulate mineralization of the bone matrix by expressing factors such as dentin matrix protein 1 (DMP-1) [59] and matrix extracellular phosphoglycoprotein (MEPE) [60] and act in an endocrine manner to control phosphate homeostasis by their release of fibroblast growth factor 23 (FGF23) [61] (refer also to Chap. 3 (Basic Aspects of Osteocyte Function)).

Bone Lining Cells

Osteoblasts that do not become terminally differentiated osteocytes or undergo apoptosis remain on the bone surface to become flattened bone lining cells. Lining cells are characterized by flat nuclei and the ability to synthesize only small amounts of protein and, like other cells of the osteoblast lineage, connect with each other via gap junctions [62].

Although long regarded as a protective cell population covering the bone surface that is "resting," or "quiescent", bone lining cells, like osteoblasts, express receptors for endocrine and paracrine agents. Their contraction from the bone surface in response to PTH [63] was suggested to

allow osteoclasts access to the bone surface [64]. It has been suggested that this lifting of the bone lining cell layer occurs not only in response to PTH treatment but also at the commencement of bone remodeling to generate a temporary canopy [65]. Such a canopy was previously suggested as a mechanism that encloses the bone remodeling activity, separating it from the rest of the bone marrow microenvironment [66], thereby providing a controlled locale in which osteoblast lineage cells, osteoclasts, and other contributing marrow cells, may exchange factors. This canopy is also closely associated with blood vessels, which can thereby readily provide both osteoblast and osteoclast precursors for the bone remodeling process [67, 68].

In addition to forming a canopy, bone lining cells are capable of reactivation to form active matrix-producing osteoblasts. This was first hypothesized when intermittent PTH administration increased osteoblast number on the bone surface without increasing osteoblast proliferation [69]. This mechanism has now been verified by lineage-tracing studies where intermittent administration of PTH reactivated quiescent lining cells to mature osteoblast in vivo [70]. Such reactivation of lining cells has also been demonstrated after mechanical loading [71] and after treatment with anti-sclerostin, a therapeutic stimulus of bone formation [72]. This reactivation is in addition to the proposal that these cells form a proliferating progenitor population during adulthood [21] and may provide a more rapidly inducible partially differentiated source of osteoblast precursors.

Bone Formation: Osteoid Production and its Mineralization

Bone is a heterogenous compound material. The mineral phase, in the form of modified hydroxyapatite (bioapatite) crystals, contributes about two-thirds of its weight. The remaining organic matrix consists largely of type I collagen (~90%) [73, 74], with small amounts of lipid (~2%), ~5% non-collagenous proteins, proteoglycans, and water [75]. Non-collagenous proteins within the bone matrix include substances that act as signaling molecules (such as transforming growth factor β (TGFβ) and insulin-like growth factor 1 (IGF1)) and substances that regulate mineralization (such as osteocalcin and DMP-1).

While a range of cell types are capable of depositing mineral, particularly in cell culture conditions or in pathological circumstances (such as vascular calcifications), it is only the osteoblast that is capable of bone formation. Osteoblasts are responsible for the deposition of bone matrix on a range of surfaces and in a number of different contexts. During endochondral bone formation, osteoblasts deposit bone on a cartilage template. This process occurs both in skeletal development and in fracture healing. In these instances, osteoblasts attach to the cartilage template and deposit osteoid, which becomes mineralized, according to processes described below. During intramembranous bone development, bone is formed directly by mesenchymal precursors with no underlying template. This process occurs largely during skeletal development, particularly of the calvarial bones, and occurs during the formation of the periosteal collar at the diaphysis (midshaft) of bones that form by endochondral ossification. During bone remodeling, bone mass is maintained by osteoblasts that form sufficient bone to replace bone that was recently removed by osteoclasts. In contrast, during bone growth, periosteal expansion occurs by modeling, where osteoblasts form bone on a bone surface that has not been previously resorbed. There are also pathological conditions, where bone is formed in locations where it is not normally found, e.g., in heterotopic ossifications in the muscle in the context of injury [76] or in rare genetic conditions [77]. In all of these processes, bone formation occurs as follows.

Osteoblasts do not produce "bone" *per se*, but synthesize a collagen-rich osteoid matrix. The osteoid matrix serves as a template for the subsequent deposition of mineral in the form of bioapatite which contributes to the hardness of bone. The balance between osteoid and mineral content determines bone strength: essentially, the collagen provides flexibility, while the mineral provides hardness. The process of mineralization is

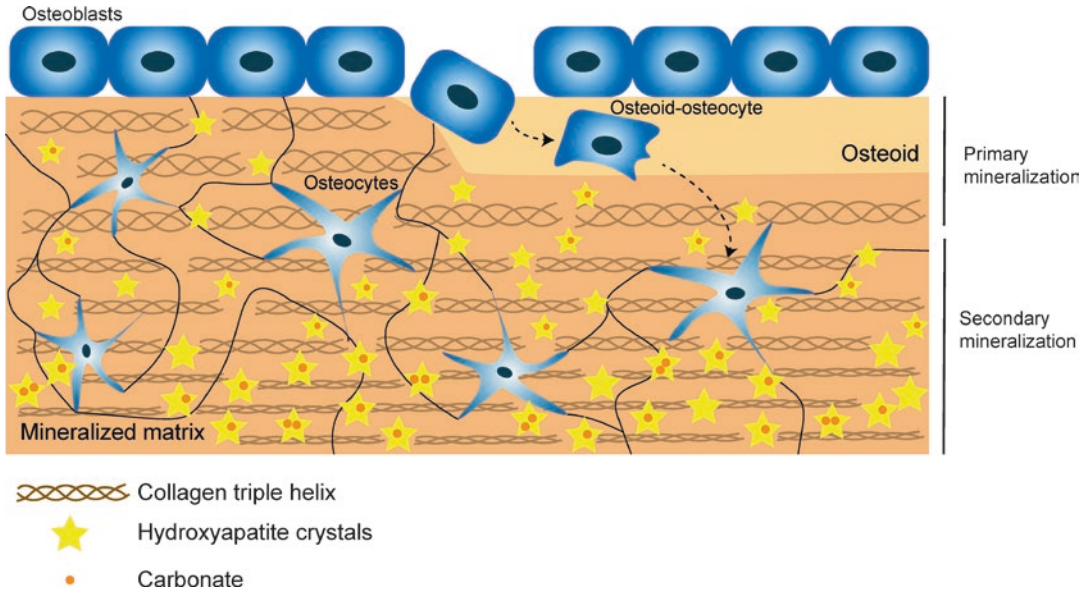

Fig. 1.2 The process of bone matrix production and mineralization. Mature osteoblasts on the bone surface deposit newly formed matrix, known as osteoid (1), largely comprised of type I collagen (a triple helical structure). After collagen deposition, the matrix becomes progressively mineralized by the accumulation of hydroxyapatitic bioapatite crystals (2). This mineralization process has two phases. Within ~5–10 days, osteoid undergoes rapid primary mineralization, and over subsequent weeks, months, and years, secondary mineralization occurs. The bioapatite crystals grow and accumulate carbonate in the more mature regions of bone, and collagen fibers become more condensed (compact) presumably due to steric hindrance caused by the presence of the growing crystals

controlled by non-collagenous proteins produced by late-stage osteoblasts and osteocytes. We will describe each of these processes in turn (Fig. 1.2).

Osteoid Deposition

When osteoid is deposited by osteoblasts, it has two potential forms depending on its collagen orientation and speed of production: woven and lamellar bone. During bone development and fracture healing, woven bone is deposited rapidly: this substance contains disordered, seemingly randomly oriented collagen fibers. In contrast, lamellar bone is highly organized. Fibers are more slowly deposited, predominantly oriented longitudinally, and create a defined, ordered structure [78]. Collagen fibers in lamellar bone are oriented in perpendicular planes in adjacent lamellae [79], adding strength of the substance. The loose structure and random orientation of woven bone suggest that it is mechanically weaker than lamellar bone. This has been tested in human fetal bone, where younger, more woven bone was associated with lower elasticity and lower resistance to penetration (microhardness) [80].

How osteoblasts are instructed to form either woven or lamellar bone is not known, but ultra-high voltage electron microscopy studies suggest that even during lamellar bone formation, collagen fibers are deposited sparsely and randomly, but as the osteoblast becomes more distant due to further deposition, the fibres begin to reoirent parallel to the direction of growth and become thicker [81]. This suggests that as-yet unidentified events after initial collagen secretion may be responsible for the woven or lamellar nature of bone. Adding to these observations, live cell imaging of osteoblasts engineered to deposit fluorescent-labeled collagen has recently revealed that osteoblasts constantly move during the collagen assembly process, and actively exert forces on the fibrils, physically shaping the collagen

matrix and potentially guiding the formation of osteocyte lacunae [82].

Type I collagen comprises a triple-helix structure of two α1 and one α2 polypeptide chain [83]. In osteoblasts, single pro-α chains are synthesized in the endoplasmic reticulum, which assemble into procollagen triple helices, and are released by exocytosis into the extracellular space, where the N- and C-termini are cleaved, allowing the formation of fibrils [84]. Multiple intracellular posttranslational modifications, including hydroxylation of proline and lysine residues, and glycosylation, stabilize the collagen triple helical structure [85]. After secretion, collagen fibers are stabilized and bone is strengthened further by the formation of inter- and intra-molecular cross-links, through the action of lysyl oxidase [86]. Other modifications such as advanced glycation adversely affect the mechanical properties of the bone matrix, particularly during ageing [87]. Defects not only in the proteins coding collagen itself but also in the many different aspects of collagen fibril assembly, including collagen folding, secretion, cross-linking, and posttranslational modifications, have been described in the diverse family of skeletal fragilities observed in osteogenesis imperfecta [88].

Matrix Mineralization

After collagen is deposited, it becomes progressively mineralized by the accumulation of bioapatite crystals. This mineralization process has two phases. Within ~5–10 days, osteoid undergoes rapid primary mineralization, and over subsequent weeks, months, and years, secondary mineralization occurs [89]. During primary mineralization, the tissue usually reaches ~50–70% of its final mineral content [90, 91]. During secondary mineralization, mineral continues to accumulate at a slower rate [92], the crystals beome larger [89], and carbonate is substituted for phosphate groups within the matrix [93, 94]. In addition, as mineral is deposited, the surrounding collagen fibers of bone also change, becoming more compact, possibly in response to the growing crystals [93, 94] (Fig. 1.2).

The final stage of mineralization achieved in the bone substance varies locally within the bone matrix and depends on the species, sex, age, and anatomical location of the bone [95]. Mineralization involves the release of matrix vesicles, which are cell-derived extracellular membrane-enclosed particles of poorly crystalline bioapatite mineral [96, 97]. The mineral crystals become ordered (a process termed nucleation) by a process driven by contact with collagen, local availability of calcium and phosphate, and by apatite nucleators such as DMP-1 and osteopontin [98, 99]. The importance of phosphate-regulating proteins is clearly illustrated by the association of human and murine genetic insufficiencies in phosphate regulators with impaired bone mineralization [100–102].

Mineralization initiation, accrual, and crystal maturation are controlled, not only by apatite nucleators but also by a range of multifunctional non-collagenous proteins secreted by mature osteoblasts and osteocytes. Osteoblasts and osteocytes express proteins that support mineralization such as alkaline phosphatase, PHOSPHO1, phosphate-regulating neutral endopeptidase, X-linked (PHEX), and bone sialoprotein/integrin-binding sialoprotein. Osteoblasts and osteocytes also express proteins that inhibit mineralization, such as osteocalcin [103], MEPE, and PC-1 (*Enpp1*) [104]. An illustration of the fine control exerted by osteoblasts on mineralization is their ability to control local levels of inorganic phosphate through alkaline phosphatase (ALP) and plasma cell membrane glycoprotein-1 (PC-1). Hydroxyapatite nucleation depends on a high ratio of inorganic phosphate (P_i), which promotes mineralization, to inorganic pyrophosphate (PP_i), which inhibits it. Alkaline phosphatase (ALP) positively regulates this balance by hydrolyzing PP_i to form the P_i required for hydroxyapatite crystal nucleation; insufficiency of ALP leads to poor mineralization, as observed in individuals with hypophosphatasia [100]. In contrast, PC-1 inhibits mineralization by producing inorganic pyrophosphate; insufficiency of PC-1 therefore leads to excessive mineralization [104].

The Osteoblast Lineage Supports Osteoclast Formation, Attachment, and Bone Resorption

The function of the osteoblast lineage is not restricted to bone formation. Osteoblast lineage cells also control the differentiation of osteoclasts, the cells responsible for bone resorption. There are three major ways in which cells of the osteoblast lineage carry out this role: (1) by producing RANKL and OPG in response to paracrine and endocrine agents, (2) by releasing chemoattractants that draw osteoclast precursors to the bone surface, and (3) by preparing the bone surface for osteoclast attachment. We will discuss each of these actions in turn.

Production of RANKL and OPG

A range of locally acting cytokines, including interleukin-11 (IL-11), prostaglandin E_2, PTHrP, and oncostatin M, stimulate osteoclast formation, but do not achieve this by direct action on osteoclast precursor themselves. Instead, these agents, and endocrine factors like PTH and 1,25-dihydroxyvitamin D, stimulate osteoclast formation indirectly, by acting on osteoblast lineage cells to stimulate expression of RANKL and CSF-1 (M-CSF), two regulatory molecules that are both required for osteoclastogenesis [105–110]. It is the interaction of RANKL with its receptor (RANK), expressed on the cell surface of mononuclear hemopoietic osteoclast precursors, that triggers osteoclast formation (Fig. 1.3).

The necessity for RANKL and RANK for osteoclastogenesis was demonstrated by the generation of genetically altered mice that lack either RANKL or RANK and exhibited a lack of osteoclasts and severe osteopetrosis [111, 112]. Osteoblast lineage cells also express a soluble protein that is a non-signaling decoy receptor for RANKL, known as osteoprotegerin (OPG). OPG acts as a “brake” on osteoclast differentiation by blocking the interaction of RANKL and RANK [113, 114], and through modulation of RANKL and OPG expression, osteoblasts can precisely regulate the formation of osteoclasts.

RANKL is expressed at all stages of osteoblast differentiation, including in precursors, matrix-producing osteoblasts, bone lining cells, and osteocytes [115]. RANKL production is not exclusive to osteoblast lineage cells. T-cells and natural killer (NK)-cells also express RANKL and are capable of promoting osteoclast formation [116, 117]. It appears that expression of RANKL by T-cells is dispensable for normal bone development and maintenance [118]. In contrast, in mice that lack RANKL in the osteoblast lineage, severe osteopetrosis is observed [119]. However, the most important stage in osteoblast differentiation for production of RANKL is not known, and whether the key source of RANKL is the osteocyte, the bone lining cell, or the preosteoblast remains controversial [21, 119–122]. One important concept to consider is that direct contact between the RANKL-expressing osteoblast lineage cells and the RANK-expressing haemopoietic osteoclast precursors is absolutely required for osteoclast formation in vitro [123, 124], and the same situation is likely to be true in vivo (Fig. 1.3). While recombinant soluble RANKL certainly promotes osteoclast formation from precursors in vitro [125], and in vivo [126], there remains no convincing evidence that soluble RANKL, produced by osteoblast lineage cells, can substitute for the membrane form, nor is there any convincing evidence of a physiological role for circulating RANKL. This means it is important to consider the location of the osteoblast lineage cells most likely to support osteoclast formation. Cells in the marrow, or in direct contact with it, such as osteoblast precursors and bone lining cells, rather than embedded osteocytes, are more likely to come into contact with osteoclast precursors, and therefore more likely to support osteoclast formation in normal remodeling. It has been difficult to understand how osteocytes, from within the matrix, could control RANKL availability to osteoclast precursors in the bloodstream through a contact-dependent mechanism although it has been suggested that osteocyte processes extend into the marrow space [127]. However, even

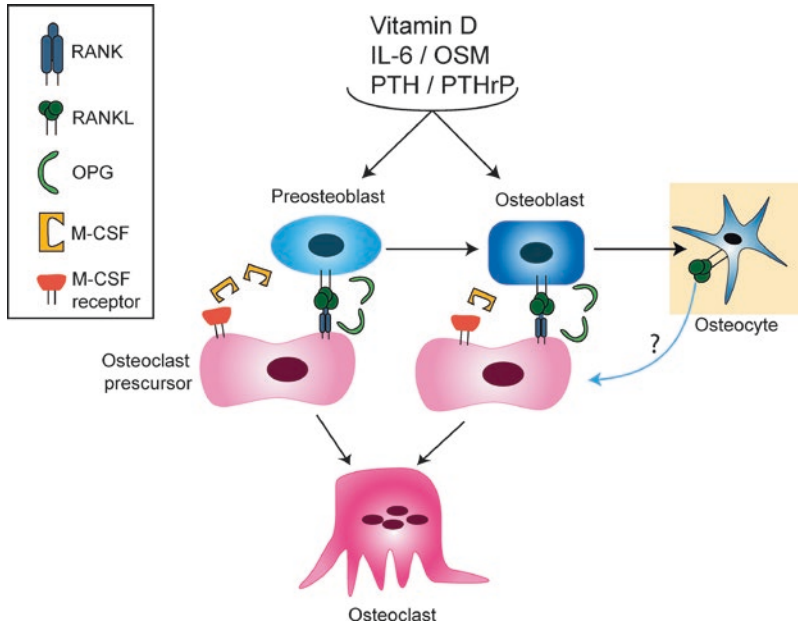

Fig. 1.3 The osteoblast lineage supports osteoclastogenesis. Osteoblast lineage cells control the differentiation of osteoclasts in response to paracrine and endocrine agents and locally acting cytokines such as vitamin D, interleukin-6 (IL-6), oncostatin M (OSM), and parathyroid hormone (PTH) / parathyroid hormone-related protein (PTHrP). These agents and factors act on the osteoblast lineage to stimulate expression of RANKL and M-CSF which each promote osteoclast formation. M-CSF is soluble. Receptors for both RANKL and M-CSF are expressed on the cell surface of mononuclear hemopoietic osteoclast precursors. Direct contact between membrane-bound RANKL and its membrane-bound receptor (RANK) triggers osteoclast formation. Osteoblast lineage cells also express a decoy receptor for RANKL, known as osteoprotegerin (OPG), which blocks the interaction of RANKL and RANK. Through their modulation of RANKL and OPG expression, osteoblasts can precisely regulate the formation of osteoclasts. Osteocytes also express RANKL, but the mechanism by which this reaches the osteoclast precursors remains undefined

when osteocytes were cultured in direct contact with osteoclast precursors and stimulated with appropriate stimuli, only binucleated "osteoclasts" formed [120].

RANKL production by osteoblast-lineage cells is also stimulated by microdamage within the bone matrix. Microdamage or microcracks are small defects in the bone matrix that occur in both pathological conditions and with normal skeletal loading [128]. Experimental loading, which causes a higher level of microdamage, initiates bone resorption [129], and indeed, resorption and replacement of the bone compromised by this damage is one of the important mechanical functions of bone remodeling [128]. It has been suggested that the microdamage site "steers" those osteoclasts already functioning on the bone surface toward the site of damage [130]. Microdamage within the bone is sensed by osteocytes, which are terminally differentiated osteoblasts that reside within the bone matrix, and sense changes in pressure within the matrix. Anatomical studies of rat bone in which microcracks were induced by ex vivo loading demonstrated that osteocytes located near to microcracks are more likely to be apoptotic compared to sites more distant to the microcrack [131]. Mechanical loading of human bone ex vivo and of rat bone in vivo increases osteocyte apoptosis [132, 133], and osteocytes surrounding the dying cell increase their production of RANKL to initiate resorption [134]. In support of this, short-term deletion of osteocytes in vivo resulted in a rapid increase in expression of RANKL mRNA in the bone, presumably by osteoblast lineage cells, and an increase in osteoclast formation [135].

Another factor produced by the osteoblast lineage and required for osteoclast formation is CSF-1/M-CSF [136, 137]. Together, RANKL and CSF-1 are all that is required to support osteoclast formation from bone marrow precursors in vitro. Just as observed in RANKL null mice, mutant mice lacking CSF-1 also exhibit severe osteopetrosis due to lack of osteoclast formation [138]. While RANKL is membrane bound and acts to promote osteoclast precursor fusion, CSF-1 is secreted by osteoblasts and promotes osteoclast precursor proliferation [139].

Release of Chemoattractants

Another mechanism by which osteoblasts control osteoclast differentiation is by controlling the movement of osteoclast precursors toward each other (allowing fusion) and to the bone surface (allowing attachment) through their release of chemoattractants. These factors may be deposited in the bone matrix itself during bone formation; they may be released by active osteoblasts or may be released from apoptotic osteocytes. Some bone matrix-derived factors, suggested to act as chemoattractants for monocytic osteoclast precursors, include osteocalcin, fetuin-A, and collagen-I fragments [140]. Thus, attraction of osteoclast precursors to the bone surface may be determined by the specific content of the bone to be resorbed; this is supported by studies of ageing bone. As bone ages, collagen-I is isomerized, and aged bone, which has a higher ratio of α/β collagen isomers, supports the formation of many more osteoclasts in vitro than younger bone [141], supporting a role for matrix constituents, deposited by osteoblasts, in the control of osteoclast formation.

Production of a range of chemokines (including stromal-derived factor-1 (SDF-1/CXCL12); chemokine-ligands 3, 5, and 7 (CCL3, CCL5, CCL7) [142]; chemokine (C-X-C motif) ligand 1 (CXCL1) [143]; and monocyte chemoattractant protein-1 (MCP-1) [144]) by osteoblast-lineage cells is stimulated by osteoclastogenic factors including the cytokines interleukin-1β (IL-1β), tumor necrosis factor α (TNF-α), and PTHrP. Such factors have been shown in vitro to act on osteoclast precursors (monocyte macrophages) to stimulate their chemotaxis and fusion [143, 145, 146], and it is likely that they have similar roles in vivo.

Preparing the Bone Surface for Osteoclast Attachment and Resorption

To commence resorption, the multinucleated osteoclast attaches to the bone matrix via the interaction of integrins with arginine-glycine-aspartic acid (RGD) sequences in non-collagenous matrix proteins including osteopontin and bone sialoprotein [147]. These proteins were laid down by osteoblasts during the previous cycle of bone formation. So, at some distance, it could be said that osteoblasts regulate osteoclast attachment by their control of the bone matrix itself. Intriguingly, mice lacking bone sialoprotein or osteopontin demonstrate, respectively, reduced osteoclast surface and reduced response to osteoclastogenic stimuli [148, 149]. However, this appears to be an indirect result of the reduced osteoblast numbers (and therefore reduced osteoblast-derived RANKL and M-CSF), or a requirement for intracellular osteoclastic osteopontin [150], rather than it relating to attachment to the bone matrix. Further work is required to determine how the bone matrix itself regulates osteoclast attachment; however, it should be noted that this is unlikely to be a method that precisely controls bone resorption, given the time delay between bone formation and subsequent resorption; more likely it is a mechanism that may exist in different types of bone that are responsible for biological variation in the level of bone resorption.

Concluding Remarks

The osteoblast lineage includes a range of cell types: multipotent precursors, matrix-producing osteoblasts, osteocytes, and bone lining cells; each of these stages of the lineage has distinct functions which we are only beginning to fully

understand. The most well-known role of the osteoblast lineage is the production of bone matrix and the control of its mineralization by non-collagenous proteins. The osteoblast lineage controls both the progression of differentiation of its own lineage and the formation of osteoclasts, the cells that resorb bone. As such the lineage is central to the control of bone mass, both by forming it and by controlling its destruction.

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Basic Aspects of Osteoclast Differentiation and Function

2

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Key Points

- Osteoclasts are the only cell type known to resorb bone, and their activity is essential for normal skeletal development and remodeling to repair skeletal microdamage.
- Osteoclast differentiation from myeloid precursors requires two key cytokines, MCSF and RANKL, as well as a second signal that is initiated by activation of an ITAM-associated receptor. During differentiation, osteoclast precursors fuse through a poorly understood mechanism to form mature multinucleated osteoclasts.
- Osteoclasts form a tight connection to bone, termed the sealing zone, and secrete acid and degradative enzymes through a specialized membrane-rich ruffled border into the resorption lacunae.
- Increased osteoclast activity in states of estrogen deficiency or inflammation contribute to osteoporosis. In contrast, genetic mutations, which impair osteoclast formation or activity, result in diseases such as osteopetrosis and pycnodysostosis.

Osteoclasts in the Bone Landscape

Osteoclasts are highly specialized hematopoietic cells that reside on and resorb the bone surface. Osteoclasts have some similarities to macrophages in their shared myeloid lineage and in that they are also functionally specialized for "digestion." In contrast to macrophages, osteoclasts do not phagocytose but rather exert their digestive function outside the cell through a process called lysosomal exocytosis, in which the lysosome fuses with the plasma membrane and releases its content in the extracellular space. A further distinguishing characteristic of osteoclasts is that they are multinuclear, forming from fusion of

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mononuclear precursors. Normal skeletal development and remodeling require the action of osteoclasts, which are the only cells definitively shown to resorb bone. Balance between osteoclast and osteoblast activity is critical to maintain the skeleton and either over- or underactive osteoclast function can result in skeletal disease. The classic and most common disease of excess osteoclast activity is osteoporosis. As such, it is helpful for clinicians treating osteoporosis or other bone diseases to understand where osteoclasts come from, the stimuli that drive their differentiation, and the mechanism by which they resorb bone.

Osteoclasts differentiate from myeloid precursors in the presence of the key osteoclastogenic cytokine, RANKL (receptor activator of NF-κB ligand) and survival factor MCSF (macrophage colony-stimulating factor). As with many hematopoietic cells, differentiation requires a second signal, in this case provided by any one of several immunoglobulin receptors that signal through an associated immunoreceptor-based activation motif, or ITAM-containing adapter. A number of signaling pathways are activated downstream of stimulation of RANK, the receptor for RANKL, and ITAM-associated receptors, which converge to drive expression of the transcription factor NFATc1 (nuclear factor of activated T cells), the master regulator of osteoclastogenesis. NFATc1, in conjunction with the transcription factor AP-1, drives expression of a number of molecules that are required for osteoclast resorptive function, such as the protease cathepsin K and tartrate-resistant acid phosphatase (TRAP). The history of the discovery of osteoclasts as cells of the myeloid lineage and the identification of specific osteoclast precursors is discussed in detail in section "Cellular Origins of Osteoclasts." The events of osteoclast differentiation and fusion, including an extensive discussion of the receptors, ligands, signaling pathways, and transcription factors involved, are covered in section "Osteoclast Differentiation".

Osteoclasts do not function in isolation, but rather work in proximity with osteoblasts and osteocytes in what is termed the bone multicellular unit (BMU), diagrammed in Fig. 2.1. Within the BMU, osteoclasts and osteoblasts work in series to remodel bone. In the activation phase, remodeling can be stimulated by mechanical stress, microfractures, microischemic, or other events which release factors "trapped" in the bone microenvironment including TGFβ and IGF-1 [1]. These factors activate lining osteoblasts which can then recruit migratory mature osteoclasts as well as drive maturation of osteoclast precursors through the expression of RANK. Mature osteoclasts undergo cytoskeletal rearrangement, becoming highly polarized and form a specialized structure called the sealing zone which isolates the space between the

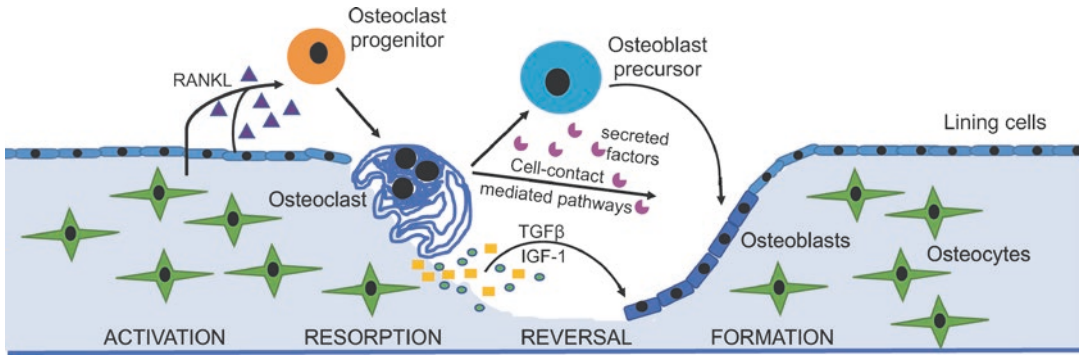

Fig. 2.1 Osteoclasts in the bone multicellular unit. Osteoclasts and osteoblasts work in series to remodel the bone in the bone multicellular unit (BMU). The process of remodeling consists of four sequential and distinct phases of cellular events depicted above: activation, resorption, reversal, and formation. The coupling of osteoclasts to osteoblasts is mediated by the liberation of growth factors by process of resorption, cell contact-mediated pathways, and secreted factors produced by osteoclasts

osteoclast and underlying bone from the surrounding environment. Acidification and exocytosis of hydrolases, including the protease cathepsin K, into this space results in dissolution of the mineral and digestion of the organic matrix of bone. The process of bone resorption is reviewed in section "Functions of Osteoclasts".

In the reversal phase, the BMU switches from resorption to formation in what is termed the reversal phase. During this phase, digestion of bone by osteoclasts releases other factors trapped in the bone matrix, including BMPs, TGFβ, and IGF-1, which are thought to stimulate osteoblasts to form bone [2, 3]. Osteoclasts also modulate osteoblast function both through cell contact-mediated interactions and secreted factors, a regulatory function of osteoclasts discussed in section "Functions of Osteoclasts". In the formation phase, mature osteoblasts deposit osteoid, demineralized bone matrix, followed by deposition of hydroxyapatite to generate mineralized bone (see Chap. 5).

The importance of osteoclast function for bone health is underscored by the variety of genetic diseases that map to the osteoclast, covered in section "Genetic Diseases of Osteoclast Dysfunction". Excessive osteoclast activity also contributes to bone pathology in post-menopausal osteoporosis and inflammatory arthritis, among other conditions. In these settings, osteoclast differentiation and activity are modulated both directly by a variety of cytokines and indirectly by enhanced RANKL expression. The influence of microenvironment on osteoclasts is explored in section "Regulation of Osteoclasts By Their Environment". Overall, this chapter attempts to provide a broad review of the cellular and molecular aspects of osteoclast differentiation and function.

Cellular Origins of Osteoclasts

A distinct multinucleated cell type associated with bone was reported as early as 1849, though the first use of the term osteoclast was not until 1873 [4, 5]. It was not until the 1960s, however, that it was conclusively demonstrated that osteoclasts resorb bone [6–8]. Osteoclasts were proposed to be derived from leukocytes as early as 1911, based on their morphologic similarity to foreign body giant cells [9]. A series of elegant parabiosis and chimera experiments performed by Walker in the 1970s conclusively demonstrated the hematopoietic origin of osteoclasts [10–13]. A myeloid origin for osteoclasts was proposed early on because of the morphologic and functional similarities with macrophages and giant cells and confirmed by experiments in which labeled peripheral blood monocytes injected into mice resulted in generation of labeled osteoclasts [14].

Osteoclast progenitors have subsequently been more precisely defined though the in vitro assessment of the ability of various subsets of bone marrow or peripheral blood cells to differentiate into osteoclasts in the presence of RANKL. Each of these studies have used a variety of myeloid cell surface markers to define the osteoclast progenitor. Arai and colleagues performed the seminal studies in this area, demonstrating that the bone marrow $CD11b^{lo}$ $CD117^{+}$ (c-Kit) population contained precursors that could differentiate into osteoclasts in the presence of MCSF and RANKL [15]. Several groups have identified early myeloid progenitor populations in the bone marrow that are highly enriched for osteoclast progenitors and are distinct from the progenitors for monocytes and dendritic cells [16–19]. Peripheral blood monocytes from both mice and humans can differentiate into osteoclasts in the presence of MCSF and RANKL. Using purification based on cell surface markers in conjunction with in vitro osteoclast differentiation assays, a population of peripheral osteoclast progenitors sharing many features of classical circulating monocytes was identified in mice [17]. Circulating osteoclast progenitor populations in humans have similarly been identified as having markers overlapping with classical monocytes [20, 21]. Although both bone marrow and circulating progenitor populations efficiently differentiate into osteoclasts, the relationship between these progenitor pools and relative contribution of these progenitors to maintaining osteoclast formation is unknown.

Osteoclast Differentiation

Overview

Osteoclasts represent a terminally differentiated cell in the myeloid lineage. Similar to other differentiated myeloid cells, key cytokine stimuli are required to activate specific intracellular signaling pathways to initiate specific transcriptional programs. The master transcriptional regulator of osteoclasts, the transcription factor NFATc1, is essential for osteoclast differentiation and function [22]. The process of osteoclast differentiation from immature myeloid precursor cells is highly regulated by both positive and negative stimuli emanating from surrounding bone and immune cells. These signals orchestrate a coordinated signaling cascade that initiates precursor proliferation, fusion to multinucleated cells, cellular polarization, adherence to bone, and activation of functional resorption (Fig. 2.2).

Receptors

RANK/RANKL Signaling Is Essential for Osteoclast Differentiation

The key cytokine required to stimulate osteoclast differentiation is RANKL[23, 24] which was originally described under several names including OPGL (osteoprotegerin ligand) [25], ODF (osteoclast differentiation factor) [26] and TRANCE (TNF-related activation-induced cytokine) [27]. RANKL is in the TNF (tumor necrosis factor) cytokine family (TNFSF11) and is produced by osteoblasts, stromal cells, osteocytes, and activated immune cells as both a type II transmembrane protein and a secreted cytokine. RANKL binds to RANK (receptor activator of NF-κB) on myeloid cell precursors and serves as the key stimulus for osteoclast differentiation and activation. Studies of mice genetically deficient in RANK or RANKL demonstrated that in the absence of RANK signals, no osteoclasts are

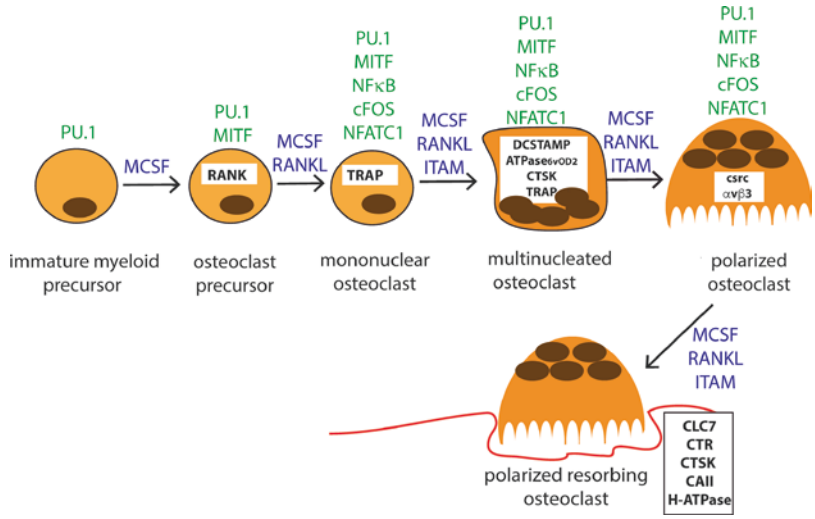

Fig. 2.2 Osteoclast differentiation. Osteoclasts develop from immature myeloid precursors. When stimulated by MCSF, they upregulate RANK, and then under the stimulation of MCSF and RANKL, they initially form mononuclear osteoclasts that fuse into multinucleated cells. The multinucleated osteoclasts polarize and adhere to bone and become functionally bone resorbing through secretion of metalloproteinases, acid, and cathepsin K (white box lower right shows osteoclast-specific genes). The figure shows in blue: Stimuli required to progress in osteoclast differentiation, in green: key transcription factors upregulated at each stage during osteoclast differentiation, in boxes within the cell: key osteoclast genes upregulated at each stage. Abbreviations: MCSF macrophage colony-stimulating factor, RANK receptor-activating NFkB, MITF microphthalmia-associated transcription factor, DC-STAMP dendritic cell-specific transmembrane protein, ECM extracellular matrix, GM-CSF, CTSK cathepsin K, TRAP tartrate resistant acid phosphatase, ITAM immunoreceptor tyrosine-based activating motif, CLC7 voltage-gated chloride channel 7, CTR calcitonin receptor, CTSK cathepsin K, CAII carbonic anhydrase II

generated. Mice genetically deficient in RANK or RANKL have bones that are severely osteopetrotic, and the animals are toothless due to their inability to erupt teeth in the absence of osteoclastic degradation of the mandible [28–31]. RANKL also binds to a soluble decoy receptor OPG (osteoprotegerin or "bone protector") which serves to prevent RANKL from interacting with RANK. Mice deficient in OPG are osteoporotic, and transgenic mice that overexpress OPG have few osteoclasts and are severely osteopetrotic [32–36]. The ratio of RANKL and OPG expression in the vicinity of osteoclast precursors is therefore important in determining the osteoclast differentiation response. Expression of RANKL and OPG is both highly regulated, and their production by osteoblasts/stromal cells is regulated by endocrine factors such as PTH and $1,25(OH)_2D_3$ and inflammatory cytokines such as TNF and IL-1 [37]. Many cytokines, hormones, and growth factors regulate osteoclastogenesis indirectly, through regulation of RANKL and/or OPG expression on other cell types (Table 2.1). RANKL stimulation is required for osteoclastogenesis but is also required to activate functional resorption by mature osteoclasts, while lack of RANKL stimulation impairs osteoclast survival [37]. Given this critical role in osteoclast generation and function, RANKL was identified as an ideal therapeutic target. Denosumab (see Chap. 17) is an anti-RANKL antibody currently FDA approved for a number of indications, including treatment of postmenopausal women and men with osteoporosis at high risk for fracture, bone loss during cancer hormone ablation therapy, glucocorticoid-induced osteoporosis, and skeletal lesions in patients with bone metastases from solid tumors and giant cell tumors of the bone [38–40].

RANKL and OPG are expressed by osteoblasts, stromal cells, and osteocytes; however, the relative importance of each source has only recently been redefined. Osteoblasts lining the bone surface were previously thought to be the primary source of RANKL during osteoclastogenesis. However, osteocytes, the cells residing deep within the bone, were found to express high levels of RANKL, and osteocyte-derived RANKL can reach the bone surface through osteocyte canaliculi to interact with precursor cells and

Table 2.1 Modulators of RANKL and OPG expression

	RANKL	OPG	References
PTH	Increased	Decreased	[173, 174]
PTHrP	Increased	Decreased	[175, 176]
1,25(OH)2 Vitamin D3	Increased	Decreased	[177, 178]
Wnts	Decreased	Increased	[179]
Estradiol	No change	Increased	[180]
Glucocorticoid	Increased	Decreased	[181]
Prostaglandin E2	Increased	Decreased	[182]
VEGF	No change	Decreased	[183]
IGF-1	Increased	Decreased	[184]
PDGF receptor ββ inhibitors (imatinib nilotinib)	Decreased	Increased	[185]
Oncostatin M	Increased	Increased	[178]
IL-1	Increased	Increased	[177, 181]
IL-6	Increased	Increased	[178]
IL-11	Increased	No change	[186, 187]
IL-17	Increased	Decreased	[186]
IL-18	Increased	Decreased	[188]
IFNγ	Increased	Increased	[186]
TNF	Increased	Increased	[188, 177, 181]
TGFβ	Decreased	Increased	[189, 190]
CD40L	Increased	Not tested	[35]
BMP-2	Not tested	Increased	[177]

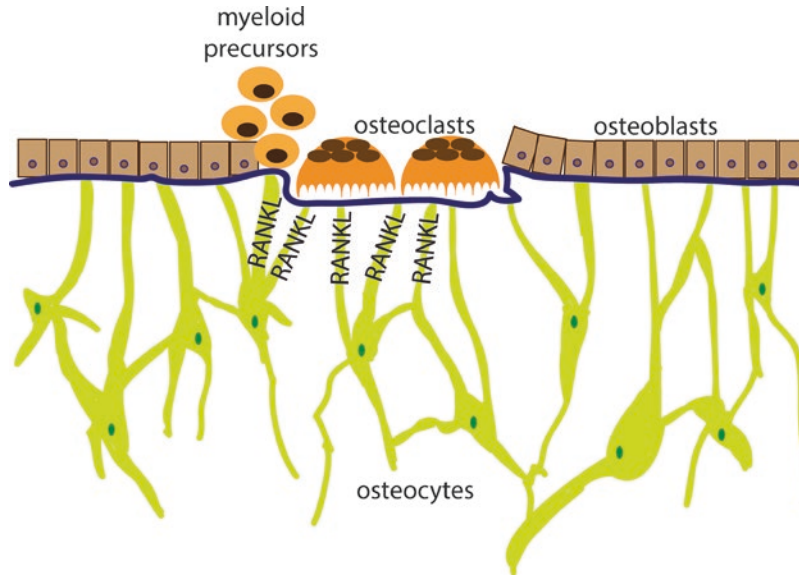

Fig. 2.3 Osteocytes secrete key regulator of osteoclasts. Osteoclasts differentiate under the stimulation of MCSF and RANKL. While a number of cell types produce these cytokines, including osteoblasts, stromal cells, and T cells, the cell type responsible for RANKL production important in maintaining bone homeostasis is the osteocyte. Osteocytes are highly differentiated osteoblasts imbedded in the bony matrix. Shown in the bone remodeling unit in which cells are connected to each other and the cell surface through a canalicular network that allows osteocyte cells to interact with cells at the surface of bone. Using mice deficient in RANKL only in osteocytes, it was shown that osteocytes supply RANK ligand for osteoclastogensis in both homeostatic and pathologic conditions such as low-calcium diet and estrogen deficiency

stimulate differentiation (Fig. 2.3) [41]. An osteocyte-specific deletion of RANKL leads to a significant osteopetrotic bone phenotype in mice, demonstrating the importance of osteocyte-derived RANKL for basal bone remodeling [42–44]. Osteocytes also express OPG, which can diffuse through the lacuno-canalicular system to downregulate osteoclastogenesis. Under pathologic conditions such as mechanical unloading or "weightlessness," osteocytes increase production of sclerostin, a Wnt inhibitor, which leads to decreased OPG and increased RANKL production to stimulate osteoclastogenesis [45, 46]. Osteocyte-derived RANKL has also been shown to be critical for the increased osteoclast formation and bone loss due to a low-calcium diet [47] and estrogen deficiency [48]; thus osteocytes are a critical source of RANKL in a variety of homeostatic and pathologic states [41]. RANKL is produced as a membrane-bound protein on the cell surface that is cleaved at the surface by enzymes (such as matrix metalloproteinase 14) to generate a soluble form. The relative importance of membrane and soluble RANKL was examined using genetically modified mice that produced a form of RANKL that could not be cleaved. The lack of soluble RANKL in adult mice led to increased cancellous bone mass and decreased osteoclast numbers, suggesting that soluble RANKL is in important ongoing osteoclast formation. However, lack of soluble RANKL did not affect bone mass in developing mice or bone loss due to estrogen deficiency suggesting that membrane RANKL is sufficient for osteoclastogenesis under other conditions [49].

Second Signals: Co-stimulatory Receptors in Osteoclast Differentiation

Similar to other immune cells, osteoclasts require simultaneous stimulation through multiple receptor signals to initiate the cellular differentiation program (Fig. 2.4). While signaling through the RANK receptor is the key specific osteoclastogenic signal, a critical co-stimulatory signal is

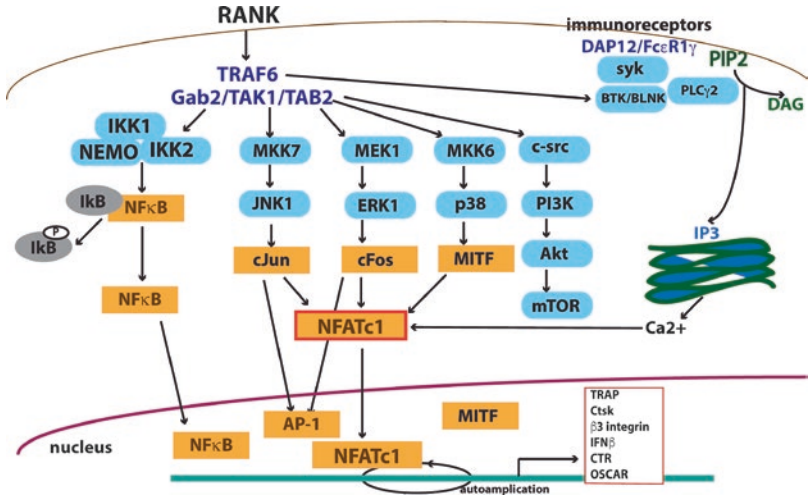

Fig. 2.4 RANK signaling interactions. RANK stimulation leads to binding of TRAF6 which forms a central scaffold with Gab2/TAK1/TAB2 and subsequent activation of a number of pathways including NFκB and several MAPK intracellular signaling cascades (JNK1, p38, ERK1, PI3K) and interaction with the immunoreceptor ITAM signaling pathway. In the figure receptors are shown in black, adapter proteins in blue, enzyme intermediates in signaling cascade in blue boxes, and activation of transcription factors in orange boxes, with the master regulator of osteoclastogenesis NFATc1 in the orange box outlined in red. Cooperation with the ITAM signaling pathway is shown on the right, where the interaction provides the intracellular calcium flux needed for NFATc1 translocation. Osteoclast-specific genes downstream from NFATc1 are shown in the white box lower right

directed by innate receptors that utilize ITAM (immunoreceptor tyrosine-based activation motif) signaling adapters, DAP12 (DNAX-associated protein 12kD size), and FcRγ (FcεR1γ chain) [50, 51]. The ITAM motif was initially recognized as a common sequence in the cytoplasmic tails of the signaling chains associated with the T cell receptor and B cell receptor but has since been identified in a number of receptor-associated cytoplasmic domains, where it is used to link receptor activation to downstream signaling cascades. The ITAM adapter chains transduce signals from a variety of ligand-binding immunoreceptors on osteoclasts. Signaling through ITAM adapter chains in osteoclast precursors initiates the calcium flux that leads to the activation of NFATc1, the master transcriptional regulator required for osteoclastogenesis [52]. Innate immunoreceptors generally function to activate cells in response to local microenvironmental change, and it is likely that the combined input of a number of coreceptors on osteoclast precursors fine-tunes osteoclast differentiation and functional response. Each of the ITAM signaling chains pairs with specific immunoreceptors, with the best known pairs being TREM2-DAP12 and OSCAR-FcRγ [53]. Ligands that stimulate these receptors in the bone microenvironment are not well defined, though potential ligands include collagen fragments for OSCAR and apoptotic cells for TREM2 53].

Mice deficient in both of the ITAM adapter chains, DAP12, and FcRγ are severely osteopetrotic with no osteoclasts in the long bones [50, 51]. However, these mice are distinct from RANK- or RANKL-deficient mice, in that mice deficient in both DAP12 and FcRγ have teeth, because they can develop osteoclasts in the jaw needed for tooth eruption [51]. Surprisingly, despite the lack of osteoclasts in the long bones under basal conditions, following a bone-remodeling stimulus such as estrogen deficiency, $DAP12^{-/-}$/ $FcR\gamma^{-/-}$ mice lose significant amounts of bone and are able to generate osteoclasts in vivo [54]. These studies suggest that the requirement for specific coreceptors can be bypassed under specific microenvironmental conditions, either due to the usage of additional

coreceptors or alterations in other regulatory signals.

One additional signal comes through the MCSF receptor (CSF-1R or cFms), a tyrosine kinase-based growth factor receptor that is required for osteoclastogenesis. The identification of a mutation in the coding region of MCSF (also known as CSF-1) in the osteopetrotic *op/op* mice demonstrated the essential nature of MCSF receptor signals for osteoclast development [55, 56]. MCSF stimulation promotes the proliferation, survival, and differentiation of a number of myeloid cells and is similarly important during osteoclastogenesis. MCSF is produced by osteoblasts, stromal cells, and osteocytes, similar to RANKL. In osteoclasts, MCSF also stimulates cytoskeletal organization, cellular spreading, and migration [56].

Osteoclasts also interact with their surroundings through cell surface receptors, which is important for differentiation of osteoclasts to a polarized, bone degrading cell. Osteoclast-expressed integrins interact with bone matrix through $\alpha_v\beta_3$ binding to RGD peptides in the extracellular matrix. This interaction polarizes the osteoclast cell and initiates actin ring formation, creating the characteristic ruffled border [57–59]. The osteoclast forms an external phagolysosome adherent to the bone at the actin ring which organizes the sealing zone underneath the osteoclast where enzymatic and acidic bone degradation can take place [58–60]. Mice deficient in the β_3 integrin subunit cannot efficiently organize their cytoskeleton for resorption and have an osteopetrotic phenotype with hypocalcemia [61]. Matrix interaction with the $\alpha_v\beta_3$ integrin induces phosphorylation of DAP12 and formation of an ITAM/Syk/Src/$\alpha_v\beta_3$ signaling complex [57, 62]. The importance of these interactions for osteoclast function is seen in β_3/DAP12 double-deficient ($DAP12^{-/-}\beta3^{-/-}$) mice that are profoundly osteopetrotic, reflecting a severe degree of osteoclast dysfunction, which is not seen in mice lacking either $\alpha_v\beta_3$ or DAP12 alone [63]. These examples suggest that multiple receptor inputs are also required to fully activate osteoclast adherence and functional bone resorption, mimicking the need for the multiple co-stimulatory signals required in the early stages of osteoclastogenesis. Functional activation of osteoclasts is therefore a final step in the process of specialized cellular differentiation to form mature terminally differentiated osteoclasts.

Fusion and the Formation of Multinucleated Osteoclasts

One of the most unique and distinctive properties of osteoclasts is multinucleation. Multinucleation has typically thought to be a requirement for resorptive activity in higher vertebrates, although some fish species have mononuclear osteoclasts. As early as the 1980s, it was appreciated that this multinucleation occurred through fusion of mononuclear cells rather than by endoreplication [14, 64]. The precise mechanism by which homotypic membrane fusion of osteoclast precursors occurs is not known, though a number of proteins important for osteoclast fusion have been identified.

Three cell surface molecules induced by RANKL are strongly implicated in osteoclast fusion. These molecules are the multi-pass transmembrane proteins known as DC-STAMP and OC-STAMP (dendritic cell- and osteoclast- specific transmembrane protein) and ATP6V0d2 (ATPase, H+ transporting, lysosomal 38 kDa, V0 subunit d2). DC-STAMP and OC-STAMP are required for multinucleation of osteoclasts [65–69]. Only one cell in a cell-cell fusion needs to express STAMPs, as wild-type monocytes can fuse with STAMP-deficient monocytes. Loss of DC-STAMP results in mononuclear osteoclasts and also defective resorptive function, leading to increased trabecular bone [68]. Loss of OC-STAMP also results in mononuclear osteoclasts with diminished resorptive activity in vitro, but OC-STAMP-deficient mononuclear osteoclasts appear to function adequately in vivo as the mice have no bone phenotype [67, 70]. ATP6V0d2, a subunit of the V-ATPase complex essential for extracellular acidification, is also essential for osteoclast fusion. As with DC-STAMP, *Atp6v0d2*$^{-/-}$ mice have increased bone mass [71].

A number of additional molecules have been implicated as regulators of osteoclast fusion,

though none are essential for fusion, and mice lacking these molecules have modest or no bone phenotype. These molecules include CD47 and SIRPα (signal regulatory protein alpha), tetraspanins, CD44, and ADAM8 (a disintegrin and metalloprotease 8) [72]. Although identification of molecules involved in fusion of mononuclear precursors to a mature, multinucleated osteoclast has provided insight into the requirements for fusion, we have little mechanistic insight into the fusion process, and much remains to be learned.

Downstream Events: Signaling Cascades and Transcriptional Activation

Osteoclastogenesis requires the activation of a number of transcription factors to induce the transcriptional program that defines the osteoclast, including expression of TRAP, integrin β_3, cathepsin K, matrix metalloprotease 9, and calcitonin receptor [73]. RANKL stimulation leads to the upregulation and activation of NFATc1, the master regulator of osteoclast differentiation [22], through activation of a number of signaling pathways, including the canonical NF-κB and AP-1 pathways and facilitation of calcium signaling by ITAM-associated receptors. The complexity of RANK-induced signaling is outlined in Fig. 2.4. While significant advances have been detailed by numerous studies, these complex interactions remain incompletely understood, and new key signaling factors are still being described [73]. The delineation of intracellular signaling during osteoclastogenesis has been a topic of considerable interest given that identification of critical signaling intermediates may suggest new therapeutic targets to block bone loss and will also further our understanding of how these pathways are dysregulated by medications or pathologic or inflammatory disease states.

Signaling Cascades in Osteoclast Differentiation

RANKL interaction with the RANK receptor initiates a signaling cascade beginning with the binding of the adapter molecule TRAF6, which forms scaffolds that lead to activation of JNK, p38, and NF-κB [73, 74] (Fig. 2.4). While there are multiple TRAF adapters, the key role for TRAF6 in osteoclastogenesis was shown when the TRAF6-deficient mouse was found to develop severe osteopetrosis with impaired osteoclast differentiation and bone resorption [75].

RANK/TRAF6 signaling recruits IKK-α (IκB kinase alpha) and IKKβ- (IκB kinase beta), also known as IKK1 and 2, an upstream enzyme complex in the NF-κB signaling cascade. The α- and β-subunits together are catalytically active as a serine-threonine kinase and are modified by IKK3/IKK-β or NEMO (NF-κB essential modifier of NF-κB kinase), a subunit of the IKK complex that serves a regulatory function. Activation of the IKK complex leads to binding of NEMO to IKK-α and IKK-β with subsequent serine phosphorylation of IκB, which binds NF-κB and retains it in the cytoplasm [76]. Phosphorylation of IκB leads to its ubiquitination and degradation by the proteasome, releasing NF-κB and allowing its translocation to the nucleus where it initiates gene transcription. Mice lacking NF-κB subunits develop osteopetrosis due to a severe defect in osteoclast differentiation [77]. In the NF-κB-null mice, development of macrophages and osteoclast precursors is preserved, suggesting that NF-κB is not essential during early osteoclast differentiation [78, 79]. Gene targeting studies have demonstrated that different transcription factors are required at different stages of osteoclast differentiation and therefore differentially affect other myeloid lineages (Fig. 2.2) [73].

TRAF6 also links RANK to multiple MAPK (mitogen-activated protein kinase) pathways: ERK, JNK and p38, through formation of complexes with TAK1 (TGF-β-activated kinase), TAB1 and TAB2 (TAK-1-binding proteins 1 and 2) [73]. Ablation of TAK1 in myeloid cells results in defective osteoclastogenesis and development of osteopetrosis in mice [80]. Interestingly, TAK1 deficiency alters signaling through NF-κB, p38 MAPK, and Smad1/5/8 and has been shown to alter expression of multiple transcription factors, including PU.1, MITF, c-Fos, and NFATc1, suggesting that TAK1 acts as a regulator at multiple points during osteoclast differentiation [80].

RANK stimulation of MAPK activation leads to activation of downstream targets of ERK, JNK, and p38 in osteoclast precursors, which include c-Fos, AP-1 transcription factors, and MITF, respectively [73]. AP-1 (activator protein-1), which is composed of a protein complex of Fos (c-Fos, FosB, Fra-1 and Fra-2) and Jun (c-Jun, JunB, and JunD) proteins, is critical during osteoclastogenesis, because genetic deletion of c-Fos also abrogates osteoclastogenesis resulting in osteopetrosis [81]. Interestingly, cFos-deficient animals have increased macrophages; thus AP-1 regulation of osteoclast and macrophage differentiation is in opposing directions [81]. Transgenic mice expressing dominant negative c-Jun in the osteoclast lineage also demonstrate severe osteopetrosis with defective osteoclastogenesis [81]. The role of p38 MAPK is more complex as, although p38-deficient cells have defective osteoclastogenesis and p38 MAPK inhibitors can inhibit in vitro osteoclastogenesis, p38 MAPK deficiency in monocytes led to only a minor increase in bone mass in young animals, while older animals developed osteoporosis and an increase in osteoclastogenesis. The absence of p38 led to increased monocyte proliferation and increased size of the osteoclast progenitor pool in the aged mice, demonstrating a complex role for p38 that varies with age [82, 83]. ERK1 positively regulates osteoclast development and bone resorption, and genetic deletion of ERK1 in hematopoietic cells resulted in reduced osteoclast progenitor cell number, decreased osteoclast function with defective pit formation, and diminished MCSF-mediated adhesion and migration [84].

RANK also activates the PI3K (phosphoinositide 3-kinase)/AKT pathway. PI3K activation leads to the production of phosphatidylinositol-(3,4,5)-phosphate (PIP3) at the plasma membrane, where it recruits AKT. The critical nature of PI3K/AKT for osteoclasts was demonstrated by deletion of the p85 regulatory subunit of the Class IA PI3K, which results in an osteopetrotic phenotype caused by a defect in osteoclast resorption of bone. Class IA PI3K was found to be required to initiate ruffled border formation and vesicular transport, but not for the formation of the sealing zone [85]. p85α/β doubly deficient osteoclasts showed defective AKT activation and loss of resorption, which could be recovered by expression of activated AKT. Simultaneous blockage of both AKT and MEK1/2 causes rapid apoptosis of nearly all osteoclasts, which suggests a role for PI3K in osteoclast survival. In keeping with this finding, PI3K inhibitors can also lead to rapid osteoclast apoptosis [86].

Activation of PI3K/AKT by RANK is modulated by Src kinase activity, thus integrating RANK and ITAM-associated receptor signaling. This collaborative activation of PI3K/AKT is demonstrated by the loss of RANKL-mediated AKT activation in cells genetically deficient for c-Src. PI3K is also activated downstream of $\alpha_v\beta_3$ integrin and CSF-1 receptor, which may be of importance in regulation of osteoclast function [73]. AKT activation requires PIP3 production, which is negatively regulated by PTEN (phosphatase and tensin homolog) and SHIP1 (SH2-containing inositol phosphatase 1). As would be predicted, both PTEN and SHIP1 negatively regulate osteoclast differentiation, with deficiency of either SHIP1 or PTEN, leading to increased osteoclastogenesis and severe osteoporosis in mice [87, 88].

Transcription Factors in Osteoclast Differentiation

The transcription factor PU.1, an ETS-domain transcription factor, is expressed at all stages of osteoclast differentiation but plays a critical role early in osteoclastogenesis and is essential for development of all myeloid lineage cells. In osteoclast precursors, PU.1 regulates expression of the CSF-1 receptor and RANK which are required for osteoclastogenesis. Consistent with this, PU.1 deletion in mice causes osteopetrosis and lack of both osteoclasts and macrophages [89, 90]. PU.1 also cooperatively regulates gene transcription with other key osteoclastogenic transcription factors MITF and NFATc1 and thus plays a role in later osteoclast differentiation as well [89]. MITF plays a later role in osteoclast differentiation, around the time of precursor cell fusion to multinucleated cells. Mutations in MITF lead to osteopetrosis with formation of only mononuclear osteoclasts that are defective

in bone resorption with a lack of ruffled border formation on bone [91–94].

NFATc1 was termed the master switch for regulating the terminal differentiation of osteoclasts because ectopic expression of NFATc1 in precursor cells led to efficient differentiation to osteoclasts in the absence of RANKL signaling [22]. NFATc1-deficient embryonic stem cells also failed to differentiate into OCs in response to RANKL stimulation; thus the expression of NFATc1 was both necessary and sufficient to drive osteoclastogenesis [22]. NFAT transcription factors are regulated primarily by intracellular calcium signaling. Signals through the ITAM adapters initiate calcium signaling that is required in the basal state to drive osteoclastogenesis and NFATc1 activation [50, 51]. In osteoclast precursors, stimulation of ITAM-associated receptors leads to phosphorylation of the tyrosine residues in the ITAM motif through the action of Src family kinases. The activated ITAM motif then recruits the tyrosine kinase Syk which initiates a signaling cascade involving the intermediates BTK/Tec, BLNK (B cell linker)/SLP76 and phospholipase C-γ2 [51, 52, 95. PLCγ2 is activated through phosphorylation which increases its catalytic function to hydrolyze phosphatidylinositol-4,5 bisphosphate into inositol-1,4,5-triphosphate (IP3) and diacylglycerol. IP3 then activates receptors on the endoplasmic reticulum to stimulate Ca^{2+} release from the endoplasmic reticulum to the cytoplasm [52, 96]. The increase in cytoplasmic Ca^{2+} activates calcineurin, a cytoplasmic phosphatase that dephosphorylates NFATc1, allowing it to translocate to the nucleus to initiate and regulate gene transcription. Consistent with this, calcineurin inhibitors such as FK506 and cyclosporin A strongly inhibit osteoclastogenesis [52]. NFATc1 also autoamplifies its own gene, possibly by binding to its own promoter, and associates with AP-1 to initiate gene transcription of essential osteoclast genes such as TRAP, calcitonin receptor, cathepsin K, and β3 integrin [97].

The transcription factor c-MYC is strongly upregulated by RANKL stimulation and promotes osteoclastogenesis in vitro. Recent studies examining the role of MYC have highlighted the role of cellular metabolism in osteoclastogenesis [98]. MYC has been shown to function to drive metabolic reprogramming during osteoclast differentiation, and switching cellular metabolism to an oxidative state enhances both osteoclastogenesis and function [98]. Osteoclasts contain abundant mitochondria and undergo metabolic adaptation during the course of differentiation to meet the bioenergetic demands required for functional resorption of bone. PGC-1β (PPARγ coactivator-1β) is induced during osteoclast differentiation by CREB via reactive oxygen species (ROS) and also stimulates mitochondrial biogenesis [99]. During this switch MYC induces estrogen receptor-related receptor α (ERRα), a nuclear receptor that cooperates NFATc1 to drive osteoclastogenesis [98]. While a complex array of transcriptional activators must be engaged through RANK/RANKL stimulation to drive osteoclast differentiation, an important additional function of RANK stimulation on osteoclast precursors is to downregulate expression of transcriptional repressors to enable osteoclastogenesis to take place [100].

Negative Regulators of Osteoclast Differentiation

A host of negative regulatory mechanisms exist to ensure that osteoclasts are generated only in the correct time and place. Downregulation of transcriptional repressors during RANK stimulation is required for osteoclastogenesis to proceed. Repressors of gene transcription that are downregulated during osteoclastogenesis include Ids (inhibitors of differentiation/DNA binding), Eos, MafB (v-maf musculoaponeurotic fibrosarcoma oncogene family protein B), C/EBPβ (CCAAT-enhancer-binding protein β), IRF-8 (interferon regulatory factor 8), and Bcl-6 (B cell lymphoma 6) [100, 101]. The negative regulatory transcription factors also inhibit osteoclastogenesis at specific points during differentatiation; Ids, IRF-8, and MafB are inhbitiory during early osteoclastogenesis (within 24 h after RANKL stimulation), while Eos and Bcl6 expression are inhibitory at later time points during osteoclast development.

MafB expression is downregulated following RANKL stimulation during osteoclastogenesis and MafB has since been shown to negatively regulate osteoclast formation. MafB is a basic leucine zipper transcription factor that plays an important role in the regulation of lineage-specific hematopoiesis, and overexpression of MafB inhibits the formation of $TRAP^+$ multinuclear osteoclasts. In osteoclasts, MafB abrogates NFATc1 expression and interferes with the DNA binding of cFos, Mitf, and NFATc1 transcription factors [54].

Similarly, RANKL stimulation downregulates the Ids helix-loop-helix (HLH) transcription factors encoded by the *Id1*, *Id2*, and *Id3* genes. Overexpression of the three *Id* genes negatively affects osteoclast differentiation [102] Overexpression of Eos also leads to defective osteoclast differentiation, with selective repression of transcription of MITF/PU.1 targets such as *Ctsk* (encoding cathepsin K) and *Acp5* (encoding TRAP) [103] Eos forms a complex with MITF and PU.1 at their target gene promoters and suppresses transcription through recruitment of corepressors. In myeloid progenitors prior to the initiation of osteoclast differentiation, Eos directly interacts with MITF and PU.1 to suppress transcription. Later in osteoclast differentiation, Eos association, for example, at *Ctsk* and *Acp5* promoters, decreases significantly allowing transcription to proceed.

IRF-8 is a transcription factor critical for lineage commitment in the maturation of myeloid precursors [104]. IRF-8 is expressed in macrophages derived from bone marrow and spleen, and downregulation of IRF8 is required for these cells to initiate osteoclastogenesis. IRF-8 suppresses osteoclastogenesis by inhibiting NFATc1 expression and physically interacts with NFATc1 to inhibit its function [105].

The downregulation of these negative regulators of osteoclastogenesis is in fact controlled by RANK stimulation. RANKL induces expression of Blimp1 (B lymphocyte-induced maturation protein-1) via NFATc1 during osteoclastogenesis. Blimp1 functions as a transcriptional repressor of anti-osteoclastogenic regulators such as IRF-8, MafB, and Bcl6. Overexpression of Blimp1 leads to an increase in osteoclast formation, while deficiency of Blimp1 leads to defective osteoclast differentiation. Thus, while Blimp1 is a positive regulator of osteoclastogenesis in itself, its primary function is to suppress the transcription of negative regulators. Mice with an osteoclast-specific deficiency of Blimp1 exhibit a high bone mass phenotype caused by a decreased number of osteoclasts [101]. In the absence of Blimpl1, osteoclastogenesis is impaired through increase of Irf8 and MafB and by upregulating Bcl6. Bcl6 suppresses expression of osteoclastic genes downstream of NFATc1 which includes cathepsin K, dendritic cell-specific transmembrane protein (DC-STAMP), and NFATc1 itself [106]. RANKL also induces the IFN-β (interferon-beta) gene in osteoclast precursor cells. In a negative regulatory feedback loop, IFN-β then functions to limit osteoclastogenesis by interfering with the RANKL-induced expression of c-Fos [107].

Signaling during osteoclastogenesis is also regulated by ubiquitination of specific substrates. RANK regulates the de-ubiquitinase CYLD, which inactivates TRAF6 by removal of polyubiquitin chains, resulting in inhibition of osteoclast formation. CYLD deficiency leads to severe osteoporosis and osteoclasts that are hyperresponsive to RANK stimulation [108]. NUMB/NUMB-like (NUMBL) is an intracellular adapter protein that directly interacts with TRAF6 and NEMO and induces their ubiquitination and proteasomal degradation. NUMBL has been shown to be downregulated by RANKL stimulation, and its presence inhibits osteoclast differentiation and function [109]. Downstream of RANKL, TAK1 is also important in inhibiting expression of NUMBL because the TAK1-deficient mouse showed increased NUMBL expression. The TAK1/TAB2 complex mediates the polyubiquitination of NUMBL which marks it for proteasomal degradation [80]. NUMBL has also been shown to regulate NOTCH signaling and with increased NUMBL expression in myeloid cells, there is increased degradation of NICD and subsequent accumulation of RBPJ. In other studies RBPJ has been shown to be a significant inhibitor of osteoclast differentiation [110]. Thus, NUMBL

acts as an endogenous negative regulator of NF-κB signaling in osteoclasts by targeting the TAK1/TRAF6/NEMO complex which leads to indirect negative regulation of RBPJ [109]. RBPJ negatively regulates osteoclastogenesis induced by both RANKL and TNF and may be of particular importance in inflammatory bone loss. RBPJ inhibits activation of PLCγ2 downstream from the ITAM-associated receptors and has been demonstrated to function as a negative regulator of osteoclastogenesis by suppressing induction of NFATc1, BLIMP1, and c-Fos [110].

As evident from the discussion above, osteoclast formation is a highly regulated process, requiring MCSF stimulation of CSF-1R for precursor survival, RANKL-RANK pathway stimulation and a second signal through and an ITAM-associated immunoreceptor, with further modulation by negative regulatory pathways. The ability to differentially regulate the combination and balance of these signals, as well as the potential for site and/or condition-specific ligand expression for ITAM-associated receptors, results in a highly tunable program of osteoclastogenesis. This likely allows for location- and environment-specific regulation of osteoclast formation and function.

Functions of Osteoclasts

Bone resorption is the canonical function of osteoclasts and they are the only cells capable of resorbing bone. Their resorptive function is essential for the formation of the bone marrow cavity during skeletogenesis, and they actively remodel bone throughout life, resorbing approximately 10% of skeletal bone annually by some estimates. However, it has increasingly been appreciated that osteoclasts are more than just bone resorbing cells. Osteoclasts are able to regulate other biological processes through the production of cytokines and heterocellular signaling [111]. Moreover, several lines of evidence support the idea that communication between osteoclasts and osteoblasts, referred to as coupling, is bidirectional, with osteoclasts actively promoting osteoblast function [112]. Thus, one can divide osteoclast functions into canonical bone resorptive/remodeling functions and what might be termed "regulatory functions," consisting of regulation of bone formation through coupling, autocrine regulatory pathways, and angiogenesis [113].

Bone Resorbing Function

Within bone, osteoclasts reside on the periosteal and trabecular surfaces and in Haversian canals. Osteoclasts are highly motile cells, migrating along the bone surface to resorb bone at multiple sites. The mature differentiated osteoclast, after reabsorbing bone in a specific area, is able to adopt a migratory state to move to a new site of resorption. The migratory osteoclast has a lamellipodic front to back "horizontal" migratory polarity with the majority of the cytoplasm at the leading edge. When it reaches a new resorption site, attracted by cytokines released by osteoblasts, the osteoclast changes its morphology to a static conformation that facilitates bone reabsorption [114, 115]. The hallmark of a resorbing osteoclast is the reorganization of the cytoskeleton to form a "vertical" polarized cell. The cytosol is reorganized, with the new position of the organelles inside the cells reflecting the different activity of the opposing surfaces of the osteoclast. The nuclei, Golgi apparatus, and the rough endoplasmic reticulum are on the basolateral side of the cell, in contact with the microvasculature. The lysosomes together with mitochondria and components of the endocytic compartment move close to the apical side of the cell, juxtaposed to bone. This polarization reflects the different activities that occur at the two cell surfaces, with apical surface producing degradative enzymes to deliver into the reabsorption lacunae, whereas the basolateral surface is in charge of "packing" the products of reabsorption and delivering them into the main circulation (Fig. 2.5) [113, 116, 117].

The functional domains of the active reabsorbing osteoclast can be divided into the sealing zone, the membrane-rich ruffled border, the functional secretory domain, and the basolateral domain. The sealing zone has the key function of mediating the attachment of the osteoclasts to the underlying bone matrix, forming a distinctive

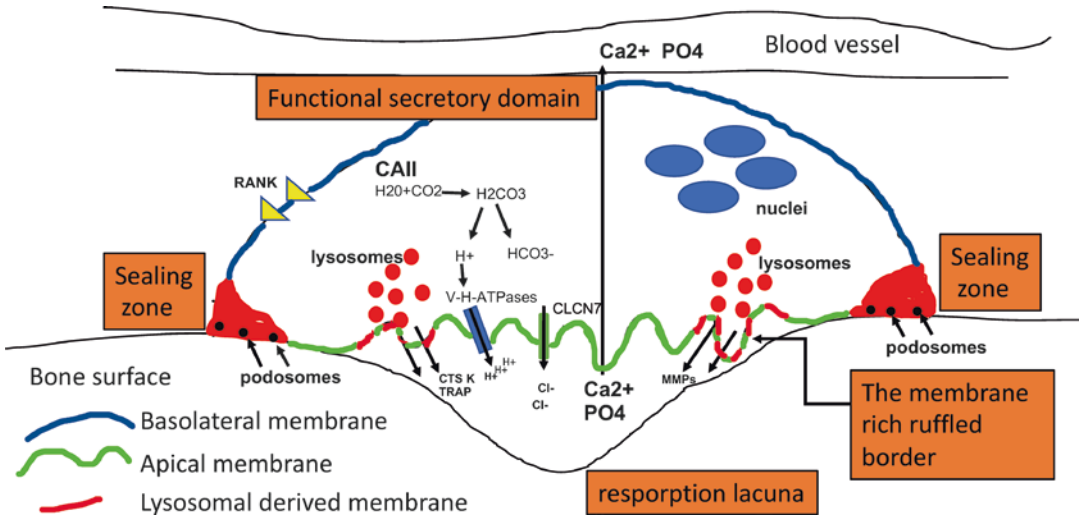

Fig. 2.5 Functional polarization in the osteoclast. Resorbing osteoclasts are highly polarized, with the resorption machinery located on the apical surface (highlighted in green) adjacent to the bone surface, while the basolateral membrane (highlighted in blue) transports resorbed molecules to the adjacent circulation. The cytoskeleton is reorganized to form a specialized actin structure, the podosome. The acid and hydrolases required for resorption are isolated from surrounding bone and cells by the formation of a tightly bone adherent sealing zone. The apical surface of the osteoclast has a highly invaginated membrane or ruffled border that greatly expands the membrane surface available for transport. The V-H-ATPase required for acidification and ion channels required to maintain intracellular electroneutrality are located in the ruffled border. Degradative enzymes, including cathepsin K (CTS K), TRAP, and matrix metalloproteinases (MMPs), are exocytosed into the resorption lacuna via fusion of lysosomal membrane (highlighed in red) with the ruffled border

and isolated "pouch" called the Howship or resorption lacuna, into which the osteoclast pumps protons and degradative enzymes to digest the bone matrix. The extremely tight connection between the apical surface and bone is mediated by a structure called the podosome ring. Podosomes are highly specialized adhesions that consist of actin microfilaments and integrins, together with several other regulatory proteins. Individual podosomes cluster into groups and then migrate to encircle the outside circumference of the sealing zone forming the so-called podosome belt. The formation of the podosome belt is the hallmark of a sealed attachment of the osteoclast to the bone [113, 115, 118, 119].

The membrane-rich ruffled border is centrally positioned relative to the sealing zone and is composed of an irregular array of membrane expansions. The ruffled border is divided into functional subdomains: the outer "secretive zone" and the inner "reuptake zone." The secretive zone is characterized by secretion of lysosomal enzymes into the resorption lacuna and the presence of ion transporters to discharge protons resulting in acidification of the lacuna. The reuptake zone is specialized for the reuptake of the calcium, phosphorus, and other bone components digested by the released enzymes. The functional secretory domain of the basolateral surface of the cell is connected with the microvasculature and is important for the passage of the reabsorption products into the general circulation [113, 115]. The functional secretory domain is anatomically connected with the sealing zone by what is referred to as the basolateral domain [115].

This complex reorganization creates a lacunar "pouch" between the osteoclast and the underlying bone that is isolated from the surrounding environment. Now the osteoclast can safely secrete protons, driven by V-H-ATPases, to acidify the lacuna and dissolve the inorganic hydroxyapatite component of the bone. Removal of mineral unmasks the organic component of the bone, mostly composed of type 1 collagen. The

organic matrix is then digested by a number of hydrolases secreted into lacuna via lysosomal exocytosis. Acidification of the lacuna not only unmasks the organic component of the bone but also creates the acidic environment needed for optimal hydrolase activity, as lysosomal enzymes perform best between pH 4.0 and 5.0 [113].

The critical nature of the highly specialized resorptive apparatus described above is revealed by the causal loss-of-function mutations described for several human bone diseases. Many genetic diseases involving the osteoclasts are characterized by abnormal function of the ruffled border, as detailed in the section "Genetic Diseases of Osteoclast Dysfunction."

Regulatory Functions

Osteoclasts are reported to secrete cytokines that act in an autocrine fashion to either promote or inhibit osteoclastogenesis. The IL-6 family member cardiotrophin-1 (CT-1) is produced by and stimulates osteoclast formation and has also been hypothesized to have paracrine effects promoting osteoblast formation [120]. Stimulation of osteoclasts with autoantibodies to citrullinated peptides that are found in rheumatoid arthritis was reported to induce an autocrine loop of IL-8-stimulated osteoclastogenesis [121]. On the other hand, osteoclast progenitors have been reported to express OPG, which would inhibit RANKL-stimulated osteoclast formation [122] Paracrine functions of osteoclasts include secretion of platelet-derived growth factor-BB (PDGF-BB) by osteoclast progenitors. PDGF-BB induces Type H capillary formation in bone; thus one paracrine action of osteoclasts appears to be promoting the coupling of angiogenesis and osteogenesis mediated by Type H capillaries [123].

The term "clastokine" has been coined and is often used to describe factors secreted by osteoclasts with putative paracrine actions on osteoblasts. While osteoblasts are widely accepted to modulate osteoclast formation and function through expression of RANKL and OPG, osteoclasts traditionally have been thought to contribute to osteoblast regulation through liberation of previously trapped cytokines from the bone matrix. More recently the concept of osteoclast-secreted "clastokines" has emerged. Clastokines are hypothesized to attract and facilitate the maturation of pre-osteoblast cells into mature bone-forming osteoblasts. A number of putative clastokines, including collagen triple repeat containing 1 (CTHCR1), sphingosine-1-phosphate (S1P), and complement factor 3a (C3a), have been described in vitro though relative in vivo significance of these putative clastokines is not entirely clear (reviewed in [111, 112]), and the osteoclast-specific expression of CTHRC1 in bone has been challenged [124]. The axon guidance molecule SLIT3 was recently proposed to act as a clastokine [125], though similar to CTHRC1, the source and cellular target of SLIT3 in bone are a source of debate. Loss of SLIT3 results in decreased bone mass, though whether this is via osteoclast-derived SLIT3 actions to promote osteoblast proliferation and migration via activation of the beta-catenin pathway [125] or via osteoblast production of SLIT3 promoting the development of the Type H vascular endothelium in bone [126] is not settled. The concept, however, is particularly exciting as it suggests the possible existence of novel mechanisms to stimulate bone anabolic activity which might prove attractive therapeutic targets.

Osteoclast regulation of osteoblast lineage cells can also occur via cell contact-mediated mechanisms. Semaphorin 4D expressed by osteoclasts inhibits osteoblast migration by binding to Plexin-B1, its cognate receptor on osteoblasts. In contrast, Ephrin B2 expressed on osteoclasts stimulates bone formation through binding EphB4, its receptor on osteoblasts [127, 128] Recently, osteoclast RANK stimulation of reverse signaling through RANKL on osteoblasts was proposed as a key mechanism coupling bone resorption and formation. Although reverse signaling could be stimulated through a cell contact-mediated mechanism, RANK was shown to be released from osteoclasts in small extracellular vesicles and thus likely acts in a paracrine fashion [129]. In summary, osteoclast regulatory functions have broad impact on the local bone microenvironment, influencing bone resorption, formation, and angiogenesis. However, future

research is needed to clarify the roles of many of the aforementioned coupling factors in human bone remodeling.

Genetic Diseases of Osteoclast Dysfunction

The identification of causative mutations underlying monogenic traits responsible for bone syndromes has added greatly to our understanding of osteoclast biology. Recognizing the gene/protein impaired in a specific rare bone disease had revealed the role of several proteins involved in the maturation and/or reabsorption machinery of the osteoclasts. Table 2.2 provides a list of genes and corresponding diseases. The genetic diseases primarily involving the osteoclast can be divided into two broad categories: those with normal or increased osteoclast function and those with decreased osteoclast function.

The spectrum of mutations in *TNFRSF11A (RANK*) are emblematic of these categories. Loss-of-function mutations in *TNFRSF11A* are responsible for osteopetrosis type VII, in which osteoclast differentiation and thus bone resorption are impaired and therefore active [130]. In contrast, gain-of-function mutations in *TNFRSF11A* are responsible for two diseases, expansile skeletal hyperphosphatasia and familial expansile osteolysis; these conditions are characterized by increased osteoclast activity which in turn leads to an excessive immature and disorganized bone formation [131]. A disease characterized by focal lesions with increased osteoclast activity is Paget's disease of bone (PDB), hereditary forms of which have been linked to heterozygous loss-of-function mutations of genes important for osteoclast maturation, *SQSTM1* and *VCP* [132, 133]. *SQSTM1* encodes sequestosome 1, a scaffolding protein important for RANK signaling. A *SQSTM1* mutation commonly associated with PDB has been shown to impair association with the TRAF6 deubiquitnase CYLD described above, resulting in increased poly-ubiquitinated TRAF6, RANK signaling, and osteoclastogenesis [134].

Table 2.2 Genetic diseases affecting the osteoclast

Osteoclast-specific mutation categories	Gene	Disease nomenclature
Normal or increased resorption activity (gain-of-function mutations)		
	TNFRSF11A (RANK)	Expansile skeletal hyperphosphatasia
		Familiar expansile osteolysis
	SQSTM1	Paget's disease of the bone
	VCP	
Decreased resorption activity (loss-of-function mutations)		
Impaired osteoclast differentiation		
	TNFRSF11A/RANK	ARO type VII
	IKBKG	Anhidrotic ectodermal dysplasia
Impaired osteoclast function		
Cytoplasmic defects	*CAII*	Aro type III with renal tubular acydosis
Podosome formation defects	*KIND-3*	Leucocyte adhesion deficiency with osteopetrosis
Lysosomal defects	*CTSK*	Pycnodysosotosis
Lysosomal protease defects	*ACP5 (TRAP)*	Spondyloenchondro-dysplasia
	MMP 9, MMP13	Metaphyseal dysplasya
Defects ruffled border maturation/ impaired lysosome fusion	*TCIRG1*	ARO type I
	CLCN7	ARO type IV/ ADO type II (Albers-Schonberg disease)
	OSTM1	ARO type V
	PLEKHM1	ARO type VI
	SNX10	ARO type VIII

Monogenic diseases with decreased osteoclast function present with a phenotype of osteopetrosis, or "stone bone," with dramatically increased bone density and loss of bone marrow cavity. Osteopetroses are divided in three categories based on the mechanism of transmission: autosomal-dominant osteopetrosis (ADO), autosomal-recessive osteopetrosis (ARO), and X-linked osteopetrosis. ADOs are usually more benign and occur in adulthood or in some cases represent an incidental finding in radiographic exams, whereas AROs result in severe skeletal involvement, are diagnosed in early childhood, and result in more morbidity. ADOs and AROs can develop from a heterozygous or homozygous mutation of the same gene; it is the involvement of one or both alleles that determine the severity of the disease [135, 136]. This is the case for mutations in the chloride channel *CLCN7*; heterozygous mutations in *CLCN7* cause ADO type II or Albers-Schonberg disease, whereas a homozygous mutation or a composite heterozygous mutation is responsible for ARO type IV [137, 138]. The only known X-linked osteopetrotic syndrome involves the gene *IKBKG* necessary for translocation of the transcription factor NF-κB into the nucleus [139].

A more biologically based approach to classifying osteopetrosis is considering whether osteoclasts do not form (osteoclast-poor osteopetrosis) or form but do not function (osteoclast-rich osteopetrosis). In the category of osteoclast-poor osteopetrosis are mutations involving *TNFRSF11A (RANK*) causing ARO type VII [130] and X-linked *IKBKG* mutations (anhidrotic ectodermal dysplasia, lymphedema, and immunodeficiency), underscoring the importance of the NF-κB pathway (described in the section "Osteoclast Differentiation") downstream of RANKL-RANK signaling in the development of the mature osteoclast [139]. In this category, we can also include mutations in genes expressed primarily by osteoblasts which indirectly alter osteoclast maturation and differentiation. These include mutations in *TNFSF11* encoding RANKL and *TNFRSF11B* encoding OPG, resulting in ARO type II and juvenile Paget's disease, respectively [140, 141].

Osteoclast-rich osteopetrosis can be subdivided into diseases with defects in cytoplasmic proteins, podosome formation, or lysosomal defects caused either by mutations in lysosomal proteins or defects in ruffled border maturation. The only identified defect in a cytoplasmic protein described to date is mutation in the gene encoding carbonic anhydrase type II, the enzyme necessary to maintain intracellular neutrality during acidification of the lacuna. Patients with this mutation not only manifest osteopetrosis but also renal tubular acidosis since the same isoform is present in tubular cells [142]. Mutation of *KIND3*, which encodes KINDLIN-3, impairs podosome assembly into the sealing zone and results in leucocyte adhesion deficiency with osteopetrosis [143].

Mutations in lysosomal proteases can cause bone disease even if the ruffled border formation is normal. Mutations of the gene encoding the abundant osteoclast protease cathepsin k results in pycnodysostosis, characterized by short stature with increased bone density [144]. Mutation of other lysosomal proteases cause bone disease, though not osteopetrosis. Patients with mutations in metalloproteinases 9 and 13 and tartrate-resistant acid phosphatase (TRAP) have been described and cause recessive and dominant metaphyseal dysplasia and spondyloenchondrodysplasia, respectively [145]. Mutations that impair fusion of lysosomes with the ruffled border comprise the largest subtype of osteopetrosis and are characterized by the inability of the abnormal ruffled border to acidify and secrete enzymes into the resorption lacuna. Mutations in *TCIRG1* cause ARO type I, the most common of the ARO types with more than 50% of ARO patients carrying a mutation in *TCIRG1*. *TCIRG1* encodes the subunit a3 of the V-ATPase complex, which is necessary to localize the V-ATPase complex in the ruffled border. Absence of this subunit impairs acidification of the resorption lacuna. ARO IV, also relatively frequent, is characterized by mutations in *CLCN7*, which encodes a Chloride-hydrogen antiporter in the lysosomal membrane. Mutations in *OSTM1*, encoding for a protein that binds CLCN7, is responsible for ARO type V. Mutations in *PLEKHM1*, which

encodes a protein that interacts with the vesicular trafficking protein RAB-7, and mutations in *SNX10*, which encodes a protein involved in intracellular endosomal trafficking, cause much rarer types of ARO [146, 147] These monogenic diseases affecting osteoclast function not only illuminate aspects of osteoclast biology, they provide indisputable evidence for the essential function of osteoclasts in skeletal biology.

Regulation of Osteoclasts by their Environment

A variety of perturbations in the physiologic state can promote osteoclastogenesis and bone loss, with perhaps the best characterized being inflammatory states such as rheumatoid arthritis. Inflammation promotes osteoclast formation at many levels, including through increasing the abundance of osteoclast progenitors. In mice, an increase in bone marrow osteoclast progenitors and in differentiation of circulating monocytes to osteoclasts is enhanced in inflammatory arthritis [16, 148–150] Circulating monocytes from patients with inflammatory arthritis, including rheumatoid arthritis, psoriatic arthritis, and ankylosing spondylitis, generate more osteoclasts in vitro cultures, suggesting an increase in circulating osteoclast progenitors within the monocyte pool [151–154]. Thus, the enhanced osteoclast activity seen in inflammatory arthritis may result in part from an increase in or skewing of progenitors toward an osteoclast fate.

The local microenvironment further regulates osteoclast differentiation through the relative expression of RANKL and OPG. The degree of osteoclast differentiation is likely further tuned by differential expression of the ligands that activate the ITAM-associated receptors essential for osteoclast differentiation. Superimposed on this is an additional layer of regulation by cytokines, which can affect osteoclast differentiation and activity both directly and indirectly by enhanced RANKL expression.

A number of inflammatory cytokines promote osteoclastogenesis, with TNF being the canonical example. TNF-induced bone resorption is implicated in both post-menopausal bone loss and the formation of bone erosions and generalized osteopenia of inflammatory arthritis. TNF acts directly to promote osteoclastogenesis by inducing expression of RANK on progenitors and through TNF receptor-mediated NF-κB activation [155, 156]. A large body of evidence has demonstrated that TNF has a critical role in pathologic osteoclastogenesis through promoting RANKL expression by osteoblasts, osteocytes, and synovial cells in inflammatory arthritis [157]. In mouse models, TNF contributes significantly to estrogen deficiency-induced bone loss, as demonstrated by the effectiveness of TNF inhibitor treatment or genetic deficiency in TNF or TNF receptor p55-deficient mice in preventing ovariectomy-induced bone loss [158, 159].

Other inflammatory cytokines that promote osteoclastogenesis include IL-1 and IL-6. Similar to TNF, IL-1 both directly stimulates osteoclast progenitors and increases expression of RANKL by the environment [160]. IL-6 and IL-6 family members promote osteoclastogenesis indirectly via enhancing RANKL expression, and also appear to have direct effects on progenitors, though whether IL-6 stimulates or inhibits differentiation is controversial [161]. IL-6 is thought to be increased by estrogen deficiency and is a putative mediator of post-menopausal bone loss. However, blocking IL-6 did not prevent bone loss in a mouse ovariectomy model, and there are conflicting reports on the effect of IL-6 deficiency on ovariectomy-induced bone loss [158, 162, 163]. Other cytokines, particularly Th2 cytokines, inhibit osteoclast differentiation either by promoting OPG expression or by direct actions on osteoclasts. These cytokines include IL-4, IL-13, IL-33, and IL-10 [164–167]. See also Table 2.1 for list of the effect of various cytokines on RANKL and OPG.

Estrogen deficiency may also promote bone loss through expansion of a T cell subset termed Th17 for their production of IL-17. Two mechanisms explain the pro-osteoclastogenic effect of T cells: IL-17 induces RANKL expression and Th17 cells themselves express RANKL. IL-17 also induces TNF and IL-1, further promoting a pro-osteoclastogenic environment [168]. Although

Th17 cells function as an osteoclastogenic helper cell T cell subset, other T cell subsets are inhibitory. Activated Th1 $CD4^+$ T cells produce IFNγ, a potent inhibitor of osteoclastogenesis [169]. Regulatory T cells directly inhibit osteoclast differentiation through their expression of CTLA-4. CTLA-4 on regulatory T cells interacting with CD80 and CD86 on osteoclast precursors induces reverse signaling in the myeloid cells to induce IDO (indolamine oxidase) which inhibits osteoclastogenesis [170–172]

Many stimuli discussed here play multiple roles in the immune system and on myeloid cell development. Therefore, the impact of any of the individual regulatory pathways on osteoclastogenesis likely depends mostly on the homeostatic or pathologic state in which they are deployed. Similar to other immune cells, osteoclasts are differentiated and activated in response to their environment and in pathologic or inflammatory disease, and the effect of specific positive and negative regulatory stimuli differs depending on the situation. While the focus of our discussion of regulation of osteoclasts has centered on differentiation, osteoclast survival and function are also of importance and likely have additional regulatory elements that center on the fine-tuning of the bone degradation response.

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Basic Aspects of Osteocyte Function

3

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Key Points

- Osteocytes, the most abundant cells in bone, differentiate from osteoblasts and live in the mineralized bone matrix, where they establish multiple connections with surrounding osteocytes and cells on the bone surface and the bone marrow.
- Osteocytes integrate mechanical signals and control bone homeostasis by secreting autocrine/paracrine factors (Sclerostin, Rankl) that regulate the activity of other bone cells.
- Osteocytes also secrete hormones (FGF23, Sclerostin) that affect distant tissues (kidney, liver, peripheral fat) by endocrine mechanisms.
- Osteocyte life span and function are altered during aging and in several skeletal diseases and cancers that grow in bone, thus contributing to the pathophysiology of several bone disorders.
- The improvement in the understanding of osteocyte biology has led to the development of novel therapeutic approaches targeting osteocytes and their derived factors to improve skeletal health in rare and common diseases.

Introduction

It has long been recognized that osteocytes are the most abundant cells in bone and that, in contrast to the short life span of osteoblasts and osteoclasts on bone surfaces, osteocytes are permanent residents of the mineralized bone matrix where they live for decades. However, it was only recently that the magnitude of the role that osteocytes play in bone homeostasis become evident. This revolution in the knowledge about osteocyte functions started in the late 1990s, and it was harnessed by the concomitant discovery of rare human diseases of bone caused by altered expression of osteocyte-derived proteins, the development of osteocytic cell lines, and the ability to alter the mouse genome in vivo by manipulating gene expression in osteocytes. Today, there

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is ample evidence demonstrating that osteocytes orchestrate the function of osteoblasts and osteoclasts, sense and transmit mechanical and hormonal signals, and regulate the function of bone and bone marrow cells by paracrine mechanisms as well as the function of cells in other tissues by endocrine mechanisms. In addition, the boom in research centered on osteocytes has extended our knowledge of osteocyte biology towards the role of these cells in pathophysiologic process, opening new avenues to treat diseases of bone by targeting osteocytes and their products to improve bone mass and strength.

Osteocytogenesis

Osteoblast to Osteocyte Differentiation

Osteocytes, the most abundant cells in bone, are master signal sensors, integrators, and transducers of the skeleton and therefore orchestrate growth, maintenance, and healing of bone. Osteocytes differentiate from osteoblast present on the bone surface that become surrounded by matrix proteins that they produce [1, 2]. It is estimated that 5–20% of osteoblasts differentiate into osteocytes, while the rest either undergo apoptosis or become quiescent bone lining cells. Although the mechanisms regulating osteoblast fate remain uncertain, it is known that the transition from osteoblast to osteocyte is accompanied by changes in gene expression that ultimately modify the morphology and function of osteoblasts. Major changes in gene expression during this process are related to the development of dendritic processes and the formation of the canalicular network, and the regulation of phosphate metabolism, bone formation, and bone resorption [2]. During osteoblast to osteocyte differentiation, the number of organelles markedly decreases, and osteoblasts acquire the characteristic star-like morphology that defines osteocytes. Further, osteocytes develop long cytoplasmic processes that run through canaliculi allowing osteocytes to physically interact and distribute osteocyte-derived molecules to neighboring osteocytes and other cells in the bone/bone marrow microenvironment. Several genes have been identified as mediators of the development of this canalicular network. E11, also known as podoplanin, is expressed in newly embedded osteocytes [3] and has been associated with osteocytic dendrite branching [4–6]. Dentin matrix protein 1 (DMP1) expression increases as osteoblasts differentiate toward osteocytes [7] and is also essential for proper osteocyte maturation [8]. The expression of collagen-degrading matrix metalloproteinases (MMPs) also increases as osteoblasts differentiate into osteocytes, allowing the formation and extension of osteocytic cytoplasmic projections [9, 10]. Another important molecule for the functionality of the osteocyte-canalicular network is connexin 43 (Cx43), which allows communication within the osteocyte network and the bone marrow, maintains osteocyte viability, and mediates osteocyte response to mechanical signals [11–13].

During osteocyte differentiation there is also an increase in the expression of genes related to phosphate metabolism and matrix mineralization [7]. Fibroblast growth factor 23 (FGF23) is produced by osteocytes and regulates phosphate metabolism by binding to FGF receptors and the Klotho co-receptor in the renal proximal tubular cells [14]. FGF23 expression in osteocytes is regulated by other osteocyte-derived factors. For example, human inactivating mutations of DMP1 or phosphate-regulating neutral endopeptidase, X-linked (PHEX), lead to high FGF23 circulating levels and hypophosphatemia [15]. As mentioned above, DMP1 is required for proper osteocyte differentiation and bone mineralization, whereas PHEX deletion in mice results in osteomalacia and an abnormal osteocytic lacuna-canalicular system [16]. Another osteocytic product is matrix extracellular phosphoglycoprotein (MEPE), a mineralization inhibitor that when deleted from the mouse genome results in increased bone mineral density [17]. Importantly, altered expression of some of the osteocytic genes described above causes disorders of phosphate homeostasis in humans [14, 18–21].

Work over the last several years has demonstrated that the osteocyte is a major source of the master regulator of osteoclastogenesis *receptor* activator of nuclear factor kappa-B ligand (RANKL) and the Wnt signaling antagonist and

inhibitor of bone formation SOST/Sclerostin [22–26]. RANKL is produced by multiple cells in the bone/bone marrow niche, including osteoblasts, osteocytes, and T-cells [27]. However, deletion of the RANKL gene in osteocytes markedly decreased osteoclast number in cancellous bone and increased cancellous bone mass [24–26]. The large reduction in osteoclast number in mice lacking RANKL in osteocytes compared to the one resulting from the deletion of RANKL in osteoblasts supports that osteocytes are the main source of RANKL in adult bones. In addition, immunohistochemical approaches have shown that SOST/Sclerostin is expressed by mature osteocytes, but not by osteoblasts or lining cells, and its expression progressively increases as osteocytes mature. Indeed, high Sclerostin expression is found in osteocytes surrounded by mineralized bone [28–31]. Further, recent findings have shown that Sclerostin could also stimulate RANKL expression in osteocytes [32, 33]. However, the regulation of RANKL and SOST/Sclerostin expression in osteocytes is not fully understood and might involve several transcription factors as well as epigenetic mechanisms (see section "Epigenetic Regulation of Osteocytic Gene Expression"). This compelling evidence shows that through the production of RANKL and SOST/Sclerostin, osteocytes control the rate of bone remodeling by regulating osteoclastogenesis as well as osteoblast differentiation and function [34, 35].

Epigenetic Regulation of Osteocytic Gene Expression

Osteoblasts and osteocytes originate from mesenchymal stem cells (MSCs), which can also differentiate into adipocytes and myogenic cells, as well as chondrocytes. Thus, the differentiation process is tightly regulated to enable lineage-specific differentiation of MSCs. Epigenetic mechanisms play an important role in osteocyte differentiation by regulating the expression levels of key genes [36, 37]. DNA methylation is the most studied epigenetic mark. Methylation at proximal promoter CpG sites is associated with silencing of gene transcription [38]. Histone modification epigenetic marks are divided into those that activate transcription (mainly acetylation and phosphorylation) and those that repress it (methylation, ubiquitination, and sumoylation) [39], whereas miRNAs bind to RNAs and induce mRNA cleavage or translational repression, depending on the degree of complementarity [40]. As mentioned above, the cell shape change from the polygonal bone-forming osteoblasts to the dendrite-rich stellate osteocytes is regulated by E11/podoplanin, which expression is controlled by a cooperative crosstalk between DNA methylation and histone modification in osteoblastic cells [41]. Alkaline phosphatase (ALPL), an enzyme required for bone mineralization, is highly detected in osteoblasts, but its expression is reduced in osteocytes [42]. This change in ALPL expression is mediated by DNA methylation, as osteoblasts and osteocytes present opposite methylation profiles in the ALPL proximal promoter, hypomethylated and hypermethylated, respectively [43]. DNA demethylation at the SOST proximal promoter also occurs during osteoblast-osteocyte transition, allowing osteocytes to express SOST [44]. In addition to DNA methylation in the proximal promoter, several regulatory elements and transcription factors tightly regulate SOST transcription in bone, including the evolutionarily conserved region 5 (ECR5) and the myocyte enhancer factor-2 (MEF2C), a transcription factor that binds to ECR5 [45–47]. Other genes influencing osteogenesis, such as osterix, the osteogenic protein Dlx-5, aromatase, RANKL, and the estrogen receptor, are also regulated by DNA methylation [36, 37, 48]. Chromatin remodeling plays also an important role in osteocyte differentiation and function. For instance, it has been shown that histone modifications regulate the expression of Runt-related transcription factor 2 (*RUNX2*), Activator protein 1 (*AP-1*), Activating transcription factor 4 (ATF4), and SMADs [49], all key factors involved in osteoblast maturation. In addition, sirtuin 1, a histone deacetylase, directly regulates SOST expression [50]. Similarly, HDAC5, a class IIa histone deacetylase, was identified as a negative regulator of MEF2C-driven *SOST* expression, through a

mechanism that involves salt inducible kinase 2 [51, 52]. miRNAs are also actively involved in the regulation of osteocytic gene expression. miR-26a has been shown to negatively regulate Smad1, resulting in a decreased expression of various osteoblast markers, such as ALPL, osteoclacin, osteopontin, and collagen 2A1 (COL2A1) [53]. Recent studies demonstrated that miR-206 inhibits Cx43 expression and potentially impairs osteoblast differentiation [54], and miR21 regulates osteocyte apoptosis downstream Cx43 in osteocytes [55].

Osteocyte Apoptosis

Osteocytes do not proliferate in vivo. Thus, their number is controlled by the rate at which they are generated from mature osteoblasts and by their rate of apoptosis. One of the first pieces of evidence demonstrating that osteocytes perceive changes in the level of both physical stimuli and circulating factors was provided by studies on the regulation of osteocyte life span [56–58]. Although osteocytes are long-lived cells, they die by apoptosis as other bone cells. Early work showed an association between osteocyte apoptosis and estrogen withdrawal [59], and this work was followed by multiple studies demonstrating the role of estrogen and SERMS preserving osteocyte viability and the signaling pathways involved [60–69]. Osteocyte viability is now known to accompany the bone fragility syndromes of estrogen and androgen deficiency, as well as glucocorticoid excess, mechanical disuse, and aging [70–72]. Conversely, preservation of osteocyte viability results from physiological levels of mechanical stimulation [71, 73] and maintained by treatment with sex steroids [63, 64] or bisphosphonates [74].

Regulation of Osteocyte Apoptosis by Mechanical Forces

Mechanical stimulation prevents apoptosis induced by death stimuli including glucocorticoids in both cultured osteocytic cells and authentic osteocytes [75, 76]. In vitro work showed that osteocytes transduce mechanical forces into intracellular anti-apoptotic pathways by integrins, cytoskeletal proteins, the focal adhesion kinase (FAK), and the Src kinase assembled at caveolin-rich domains of the plasma membrane, which ultimately activate survival kinases including ERKs [75]. Mechanical loading also activates the Wnt signaling pathway [69, 77–80]. The protective ERK nuclear translocation and anti-apoptotic signals induced by mechanical stretching or fluid flow are abolished by antagonists of the canonical Wnt pathway, such as DKK1 and the stimulator of β-catenin degradation Axin2 [81]. Conversely, glycogen synthase kinase 3β (GSK3β) phosphorylation and β-catenin accumulation induced by mechanical cues are abolished by silencing caveolin-1 or by pharmacologically inhibiting ERKs. These findings suggest a bidirectional crosstalk between the caveolin-1/ERKs and the Wnt/β-catenin pathways in mechanotransduction [81].

Consistent with the regulation of apoptotic pathways in vitro, mechanical forces also regulate osteocyte life span in vivo. Increased prevalence of apoptotic osteocytes is found in unloaded bones [71] or in bones exposed to high levels of mechanical strain [56, 82, 83]. In both cases, increased osteocyte apoptosis precedes osteoclastic resorption and apoptotic osteocytes accumulate in areas subsequently removed by osteoclasts [71]. Together these findings suggest that dying osteocytes are beacons for osteoclast recruitment to the vicinity and the resulting increase in bone resorption [84]. Consistent with this notion, targeted ablation of osteocytes by genetic means is sufficient to induce osteoclast recruitment and increase resorption leading to bone loss [85]. Recent studies demonstrate that osteocyte apoptosis, induced by either unloading or overloading leading to fatigue damage, increases the expression of RANKL in osteocytes of adjacent areas [86–88]. Blocking apoptosis with a pan inhibitor prevents both the increase in RANKL expression and the proresorptive effect of either unloading or overloading [86, 87]. In contrast, inhibiting osteocyte apoptosis with a bisphosphonate that targets osteo-

cytes and osteoblasts prevented the increase in RANKL but was not able to prevent the bone loss induced by unloading [88]. Taking together, these findings confirm the relationship between osteocyte apoptosis and RANKL expression between neighboring osteocytes. In addition, this evidence suggests that, in some but not all cases, increased RANKL expression in osteocytes leads to targeted resorption. Therefore, it is possible that more than one osteocytic mediator regulates osteoclast precursor recruitment to specific areas of bone to initiate resorption.

Other potential candidates mediating this phenomenon are osteoprotegerin (OPG), the decoy receptor for RANKL, which is expressed in osteocytes at least at similar levels than in osteoblasts [89], and the osteoclast chemotactic factor high-mobility group box 1 (HMGB1) protein [90], which is released by osteocytes undergoing apoptosis and upregulates the expression of RANKL, TNF and, IL-6 and also decreases OPG expression. Further, in overloaded rat bones, dead osteocytes are surrounded by still-living osteocytes in which the expression of VEGF (besides RANKL) is elevated [91], suggesting that signals emanated from apoptotic cells alter the expression of molecules that influence angiogenesis and potentially osteoclast precursor recruitment by acting on neighboring cells.

Mechanical loading is critical for the maintenance of bone mass, whereas skeletal unloading, as occurs with reduced physical activity with old age, immobilization of bed rest, and total or partial motor paralyses, causes bone loss leading to disuse osteoporosis [92]. Further, the bone loss that ensues under microgravity conditions represents the most significant hindrance for long-term space flying [93]. There is a rapid decrease in osteocyte viability with unloading suggesting that osteocytes are critical skeletal responders to changes in mechanical forces [71]. Consistent with this notion, mice depleted from osteocytes are protected from the bone loss induced by tail suspension, suggesting that in the absence of osteocytes, the skeleton is unable to elicit a normal osteoclastogenic response [85]. Mice with conditional deletion of RANKL in osteocytes are also protected from unloading-induced elevation in osteoclasts and bone loss [25], suggesting that osteocytes provide the required RANKL for osteoclast formation during skeletal disuse. Together, these findings confirm that osteocytes are the primary culprit of the negative bone balance that ensues with weightlessness.

Regulation of Osteocyte Apoptosis by Sex Steroids and Bisphosphonates

Loss of sex steroids leads to increased prevalence of osteocyte apoptosis. In contrast, estrogens and androgens inhibit apoptosis of osteocytes as well as osteoblasts [64, 69]. This anti-apoptotic effect is due to rapid activation of the Src/Shc/ERK and PI3K signaling pathways through non-genotropic actions of the classical receptors for sex steroids [64, 94]. Bisphosphonates also preserve viability of osteocytes (and osteoblasts) in vitro and in vivo, through a mechanism that involves opening of Cx43 hemichannels and ERK activation [12, 57, 74, 95]. The fact that apoptotic osteocytes trigger bone resorption, taken together with the evidence that osteocyte apoptosis is inhibited by estrogens and bisphosphonates, raises the possibility that preservation of osteocyte viability contributes to the anti-remodeling properties of these agents.

Regulation of Bone Formation by Osteocytes

Due to their location in the bone matrix and their extensive connections with other osteocytes and cells in the bone marrow, osteocytes were traditionally considered the main mechanosensors in the skeleton, capable of sensing mechanical forces in bone and translating them into biochemical signals promoting bone formation [96, 97]. Supporting this notion, targeted deletion of osteocytes results in bone loss and lack of anabolic response to mechanical loading [85]. More recently, the discovery of loss-of-function mutations in several components of the Wnt signaling and their dramatic effects in bone mass attracted considerable attention

to this pathway [98]. Clinical and animal data have shown that Wnt/β-catenin signaling in bone plays a significant role in the control of osteoblast differentiation, survival, and function [98]. Now we know that osteocytes negatively regulate osteoblast viability and function by secreting Wnt signaling antagonists, including DKK1 and Sclerostin [34, 99], which block the binding of Wnt ligands to Frizzled receptors and low-density lipoprotein receptor–related proteins (LRP) 5 and 6 [34, 98]. Genetic deletion of SOST, the gene encoding Sclerostin, in mice increases bone formation and bone mass, recapitulating the high bone mass phenotype exhibited by patients with mutations in this gene [98, 100]. Moreover, SOST/Sclerostin expression is modulated by anabolic stimuli and has become a promising target for the treatment of skeletal diseases associated with low bone formation. Specifically, Sclerostin is downregulated by parathyroid hormone (PTH), a Food and Drug Administration (FDA)-approved anabolic agent for osteoporosis in the USA [101–105] (*see section* "Osteocytes and the Actions of the PTH Receptor"). In addition, the increase in bone formation in response to mechanical loads is mediated by the downregulation of Sclerostin in osteocytes [106, 107]. Deletion or pharmacological inhibition of LRP4, a chaperone required for the inhibitory actions of Sclerostin, also increased bone formation and bone mass [108]. Opposite to LRP4 and SOST/Sclerostin inhibition, deletion of DKK1 has minor effects on the skeleton. It was recently shown that DKK1 inhibition increases SOST/Sclerostin expression, suggesting a potential compensatory mechanism that could account for the weak anabolic effects of DKK1 suppression [109]. Supporting this notion, a robust anabolic response to DKK1 deletion was found when SOST/Sclerostin signaling was impaired. In the past decade, several neutralizing antibodies against DKK1, Sclerostin, and LRP4 have been developed and have shown promising therapeutic outcomes for patients with osteoporosis and other skeletal diseases (*discussed in section* "Neutralizing Antibodies Against Sclerostin").

Although the effects of Wnt signaling activation on bone mass are well documented, the cell responsible for orchestrating the Wnt anabolic actions had remained elusive. Indeed, activation of Wnt/β-catenin signaling in pre-osteoblasts or osteoblasts inhibits resorption without increasing bone formation [110]. In contrast, genetic activation of canonical Wnt signaling in osteocytes increases bone mineral density and bone volume, osteoblast number, bone matrix production, periosteal bone formation rate, and activates Notch signaling in bone, without affecting survival [33]. These results contrast with those observed in mice expressing the same dominant active β-catenin transgene in osteoblasts, which exhibit decreased resorption and perinatal death from leukemia [111], and identify osteocytes as the central target cell coordinating the anabolic actions of canonical Wnt/β-catenin signaling in bone.

Finally, osteocytes also affect osteoblasts through physical interactions. It has been shown in vitro that direct cell-to-cell contact between osteocytes and osteoblasts increases the expression of genes involved in osteoblast differentiation (COL1A, RUNX2, ALPL) [112]. In addition, Notch signaling, a pathway that mediates cell-to-cell communication upon interactions between Notch receptors and Notch ligands, regulates both osteocyte and osteoblast function [113, 114] (*see section* "Targeting Notch Signaling in Osteocytes").

Regulation of Bone Resorption by Osteocytes

The osteopetrotic phenotype of adult but not growing mice lacking RANKL in osteocytes demonstrates that osteocytes are a major source of RANKL controlling adult remodeling bone [25, 26]. However, the contribution of osteocytic membrane-bound or soluble RANKL to osteocyte-driven bone resorption had remained unclear. Recent findings demonstrated that the membrane-bound form of RANKL is sufficient for most functions of this protein but that the soluble form contributes to physiological bone

remodeling in adult mice [115]. Thus, mice lacking soluble RANKL exhibit normal bone mass and structure during growth but reduced osteoclast number and increased cancellous bone mass in adulthood. In addition, bone loss, induced by estrogen deficiency, as well as lymphocyte number, lymph node development, and mammary gland development, is normal in mice lacking soluble RANKL. The similar phenotypes between mice lacking RANKL in osteocytes and mice lacking soluble RANKL in all cells suggest that osteocytes regulate bone resorption at least in part via soluble RANKL. However, the decrease in osteoclast number in mice lacking soluble RANKL was not as accentuated as in mice lacking osteocytic RANKL, suggesting that osteocytes also utilize membrane-bound RANKL to communicate with osteoclast progenitors. Intriguingly, most osteocytes are not in direct contact with the blood vessels or the bone marrow, in particular in larger animals exhibiting true osteonal remodeling. Therefore, the mechanisms by which membrane-bound RANKL in osteocytes make direct contact with osteoclast progenitors remain to be clarified.

As mentioned above, osteocytes also secrete OPG, which competes with RANKL for its receptor RANK on osteoclast precursors. In osteocytes, as in osteoblasts, OPG secretion is regulated by the Wnt/β-catenin pathway, and mice lacking β-catenin in osteocytes are osteoporotic due to increased osteoclast numbers and bone resorption [89]. In addition, emerging evidence also points to osteocytes as an additional source of secreted M-CSF in bone [116].

Together, these novel findings suggest that osteocytes have the potential to control bone resorption through regulation of osteoclast differentiation and function under both physiological and pathological conditions.

Osteocytes and Aging

One of the functions attributed to the osteocyte network is to detect microdamage and trigger its repair [1, 117]. During aging, there is accumulation of microdamage and a decline in osteocyte density accompanied by decreased prevalence of osteocyte-occupied lacunae, an index of premature osteocyte death [118]. Reduced osteocyte density might be a direct consequence of increased osteoblast apoptosis. However, the prevalence of apoptotic osteocytes might result from the decline in physical activity with old age leading to reduced skeletal loading, accumulation of reactive oxygen species (ROS) in bone [119], and/or increased levels of endogenous glucocorticoids with age [120]. Nevertheless, the loss of osteocytes is at least partially responsible for the disparity between bone quantity and quality that occurs with aging.

Cx43 expression in osteocytes is required in a cell-autonomous fashion to preserve osteocyte viability, as well as to control in osteocytes the levels of proteins that regulate osteoclastogenesis [11, 121]. Anatomical mapping of apoptotic osteocytes, osteocytic protein expression, and resorption and formation in bones from Cx43-deficient mice suggests that Cx43 controls osteoclast and osteoblast activity by regulating OPG and Sclerostin levels, respectively, in osteocytes located in specific areas of cortical bone. Furthermore, cultured osteocytic cells lacking Cx43 exhibit increased rate of apoptosis, decreased OPG, and increased RANKL expression [11, 122]. Similar molecular changes are observed in bones of mice lacking Cx43 in osteocytes. Moreover, these conditional knockout mice display increased endocortical resorption and exaggerated periosteal bone apposition resulting in altered cortical bone geometry. As a consequence, long bones from mice deficient in Cx43 in osteocytes exhibit enlarged bone marrow cavities and increased cross-sectional diameter [11, 122, 123]. Accumulation of apoptotic osteocytes and empty lacunae, increased endocortical resorption, and periosteal expansion of the long bones resemble bones from aging rodents and humans [72, 124]. The expression of Cx43 channel/hemichannel protein decreases with age [125]. Therefore, reduced Cx43 expression might mediate at least some of the changes induced by aging in the skeleton.

Effects of Glucocorticoids (GC) on Osteocytes

GCs have profound effects on cells of the osteoblastic lineage and, in particular, in osteocytes. Endogenous GC activity is regulated by two enzymes: 11β-HSD type 1 and type 2. 11β-HSD1 converts inert 11-ketometabolites into biologically active GC, whereas 11β-HSD2 converts active GC into inactive metabolites. By overexpressing 11β-HSD2 under the control of promoters active at different stages of differentiation of the osteoblastic lineage, GC action can be blocked in a cell-specific manner. Blocking GC action by overexpressing 11β-HSD2 in immature and mature osteoblasts versus late osteoblasts and osteocytes demonstrated that GC signaling in early osteoblastic differentiation stages, but not in late osteoblastic or osteocytic stages, is required for optimal bone mass acquisition during bone growth [126].

A hallmark of GC excess on mature osteoblasts and osteocytes is the promotion of apoptosis [127, 128]. The increase in the prevalence of osteoblast apoptosis partially explains the reduced osteoblast number and decreased bone formation induced by GC. Further, accumulation of apoptotic osteocytes contributes to osteoporosis of GC excess. Mice overexpressing 11β-HSD2 under the control of the murine osteocalcin gene 2 promoter, which is active only in mature osteoblasts and osteocytes, were protected from GC-induced apoptosis of these cells [129, 130]. Prevention of osteoblast/osteocyte apoptosis preserved cancellous osteoblast function and osteoid production, thus preventing the decrease in bone formation. Accordingly, the apoptotic effect of GC observed in vivo is readily reproduced in vitro in cultured osteoblasts and osteocytes and depends on the expression of the glucocorticoid receptor (GR) [131, 132]. Importantly, bone strength was preserved in these transgenic mice despite loss of bone mass, suggesting a potential effect of osteocyte viability in preserving bone strength. In addition, the initial rapid bone loss induced by GC was not prevented by blocking GC action in osteoblasts and osteocytes, strongly suggesting that the early phase of bone loss is due to direct actions of GC on osteoclasts [133].

Binding of GC to the GR is followed by cis- or trans-interactions between the ligand-bound receptor with DNA and induction or repression of gene transcription [134, 135]. In addition, GC exert "non-genomic" actions mediated by the GR that do not involve direct GR interaction with the DNA, but that result from modulation of the activity of intracellular kinases such as ERKs, the c-Jun N-terminal kinase (JNK), and the proline-rich tyrosine kinase 2 (Pyk2) [136–141]. Pyk2, also known as related adhesion focal tyrosine kinase (RAFTK), cellular adhesion kinase β (CAKβ), and calcium-dependent tyrosine kinase (CADTK), is a member of the focal adhesion kinase (FAK) family of non-receptor tyrosine kinases [142, 143]. Despite their high homology, Pyk2 and FAK exhibit opposite effects on cell fate as FAK activation promotes cell spreading and survival and Pyk2 activation induces cell detachment and apoptosis [142, 144]. FAK and Pyk2 control survival of osteocytes and osteoblasts by regulating cellular interactions through focal adhesions with the extracellular matrix [145–147]. At the focal adhesions, integrins connect extracellular matrix proteins with cellular structural and catalytic molecules by bidirectional signaling. Outside-in signaling is the one activated by extracellular matrix proteins that induces integrin engagement and activates intracellular signaling; and inside-out signaling is the one triggered from inside the cell and regulates the interaction of integrins with extracellular matrix proteins [148, 149]. Association of integrins with the extracellular matrix leads to survival, while loss of this interaction causes detachment-induced apoptosis or anoikis [147]. For osteocytes, integrin engagement mediated by FAK is potentiated by mechanical signals and maintains osteocyte survival [75]. GCs oppose this integrin-/FAK-dependent survival signaling by activating Pyk2, which in turn activates inside-out signaling resulting in cell detachment and leads to anoikis. Pyk2 activation also activates pro-apoptotic JNK signaling [132]. Remarkably, although this action of GC is exerted via a receptor-mediated mechanism, it is independent

of new gene transcription [132]. Changes in FAK and Pyk2 kinase signaling induced by GC, combined with downregulation of genes that prolong survival, such as interleukin-6, insulin growth factors, transforming growth factor β, collagenase type I, and integrin β1 [134, 150–153], could result in the increase in osteocyte and osteoblast apoptosis observed in vivo. This evidence highlights the importance of rapid kinase signaling in GC action in bone and opens new avenues for the design of GC analogs with the ability to activate transcription-mediated versus kinase-mediated actions of the GR.

GCs also increase reactive oxygen species (ROS) production in bone in vivo and in osteoblasts in vitro [154]. Although it has not been determined whether this occurs in osteocytes, the global effect of GC on ROS could contribute to the effects of the steroids in bone in vivo. Endoplasmic reticulum (ER) stress is associated with increased ROS, resulting from accumulation of misfolded/unfolded proteins, and can trigger apoptosis. ER stress is alleviated by phosphorylation of eukaryotic translation initiation factor 2α (eIF2α), which slows the global rate of protein translation to provide time for the ER to recover from the excessive protein load, thus allowing the cell to escape from apoptosis [155, 156]. Consistent with a role for ROS/ER stress, GC effects are prevented by the compounds, salubrinal and guanabenz [157], eIF2α dephosphorylation inhibitors that block ROS-induced ER stress [158, 159]. Salubrinal and guanabenz prevented the pro-apoptotic effect of GC on osteoblasts and osteocytes in vitro as well as the decrease in differentiation induced by GC in osteoblastic cell cultures. Further, salubrinal prevented apoptosis of osteoblasts and osteocytes in vivo and blunted the decrease in bone mass and bone formation induced by GC. Salubrinal increased the number of alkaline phosphatase-positive colonies in bone marrow cell cultures [160] and osteocalcin expression in osteoblastic MC3T3-E1 cells [161]. Conversely, in vitro exposure to thapsigargin or tunicamycin induces elevated ER stress and increased apoptosis of osteoblasts and changes in osteoblast differentiation [162, 163]. Increased ER stress appears to have a time-dependent biphasic effect inducing rapid increase in osteoblast markers Runx2 and osterix, followed by a reduction in the expression of these transcription factors as well as osteocalcin [162].

GCs not only induce osteocyte apoptosis but also alter their gene expression profile. GCs increase the expression of SOST/Sclerostin in bone and the number of Sclerostin-positive osteocytes, an effect accompanied by decreased expression of genes associated with both anti-catabolism, including OPG, and anabolism/survival, such as cyclin D1 [164]. GC decreased the mass, deteriorated the microarchitecture, and reduced the structural and material strength of bone in wild-type mice, but not in mice with genetic deletion of SOST. Mechanistic studies showed that the preservation of bone mass and strength in SOST KO mice was due to prevention of GC-induced bone resorption and not to restoration of bone formation [164]. These results support the notion that activation of Wnt/β-catenin signaling by inhibiting SOST/Sclerostin signaling maintains bone integrity by opposing bone catabolism, despite the reduction in bone formation and increased osteoblast/osteocyte apoptosis induced by GC.

Osteocytes and the Actions of the Parathyroid Hormone (PTH) Receptor

PTH has profound effects on the skeleton, and its elevation in the circulation can generate catabolic and anabolic effects on bone depending on the temporal profile of its increase. Chronic excess of PTH, as in primary hyperparathyroidism or secondary to calcium deficiency, increases the rate of bone remodeling and can result in loss of bone mass. In contrast, intermittent PTH elevation, as achieved by daily injections, stimulates bone formation to a greater degree than resorption and is a current bone anabolic therapy to increase bone mass in the setting of osteoporosis. High bone remodeling rates and bone loss with chronic PTH elevation are associated with excessive production of osteoclasts coupled to increased osteoblasts, with a negative balance between formation and

resorption within each bone multicellular unit. Instead, the primary effect of intermittent PTH elevation is a rapid increase in osteoblasts and bone formation, attributed to the ability of PTH to promote proliferation of osteoblast precursors, to inhibit osteoblast apoptosis, to reactivate lining cells to become matrix-synthesizing osteoblasts, or to a combination of these effects [165, 166]. Daily PTH injections in humans stimulate bone formation by increasing bone remodeling rate and the amount of bone formed by each remodeling unit, named "remodeling-based formation" [167]. PTH also stimulates bone formation not coupled to prior resorption, referred to as "modeling-based formation" [165, 167, 168].

Osteocytic PTH Receptor and Skeletal Actions of PTH

Work of the last few years established that some of actions of PTH on the skeleton are mediated by direct effects of the hormone on osteocytes [169, 170]. PTH downregulates the expression of SOST/Sclerostin [101, 102], and increases the expression of FGF23, which regulates phosphate reabsorption in the kidney, thus contributing to mineral homeostasis [169, 171, 172]. Moreover, the major skeletal effects of PTH are recapitulated in transgenic mice expressing a constitutively active PTH receptor in osteocytes (DMP1-caPTH1R^{Ot}) [169, 173–175], which display marked increase in bone mineral density and increased bone formation rate and osteoblasts in cortical and cancellous bone surfaces. In addition, expression of SOST/Sclerostin is reduced and Wnt signaling is activated in DMP1-caPTH1R^{Ot} mice. Consistently, mice with conditional deletion of the PTH receptor 1 (PTH1R) in osteocytes using the DMP1-8 kb promoter or that DMP1-10 kb promoter to drive Cre expression (cKO-PTH1R) exhibit decreased resorption, lower RANKL/OPG ratio and a modest increase in cancellous bone volume [176, 177]. In contrast, the elevated bone resorption and bone loss induced by endogenous elevation of PTH in mice fed a low calcium diet was similar in cKO-PTH1R mice and control littermates, in both cancellous and cortical bone. There results suggest that cells other than osteocytes in the bone/bone marrow microenvironment might mediate the skeletal actions of chronic PTH elevation. On the other hand, cKO-PTH1R mice failed to fully respond to daily PTH injections, as the increase in bone mass and bone formation rate in all bone surfaces (cancellous, endocortical, and periosteal) observed in control mice was reduced or absent in cKO-PTH1R mice. Together, these findings demonstrate that PTH1R signaling in osteocytes is required to maintain basal levels of bone resorption. Further, osteocytic PTHR signaling is dispensable for the catabolic actions of chronic PTH elevation; however, it is essential for the full anabolic actions of daily PTH injections [176]. The evidence that direct PTHR signaling in osteocytes is not needed for the catabolic actions of the hormone suggests that chronic elevation of PTH may induce osteocytic RANKL production indirectly acting on other cells of the bone/bone marrow microenvironment. Indeed, T-cell-null mice and mice with conditional deletion of PTHR in T cells fail to induce bone loss with chronic elevation of PTH [178, 179]. Further, recent evidence demonstrates that chronic PTH elevation fails to induce bone loss and causes less bone resorption in mice lacking the IL-17A receptor in osteocytes (with DMP1-8 kb-Cre) [180]. In addition, deletion of IL-17A receptor signaling in osteocytes blunts the capacity of chronic PTH to stimulate osteocytic RANKL production. Therefore, direct IL-17A receptor signaling in osteocytes is required for PTH to exert its bone catabolic effects and chronic PTH increases RANKL expression in osteocytes indirectly, via an IL-17A/IL-17RA-mediated mechanism. In summary, osteocytic production of RANKL and T-cell production of IL-17A are both critical for the bone catabolic activity.

Osteocytic PTH Receptor and Regulation of Bone Formation

Similar to the requirement of the PTH1R for the full anabolic response to PTH, periosteal bone formation induced by axial ulna loading

was reduced in cKO-PTH1R mice compared to controls [176]. Consistently, mechanical loading decreased SOST and increased Wnt target gene expression in WT mice but not in cKO-PTH1R mice [176]. Both mechanical stimulation and daily PTH injections decrease SOST/Sclerostin expression [101, 181], thus suggesting that osteocytic PTH1R controls bone anabolism in response to both stimuli through downregulation of SOST expression, unleashing bone formation. Recent studies have examined the requirement of SOST downregulation for the anabolic response to PTH and mechanical loading using DMP1-8 kb-SOST mice overexpressing in osteocytes a human SOST transgene that cannot be downregulated. The inability to downregulate SOST abolished the increase in bone formation and Wnt/β-catenin signaling induced by loading in DMP1-8 kb-SOST mice [106]. However, DMP1-8 kb-SOST mice exhibited a similar bone anabolic response to daily PTH injections compared to control littermates, regarding bone mass, bone formation rate, and activation of Wnt/β-catenin signaling [176]. Taken together, these findings support that expression of the PTH1R in osteocytes is required to stimulate Wnt/β-catenin signaling and to elicit bone gain resulting from daily injections of PTH and mechanical loading. However, whereas downregulation of SOST/Sclerostin expression is required for loading-induced bone formation, it is dispensable for PTH-induced anabolism. Therefore, mechanisms downstream of PTH1R other than inhibition of SOST/Sclerostin are responsible for PTH bone anabolic effects driven by osteocytes.

PTH Receptor Signaling in Osteocytes and Bone Resorption

It has been long recognized that PTH upregulates RANKL expression in cells of the osteoblastic lineage, but the precise differentiation stage of the PTH target cell responsible for RANKL-mediated stimulation of bone resorption had remained undefined. Recent work demonstrates that PTH signaling in osteocytes directly upregulates the RANKL gene [182]. Deletion of the distal control region (DCR) responsible for the increase in RANKL by PTH (DCR$^{-/-}$ mice) decreased the high resorption exhibited by mice with constitutive active PTH1R signaling in osteocytes (DMP1-caPTH1R^{Ot}) [182]. Furthermore, DCR deletion decreased the elevated RANKL expression in osteocytes exhibited by DMP1-caPTH1R^{Ot} to WT levels. These findings demonstrate that PTH1R signaling in the adult skeleton requires direct regulation of the RANKL gene in osteocytes [182].

More recent studies demonstrated that matrix metalloproteinase 14 (MMP14) is a novel target gene of PTH in osteocytes, and inhibition of its activity regulates PTH-induced bone resorption [183]. Bones from DMP1-caPTH1R^{Ot} mice exhibit elevated expression MMP14, and MMP14 expression is increased in WT mice injected daily with PTH, but not in bones from PTH1R cKO in osteocytes, suggesting that MMP14 is a new target for PTH1R signaling in osteocytes. Mechanistic studies demonstrated that MMP14 increases soluble RANKL production, which, in turn, stimulates osteoclast differentiation and resorption. Pharmacological inhibition of MMP14 inhibited bone resorption and allowed full stimulation of bone formation, thus potentiating the increase in cancellous bone induced by daily PTH. Thus, MMP14 is a new member of the intricate gene network activated in osteocytes by PTH1R signaling that can be targeted to adjust the skeletal responses to PTH in favor of bone preservation [183].

Osteocytes and Diabetes Mellitus (DM)

DM is a highly prevalent disease that affects 200 million people worldwide, and it is associated with increased fracture risk [184]. In subjects with type 1 DM (T1D), bone mass is consistently decreased, whereas in patients with type 2 DM, bone mass is usually normal or even high; but in both cases, there are concomitant alterations in bone structure and strength that compromise bone quality [185]. Diabetic patients show decreased bone formation and increased resorption due to complex and yet ill-defined

mechanisms, including hyperglycemia and accumulation of advanced glycation end products. The morbidity and mortality associated with bone fractures are especially aggravated by impaired fracture healing [186], likely due to disrupted function of bone cells. Recent findings demonstrate that DM affects the osteocyte in bone. Mice with DM induced by streptozotocin, a non-autoimmune model of type 1 DM, exhibit low bone mass, inferior mechanical and material properties, increased osteoclasts and bone resorption, and decreased osteoblast bone formation, which were accompanied by increased expression of the osteocyte-derived Wnt antagonists Sclerostin and DKK1 and by increased RANKL/OPG expression in bone [187, 188]. The changes in bone formation and resorption and the bone loss induced by DM were corrected by treating with two different peptides derived from PTH-related peptide (PTHrP):(1–37)and (107–111), which, respectively, act through the PTH1R or cross-activate the VEGF receptor [187]. Reversal of the bone loss in DM was also reported with similar doses of PTH [188]. The changes in gene expression were also reversed by the PTHrP fragments, except for the increased DKK1 expression. DM mice also display increased prevalence of osteocyte apoptosis, which was inhibited by the PTHrP peptides [187], in particular [1–37]. Osteocytic MLO-Y4 cells cultured in high glucose also showed increased apoptosis. Moreover, PTHrP [1–37] prevented the increase in osteocytic cell apoptosis and increased Bcl2 levels, activated the survival kinases ERKs, and induced nuclear translocation of the canonical Wnt signaling mediator β-catenin. Further, in a recent study, Bonnet and colleagues studied the role of Peroxisome proliferator-activated receptor γ (PPARγ) in osteocytes on body composition and glucose metabolism using a high-fat diet model [189]. Mice lacking PPARγ in osteocytes had less fat, enhanced insulin sensitivity, and energy expenditure compared with wild-type mice. When fed with a high-fat diet, mice lacking PPARγ in osteocytes retain glycemic control, suggesting that osteocytes regulate glucose homeostasis through a PPARγ-dependent mechanism. These findings suggest a crucial role of osteocytes in the harmful effects of diabetes on bone and raise the possibility of targeting these cells as a novel approach to treat skeletal deterioration in DM.

Osteocytes and Cancer

The skeleton is a preferred site for cancer metastasis. Current knowledge supports that multiple cells within the bone and bone marrow niche contribute to the development and progression of cancer in bone [190]. For many years, osteocytes have been the forgotten bone cells and considered inactive spectators in the bone/cancer niche. However, accumulating evidence over the last years indicates that osteocytes contribute to generate a microenvironment that is conducive to tumor growth, skeletal destruction, and bone pain [191, 192]. The first evidence suggesting a potential role of osteocytes in cancer in bone was provided by Giuliani and colleagues, who found an increase in apoptotic osteocytes in bones from multiple myeloma (MM) patients [193]. Consistent with this finding, analysis of murine models of MM bone disease revealed that the number of apoptotic osteocytes is increased in bone areas infiltrated with MM cells [194]. Mechanistic studies demonstrated that MM cells activate Notch signaling in osteocytes, which in turn triggered caspase-3-mediated apoptosis [194]. In addition, MM-derived tumor necrosis factor α (TNFα) sustains/amplifies osteocyte apoptosis, a second mechanism by which these cancer cells decrease the life span of osteocytes [194]. More recently, it has been suggested that MM cells can also stimulate osteocyte apoptosis by inducing autophagy [195].

Giuliani and colleagues also found a positive correlation between death osteocytes and number osteoclasts in bone samples from MM patients [193], suggesting that apoptotic osteocytes could target bone resorption to particular areas of the bone infiltrated with MM cells. This notion is supported by in vitro experiments showing that apoptosis increases RANKL expression and enhances the ability of osteocytes to attract osteoclast precursors and stimulate osteoclastogenesis

[193, 194]. Other factors released by osteocytes, including interleukin (IL)-11, could also play a role in the increased resorption induced by the growth of MM cells in the bone [193]. In addition, the expression of the Wnt target gene osteoprotegerin (OPG) is also decreased in osteocytes cultured with MM cells, thus increasing even further the RANKL/OPG and their osteoclastogenic potential [194].

Moreover, osteocytes also participate in the suppression of osteoblast differentiation and new bone formation induced by cancer cells by increasing the levels of Sclerostin in the microenvironment [194, 196]. Osteocytes overproduce SOST/Sclerostin in MM-colonized bones, leading to Wnt signaling inhibition and impairing osteoblast differentiation [194]. In addition, it has been shown that Sclerostin could also promote breast cancer cell migration, invasion, and bone osteolysis [197]. Importantly, genetic and pharmacologic inhibition of SOST/Sclerostin signaling increases osteoblast number and bone formation and prevents bone destruction in several preclinical models of cancer-induced bone disease [197–200] (*see section* "Neutralizing Antibodies Against Sclerostin"). Importantly, inhibition of Sclerostin did not alter tumor growth in MM mouse models, whereas it decreased tumor proliferation in breast cancer models. Further studies are required to fully understand the contribution of SOST/Sclerostin signaling to tumor growth.

Osteocytes also can support the growth of cancer cells. Osteocytes activate Notch signaling in MM cells and stimulate tumor growth [194]. Further, osteocyte-induced bone resorption could also enhance the release of matrix factors stimulating tumor growth. Moreover, osteocytes produce chemokine (C-C motif) ligand 5 in response to mechanical signals (pressure) from the growth of prostate cancer cells in the bone marrow niche, which favors the growth and invasion of prostate cancer cells into bone [201]. Also, recent findings suggest that osteocytes, in addition to osteoblasts, may act as a cell of origin for osteosarcoma [202]. Interestingly, recent data suggest that osteocytes can communicate with sensory nerves and also can contribute to cancer-induced bone pain [203].

Regulation of Body Composition and Whole-Body Metabolism by Osteocytes

The skeleton has recently emerged as an endocrine organ implicated in the regulation of whole-body composition and energy metabolism [204]. This function of bone has been attributed mainly to osteoblast-derived osteocalcin and lipocalin, which control insulin sensitivity and secretion, body composition, appetite, and energy expenditure [205, 206]. Growing evidence supports that osteocytes also play a part in the homeostasis of remote organs. For instance, ablation of osteocytes in mice leads to severe lymphopenia and complete loss of white adipose tissues (WAT), suggesting osteocytes can act as regulators of multiple organs [207]. Similarly, Ohlsson and colleagues showed that body weight regulates fat mass in an osteocyte-dependent manner, as depletion of osteocytes impaired the suppression of body weight induced by increased loading [208].

Another area of recent interest is the potential role of circulating osteocyte-derived Sclerostin in the regulation of adipose tissue and energy metabolism. In vitro, Sclerostin positively regulates the differentiation of cells of the adipocyte lineage and marrow adipocytes [209, 210]. Moreover, in vivo studies have shown that radiation increases Sclerostin expression and the number of bone marrow adipocytes, and this effect is blocked by anti-Sclerostin antibodies or genetic deletion of SOST [211]. Further, overexpression of Sclerostin increases peripheral fat and impairs glucose metabolism, whereas SOST knockout mice exhibited decreased peripheral fat weight [212]. Additionally, genetic and pharmacologic inhibition of SOST/Sclerostin partially prevented the increase in fat and alteration of glucose metabolism induced by administration of a high-fat diet [212]. Similarly, mice with constitutive activation of b-catenin or conditional deletion of LRP4 in osteocytes exhibit increased serum levels of Sclerostin, an effect accompanied by increased body fat, peripheral WAT mass, and impaired glucose metabolism [213]. In support of a potential role of serum Sclerostin in the regulation of body metabolism, clinical data

has shown that serum Sclerostin is increased in T2DM and correlates with body mass index and fat mass in both DM patients and healthy adults [214–216]. Further, altered fat distribution is found in patients with mutations in LRP5 that affect the interaction of Sclerostin with this Wnt co-receptor [217]. In a recent study, loss of the stimulatory subunit of G-proteins Gsα in osteocytes was associated with a progressive loss of WAT in gonadal and inguinal fat depots, even when these mice displayed high levels of serum Sclerostin [218]. These results suggest that Gsα controls other factors in osteocytes that could affect adipocytes differently than Sclerostin. Nevertheless, together, these findings support a novel endocrine function for osteocyte-derived Sclerostin that facilitates communication between the skeleton and distant organs and justify future investigations to explore the potential role of Sclerostin as an endocrine regulator of energy and fat metabolism. Whether Sclerostin regulates body fat by binding to LRP receptors and inhibiting Wnt signaling in adipocyte progenitors/adipocytes or stimulates the expression of circulating pro-adipogenic cytokines in other cells remains an open question. Thus, further investigation is required to determine the specific mechanisms by which Sclerostin regulates adipocyte biology.

Osteocyte as Therapeutic Targets for Skeletal Diseases

Bisphosphonates

Bisphosphonates stop bone loss by inhibiting the activity of bone-resorbing osteoclasts. However, the effect of bisphosphonates on bone mass cannot fully explain the reduction in fracture incidence observed in patients treated with these agents. Research efforts provided an explanation to this dichotomy by demonstrating that part of the beneficial effect of bisphosphonates on the skeleton is due to prevention of osteoblast and osteocyte apoptosis [57]. This pro-survival effect is strictly dependent on the expression of Cx43, as demonstrated in vitro using cells lacking Cx43 or expressing dominant negative mutants of the protein as well as in vivo using Cx43 osteoblast/osteocyte-specific conditional knockout mice [121]. Remarkably, this Cx43-dependent survival effect of bisphosphonates is independent of gap junctions and results from opening of Cx43 hemichannels [12, 74]. Hemichannel opening leads to activation of the kinases Src and extracellular signal-regulated kinases (ERKs), followed by phosphorylation of the ERK cytoplasmic target $p90^{RSK}$ kinase and its substrates BAD and C/EBPβ, resulting in inhibition of apoptosis. The anti-apoptotic effect of bisphosphonates is separate from the effect of the drugs on osteoclasts, as analogs that lack anti-resorptive activity are still able to inhibit osteoblast and osteocyte apoptosis in vitro [219]. Furthermore, a bisphosphonate analog that does not inhibit osteoclast activity prevented osteoblast and osteocyte apoptosis and the loss of bone mass and strength induced by glucocorticoids as well as apoptosis induced by unloading in mice [88, 220]. Preservation of the bone-forming function of mature osteoblasts and maintenance of the osteocytic network, in combination with lack of anti-catabolic actions, could open new therapeutic possibilities for bisphosphonates in the treatment of osteopenic conditions in which decreased bone resorption is not desired.

Neutralizing Antibodies Against Sclerostin

The preclinical findings showing that osteocyte-derived Sclerostin is a potent negative regulator of bone formation led to development of neutralizing antibodies against Sclerostin as a novel bone anabolic therapeutic approach [221–224]. Clinical data showed that treatment with anti-Sclerostin antibodies stimulates bone gain by enhancing osteoblast function while inhibiting osteoclasts, thus uncoupling bone formation and bone resorption. Anti-Sclerostin therapy has shown beneficial skeletal outcomes in osteoporotic patients. However, the anabolic effects of anti-Sclerostin attenuate with time, and after therapy discontinuation, BMD eventually returns

to pretreatment. Further, recent concerns have been raised about the development of serious cardiovascular adverse events in patients treated with anti-Sclerostin compared to those treated with alendronate or placebo [225, 226]. Of note, a bispecific antibody targeting both Sclerostin and DKK1 was developed and led to synergistic bone formation in rodents and nonhuman primates [227]. However, the performance of this bispecific antibody in humans is currently unknown. Thus, despite its beneficial effects on bone mass and fracture risk reduction, the FDA has not yet approved the use of anti-Sclerostin for the treatment of osteoporosis.

Another group of patients that could benefit from anti-Sclerostin therapy is the cancer population. High serum Sclerostin levels have been detected in patients with different types of cancers that grow in bone [196]. Further, preclinical data has demonstrated that treatment with anti-Sclerostin antibodies prevents cancer-induced bone loss and induces new bone formation [197–200], raising the possibility of using neutralizing antibodies against Sclerostin as new therapeutic approach to treat cancer-induced bone disease. Further studies are required to determine the source of Sclerostin (osteocytes vs cancer cells) and the mechanisms underlying its aberrant secretion. Without doubt, a deeper understanding of the pathophysiology of SOST/Sclerostin will lead to improve current therapies and the identification of novel therapeutic targets to combat skeletal diseases.

Neutralizing Antibodies Against RANKL

As mentioned above, accumulating evidence supports that RANKL production increases with age and its expression is altered in several skeletal diseases, explaining, at least in part, the imbalance between bone formation and bone resorption that lead to bone loss. In order to block RANKL signaling, denosumab, a fully human monoclonal antibody against RANKL, was developed as a novel therapeutic agent to inhibit RANKL-induced bone resorption [228, 229]. Denosumab binds to RANKL and blocks downstream signaling, thus inhibiting osteoclast differentiation and function and decreasing bone resorption. Given that osteocytes are a major source of RANKL in adult bones, it is likely that the potent anti-resorptive effects of denosumab on bone are due to the inhibition of osteocyte-derived RANKL. Ongoing long-term studies suggest that treatment with denosumab leads to bone density gains, even after 5 years of treatment [230]. However, therapy discontinuation is associated with bone loss, which can be attenuated by bisphosphonate administration [231]. Currently, denosumab is FDA approved for the treatment of postmenopausal women with osteoporosis at high risk for fracture, for the treatment of bone loss in patients with prostate or breast cancer undergoing hormone ablation therapy, as a treatment to increase bone mass in men with osteoporosis at high risk for fracture, and for the treatment of glucocorticoid-induced osteoporosis in men and women at high risk of fracture. In a recent study, Roodman and colleagues assessed the efficacy and safety of denosumab for the prevention of skeletal-related events in patients with newly diagnosed MM [232]. Denosumab showed non-inferiority for the prevention of skeletal-related events when compared to zoledronic acid, the mainstay therapy for MM patients. This study also provided suggestive clinical evidence of a potential anti-MM effect based on RANKL inhibition that requires further investigation. Based on these promising results, denosumab could soon become an additional option for the standard of care for patients with MM with bone disease. However, further studies are required to determine the specific contribution of osteocyte-derived RANKL to cancer-induced bone disease.

Proteasome Inhibitors

The proteasome is involved in the rapid degradation of proteins targeted with a chain of ubiquitin marks [233]. Cancer cells, in particular MM cells, are sensible to proteasome inhibitors (PI) due to their high proliferation and high pro-

tein synthesis rate. In fact, inhibition of the proteasome in MM cells leads to cell cycle arrest and apoptosis [234]. Preclinical and clinical data demonstrated that in addition to inhibiting tumor growth, PIs also affect the function of bone cells [235]. PIs decrease osteoclast formation and resorption capacity by inhibiting RANKL production in osteoblastic cells and NF-κB signaling in osteoclasts [236]. Further, treatment with PIs also increases bone formation and bone mineral density [236]. More recently, it was reported that Bortezomib, a PI use in the clinic for the treatment of MM, decreased the elevated osteocyte apoptosis seen in bones infiltrated with MM cells [195]. Further, the elevated circulating Sclerostin levels found in MM patients decreased by 50% after treatment with Bortezomib [237]. Together, these findings suggest that osteocytes are possible targets of therapies based on PIs; however, future studies are needed to determine if direct effects of PIs on osteocytes regulate their function and Sclerostin production.

Targeting Notch Signaling in Osteocytes

Notch signaling plays a critical role in cell-to-cell communication among bone and bone marrow cells under physiological conditions, and it favors growth and survival of cancer cells in bone. However, manipulation of this pathway results in different bone phenotypes depending on the Notch component (ligands, receptors, target genes), the cell lineage, or differentiation stage being targeted. In addition, the skeletal phenotypes of mice with alterations in Notch-related genes result from combined developmental and postnatal effects. In particular, the effects of Notch signaling in osteocytes remain conflicting. Overexpression of the intracellular domain of Notch receptor 1 (NICD1) in osteocytes increases trabecular bone volume due to a decrease in osteoclast number, a phenotype that evolves as mice mature [114]. Surprisingly, conditional deletion of Notch receptor 1/2 also increases trabecular bone volume and decreases osteoclasts, [238]. Mechanistically, Notch signaling in osteocytes increases OPG expression and activates Wnt signaling via activation of the Notch canonical transcription factors recombination signal-binding protein 1 for j-kappa (RBPJK) [238, 239]. To circumvent potential developmental issues induced by Notch signaling activation, Zaidi and colleagues conditionally activated Notch signaling in osteocytes in adult mice. Results showed that activation of Notch signaling in this scenario increases bone formation and prevents both age-associated and ovariectomy-induced bone loss [240]. Further, Canalis and colleagues showed that PTH signaling in osteocytes decreases Notch signaling [241]. In contrast, it was recently reported that constitutive activation of PTH signaling in osteocytes and intermittent administration of PTH increase Notch signaling in bone [242]. Moreover, conditional deletion of RBPJK in osteocytes further increases the elevated bone resorption induced by PTH signaling in osteocytes [242], suggesting that canonical Notch signaling in osteocytes restrains bone resorption and facilitates bone gain induced by PTH. Additionally, cell-to-cell interactions with MM cells activate Notch signaling in osteocytes, which in turn results in osteocyte apoptosis [194]. Similarly, in vitro, osteocytes overexpressing NICD1/NICD2 or cultured on plates coated with the Notch ligand DLL1 exhibited increased cell death [194], supporting that Notch signaling regulates osteocyte life span.

Pharmacologic inhibition of Notch signaling also results in disparate outcomes. For instance, pharmacologic inhibition of the Notch ligand Jagged1 inhibits mineralization [240]. In contrast, inhibition of Notch signaling using a novel bone-targeted gamma secretase inhibitor increases bone mass by decreasing osteoclast number and bone resorption, without affecting the rate of bone formation. Indeed, bone-targeted inhibition of Notch preserves the increase in bone formation and enhances the bone gain induced by intermittent PTH administration [243]. The discrepancy of these genetic and pharmacologic approaches highlights the complexity of the Notch signaling pathway in bone and its cell- and context-dependent nature. Future research is warranted to define the specific effects of Notch signaling in osteocytes and to determine the

effectiveness of targeting Notch signaling to treat skeletal diseases.

Conclusions and Future Directions

Recent advances have provided compelling evidence supporting the notion that osteocytes are multifunctional cells that regulate skeletal homeostasis by controlling osteoblast and osteoclast activity (Fig. 3.1). Identification of some of the molecular mechanisms by which osteocytes control osteoblasts and osteoclasts provides the mechanistic basis to develop novel therapeutic approaches to treat skeletal maladies. Further, osteocytes, at least, in part, mediate some of the positive effects of common treatments for bone diseases (PTH, bisphosphonates, or sex steroids). In addition, now we know that osteocytes play a major role in several bone disorders, including those secondary to diabetes and the growth of cancer cells in bone (Fig. 3.1). Another emerging and exciting area of study is the potential role of osteocytes in the regulation of body composition

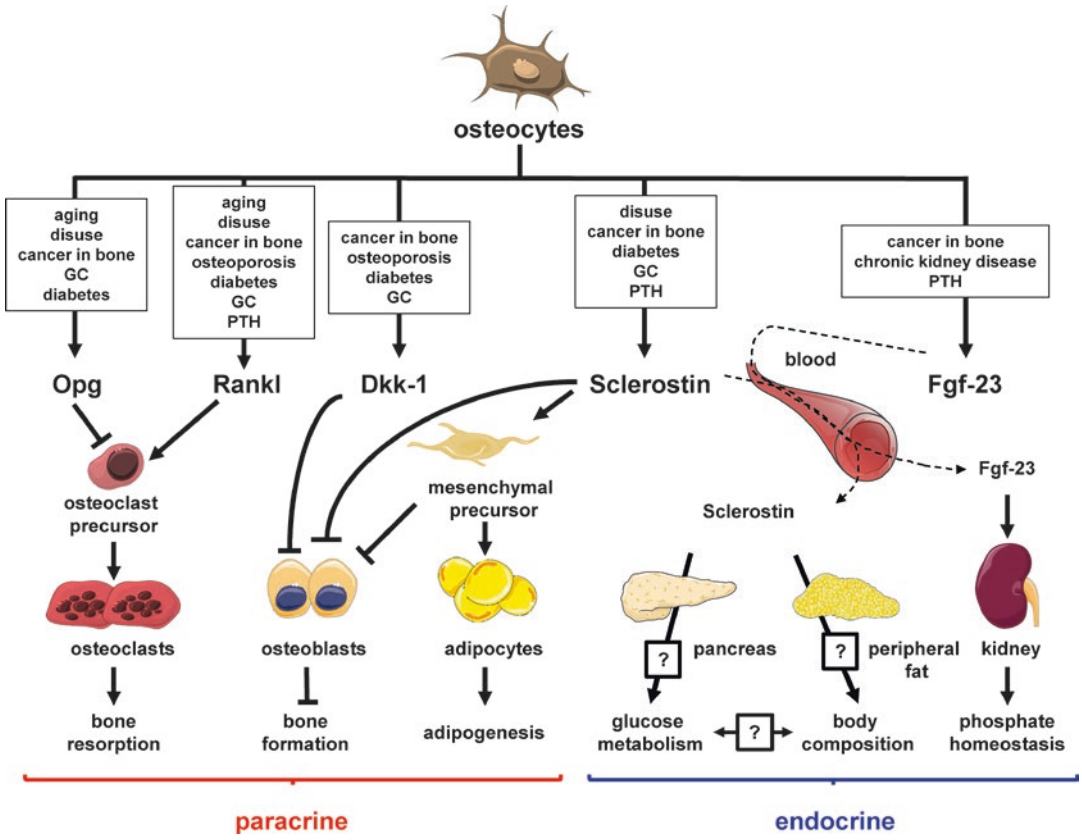

Fig. 3.1 Actions of osteocytes and their derived factors in bone physiology and pathophysiology. Osteocytes regulate bone resorption and bone formation in response to both physical and hormonal stimuli through the secretion of paracrine factors. Osteocytes express the anti-osteoclastogenic cytokine OPG and are the main source of RANKL in adult bone, an essential regulator of osteoclast differentiation and survival. Through the secretion of the Wnt antagonists Sclerostin and DKK1, osteocytes negatively regulate osteoblast differentiation and function. Osteocytes also influence distant organs via secretion of circulating factors. Endocrine actions of osteocyte-derived FGF-23 regulate phosphate and vitamin D homeostasis. In addition, emerging evidence suggest that Sclerostin contributes to the regulation of whole-body composition and glucose metabolism. Importantly, the decrease in osteocyte life span and alteration of their expression profile underlie the pathophysiology of a number of skeletal disorders. Thus, secretion of several osteocytes-derived factors is dysregulated during aging, when cancer cells grow in bone, by sustained high levels of blood glucose (DM), in osteoporosis, as well as in response to elevation of both endogenous and exogenous GC and PTH levels

and energy balance (Fig. 3.1). Further studies are warranted to determine the specific contribution of these cells to whole-body metabolism and whether osteocytes and their derived factors could be suitable targets for the treatment of metabolic diseases.

Despite the remarkable advance in our understanding of osteocyte function in the last decade, several important aspects of osteocyte biology remain unclear. For instance, it is evident that the life span and genetic signature of osteocytes are altered in bone diseases. However, the mechanisms underlying these alterations and the consequences for bone homeostasis are not fully understood. Future studies involving unbiased single-cell data analysis (RNA-seq, methylome, miRNAome, epigenomics) should provide a full landscape of the molecular changes leading to the dysregulation of osteocyte function and help to identify novel therapeutic targets. Further, part of the paucity of scientific data on osteocytes is due to the difficulties in isolating these cells from bone and maintaining in vitro their characteristic in vivo phenotype. The establishment of osteocyte-like cells and identification of promoters (Dmp1) driving genetic recombination in osteocytes have furthered our understanding of osteocyte function. However, these cell lines do not entirely reproduce the phenotypic features of fully differentiated osteocytes, and the Dmp1 promoters can also target subpopulations of mature osteoblasts. Thus, future research efforts are needed to develop better in vitro and ex vivo models and new genetic tools to specifically target osteocytes in vivo. In conclusion, osteocytes play a major role on bone physiology and pathophysiology, and full understanding of the mechanisms by which they control osteoblasts and osteoclasts, and potentially other cells in distant organs, will increase the repertoire of pharmacological approaches towards better and safer treatments for bone diseases.

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Vitamin D and Bone Health: Basic and Clinical Aspects

4

Roger Bouillon and Michaël R. Laurent

Key Points

- Vitamin D, either from endogenous origin (under the influence of ultraviolet B (UVB) light) or from diet, is a precursor for 25-hydroxyvitamin D (25(OH)D) and the active hormone 1α,25-dihydroxyvitamin D (1,25$(OH)_2$D), the ligand of the nuclear receptor vitamin D receptor (VDR).
- Correction of severe vitamin D deficiency may restore intestinal calcium absorption and cure rickets and osteomalacia, while correction of more moderate vitamin D deficiency may reverse secondary hyperparathyroidism and excessive bone turnover in osteoporosis.
- Vitamin D and calcium supplements have a modest effect on fracture prevention if they are used in a population with severe vitamin D deficiency and low calcium intake, particularly in the frail elderly at high risk of falls and fractures, and if they are taken compliantly, for example, in a nursing home setting.

Introduction

Osteoporosis is defined as a structural deficit in bone mass and microarchitecture, which leads to decreased bone strength and increased fracture risk. The diagnosis of osteoporosis can be made in postmenopausal women or men aged 50 and older after they sustain a low-impact fracture, or when their bone mineral density (BMD) T-score is ≤2.5 SD compared to peak bone mass.

Osteoporosis is a disease with multifactorial origin. Indeed, many pathogenic mechanisms have been identified. The two most powerful clinical risk factors for osteoporosis are ageing and estrogen deficiency, particularly in women following menopause. Bone mass increases from birth to young adulthood (peak bone mass) and, thereafter, gradually declines with accelerated bone loss after menopause in women. A lower peak bone mass is thus a risk factor for osteoporosis later in life and, therefore, osteoporosis

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may have both genetic as well as environmental origins in childhood (see Chaps. 6 and 25).

Rickets is defined as a disorder of chondrocyte differentiation and mineralization of the growth plate as well as defective osteoid mineralization, caused by severe vitamin D deficiency and/or low calcium intake or absorption in growing children [1]. Osteomalacia is the same disorder but after the epiphyses have fused, that is, a disease characterized by excess unmineralized osteoid. Rickets and osteomalacia are eminently preventable and nutritional forms easily cured with even low doses of calcium and vitamin D [2].

There is no universally agreed definition of vitamin D deficiency. Serum 25-hydroxyvitamin D (25(OH)D) concentrations below 30 nmol/l are universally considered to represent severe vitamin D deficiency [3, 4] and, when present for sufficient time, generates a risk for rickets or osteomalacia. Most guidelines consider serum 25(OH)D levels below 50 nmol/L as deficiency [3]. The Endocrine Society guidelines also define vitamin D deficiency as 25(OH)D < 50 nmol/L but introduce "vitamin D insufficiency" if concentrations fall between 50 and 75 nmol/L [5]. The US Institute of Medicine committee defined serum 25(OH)D concentrations below 30 nmol/L as a risk factor for rickets/osteomalacia and proposed that 40 nmol/l would be sufficient for the median population requirements, whereas concentrations ≥50 nmol/L are sufficient for 97.5% of the normal human population. They also point out that secondary hyperparathyroidism occurs mainly when serum 25(OH)D concentrations falls below 40–50 nmol/L while intestinal calcium absorption does not increase further when 25(OH)D is above 20–50 nmol/L [6]. The role of vitamin D and/or calcium in peak bone mass acquisition or in middle life is less clear in epidemiological studies and randomized controlled trials (RCTs) since most long-term observational and intervention studies have focused on the role of vitamin D in the treatment of osteoporosis and prevention of fractures in older adults or the very elderly population.

We will first review the metabolism and actions of vitamin D in general. Next, we will provide an overview of the mechanisms of action of vitamin D on bone based mainly on preclinical studies. Finally, we will review the potential contributions of vitamin D for the prevention or treatment of osteoporosis.

Vitamin D Metabolism and Actions

Vitamin D Photosynthesis and Absorption from Diet

The dual origin of vitamin D, which can be derived from either nutritional sources or from sunshine exposure to the skin, was discovered about a century ago when vitamin D was identified as the agent capable of curing or preventing rickets in children or animals [7–9].

Regarding nutritional sources, vitamin D is found mostly in oily fish or egg yolk and, in minor quantities, in some other food items (Fig. 4.1). Most dietary vitamin D has the vitamin D_3 structure but vitamin D_2 is also present in some plants (e.g., mushrooms exposed to ultraviolet B (UVB) light). The nutritional intake of vitamin D is however low (below 5 μg [=200 IU]/day) in most European countries except in Scandinavian countries where the population has a high consumption of oily fish and/or cod liver oil [10]. Most other populations in the world have a low vitamin D intake unless food is regularly fortified with vitamin D (e.g., in the USA, Canada, India, and Finland [11]). Vitamin D from dietary sources is absorbed from the intestine by cholesterol-transporting proteins. It then becomes incorporated into chylomicrons for transport via the lymphatic drainage to the bloodstream. Polar metabolites such as 25(OH)D and 1α-hydroxylated metabolites are, on the other hand, absorbed via the portal venous system [12].

The epidermis of the skin is able to transform, by a photochemical reaction, 7-dehydrocholesterol (7DHC) into previtamin D and vitamin D when exposed to UVB light (280–310 nm). Most vertebrates are able to synthesize vitamin D as long as they are exposed to UVB light and have sufficient 7DHC in the irradiated cells. Felines (both

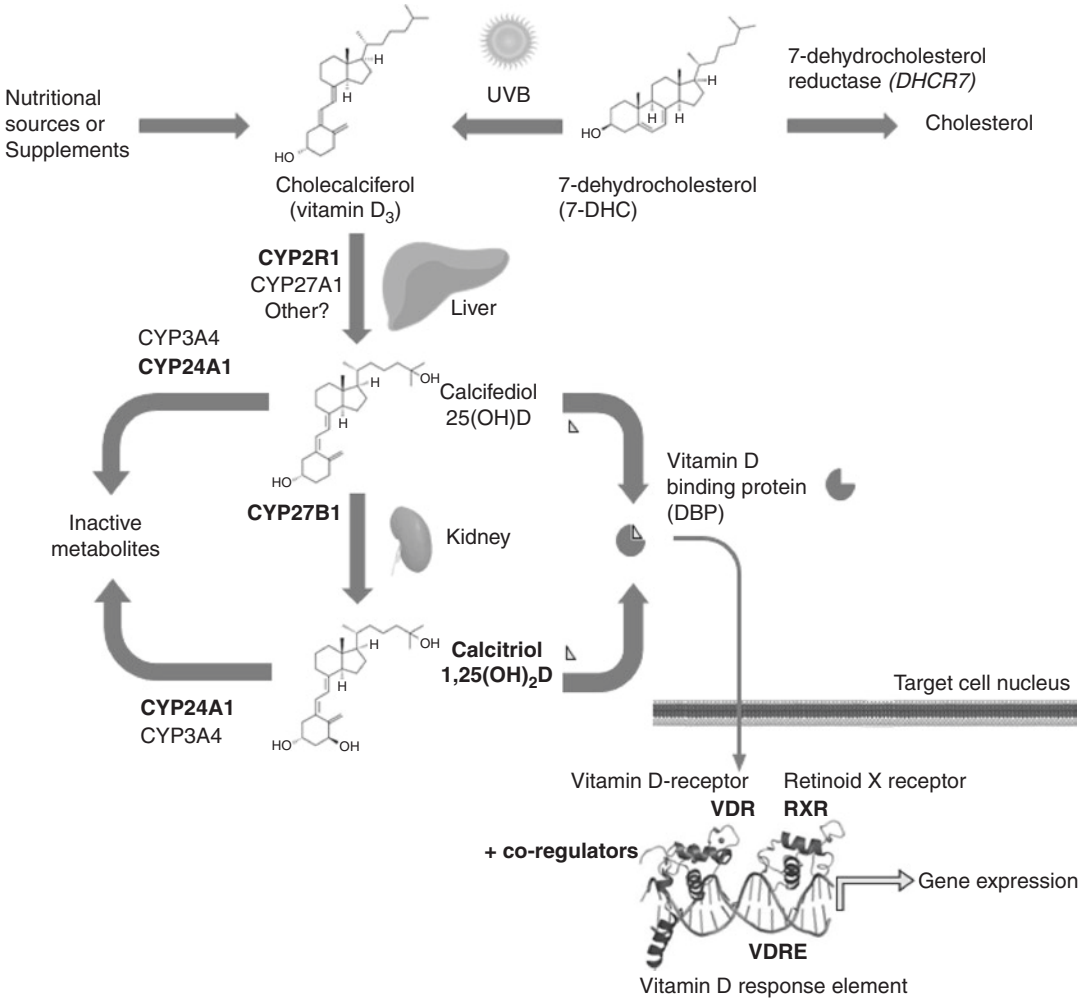

Fig. 4.1 Simplified diagrammatic representation of vitamin D metabolism and action on target cells

domestic and big cats) as well as dogs have such a low 7DHC concentration in their keratinocytes that they cannot synthesize vitamin D. For these species only, vitamin D is a true vitamin. Unfortunately, the same UVB light required for vitamin D photosynthesis also causes DNA damage to the skin which may, albeit with a long lag time, result in photoageing of the skin and increase the risk of skin cancer, including the more aggressive types like melanoma [13–15]. UVB light can thus be considered an oncogene. There is much debate on how to strike a safe balance between sufficient exposure to sunlight as to generate vitamin D and avoidance of sunlight to decrease the risk of skin diseases. The conversion of previtamin D into vitamin D is a rather slow process (hours). With even longer skin UVB exposure, previtamin D is paradoxically converted into inactive vitamin D-related compounds. As a result, exposure of the skin for longer than 15–30 min does no longer increase the net vitamin D production, while DNA damage nevertheless continues to accumulate [8]. Although previous studies have shown that prolonged sunbathing does not induce hypervitaminosis D, two recent case reports suggest that this could be the case following excessive tanning bed use (although this requires further controlled study) [16, 17].

Regulation of 25-Hydroxyvitamin D

The liver is the main tissue responsible for the conversion of vitamin D into a more polar metabolite, 25(OH)D. CYP2R1 is the major enzyme responsible for the production of 25(OH)D. It is located in the cytoplasm and metabolizes vitamin D_2 and vitamin D_3 equally well. The K_m shows a low capacity but high affinity reaction. Homozygous deficiency of this enzyme causes (type 1B) vitamin D-dependent rickets in humans [18, 19]. Interestingly, there is spontaneous clinical improvement in later childhood in this form of vitamin D-dependent rickets. Also in mice, biallelic mutations in *Cyp2r1* cause a marked but not complete decrease in 25(OH)D, which does not cause rickets in such animals [20]. This led to the realization that CYP2R1 is not the only 25-hydroxylase in the liver as at least mitochondrial CYP27A1 and possibly other microsomal cytochrome P450 enzymes are able to produce 25(OH)D [21]. So far, CYP2R1 was considered to be constitutively expressed in the liver and not regulated. More recent data however suggest that obesity, diabetes, or fasting can decrease the expression of this enzyme and contribute to the lower serum 25(OH)D concentrations found in obese or diabetic subjects (or animals) compared with their healthy counterparts [22].

Regulation of Active Vitamin D

25(OH)D is still a precursor that needs a second hydroxylation step at carbon 1 to become the active hormone, 1α,25-dihydroxyvitamin D ($1,25(OH)_2D$). There is only one enzyme, CYP27B1, capable of this metabolic activation. This enzyme is mainly expressed in the renal tubular cells and is under strict positive feedback control by parathyroid hormone (PTH) and negative feedback control by fibroblast growth factor 23 (FGF23). The 1α-hydroxylation enzyme is however also expressed at low levels in many other cells and tissues such as in keratinocytes, monocytes/macrophages and osteoclasts [23], glia cells, pancreas, testes, parathyroid cells, and bone cells of the osteoblast-lineage [24]. In these tissues, other factors (such as cytokines) regulate the enzymatic activities. It is generally believed that only the kidney exports $1,25(OH)_2D$ into the circulation whereas extra-renal $1,25(OH)_2D$ has only a local autocrine or paracrine action, except in pathological situations like severe sarcoidosis and certain hematologic malignancies [25]. Congenital absence of CYP27B1 results in (type 1A) vitamin D-dependent rickets, as it can be prevented or cured by physiologic doses of $1,25(OH)_2D$ or by pharmacologic doses of vitamin D or 25(OH)D (because 25(OH)D itself is a weak agonist for the vitamin D receptor (VDR)) [19, 26].

$1,25(OH)_2D$ has a much shorter half-life in serum (about 4 h) than 25(OH)D. Catabolism of $1,25(OH)_2D$ is mainly regulated by a single enzyme, CYP24A1, responsible for a multistep enzymatic conversion into 24R metabolites and finally calcitroic acid or lactones. CYP24A1 is expressed in many cells and is strongly upregulated by $1,25(OH)_2D$ in a negative feedback control loop. Absence of this enzyme severely impairs the degradation of 25(OH)D and $1,25(OH)_2D$ and results in excess $1,25(OH)_2D$, hypercalcemia and kidney stones, or nephrocalcinosis as demonstrated in animals and humans (idiopathic hypercalcemia of infancy or nephrocalcinosis/nephrolithiasis in adults) [27–30].

CYP3A4 also degrades 25(OH)D, which may lead to severe vitamin D deficiency and even osteomalacia/rickets in patients treated with strong enzyme inducers such as rifampin or ketoconazole [31]. Moreover, an activating *CYP3A4* mutation has recently been described as a novel (type 3) genetic form of vitamin D-dependent rickets [2, 32].

Overall, there are at least 50 known metabolites of vitamin D (including 3epi-25(OH)D and 1epi,25-$(OH)_2$) but the biological activity (if any) of most of these metabolites other than 25(OH)D and $1,25(OH)_2D$ has not been fully evaluated [33]. However, $1,25(OH)_2D$ is the most crucial metabolite as its absence alone causes rickets. Recently, however, delayed fracture healing was reported in mice lacking *Cyp24a1* [34]. Indeed, $24R,25(OH)_2D$ binds to a specific membrane receptor FAM57B2 and thereby stimulates the synthesis of a matrix protein, lactoceramide. Mice lacking this membrane receptor displayed a simi-

lar delay in fracture healing which could be corrected by administration of lactoceramide but not by administration of 24R,25$(OH)_2$D. This demonstrates that the physiological role of the many vitamin D metabolites is a topic of interest, and that the effects of the vitamin D endocrine system should also be explored in stress situations.

Role of Vitamin D-Binding Protein

The vitamin D-binding protein (DBP or GC globulin) binds vitamin D (and all other vitamin D metabolites) and functions as a carrier or chaperone protein to transport vitamin D in the bloodstream [35]. DBP influences the total 25(OH)D and 1,25$(OH)_2$D concentrations in serum, whereas free or nonprotein-bound vitamin D is believed to be the main effector on target cells. Still, the clinical usefulness of measuring free 25(OH)D or 1,25$(OH)_2$D requires further study [35, 36]. One of the functions of DBP is to prolong the circulating half-life of vitamin D metabolites and, thus, protect against vitamin D-deficiency during short-term deprivation [37]. 25(OH)D circulates in serum with a half-life of several weeks [38] due to its tight binding to DBP. There are three major variants of DBP, but despite many epidemiological studies, there is no consistent evidence that genetic variation in DBP influences any outcome other than circulating 25(OH)D concentrations [39]. Mice with total absence of DBP develop normally and have a normal bone phenotype despite extremely low serum concentrations of 25(OH)D and 1,25$(OH)_2$D. This can be explained by their normal concentrations in serum and tissues of free 1,25$(OH)_2$D, in support of the free hormone hypothesis [40].

Vitamin D Receptor Actions

The vitamin D hormone, 1,25$(OH)_2$D, acts mainly by binding to its cognate nuclear receptor, the vitamin D receptor (VDR). Nongenomic actions via binding to a putative membrane receptor have also been described, but the physiological relevance hereof remains controversial. Ligand binding to the VDR induces conformational changes, which activate a complex reaction of heterodimerization of VDR to retinoid X receptor (RXR), recruitment of cofactors, and binding to specific DNA sequences called vitamin D response elements (VDREs). Although the sequence in which these events occur remains incompletely understood, it ultimately leads to enhanced or repressed gene transcription [41]. Homozygous *VDR* deficiency causes (type 2A) vitamin D-resistant rickets, which is almost always accompanied by alopecia. In rare human or primate cases of type 2B vitamin D-resistant rickets, there is functional incapacity of the VDR, possibly due to heterogeneous nuclear riboprotein (hnRNP) overactivity, although the genetic basis of this disorder remains unknown [42, 43].

The genomic actions of 1,25$(OH)_2$D regulate a very large number of genes and it seems that about 3% of the human, mouse, or zebra fish genome is modified (directly or indirectly) by 1,25$(OH)_2$D. This is much more than potentially needed for regulation of calcium, mineral, or bone metabolism. Early in the life of zebra fish, 1,25$(OH)_2$D regulates up to 10% of its genes [44], and as in humans and mice, this includes many gene clusters related to cell proliferation, cell differentiation, immune function, and so on, whereas only a minority of the genes are really involved in calcium homeostasis. These and other preclinical and clinical data generated a plausible hypothesis that vitamin D, like many other nuclear receptor endocrine systems, not only regulates calcium and bone homeostasis but also has a wide variety of extra-skeletal actions. These extra-skeletal actions will not be discussed in this chapter but has been extensively reviewed recently elsewhere [4, 8, 45, 46]. 1,25$(OH)_2$D also clearly shows nongenomic rapid actions in many cells but their molecular mechanisms and in vivo significance remain unclear.

Vitamin D Actions on Calcium and Bone Homeostasis: Basic Biology

The action of the vitamin D metabolites on calcium homeostasis is the result of its action in all tissues with intensive calcium transport such as

the intestine, kidney, and bone (and transiently also breast and placenta during reproduction and lactation) or calcium sensing tissues (parathyroid glands).

Intestinal Calcium Absorption

In the intestine, $1,25(OH)_2D$ upregulates the calcium transport system. Specifically, it stimulates the expression of *TRPV6*, the major calcium influx channel, on the luminal side of enterocytes. This channel is constitutively open and $1,25(OH)_2D$ regulates the number of these channels via several VDREs in its promoter (Fig. 4.2) [47].

Several intracellular calcium-binding proteins (especially calbindin 9 K or CaBP9K) remove the calcium ions from the intracellular site of the TRPV6 channels to allow these channels to transport new calcium ions. CaBP9K is also strongly upregulated by $1,25(OH)_2D$ and probably functions as a calcium shuttle between the mucosal and serosal site of the intestine. Deletion of CaBP9K however does not create a calcium/bone phenotype, suggesting that this protein is largely redundant [48]. Deletion of TPRV6 decreases the active intestinal calcium absorption and double deletion of both TRPV6 and CaBP9K even further decreases the calcium absorption but a fraction of calcium absorption still remains $1,25(OH)_2D$-dependent [48], [49]. Finally, an energy (ATP) requiring step is the transport of calcium ions over the serosal mucosa of the intestine into the blood stream by the PMCA1b pump. Expression of this gene is modestly stimulated by $1,25(OH)_2D$. Tissue-selective deletion of the PMCA1b pump has not yet been reported. However, based on available data, it seems likely that there is still a missing link in vitamin D-driven intestinal calcium absorption. $1,25(OH)_2D$ also stimulates paracellular calcium transport and the effect of vitamin D on claudins may be related to this action [49]. Although the duodenum is considered a critical site for $1,25(OH)_2D$-dependent regulation of intestinal calcium absorption, 70%–80% of ingested calcium is actually absorbed in the distal intestine, and transgenic VDR overexpression in the distal

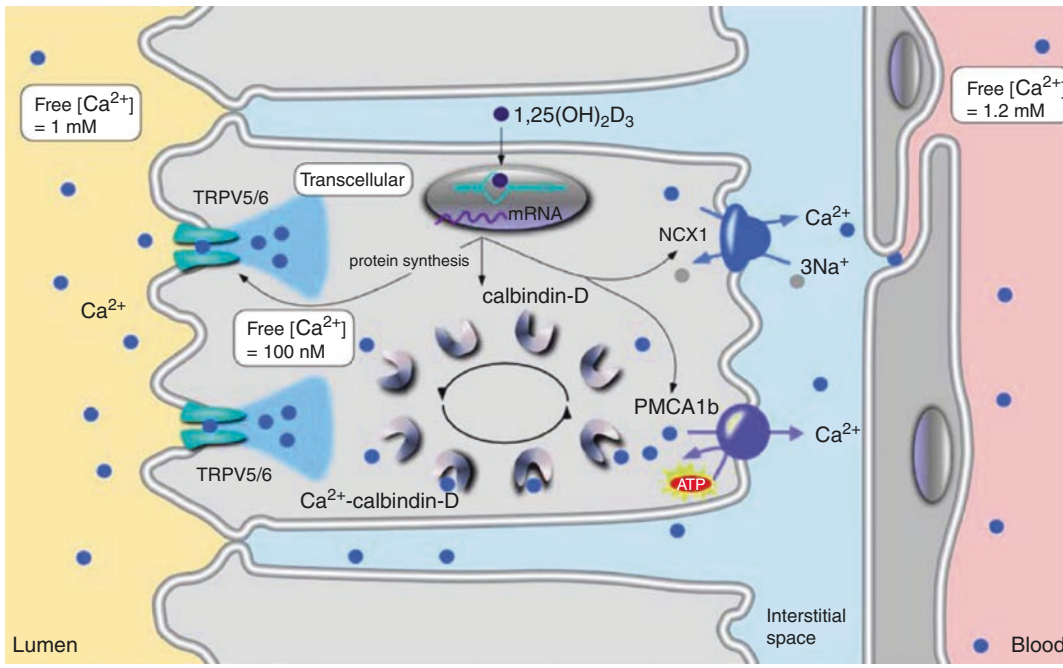

Fig. 4.2 Vitamin D actions on active transepithelial calcium transport systems in the gut and the kidneys. (Reprinted from van Abel et al. [118]. With permission from Springer Nature)

intestine also mitigates the rachitic phenotype of VDR knockout mice [50]. However, it should be noted that intestinal calcium absorption also occurs independent of vitamin D, which explains why (extremely) high-dose oral calcium supplements (if tolerated) can be used to treat vitamin D-resistant rickets caused by homozygous VDR mutations [51].

Effects of Vitamin D on the Kidneys, Placenta, Breast, and the Parathyroids

In the distal renal tubuli, $1,25(OH)_2D$ stimulates the expression of *TRPV5* (the equivalent of TRPV6 in the intestine) and other intracellular calcium transporting proteins including the sodium–calcium exchanger (NCX1) but to a relatively lower extent than in the intestine and compared to the direct effect of PTH. The net result of $1,25(OH)_2D$ is a stimulation of the relative reabsorption of calcium in the renal tubuli.

The placenta and lactating breast are major (net) calcium transporters during reproduction. Both tissues express VDR but transport of calcium in these tissues is not strongly regulated by $1,25(OH)_2D$ [52]. The parathyroid glands also express *VDR* and *CYP27B1*. $1,25(OH)_2D$ directly inhibits the synthesis and secretion of parathyroid hormone, independent from the extracellular calcium concentration. Selective deletion of VDR in the parathyroid gland results in modest secondary hyperparathyroidism which stimulates bone resorption markers. However, overall calcium and bone homeostasis remains within the normal range in this genetic mouse model [53].

Skeletal Target Cells of Vitamin D

All bone cells express the VDR and react to exposure of $1,25(OH)_2D$. This promotes mesenchymal precursor cell commitment to the osteoblast lineage and inhibits differentiation into the adipocyte lineage, at least in part via effects of VDR on the Wnt pathway [54]. In most in vitro experiments, $1,25(OH)_2D$ inhibits the proliferation and stimulates the early maturation of (pre) osteoblast. Its effect on final maturation stages is however disputed as in some experiments it inhibited, while in other studies, it stimulated mineral deposition (depending on species, culture conditions, or the maturation stage of the cells) [54]. Osteocytes also respond to $1,25(OH)_2D$, although it has a more selective cistrome in these cells [55]. It nevertheless strongly stimulates the transcription and secretion of *RANKL* (receptor activator of nuclear factor kappa B ligand, a crucial mediator of osteoclastogenesis) and *FGF23*, genes that are expressed more strongly in osteocytes than in osteoblasts [55]. Osteoprotegerin, the decoy receptor for RANKL on the other hand, is suppressed by $1,25(OH)_2D$. $1,25(OH)_2D$ is also a potent stimulus for osteoclastogenesis starting from monocytic precursor cells. The pro-osteoclastic activities of $1,25(OH)_2D$ have been clearly demonstrated in the presence of osteoblasts. However, in the absence of osteoblasts or in osteoclast-specific *Vdr* or *Cyp27b1* knock-out mice, $1,25(OH)_2D$ has anti-osteoclastic activities [56], perhaps mediated by its action on c-Fos, interferon, HIF-1a, and S1PR2, a chemorepulsive receptor [57]. Although $1,25(OH)_2D$ is a very strong inducer of osteoclastogenesis, its role in later stages of mature multinucleated osteoclasts is more disputed. Selective deletion of *Vdr* (but not *Cyp27b1*) in osteoclasts driven by a late-stage Cre promotor (cathepsin K) paradoxically generated a bone phenotype with loss of bone rather than the expected higher bone mass [56]. However, as we will discuss in the next paragraph, the effects of vitamin D on individual types of bone cells should not be considered in isolation.

Understanding the Vitamin D Conundrum: Whole-Organism Physiology and Dose-Dependent Effects

When determining the effects of vitamin D on bone based on the previously mentioned and many other studies, one may reach apparently very different conclusions at first glance (which

Table 4.1 The vitamin D conundrum: the good, the bad, and the redundant effects of vitamin D on bone health

A. Vitamin D is good for bone
Absence of $1,25(OH)_2D$ or VDR impairs mineralization and causes rickets or osteomalacia
Vitamin D can prevent or cure vitamin D-dependent or nutritional rickets/osteomalacia
Vitamin D promotes osteoblast differentiation and bone mineralization in vitro
Vitamin D inhibits mature osteoclasts and bone resorption directly [56]
Mice with transgenic overexpression of *Vdr* or *Cyp27b1* in osteoblasts have increased bone mass and strength [58, 59]
Mice treated with daily doses of $1,25(OH)_2D$ or its analogs show a marked increase in (mainly trabecular) bone mass due to suppressed bone resorption [60, 61]
B. Vitamin D is redundant for bone
High-dose calcium supplementation can rescue the bone and mineral deficits in *Vdr* knock-out mice or patients with vitamin D-resistant rickets [62]
Osteocyte-specific and some osteoblast-specific *Vdr* knock-out mice have a normal bone phenotype [49, 61]
C. Vitamin D is bad for bone
Chronic and/or high-dose vitamin D impairs mineralization [63] and causes rickets/osteomalacia [60, 64] or induces bone loss [65, 66]
High $1,25(OH)_2D$ levels impair mineralization via *Vdr* in osteocytes [67]
$Vdr^{-/-}$ bone cells show accelerated bone mineralization in vitro, and enhanced bone formation when transplanted in control animals
Mice with conditional *Vdr* knock-out in osteoblasts [68] or chondrocytes [69] have increased bone mass with lower bone resorption; thus, vitamin D stimulates osteoclastogenesis and bone resorption [70]

are still however all correct!): there are data that vitamin D is good for bone and that vitamin D is neutral or redundant for bone, or bad for bone (Table 4.1).

These apparently paradoxical results can be explained by two factors. First, vitamin D may have unfavorable skeletal effects through its direct actions on some target cells, which may however be overruled by favorable skeletal effects via other target cells. For example, $1,25(OH)_2D$ has the deleterious effect of inducing osteoclastogenesis in vitro, while in osteocytes, it upregulates RANKL and mineralization inhibitors, and in osteoblasts or chondrocytes, *Vdr* deletion has been shown to produce counterintuitive, beneficial skeletal effects [68, 69]. However, other studies show that osteoblast-specific *Vdr* deletion has no effect [61] or that *Vdr* or *Cyp27b1* overexpression in osteoblasts has favorable effects [59], and any of these effects would be superseded by the effects of vitamin D deficiency on intestinal calcium absorption and resultant hypocalcemia [67]. Surely, cell-specific gain- or loss-of-function models have tremendously increased our understanding of the target cells of some of vitamin D's actions independent of systemic dysregulations like hypocalcemia. However, to define the net effect of the vitamin D endocrine system on calcium and bone homeostasis as a whole, it might be better to evaluate the effects of global *VDR* (or *CYP27B1*) deficiency or excess.

Second, however, even when one disregards the cell-specific models, different studies have still reached opposite conclusions. This part of the conundrum can be explained by dose and threshold effects. Indeed, like many other vitamins, a beneficial effect can only be expected when correcting a deficiency, whereas a supraphysiological or toxic dose may have an opposite, counterproductive effect. For example, it is well known that BMD may rapidly and substantially increase if patients with severe nutritional or vitamin D-dependent rickets are treated with vitamin D. Conversely, BMD gains can be observed with resolution of vitamin D intoxication [66].

In our current model of understanding of the whole-organism physiological effects of vitamin D deficiency, the detrimental skeletal effects are determined by impaired intestinal calcium absorption. Indeed, the bone phenotype of intestinal $Vdr^{-/-}$ mice is more severe than that of global $Vdr^{-/-}$ mice [67]. This would lead to the dangerous situation of hypocalcemia if not corrected by secondary hyperparathyroidism, upregulation of $1,25(OH)_2D$, and compensatory stimulation of intestinal calcium absorption, renal calcium reabsorption, and net skeletal calcium efflux. In other words, because severe hypocalcemia would lead to muscle dysfunction, seizures, arrhythmias, coagulopathy, and other physiological catastrophes, the body tries to avoid this at the cost of skeletal integrity. Indeed, vitamin D is, in the first place, a plasma calcium-maintaining hormone. As such it can indeed be redundant: with intra-

venous or very high-dose oral calcium, one can completely reverse the phenotype of VDR deficient children or animals, while there is no skeletal benefit of vitamin D whatsoever if there is no intestinal calcium influx (e.g., if there is no calcium in the diet or if the intestine is compromised) [67]. In less severe circumstances, for example, in elderly vitamin D-deficient subjects with poor dietary calcium intake and absorption, secondary hyperparathyroidism increases bone turnover which results in osteoporosis. The bone loss releases calcium, which, together with more efficient renal calcium handling, compensates for the lack of intestinal influx in the face of daily obligate calcium losses. Interestingly, if calcium absorption is more severely compromised as in rickets or osteomalacia, calcium is not only released by osteoclastic bone resorption, vitamin D via the VDR in osteocytes also decreases mineralization and incorporation of calcium into bone [67]. Unfortunately, the same mechanisms can be activated by vitamin D intoxication, which can lead to hypercalcemia, osteoporosis, or even osteomalacia [67]. The latter is accomplished by $1,25(OH)_2D$ upregulation of mineralization inhibitors including osteopontin as well as pyrophosphate synthesis (by *Enpp1/3*) and transport (by *Ank*) and downregulation of pyrophosphate degradation by bone alkaline phosphatase (*Alpl*) [67].

In summary, correction of severe vitamin D deficiency may restore intestinal calcium absorption and cure rickets and osteomalacia, while correction of more moderate vitamin D deficiency may reverse secondary hyperparathyroidism and excessive bone turnover in osteoporosis. If vitamin D-dependent calcium influx is however bypassed, vitamin D becomes redundant, while at pharmacological to toxic doses, vitamin D promotes bone resorption and suppresses mineralization.

Clinical Applications of Vitamin D for Bone Health

An important distinction has to be made between the benefits of vitamin D and calcium for rickets and osteomalacia (which are clearly accepted), versus their role in bone development in utero, in children, in peak bone mass acquisition and in midlife, all of which remain unclear, and for osteoporosis in menopausal women and the elderly, where it probably plays a role, although a modest one.

Vitamin D and Calcium Supplements for the Treatment of Rickets and Osteomalacia

Nutritional rickets can either be due to severe deficiency of vitamin D, due to very low calcium intake or absorption, or due to a combination of both factors [1, 2]. Thacher et al. have shown in a randomized controlled trial (RCT) in Nigerian children with low nutritional calcium intake and moderate vitamin D deficiency that calcium alone or calcium combined with vitamin D had a superior effect compared to vitamin D alone on radiographic and biochemical healing of rickets [71]. This and other studies [72], [73] have used 300,000–600,000 IU i.m. of vitamin D_3, which may be beneficial if compliance and oral absorption is poor. However, daily or weekly oral doses are probably equally effective [73–75] and recommended first-line regimens in current guidelines [1]. Interestingly, an RCT in children from Mongolia with very severe vitamin D deficiency but without clinically overt rickets showed that daily oral vitamin D supplementation alone improved growth [76]. Importantly, another RCT in Nigeria showed that rickets can be cured with calcium alone [77]. These findings reinforce the notion that the culprit in rickets can be either low calcium intake itself [78] or vitamin D effects on intestinal calcium absorption, and that the best results can be expected with combination therapy or correction of the underlying deficit [72].

Although vitamin D- or calcium-deficiency osteomalacia is equally treatable by correction of the underlying cause and calcium−/vitamin D-supplementation based on clinical experience, only one randomized trial has demonstrated that vitamin D (in this case, alfacalcidol) efficiently corrects biopsy-proven hyperosteoidosis in osteomalacia in the elderly [79]. Only very few trials suggest BMD improvements with vitamin D and/or calcium supplements in adults at risk

for osteomalacia [80], [81]. In contrast, intestinal calcium absorption falls dramatically after derivative bariatric surgery procedures, despite optimization of vitamin D status [82]. The identification and treatment of osteomalacia in the elderly and after bariatric surgery are clearly areas where more research is needed.

Vitamin D Supplements for Optimal Peak Bone Mass

The role of vitamin D deficiency in newborns, children, and young adults outside of rickets and osteomalacia is less well established. 25(OH)D < 50 nmol/L is common in pregnant and postpartum women as well as in their newborns, particularly those who are exclusively breastfed and not receiving vitamin D supplements. Still, rickets is exceedingly rare in neonates because of efficient transplacental calcium transport [52], or infants, probably because of their high nutritional calcium intake. To better delineate the role of vitamin D deficiency in offspring bone health, the MAVIDOS (maternal gestational vitamin D supplementation and offspring bone health) trial recently randomized pregnant women to daily vitamin D_3 1000 IU/day or placebo. This RCT found no difference in the primary outcome of neonatal whole-body bone mass, although there was an interaction by birth season and a significant effect on bone mass in winter-born babies [83]. A Cochrane meta-analysis has shown that vitamin D supplementation does not influence BMD in children overall, although in children with circulating 25(OH)D < 35 nmol/L, it resulted in 1.7% faster lumbar spine BMD gains ($P = 0.04$) [84]. Regarding intestinal calcium absorption, a recent RCT in children with baseline 25(OH)D values of 70 nmol/L failed to show any effect of vitamin D doses ranging 0–4000 IU/day [85]. The long-term results of the MAVIDOS trial and other studies are eagerly awaited, although it remains unknown (and perhaps impossible to ascertain) whether greater/faster bone gains during growth are clinically meaningful and prevent fractures during the life course.

Role of Vitamin D and Calcium Supplements in Osteoporosis

As explained above, vitamin D deficiency at levels <40–50 nmol/L may trigger secondary hyperparathyroidism, whereas $1,25(OH)_2D$ and intestinal calcium absorption only becomes compromised at concentrations below 30 nmol/L [6] and, in some studies, only as low as <15 nmol/L [86]. Gallagher et al. have shown that in premenopausal women with serum 25(OH)D < 50 nmol/L, vitamin D supplementation did not influence calcium absorption [87]. In postmenopausal women with 25(OH)D < 50 nmol/L, however, the same authors observed a linear increase in calcium absorption of up to 6% using daily vitamin D supplements up to 4800 IU/day [88]. Two other recent trials have shown significant but very modest effect of vitamin D supplementation on intestinal calcium absorption in postmenopausal women. In a 10-week study, a linear increase in calcium absorption after adjusting for age, weight, and initial calcium absorption (P-trend = 0.03) [89]. Another recent one-year RCT in postmenopausal women with 25(OH)D < 75 nmol/L revealed that 50,000 IU vitamin D_3 twice monthly increased calcium absorption by 1% (10 mg/day), whereas a 2% decrease was seen with 800 IU/day ($P = 0.005$ vs. high-dose) and a 1.3% decrease with placebo ($P = 0.03$ vs. high-dose). There were however no differences in BMD, muscle or functional outcomes, supporting the author's conclusions that their threshold did not define clinically meaningful vitamin D insufficiency [90]. Still, one could speculate from these studies that the effects not only require a vitamin D-deficient population, but also a population in which intestinal calcium absorption is compromised by factors such as ageing.

With regards to the effects of vitamin D and/or calcium on BMD and fracture risk, there are numerous RCTs and meta-analyses available. Vitamin D supplements without calcium have not demonstrated significant skeletal effects in meta-analysis [91]. However, this may be because most populations were not vitamin D deficient; two recent RCTs found a significant effect on BMD in subjects with 25(OH)D < 30 nmol/L [92, 93].

Furthermore, there is no evidence to support an effect of vitamin D or calcium to prevent fractures in premenopausal women or in men [94].

Increased calcium intake from dietary sources or supplements, with or without vitamin D, has been found to produce significant 0.7–1.8% increases in BMD [95], [96]. However, this did not translate into a reduced risk of fractures overall [97]. Interestingly, the authors of this meta-analysis pointed out that "Only one trial in frail elderly women in residential care with low dietary calcium intake and vitamin D concentrations showed significant reductions in risk of fracture." This trial by Chapuy et al. randomized women with a mean age of 84 years, which reduced hip fractures by 43% and nonvertebral fractures by 32% at 18 months [98], and effect that was mitigated but maintained at 3 years [99]. This contrasts with the results from the RECORD trial, in which calcium-, vitamin D- or combined supplements did not reduce any fracture outcome compared to placebo, in ambulatory women or men aged ≥70 years with a previous low-trauma fracture [100]. However, compliance supplement was problematic, in particular for calcium. This issue is confirmed by the Women's Health Initiative (WHI) study, in which a significant reduction in hip fractures was only observed when patients who had stopped taking the supplements were censored [96]. Nevertheless, an increased risk of renal calculi was confirmed with combination supplements and later analyses—although still controversial—suggest a potential risk of adverse cardiovascular events with calcium [101] (but not vitamin D [102]) supplements.

So where does this leave us? Several meta-analyses concluded that vitamin D plus calcium resulted in a ± 15% relative risk reduction in hip and nonvertebral fractures [97, 103], [104]. However, these and other meta-analyses have clearly pointed out that the effect varies by setting: a significant reduction by about 30% is observed in institutionalized elderly, whereas the effect was <15% and not strictly significant in community-dwelling elderly [105, 106]. The US Preventive Services Task Force concluded recently that there is no role for calcium and/or vitamin D supplements to prevent fractures in community-dwelling adults without known vitamin D deficiency, osteoporosis, or prior fractures [94]. Still, there is probably a modest effect of vitamin D on fracture prevention in the elderly, which however requires (1) combination with calcium supplements, (2) targeting of a vitamin D-deficient population with poor calcium intake and/or absorption, (3) targeting of an elderly (particularly 80 years and older), frail population at high risk of fractures, (4) compliant use of supplements, and (5) avoidance of excessively high doses (which may increase the risk of falls and fractures, see the following texts) [4].

It is obvious yet important to emphasize that treatment of osteoporosis and prevention of fractures requires much *more* than just vitamin D and calcium supplements: many physicians still prescribe *only* these supplements (omitting effective pharmacologic osteoporosis treatment agents), which is clearly insufficient. Instead, guidelines on fracture prevention recommend identifying and treating reversible factors (e.g., smoking and alcohol use) and physical exercise (which probably also has myriad other health benefits). For those with high fracture risk, available antiresorptive or bone anabolic drugs reduce fracture risk much more efficiently than calcium and vitamin D supplements: by about 20–70% (depending on whether hip, nonvertebral, or vertebral fractures are examined) [107]. Defining high fracture risk is beyond the scope of this chapter, but in patients without high fracture risk, we suggest correction of low calcium intake or vitamin D deficiency if present and even more importantly, nonpharmacological measures. In those with high fracture risk, we suggest consideration of antiresorptive or bone anabolic drugs. In patients receiving these medications, we recommend correction of vitamin D deficiency by sunlight exposure or vitamin D supplements, and sufficient calcium intake (target: 1200–2000 mg/day based on IOM guidelines [6]) preferably from dietary sources but with addition from supplements if required [108], mainly to decrease the risk of hypocalcemia. In addition, most trials of osteoporosis drugs have used calcium and vitamin D supplements in their control

arms. Correction of calcium and vitamin D deficiency remains the first step in patients at risk for fractures, and combination of antiresorptive or bone anabolic drugs with calcium and vitamin D supplements remains the standard of care. Nevertheless, some data suggest that antiresorptive drugs are safe and equally effective without calcium and/or vitamin D supplements in patients without vitamin D deficiency and with sufficient calcium intake from dietary sources and without other risk factors for hypocalcemia such as chronic kidney disease [109, 110].

Apart from its role in bone, meta-analyses suggest that daily vitamin D supplements may decrease fall rate in care facilities [111]; however, in community-dwelling elderly, data are limited and inconclusive [112]. Although these trials did not demonstrate lower fracture risk, it is clear that consideration should be given to correction of vitamin D deficiency in frail older fallers. However, several RCTs have now demonstrated increased risk of falls or a lack of effect [113] with high to very intermittent doses of vitamin D [114–116] suggesting that these regimens are best avoided. Some trials have also suggested beneficial effects of vitamin D on muscle outcomes [117]. Indeed, muscle weakness is a known feature of rickets and osteomalacia, and there may also be a component in less severe calcium- and vitamin D-deficient situations as in the elderly. Furthermore, there is a need for mechanistic studies on vitamin D effects on muscle.

In summary, we can conclude that vitamin D and/or calcium supplements have the highest likelihood of having a modest positive effect on outcomes if they are used in a population with severe vitamin D deficiency and low calcium intake, particularly in the frail elderly at high risk of falls and fractures, and if they are taken compliantly, for example, in a nursing home setting.

Conclusions

Vitamin D, either from endogenous origin (under the influence of UVB light) or from diet, is a precursor for 25(OH)D and the active hormone $1,25(OH)_2D$, the ligand of the nuclear receptor VDR. VDR activated by $1,25(OH)_2D$ initiates a complex set of interactions with different proteins and DNA sequences ultimately resulting in a wide variety of genomic actions. The actions of vitamin D on calcium homeostasis are mainly based on its activation of active calcium transport across the intestine so that normal plasma calcium and phosphate concentrations allow a normal mineralization of osteoid and growth plate development. Absence of vitamin D or its subsequent metabolism or action results in rickets or osteomalacia. These diseases are still widely present around the world despite the fact that they are eminently preventable by low-dose vitamin D supplementation of common food sources.

In most circumstances, vitamin D is good for bone because of its direct actions on intestinal calcium absorption, whereas its well-documented actions on all bone cells are largely redundant if calcium supply is guaranteed. In case of severe calcium deficiency or malabsorption of calcium, plasma calcium homeostasis is defended by systemic hormones including high production of $1,25(OH)_2D$. Such high serum $1,25(OH)_2D$ concentrations are capable of stimulating bone resorption and simultaneously inhibiting mineral deposition by enhancing the production of pyrophosphate and osteopontin, two potent inhibitors of mineralization. From an evolutionary standpoint, this seems to be a clever strategy to use the calcium stores in bone as to avoid hypocalcemia and its severe consequences on muscle (cardiac, smooth, and skeletal muscles), nerve functions and coagulation, which could otherwise compromise survival. Once access to calcium can be restored, the excess osteoid can rapidly be mineralized and bone mass and strength can be restored.

There are still a number of questions such as (1) the full identification of all factors regulating calcium transport systems in the intestine, (2) the possible role of the many vitamin D metabolizing enzymes and metabolites other than $1,25(OH)_2D$, (3) the role of the vitamin D endocrine system on other tissues such as muscle, kidney, parathyroid gland, breast, and placenta, and (4) the physi-

ological significance of nongenomic vitamin D actions. This review does not cover the potential effects of vitamin D on extra-skeletal health, many of which are biologically plausible but details of such actions and the underlying mechanisms are still missing, especially their validation in rigorous RCTs in humans. The most burning issue however is probably how to measure vitamin D status and what level defines deficiency for multiple outcomes.

Conflicts of Interest *Bouillon*: Lecture fees from l'Oréal and Abiogen; co-owner of university patent on vitamin D analogs licensed to Hybrigenix

Laurent: Lecture fees and travel support from Amgen; consultancy fees from Alexion, Kyowa Kirin, Sandoz, Takeda and UCB.

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Basic Aspects of Bone Mineralization

5

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Key Points

- The regular mineralization of the bone matrix seems not only to require the proper composition and structure of the organic matrix together with the presence of sufficient calcium (Ca) and phosphate but also cellular activity for mineral transport, deposition, and removal of mineralization inhibitors.
- Mineralization of the bone matrix occurs in two phases of different time scales, which are a fast primary and a slower secondary phase reflecting the nucleation and growth of the mineral particles in length, width, and thickness.
- Due to the ongoing activity of the bone cells and the time course of mineralization of newly formed bone matrix, bone is a spatially and temporarily heterogeneous material revealing a specific pattern of mineralization which is also an important determinant of bone material stiffness/elasticity.
- Deviations in the mineralization pattern due to alterations in bone turnover and/or mineralization kinetics measured in the bone biopsy sample from the patient provide important information for the clinician about underlying pathophysiology, interpretation of densitometry data, treatment decision and monitoring as well as fracture risk assessment.

Processes of Bone Mineralization

The majority of the presented results of bone mineralization and its distribution in this book chapter will have its focus on lamellar bone, which is formed by the concerted action of osteoblasts and deposited on a preexisting bone surface, which can be either freshly resorbed (remodeling) or resting (modeling). Four of such surfaces can be distinguished: trabecular, intracortical (osteonal), periosteal, and endosteal bone surfaces. There is some evidence that the mineralization processes might be somewhat different at these surfaces, as there are important differences in either cell activities or in mineral transport distances at these bone sites [1]. Noteworthy, differences of mineral and matrix properties between periosteal and intracortical surfaces in humeri of macaques were found recently by Raman microspectroscopy [2].

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In all these cases, mineralization is associated with a clear mineralization front extending to the entire surface of a forming bone packet or osteon up to a length of several hundred microns. This transition zone between nonmineralized matrix and the area where mineral accumulation takes place can be visualized by fluorescence labeling techniques.

However, mineralization processes as occur, for example, in de novo bone formation such as in membranous and endochondral ossification might be different. Nonetheless, as many of the observations of the first events in mineralization were made, in particular, in embryonic/fetal bone from animals, we also address these results from nonlamellar bone.

Early Events of Mineralization: Mineral Nucleation

The prerequisite of bone matrix mineralization is a microenvironment of a highly supersaturated solution of calcium and phosphate ions (Pi) of a proper ratio enabling the spontaneous nucleation and following growth of bone mineral hydroxyapatite (HA) crystals. Thus, the systemic as well as the local Ca^{++} and phosphate ion (Pi) homeostasis is of crucial importance of adequate bone matrix mineralization.

Furthermore, the proper composition and organization of the organic bone matrix is important for its regular mineralization. Collagen type I is the main component of the organic matrix. It is a helical polypeptide which consists of two identical alpha 1 chains and one alpha 2 chain. This collagen matrix is formed by the osteoblasts and has to undergo a variety of intracellular and extracellular modifications before and after it is released from the osteoblast to form fibrils and fibers. In particular, investigation of the pathogenesis in brittle bone disease osteogenesis imperfecta has contributed to the understanding of the importance of these intra- and extracellular processes, including the formation of the collagen alpha-helices, their proper folding to triple-helices, the formation of fibrils, and later mineralization [3–6]. After collagen is released from the osteoblasts, the collagen molecules form fibrils of a quasicrystalline structure in a self-assembling process with the collagen molecules arranged in a staggered pattern such that there is a 35 nm gap between the termini of collinear molecules [7, 8]. This staggering results in a pattern of alternating gap and overlap zones in the collagen molecules causing the electron contrast differences in transmission electron microscopy (TEM) images, which are visible by light and dark bands in the images [9, 10].

Before mineralization starts, the newly formed matrix (osteoid) seems to require some modifications known as the osteoid maturation, which lasts about 15 days in a healthy individual [11]. It is assumed that during this time, the organic matrix is transformed to provide a scaffold or framework for mineral deposition by the formation of collagen cross-links. Moreover, specific collagen residues were identified which might be suited to act as nucleation centers and might play an important role in onset and progression of mineralization [12]. However, collagen alone is not sufficient to drive organized mineralization; rather, specific non-collagenous proteins are needed which might act as nucleators. Candidates for these are proteins from the SIBLING (small integrin-binding ligand, N-linked glycoprotein) family, including matrix extracellular phosphoglycoprotein, osteopontin, dentin matrix protein 1, and bone sialoprotein. These proteins were reported to attract the mineral and to control growth; however, the same molecules might also inhibit mineralization [13, 14]. The presence and activity of alkaline phosphatase, which transfers pyrophosphate (PP) to phosphate ions (Pi) was found to be crucial for mineralization [15]. Depending on the ratio of PP/Pi, PP can act as initiator or inhibitor of mineralization [16, 17]. In a patient with hypophosphatasia (HPP) and chronic kidney disease-mineral and bone disorder (CKD-MBD), for instance, osteomalacia, together with high levels of pyrophosphate, was observed at the bone surface [18]. This suggests that pyrophosphate is blocking the onset of mineralization [18].

It is still not fully understood how the large amounts of mineral (or its components) are transported to the mineralization front, where they

need to be quickly disposable. However, there is strong evidence that, apart from the properties of the organic matrix, the bone cells play a direct role in regulation of the mineralization process [19, 20]. It is known that the osteoblasts' differentiation to osteocytes is intimately linked to the mineralization process [20, 21]. Cryo-TEM studies revealed matrix vesicles containing calcium-phosphate particles in blood vessels and in large amounts close to the forming bone surface in growing bone from an animal model [22]. This suggests that bone-derived exosomes (i.e., matrix vesicles) might not only transport a variety of different cell proteins to the extracellular matrix [23] but might also be a carrier for mineral. Such a transport mechanism would have the advantage to prevent any ectopic mineral precipitation in other tissues (e.g., blood vessels) and to carry the mineral to the place in bone where it is actually needed [22]. Matrix vesicles have been discovered already in the 1960s in connection with the mineralization of cartilage [24]. It is assumed that these matrix vesicles are released from cells, which have taken up large amounts of calcium and phosphate ions in their mitochondria. Calcium- and phosphorus-containing mineral aggregates were found in mouse osteoblast mitochondria [25]. Others showed that the intracellular mineral granules consist of disordered calcium phosphate, which is metastable and might serve as a potential precursor of carbonated hydroxyapatite [26]. Thus, the formation of mineral crystals seems to start already within the cells in the endosomes, which are subsequently released from the cell in exosomes [27]. However, it is still unknown how the vesicles are broken up and how their mineral content is transferred to the collagen. Furthermore, it has not been shown yet that this transport mechanism also plays a role in the mineralization of the lamellar structured osteoid in human bone.

The nature of the initial mineral deposits in the mineralization process is still under debate, while that of mature bone is relatively well known as a type of carbonated hydroxyapatite. Mineralization takes place in hydrated collagen, thus, the local degree of water content might play also an important role. Transient densification stages of mineral were observed, such as a "dense liquid" phase and prenucleation clusters that form within it [28]. Transient precursors including amorphous calcium phosphate or octacalcium phosphate have been discussed for the initiation of biological apatite while others suggest that bone mineral is initiated via a very small, poorly crystalline, highly substituted hydroxyapatite (HA) mineral [29]. Both, either the existence of transient precursors [30] or the increase in apatite crystal size and crystallinity [31], might explain the differences between newly formed and mature bone apatite including chemistry, size, and solubility [29].

There is an ongoing discussion about where the mineral depositions occur within the bone matrix. As the striations of the collagen overlap-gap pattern can be also seen in mineralized tissues [9, 32, 33], it is assumed that the mineral is associated with this pattern. Using results from the mineralizing tendon, it was supposed that mineralization starts within the gap zones and the majority of mineral is located there [34, 35]. It is believed that adjacent gaps of the collagen are in contact with each other forming extended grooves which are filled by mineral [36]. During ongoing mineralization, the mineral particles might also grow beyond this space, form a continuous cross-fibrillar phase [37], and are also found associated with the fibrillar surface [38]. However, the distribution of mineral between intra- and extra-fibrillar spaces is somewhat controversial. While intra-fibrillar mineral might represent the bigger part and extra-fibrillar mineral the smaller portion of the overall mineral [39], an alternative model has also been discussed where most of the mineral is located in so-called "mineral lamellae" which are mineral-plates between adjacent collagen fibrils [40]. In any case, mineral in the inter-fibrillar space was suggested to be mechanically important as a component of "the glue" forming the connection between the mineralized collagen fibrils [41].

Generally, the mineral crystals (or particles) can be visualized individually by TEM [37, 42], atomic force microscopy (AFM) [43], or can be characterized as an average of several thousand to millions crystals by scattering techniques [44].

Scanning small-angle X-ray scattering (SAXS) together with scanning wide-angle X-ray scattering (WAXS) allows us to measure the length, the thickness, as well as the orientation of mineral particles [44]. In human bone, these types of measurements revealed mineral particle dimensions of approximately 15–200 nm with a thickness of 2–7 nm [45–47]. Furthermore, these studies have shown that the mineral particles are oriented by the collagen fibrils with the long axis of the platelets parallel to the long axis of the collagen fibril [48–50].

The Increase in Mineral Content: The Fast Primary and the Slow Secondary Phases

Once mineralization has started in the osteoid, the mineralization front proceeds with a certain speed toward the osteoid surface (termed mineral apposition rate (MAR) in histomorphometry) while osteoblasts still deposit new bone matrix. MAR is about 0.6 mm per day in cancellous bone and a somewhat higher in cortical bone [51, 52]. In addition to this spatial propagation of mineral in the bone matrix, mineral accumulation takes place with time within each mineralizing volume element of bone. This increase in mineral content thereby occurs with changing mineralization rates resulting in a specific time course of mineralization ("mineralization kinetics"). Likely the latter is not a natural constant, but it might vary with different conditions such as health or disease, skeletal site, individual's age etc. [53–56].

Until now, it is not possible to follow the accumulation of mineral directly in a specific bone volume in humans as this would require repeated in vivo measurements in the identical bone volume element. However, attempts to measure the mineralization kinetics in small animals (mice) were done recently based on micro-computed tomography (μCT) imaging [57, 58]. As the mineralization front in the osteoid is moving with a certain speed, it is possible to obtain indirectly the time course of mineralization by using techniques allowing to measure the mineral content of bone volume elements in a spatially resolved manner with increasing distances (i.e., with increasing tissue age) from the mineralization front in bone samples. A first rapid increase (primary mineralization) and a subsequent slowdown of increase (secondary mineralization) in mineral content up to a final plateau level have been observed by analyzing line profiles of mineralization perpendicular to the mineralization front (Fig. 5.1) [59–64]. Such a biphasic behavior of mineralization rates might be explained by the following hypothetical scenarios: From a physical/chemical viewpoint, during the primary fast mineralization phase, the nucleation and the growth in predominantly two dimensions of the mineral particles may occur as well as single mineral clusters may be formed, which are subsequently fusing together. In the secondary slow mineralization phase, mineral particles are growing mainly in thickness and fusing more completely together. From the viewpoint of bone cells activity, the primary mineralization might occur essentially by the action of the osteoblasts which produce the matrix vesicles for supplying rapidly the mineral components in this initial phase of mineralization, while in the secondary phase, the mineral components might be transported by the osteocytic lacunar-canalicular network [65].

In principle, the secondary mineralization process leads to a positive correlation between mineralized bone tissue age and mineral content. Thus, apart from the cement lines (which differ in their organic matrix from the other bone tissue and are generally highly mineralized [66, 67]), the highest mineral content is generally found in the oldest tissue which is interstitial bone as a remnant from bone remodeling. However, also in osteonal cortical bone, more highly mineralized areas can be observed in the center of the osteon (adjacent to the Haversian canals) compared to that in its periphery [32, 68, and own observation]. This observation might either be due to a passive deposition of calcium and phosphate ions near to the Haversian canal or it might also indicate the presence of a tertiary mineralization process at least in cortical bone. This

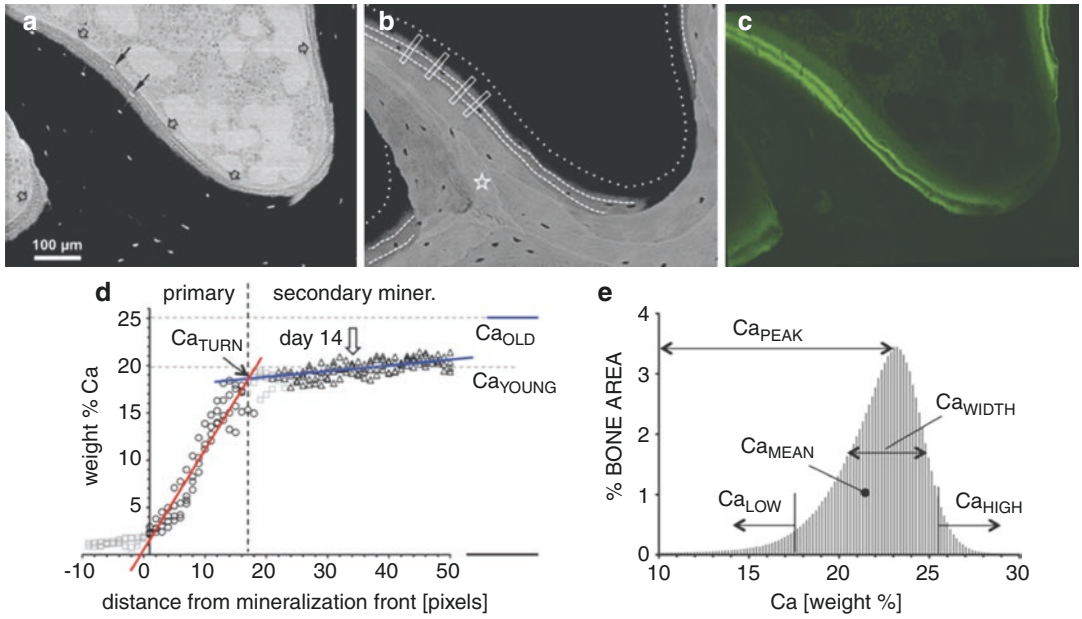

Fig. 5.1 A bone- forming site of trabecular bone— – scanning electron microscopic images acquired with a pixel resolution of 0.57 µm. (**a**) Backscatter electron image with special contrast setting showing all the mineralized matrix in black (not distinguishing between different mineral content) while the differences in grey gray level reveal the soft non-mineralized tissue embedded in PMMA (bone marrow). Black empty arrows point to lamellar osteoid, whereas black solid arrows point to preosteocytes (osteoid osteocytes). (**b**) The same site shown by the backscatter electron image with a calibrated contrast setting for quantitative backscatter electron imaging (qBEI) [63]. The grey gray levels are correlated with the local mineral content (the brighter the higher the mineral content). Bone packets of different gray levels can be seen within the trabecular feature. The newly forming ones at the surface have the lowest gray levels. White star indicates old interstitial bone. Dotted lines are indicating the borderline of the osteoid as seen in (**a**). Dashed lines are indicating the position of the fluorescence bands of tetracycline double labeling of the moving mineralization front (dynamic indices of bone formation) as obtained from (**c**). The white bars, perpendicular through the mineralization front, indicate the regions where mineralization line profiles were analyzed as shown in (**d**). (**c**) Corresponding confocal laser scanning microscope image from the identical block surface as in (**a**) and (**b**): Parallel running fluorescent double labels are visualized. In this case, the distance between the labels corresponds to 12 days and the position of the second label (latter time point) corresponds to 6.5 days before biopsy. (**d**) Mineralization line profiles pooled from the four regions indicated in (**b**) (bars): *X*-axis at zero position indicates the onset of mineralization (i.e., the mineralizing front). Red, regression line from data with circle symbols showing the fast primary mineralization phase (steep slope). Blue, regression line from data with triangle symbols showing the slow secondary mineralization phase (flat slope); gray square symbols indicate the data not included in the regression analysis. The intersection point of both regression lines defines the calcium concentration at the transition from primary to secondary mineralization (Ca_{TURN}, 18.5 wt % Ca). The empty arrow indicates the position in the center of the two fluorescent labels and corresponds to a tissue age of 14 days. Note, the labelled areas are already in the secondary phase and their corresponding Ca-content mirrors the level of early secondary mineralization named Ca_{YOUNG}, (at 20 wt % Ca,) in contrast the level of the oldest bone reflects the interstitial bone (star in (**b**)) named Ca_{OLD} (at 25 weight % Ca). Consequently, the secondary mineralization varies from 20 to 25 wt % Ca corresponding to its tissue age. (**e**) Bone mineralization density distribution (BMDD) deduced from image (B). The five derived BMDD parameters are indicated: Ca_{MEAN}, the average degree of mineralization, obtained from the integrated area of the BMDD curve; Ca_{PEAK}, the position of the peak indicating the most frequently (typical) calcium concentration within the sample; Ca_{WIDTH}, the width at half maximum of the BMDD, a parameter for the heterogeneity of mineralization, Ca_{LOW}, the percentage of areas with low (below 17.68 weight %) mineralization reflecting areas undergoing primary mineralization; Ca_{HIGH}, the percentage of areas with high (beyond 25.30 weight %) mineralization. These cut-off levels were established using the normative cancellous BMDD (see Fig. 5.2), and correspond to the 5th and 95th percentiles of calcium concentrations.

additional mineral seems to be added later to the level reached by secondary mineralization. There is some evidence that this phenomenon is associated with the higher density of osteocytic canaliculi found in the central area of the osteons [69, 70].

It is widely accepted that the mineral accumulation in the organic bone matrix is accompanied by the replacement of the free water present in the matrix [71]. For instance, this is confirmed by vibrational spectroscopy studies on plastic (polymethylmethacrylate, PMMA) embedded bone samples showing a clear decrease in the PMMA vibrational peak in tissue areas of increasing mineral content/tissue age [72, 73]. Since during the embedding process PMMA substitutes for the water in the sample, the PMMA peak is representing indirectly the water content and mirrors, therefore, also the nanoporosity of the bone material. According to experimental data on lateral spacing of the collagen molecules (1.1 nm in dry, 1.55 nm in wet, and 1.25 nm in mineralized bone conditions) in combination with theoretical model considerations, the collagen fibril could theoretically take up to a maximum of 56 vol% (volume percent) mineral corresponding to 30 wt % (weight percent) Ca until all the free water is replaced [74, 71]. However, in human bone, a maximum mineral content of only around 25 to 27 wt% Ca is found [75], which is consistent with the aforementioned 1.25 nm collagen spacing found for bone. Interestingly, this means that in reality, the mineralization seems to be limited by additional mechanisms and not only by the available space within the fibrils and moreover that water is still present in fully mineralized bone (in particular collagen-bound water [76, 77]). There is evidence that the number of nucleation centers for the mineral crystals and their growth to final size might be the determinant of the final level of mineral achieved in bone [78, 79]. An example where this was demonstrated is bone in osteogenesis imperfecta. In this disease, an increased degree of mineralization compared to healthy bone was observed [80–82]. First, this was linked to the higher amount of water present in the defective collagen which could be replaced by mineral during mineralization processes [81]. However, the degree of mineralization was increased independent of whether the patients had structurally aberrant collagen (qualitative mutation) due to the underlying collagen mutation or only a reduced quantity of structurally normal collagen (quantitative mutation) [55]. This points rather toward a scenario, where the number of nucleation centers might be a crucial determinant of the final bone mineral content [80]. Indeed, the results from X-ray scattering experiments gave evidence for normal-sized crystals in osteogenesis imperfecta suggesting that the higher bone matrix mineralization is achieved by more densely packed mineral particles [79]. In this context, it should be mentioned that the bone material has not to be considered as a nanocomposite material of two components (collagen and mineral), but rather than as a three-component system including water. Recent studies emphasized the tremendous role of the hydration status of the bone material on its mechanical performance [76, 77, 83, 84]. The more dehydrated the material is, the stiffer and less ductile are its properties. In the case of osteogenesis imperfecta, the increased mineral content as well as the reduced hydration of the collagen would explain the extreme brittleness of the material. It can be assumed that the level of about 25 wt% Ca in normal healthy bone resulting also in a certain residual hydration of the matrix might provide optimal stiffness and ductility.

Mineralization Distribution in Bone

The matrix mineralization pattern as seen in images such as Fig. 5.1b and the resulting mineralization distribution of bone can be considered as a kind of fingerprint of bone at the material level [85]. It reflects the history of bone cell activity, like conditions of low and high bone turnover rates as well as changes/abnormalities in the mineralization kinetics [86]. When visualizing bone material, for instance, in the backscatter electron mode of the scanning electron microscope, areas (so-called bone packets) with different gray levels can be seen (Fig. 5.1). These bone packets or bone structural

units (BSUs) were formed by osteoblasts during one bone formation cycle. Given the mineralization processes as described above, the mineral content of bone is dependent on its tissue age. Recently formed BSUs have lower degree of mineralization than older ones. Consequently, the mineralization distribution depends strongly on the bone formation/turnover situation. If bone formation is high, many BSUs are formed; thus, a high percentage of the bone packets will have young tissue age and correspondingly low mineral content. This is the reason why growing bone from children has on average a lower degree of mineralization compared to bone from adult individuals [78, 87]. Additionally, in the case of high bone resorption, there is low chance that a bone packet will become old and will have accordingly high mineral content as the probability for resorption is high. Thus, in high bone turnover (high formation and resorption), the overall bone tissue age is low. Vice versa, when bone turnover is low, the tissue age will be high, and thus a larger percentage of higher mineralized bone packets will be present [63, 88]. This pattern of mineralization can be described/quantified by deduction of gray-level (Ca content) histograms from the microscopic images the so-called bone mineralization density distribution (BMDD) (Fig. 5.1). For the measurement of the BMDD, spatially resolved techniques are necessary. Several methods with spatial resolution from few microns to submicron resolution, which make use of different physical mechanisms, are available for this purpose (see in the following).

Before an overview of methods for the measurement of the local mineral content and its variation in bone at the material level is given, the difference of the latter to the clinically (in vivo) measured bone mineral density (BMD) at the organ level by dual X-ray absorptiometry has to be mentioned. BMD is widely used as a surrogate measure of bone strength and is determined by the amount/volume of bone present and its material density (the latter is dominated by the calcium content). Hence, low BMD might be due to low bone volume or due to decreased bone mineral content or due to a combination of both. It is important to have this in mind when interpreting BMD data, in particular, for the evaluation of treatment effects [89].

Measurement of the Mineralization Distribution

One important technique, which measures the mineral content of bone in a spatially resolved manner, is vibrational spectroscopy (infrared and Raman microspectroscopies). It makes use of the absorption or inelastic scattering of light (infrared light or laser light of different wavelength from infrared to ultraviolet, respectively) by the bone sample [90–93]. The chemical groups of the bone sample are not stationary but undergo twisting, bending, rotation, and vibration causing absorption or inelastic scattering at specific wavelengths, which are characteristic for structure and environment of the molecules. Most commonly, the spectra are analyzed by measuring a specific absorption peak height, peak areas, peak width, and calculation of the ratios of specific peak areas (e.g., mineral to matrix ratio). The strength of these spectroscopic techniques is that both basic components mineral and organic matrix can be analyzed, however, it usually can provide only relative amounts between these components.

Other methods utilize the attenuation of an X-ray beam by the sample. The oldest method is microradiography which measures the X-ray absorption in an about 100-μm-thick bone sections [94, 95] using either photographic films or in newer systems a digital detector [96]. The resulting gray levels on the film or the measured intensities on the detector reflect the X-ray intensities transmitted through the bone slice and are evaluated by microdensitometric methods. The most modern technique is synchrotron radiation micro computed tomography (SR-μCT). It measures the X-ray absorption under different angles in similar concept as in computer tomography scanners in the clinic, however in contrast to the latter SR-μCT analyzes the gray levels for information on the bone mineralization [97, 98]. More modern techniques additionally combine the

information from X-ray tomography with phase retrieval ("holotomography") which enhances the sensitivity of mineral content measurement [99].

A further frequently applied method is quantitative backscatter electron imaging (qBEI or qBSE) which measures the intensity of the backscattered electron signal from the surface of a block bone sample [63, 100–102]. In bone, this signal is correlated to the local calcium content, which enables the calcium mapping of a sectioned bone area.

In all methods, the result is a frequency histogram of pixels (or voxels) with different calcium concentrations occurring in the sample, the so-called bone mineralization density distribution (BMDD) derived from the acquired images (Fig. 5.1) [63]. Typically, the BMDD is normalized to the measured bone area (i.e., the area under the frequency histogram is 100%). The typical BMDD is similar to a bell-shaped curve, however, shows some asymmetry with higher portion of low than highly mineralized areas. In order to perform statistical analysis between different BMDDs, special parameters deduced from the BMDD were successfully introduced describing the mean, the most frequently occurring and the variation in Ca content. Furthermore, the percentage of bone area with very low or high mineral contents is quantified (Fig. 5.1).

The measurement of the BMDD requires bone samples. For scientific purposes, these can be different types of postmortem bone samples. However, commonly these are transiliac bone biopsy samples, which were primarily obtained for histopathologic examinations for the differential diagnosis or classification of bone diseases or as part of clinical trials to analyze treatment effects. The additional histologic/histomorphometric characterization of the biopsy is an enormous advantage as it enables to interpret the BMDD data in combination with histomorphometric data. In addition, these analyses can be combined (at defined anatomical locations) with other techniques (such as Raman spectroscopy, scattering techniques, ultrasound microscopy, nanoindentation, etc.) to get detailed information on structure/function relationship of the bone material.

Bone Mineralization Distribution in Healthy Individuals

Trabecular bone was found to have a relatively low biological variation from early adulthood up to 100 years of age. The authors' own reference BMDD (based on qBEI measurements) revealed a mean calcium concentration of 22.3 ± 0.45 weight % Ca (mean ± standard deviation) measured in healthy individuals (Fig. 5.2) [103]. Comparison of the average degree of mineralization in humans showed neither significant differences between skeletal sites (iliac crest, vertebrae, patella, femoral neck, or head), nor dependency on other biological factors such as sex and ethnicity. While small increases of average calcium concentration of cancellous bone with age were observed recently [104], other studies did not find such an increase with age [95, 103]. In any case, the merely small variation of the mineralization distribution of cancellous bone in healthy adult individuals (within an age range of about 25–100 years) made it possible to establish normative data which are the basis for comparison to bone mineralization in pathologi-

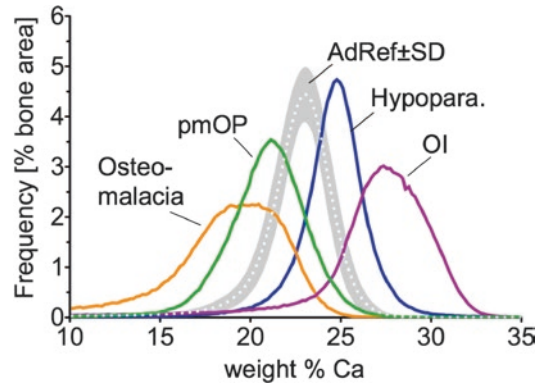

Fig. 5.2 BMDD in health and in examples of diseased bone: *AdRef* adult healthy reference of cancellous bone— – white dotted line represents the mean of each histogram bin value and the gray band its standard deviation from a cohort of 52 individuals [103], *osteomalacia* due to coeliac disease, *pmOP* postmenopausal osteoporosis (high bone turnover) [128], *Hypopara* hypoparathyroidism post surgery [133], *OI* osteogenesis imperfecta in an adult patient due to mutation in the gene region responsible for the C-terminal propeptide cleavage site of procollagen [147].

cal cases and after treatment (Fig. 5.2). Remarkably, despite these general small variations in healthy cancellous bone, the close relationship between bone turnover/formation and bone mineralization could still be detected. For example, in a cohort of healthy premenopausal women, the average degree of mineralization was negatively correlated with bone turnover (albeit within the normal range) and positively correlated with heterogeneity of bone matrix mineralization [105].

Normative BMDD data could also be established in transiliac bone biopsy samples from children aged 1.5–20 years [87]. This is extremely helpful in detecting and describing rare diseases, which are associated with a bone phenotype [106–108, 79]. For the cancellous and cortical compartment, a mean and standard deviation of 20.95 ± 0.57 and 20.31 ± 0.93 wt % Ca, respectively, were found. This level of bone mineralization is distinctly lower and its inter-individual variation is higher compared to adults, which can be explained by the higher bone formation rate and growths spurts in developing iliac crest of children.

All together the relatively constant mineralization around 22 weight % Ca is likely indicating the existence of an ideal range in degree and heterogeneity of bone matrix mineralization in relation with the trabecular bone's biological function and mechanical performance. Deviations in both directions, to lower and to higher mineralization densities, were reported to be associated with bone fragility [109]. Similar, heterogeneity of mineralization (and other properties such as lamellar orientation) has a consequence for the mechanical properties. Neither too little nor too much might be favorable as heterogeneity might hinder crack propagation while it might also facilitate crack initiation [110, 111].

Cortical compact osteonal bone, however, was found to show generally a higher average mineral content compared to cancellous bone. Additionally, differences in cortical bone mineralization itself also exist generally throughout the human skeleton [112]. However, it is remarkable that to date, systematic studies on cortical bone mineralization are rather sparse, although cortical bone represents about 80% of the entire skeleton, and is thus considered most relevant for weight bearing and also bone fragility. Skull bone (e.g., mandibles) seems to be generally more highly mineralized as the femoral midshaft or the cortex of the iliac crest [113]. Thus, intraindividual differences between cortical compartments and between cortical and trabecular compartments of the skeleton seem to exist. As explained above, the mineralization distribution of bone is closely related to both the bone turnover rate and the mineralization kinetics. It is intuitively clear that bone volumes within the thick cortex might have less probability to be remodeled compared to those in the relatively thinner trabecular struts which are closer to the surface where bone resorption takes place. Thus, cortical bone is expected to have higher tissue age, because of reduced bone turnover rates, which is reflected by in average higher degree of mineralization. Indeed, this was found for bone at the femoral neck and midshaft compared to cancellous bone [112, 114, 75]. On the other hand, it was observed that bone mineralization is clearly related to loading demands in the femoral neck, which might be accomplished by an adaption of the mineralization kinetics. Bone mineral content was found higher at the inferior compared to the superior region, which is predominantly loaded in compression, while the superior region is loaded in tension [114, 115]. Furthermore, the differences between cortical mineralization at femoral midshaft bone and cancellous bone might not be fully explained by the differences in bone turnover between these sites [56, 116]. This suggests that additionally to the variation in bone turnover, differences in mineralization kinetics among different skeletal sites and between cortical and trabecular bone might also exist due to the loading demands. Noteworthy, about 25% higher mineral/matrix ratios in human ossicles compared to femoral bone were reported albeit, it has to be mentioned that ossicles are not only comprised of lamellar bone but also woven bone and mineralized cartilage [117]. This high mineral content, however, can be considered as an adaptation to their function of sound transmission [117].

It is remarkable that the two cortical plates of transiliac bone biopsy samples were found to be very similar in mineralization with that of the corresponding cancellous bone compartment [118]. Moreover, the degree of mineralization of cortical bone was strongly correlated with that of trabecular bone. Individuals with relatively higher cancellous bone mineralization also have higher cortical bone mineralization and vice versa, which suggests a tight coupling of bone turnover in these two compartments of the iliac crest. For this reason, one should be cautious to extrapolate the BMDD findings in the iliac crest to other cortical sites. In this context, it would be helpful to establish normative BMDD reference values for different fracture relevant skeletal sites in relationship to iliac cortical bone for fracture risk prediction.

Mineralization Distribution in Diseased Bone

The aforementioned link between bone turnover and the mineralization kinetics with the bone mineralization distribution suggest that alterations in the former processes have an impact on the latter mineralization distribution. In specific diseases, the bone mineralization distribution clearly follows the deviation in bone turnover from normal, that is, high turnover is associated with low tissue age and low mineralization densities and vice versa. In other cases, however, an altered time course and/or final level of mineral accumulation within each bone packet occurs.

Postmenopausal osteoporosis (pmOP) with high fracture risk ("fracture disease") is one of the chronic diseases, which has been affecting a high portion of the elderly population with increasing incidence during the last decades [119]. To facilitate noninvasive diagnosis and assessment of fracture risk, osteoporosis is commonly diagnosed by low BMD according to the WHO classification. However, in a large portion of the patients, bone fragility is not attributable to reduced BMD. Thus, changes in bone material quality, specifically bone matrix mineralization, might affect the mechanical competence of bone.

There seems to exist some variety in bone turnover abnormalities in pmOP [120–122]; however, usually women with pmOP are diagnosed with high turnover [123, 124]. High turnover, in particular, during perimenopause and the first years after decline of estrogens, together with the imbalance of bone formation and resorption is leading to gradual bone loss, and this alters the bone mineralization distribution in pmOP by decreasing the average degree and increasing the heterogeneity of bone matrix mineralization compared to healthy individuals (Fig. 5.2) [125–131].

In addition to these findings in pmOP, a close relationship of the bone mineralization distribution with bone turnover was observed also in other pathologic conditions. For instance, patients with hyperparathyroidism reveal high bone turnover and correspondingly low bone mineralization densities [95, 132]. Vice versa, patients with hypoparathyroidism have suppressed bone turnover and increased matrix mineralization (Fig. 5.2) [133]. Low bone turnover and increased bone matrix mineralization were also reported for children with inflammatory bowel disease [134], for children after organ transplantation [135], and for young patients with chronic kidney disease and growth retardation [136], which were all associated with reduced bone formation and turnover. Deviations from normal bone mineral content and distribution were also described in association with increased bone fragility in several investigations [114, 115, 137–139].

In contrast to the aforementioned examples, where the bone mineralization distribution follows the deviation in bone turnover from normal, there exist also pathological conditions where the change of bone turnover is not predictive for the mineralization distribution. Male patients with osteoporosis and premenopausal women with idiopathic osteoporosis, for instance, were observed to have low bone turnover but also a low degree of bone mineralization [105, 140–142]. These unexpected findings might indicate that either the mineralization processes are slower or the final level of mineralization is reduced in these patients. Such modified material properties per se might be caused by altered osteoblast function in

idiopathic forms of osteoporosis associated with differences in the organic matrix and the mineralization kinetics thereof [141, 105]. The latter was also suggested for patients carrying COLIA1 Sp1 polymorphisms with increased bone fragility and reduced and more heterogeneous matrix mineralization [143].

Osteogenesis imperfecta is another example where the mineralization distribution does not follow the aforementioned correlation with bone turnover [80]. Many forms of this genetic disease have been described so far, including those with mutations in the collagen genes ("classical forms") and those more recently discovered having mutations in genes encoding for proteins which are associated with extracellular modification, cleavage of terminal endings, etc. While almost all of these forms are reported with high turnover [144], they have also in common an elevated mineral content of bone which contributes to bone brittleness [80, 145]. However, the hypermineralized bone matrix might occur in parallel with hyperosteoidosis in new forms of osteogenesis imperfecta [146–148]. This indicates that the onset of mineralization in the osteoid is delayed, but once mineralization has begun, it goes up to higher levels than normal (Fig. 5.2).

Another group of patients are those whose calcium and/or phosphate homeostasis is highly disturbed due to calcium ions uptake deficiency, kidney disease (with impaired renal phosphate excretion), and/or phosphate wasting. Both Ca and phosphate deficiency lead to mineralization defects with highly mineralized bone matrix coexisting with only weakly mineralized and nonmineralized bone matrix (Fig. 5.2). Such mineralization defects might occur in cases of renal osteodystrophy in patients with chronic kidney disease (CKD-MBD) [136], as well as in fibroblast growth factor 23 (FGF23)-induced hypophosphatemia as, for example, in patients with X-linked hypophosphatemic rickets (XLH) [149] or tumor-induced osteomalacia [54]. In a child with XLH, the transiliac biopsy sample showed areas of unmineralized bone within the mineralized bone matrix giving bone a mottled appearance [149]. Also, the mineralized bone matrix showed differences in the BMDD, revealing an increased frequency of bone areas with low calcium concentrations (i.e., low material density) in the patient's biopsy sample. Furthermore, bone mineralization abnormalities due to disturbance of calcium and phosphate metabolism might occur in celiac disease [63]. Just recently a patient with Crohn's disease and severe hypophosphatemic osteomalacia linked to iron substitution has been described (Fig. 5.2) [150].

Hypophosphatasia (HPP) which is caused by mutations in genes encoding for the tissue nonspecific alkaline phosphatase enzyme (TNSALP) is also an example where bone mineralization is disturbed [15, 151]. Clinically HPP is essentially identified by low serum alkaline phosphatase levels and increased levels of alkaline phosphatase substrates (pyrophosphate and pyridoxal-5′-phosphate). The deficiency of TNSALP activity leads to extracellular accumulation of its natural substrates including pyrophosphate which is a potent inhibitor of mineralization. The common radiographic finding in children with HPP is poorly mineralized bone [151, 152]. However, a huge range of severity in the phenotype has been described from lethal forms without mineralization of the skeleton to adults who are virtually asymptomatic [153, 154]. In general, the phenotypic severity present is related to the severity of the inherited TNSALP mutation. Bone biopsy samples from adult patients revealed (depending on the severity of HPP) the presence of osteomalacia and changes in the bone mineralization distribution [155].

Interestingly, there is strong evidence that bone matrix which has been nonmineralized for longer time in the aforementioned cases might be able to mineralize, if treatment is able to establish the proper Ca and Phosphate levels in the patient [18, 150]. The most impressive example are the children with HPP, who develop normally mineralized bone after alkaline phosphatase enzyme replacement therapy (asfotase alfa) which enables the mineralization of already formed bone matrix [152, 156]. So far, information on the mineralization changes at material level due to enzyme replacement treatment was obtained in mouse models, where increases in tissue mineral density

were reported with treatment [157, 158]. Apart from enzyme replacement treatment, an interesting observation was made in sequential biopsy samples from one adult patient with HPP (Fig. 5.3) [18]. In the first biopsy obtained from this patient, large unmineralized or poorly mineralized areas which showed diffuse fluorescence labeling were visible. In the later biopsy samples, this diffuse labeling was embedded in mineralized bone tissue, further indicating that osteoid, which does not mineralize for longer periods, might also be mineralized as soon as an appropriate environment exists and inhibitors of mineralization are removed from the matrix [18]. In this context, the case of iron treatment-induced osteomalacia in a patient with Crohn's disease should be mentioned as well [150]. The intravenous iron therapy induced a hypophosphatemia, which led to a severe osteomalacia as detected in the transiliac bone sample and contributed to a progressive decline of BMD (DXA). Cessation of iron therapy and the supplementation with phosphate

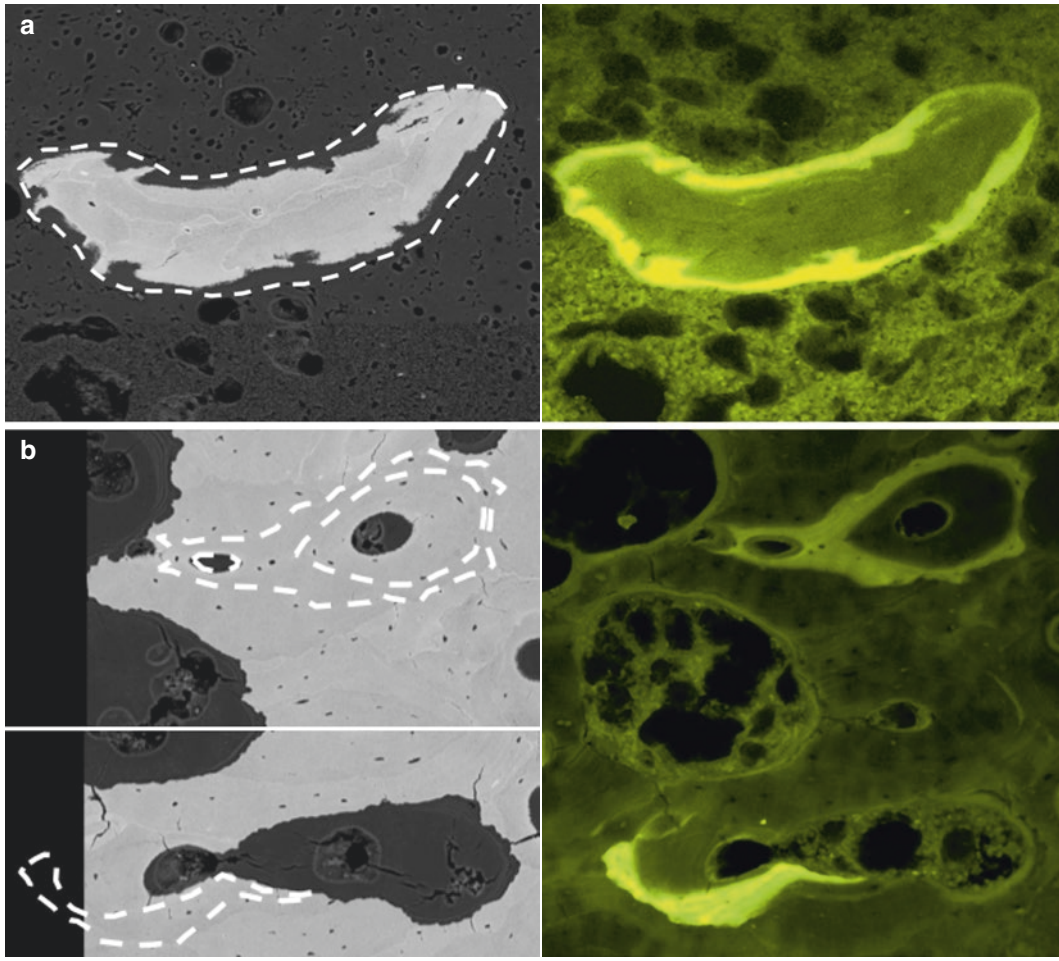

Fig. 5.3 Mineralization of aged osteoid in sequential biopsy samples from a patient with hypophosphatasia and renal failure [18]: (**a**) (left): backscatter electron (BE) image of a trabecular feature of the first transiliac biopsy sample with history of alendronate treatment and tetracycline labeling prior to biopsy: dashed white line indicates the border of the osteoid seam, which is visualized by confocal laser scanning microscopy (CLSM) of identical sample surface in **a** (right) as bright diffuse fluorescent region. (**b**) pair of BE (left) and CLSM (right) image of the second biopsy after stopping alendronate treatment (second biopsy without tetracycline labeling before). Diffuse labelled regions are now mineralized and embedded in mineralized bone tissue formed later as indicated by the dashed lines in BE.

was associated with a prompt positive response in BMD, which was likely due to filling of the osteoid matrix with mineral.

The examples mentioned above showed that information about the status of bone turnover in the individual is important for the correct interpretation of bone mineralization distribution. When increases in bone turnover rates are not associated with low bone mineral content and vice versa, alterations in the mineralization kinetics have to be taken into consideration.

Bone Mineralization Distribution after Treatment of Osteoporosis

Treatment of osteoporosis aims to decelerate bone loss and/or to increase bone volume. The different mechanisms of action of anabolic or antiresorptive agents (see Chaps. 12 and 14) are reflected in the typical changes in the distribution of bone mineralization accompanying the different types of treatment (Fig. 5.4) [159]. Noteworthy, during therapy, bone turnover/formation undergoes rapid

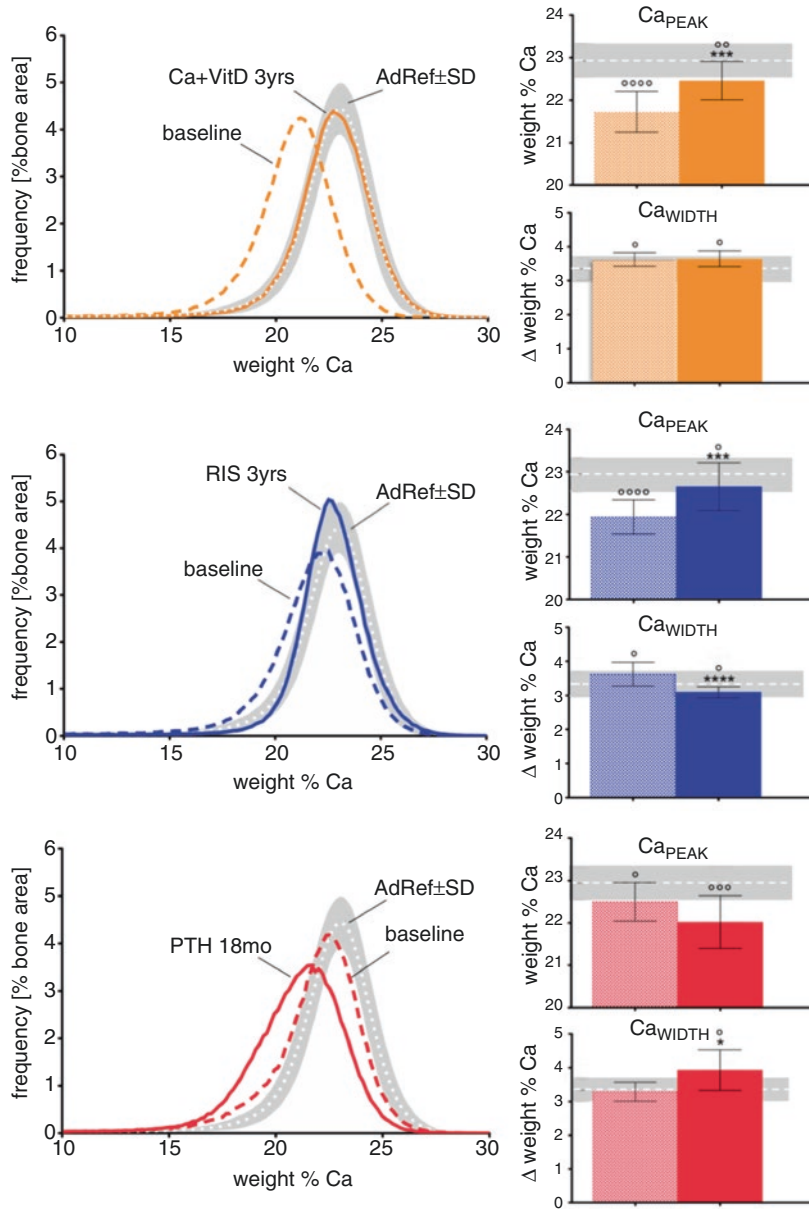

Fig. 5.4 The typical changes in BMDD of pmOP after treatment with calcium and vitamin D, RIS (risedronate) or PTH. Left column: examples of individual BMDD curves of paired biopsy samples before and after treatment. Right column: statistical analysis of BMDD-parameters Ca_{PEAK} and Ca_{WIDTH} of experimental groups before and after treatment [64, 128]. Bars indicate group mean values; error bars show standard deviations. Gray horizontal band indicates healthy adult reference data of cancellous bone (mean ± SD) [103]. The left bars show baseline values, and the right bars values after treatment.

changes as shown by the significant changes in the biochemical bone markers. For instance, a sudden drop of the C-telopeptide of type I collagen (CTX) and the intact procollagen I N-propeptide (P1NP) within as early as 1 week to few weeks depending on the type of bisphosphonate (BPs) was reported [160]. This rapid change pushes bone turnover and also the mineralization distribution out of an equilibrium stage making an observation of transient effects on the mineralization distribution possible [161] as will be described for antiresorptive treatment.

Treatment with Calcium and Vitamin D

Commonly, patients participating in a clinical trial receive calcium and vitamin D supplementation already before starting the active antiosteoporosis (or the placebo) treatment. However, the study design of the Vertebral Efficacy with Risedronate Therapy, North American trial (VERT-NA) and the Multiple Outcomes of Raloxifene Evaluation trial (MORE) provided an insight into the calcium and vitamin D effects in paired biopsy samples. Comparison of the bone matrix mineralization outcomes before and after treatment with calcium and vitamin D (and placebo) showed a shift to higher mineralization densities due to treatment [127, 128, 162]. The comparison of bone mineralization from the patients from the VERT-NA trial reference data revealed that these patients had undermineralized bone matrix at baseline [128]. This suggests that calcium and vitamin D deficiency alone is likely a cause of undermineralization, which can be offset by calcium and vitamin D supplementation (Fig. 5.4).

Hormone Replacement Therapy

Treatment with estrogen or with selective estrogen receptor modulators (SERMs) provide skeletal benefits in postmenopausal osteoporosis where estrogen deficiency is an important contributor to the pathogenesis of osteoporosis [163]. In studies where bone matrix mineralization was analyzed in postmenopausal osteoporotic patients after treatment with estrogen or SERMs, an increase in degree of mineralization or mineral:matrix ratio was reported [162, 164, 165].

Antiresorptive Treatment

Bisphosphonates (BPs) have been used for treatment of osteoporosis for several decades [166]. BPs inhibit bone resorption as they get adsorbed to mineral surfaces in bone, where they interfere with the action of the bone-resorbing osteoclasts. Their antiresorptive action is rather fast as already mentioned while the changes in mineralization are much slower (given the time of several months required for completion of one remodeling cycle [167]). Due to the sudden drop in bone resorption and formation in relation to the time which is needed for achieving a new bone turnover equilibrium, the measured effects of antiresorptive therapy on the bone mineralization distribution depend on the duration of therapy, short term (up to about 3 years) versus long term (5 years and longer). For short-term BP therapy (including alendronate, risedronate, ibandronate and zoledronic acid), a significant decrease in the heterogeneity of mineralization has been reported [130]. Moreover, the percentage of low mineralized areas is decreased and the average degree of mineralization is increased [130]. Noteworthy, these changes occur in osteoporotic bone which has generally lower degree and increased heterogeneity of mineralization than healthy bone before therapy. Moreover, part of these BP effects, in particular the reduction in mineralization heterogeneity, seem to be transient. After longer therapy duration, the heterogeneity together with the degree of bone mineralization is normalized (Fig. 5.4) [128, 168]. In the context of long-term antiresorptive treatment, it has to be noted that while no adverse effect on the bone mineralization distribution per se could be observed, the increasing occurrence of atypical femoral fractures have been reported [169]. These are therefore unlikely related to the changes in mineralization during therapy but more likely

related to the suppression of the internal fracture-repair mechanism by the decreased osteoclast activity (see also Chap. 21).

The BP effect on bone turnover and its consequences on bone matrix mineralization are well understood [63, 88, 98, 126, 128–130, 159, 161, 170–173]; however, it is unclear whether the change in turnover is also accompanied (at least to some extent) by a change in mineralization kinetics. Studies on bone from treated animals suggested no significant effect of alendronate or risedronate on the temporal course of mineral accumulation in bone [174]. However, on the other hand, it is assumed that the BP which is absorbed to the mineral might alter the chemistry and electrostatic properties of the bone surface which might be detected by osteoblasts [175]. Data from vibrational microspectroscopy suggested deviations in material properties from normal after different types of BP [176] as well as differences in the matrix formed under subsequent anabolic therapy in BP pretreated patients [177, 178].

While the effects of BP on human bone mineralization are well known as shown by the results from the numerous biopsy studies, much less is known about these effects in treatment with *denosumab*, a human monoclonal antibody to RANKL (see Chap. 15). Long-term safety and efficacy of this osteoporosis treatment have been published recently [179]; histologic evaluation of transiliac biopsy samples showed normal bone microarchitecture without evidence of adverse effects on mineralization or the formation of lamellar bone [180]. First data on bone mineralization in transiliac biopsy samples from patients were published recently [131]. In this study, bone biopsies from participants of the FREEDOM and FREEDOM extension study were analyzed. Outcomes showed an increase in the average degree and a decrease in the heterogeneity of mineralization in both cancellous and cortical compartments in denosumab versus placebo-treated patients.

There has been a debate whether therapy with *strontium ranelate* (SrR) exerts a combination of anabolic and concurrent antiresorptive action in bone [181]. Chavassieux and colleagues reported no evidence for anabolic but antiresorptive action only [182], which is in agreement with the bone matrix mineralization outcomes based on qBEI in transiliac biopsy samples [183]. Similar to above-mentioned fluoride, the element strontium gets incorporated into the bone mineral crystal; in the young bone packets formed during SrR therapy, it replaces approximately 5 at% of calcium [183, 184]. It was shown also that the strontium content of the bone matrix increases with increasing bone volume formed under therapy [185]. In contrast to fluoride, strontium seems not to change the mechanical properties of the bone material [184]. As strontium is an element with high atomic number, its incorporation into mineral, however, influences the measurement of BMD (mimics higher bone mass in DXA), bone volume by computed tomography, as well as bone mineralization and makes the evaluation of the genuine effects of SrR challenging [183].

Anabolic Treatment

Anabolic treatment with *sodium fluoride* was considered for treatment of postmenopausal osteoporosis some decades ago. However, the treatment was not widely accepted as it was recognized that despite the large increases in bone volume, bone fragility was not decreased in the treated patients [186]. It was recognized that bone formed under treatment was altered and mechanically inferior to normal bone as the element fluoride gets incorporated into the mineral resulting in a disturbance of the normal collagen–mineral relationship [187]. Abnormally large mineral particles and abnormal size distributions of the mineral particles have been observed [188, 189] together with mineralization defects [190] and abnormally high degree of bone mineralization [63]. Due to these adverse effects, systemic sodium fluoride has not gained wide use, although attempts have been undertaken to decrease the adverse effects by sustained-release sodium fluoride given on an intermittent basis [191].

The current options of anabolic therapy are treatment with *parathyroid hormone (PTH 1–84)*, *teriparatide (PTH 1–34)*, or *abaloparatide*. Significant changes in the bone mineralization

distribution with PTH or teriparatide are commonly observed. In line with an increase in bone formation, decreases in the average degree and increases in the heterogeneity of bone mineralization were reported (Fig. 5.4) [64, 192]. The decrease in average mineralization density can be explained by the increase in the percentage of low mineralized bone areas, which is a typical change in the BMDD in a situation of high bone formation/turnover. The experimental findings were confirmed by computed modeling, which revealed the occurrence of a "shoulder" in the BMDD at lower calcium concentrations after 1 year anabolic treatment [161]. Interestingly, only 1 year with PTH 1–84 was sufficient to change the mineralization distribution significantly in patients with hypoparathyroidism, a condition with suppressed bone turnover at baseline [133]. Similarly, an increase in the portion of low mineralized bone was observed after sequential treatment with bisphosphonates followed by teriparatide in postmenopausal osteoporotic patients [193]. BMDD data from combined treatment with anabolic and concurrent antiresorptive treatment are lacking so far.

More recently, treatment with *parathyroid hormone-related peptide (PTHrP, abaloparatide)* has come into focus (see also Chaps. 14 and 15) [194]. In a recent study, histologic analysis revealed no evidence of adverse effects on mineralization in bone biopsy samples from treated patients [195]; however, no data on its effect on the mineralization distribution exist so far. Similar for alternative novel anabolic agents such as *sclerostin antibody* therapy (see Chap. 16), only bone mineralization data from animal models are available yet. For treated rats [196], as well as for a treated mouse model of osteogenesis imperfecta, no significant effects on mineralization were reported [197].

Summary

The proper mineralization of the bone matrix is important for its mechanical performance. In bone from healthy individuals, relatively small variation in the distribution of cancellous bone mineralization could be observed which enabled to establish reference mineralization data that can be used for differential diagnosis. Indeed, deviations from normal have been observed in several bone diseases. Increased bone turnover associated with lowered average tissue age and lowered mineralization is found in postmenopausal osteoporosis. Antiosteoporosis therapies exert antiresorptive or anabolic mechanisms in the skeleton. Both treatment options have typical effects on the bone mineralization distribution. While antiresorptive therapy decreases bone resorption and formation resulting in higher tissue age, and thus a higher degree of mineralization, anabolic therapy increases the bone formation resulting in relatively young bone tissue having low mineralization densities. These changes might play a role in enhancing mechanical properties after treatment and have to be considered when evaluating the BMD changes in diseases and/or after treatment. In cases where predominately the Ca and Phosphate metabolism is disturbed, extreme deviations from normal BMDD can be observed showing shifts to lower calcium concentrations together with a strong increase in heterogeneity of mineralization.

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Determinants of Peak Bone Mass Acquisition

6

René Rizzoli and Jean-Philippe Bonjour

Key Points

- Peak bone mass (PBM) is a major determinant of bone mass and bone fragility later in life.
- During adolescence, increase in bone mass is mainly due to an increase in bone size rather to changes in volumetric bone density.
- Genetic factors are the main controllers of peak bone mass achievement.
- Environmental factors influencing peak bone mass achievement include physical activity, nutritional intakes (particularly protein and calcium), and chronic diseases.

Definition and Importance of Peak Bone Mass

Peak bone mass (PBM) corresponds to the amount of bony tissue present at the end of skeletal maturation [1, 2]. It is a determinant of the risk of fractures later in life, since there is an inverse relationship between fracture risk and areal bone mineral density, in women as well as in men [3]. From epidemiological studies, it can be assumed that an increase of 10% of PBM in the female population, corresponding to approximately 1 standard deviation (SD), would be equivalent to retarding menopause by 14 years and be associated with a 50% decrease in the risk of fracture [4]. Bone mineral accumulation from infancy to postpuberty can be appreciated with the availability of noninvasive techniques able to accurately measure areal (a) or volumetric (v) bone mineral density (BMD) at several sites of the skeleton by either dual X-ray absorptiometry (DXA) or quantitative computed tomography (QCT), respectively [5]. Noninvasive specific evaluations of the cancellous and cortical bone compartments, even of trabecular microstructure, are also available. These techniques allow one to capture part of the change in the macroarchitecture or geometry of the bones which, along with the mineral mass, strongly influence the resistance to mechanical strain. This chapter attempts to summarize knowledge on the characteristics of normal bone mass development from infancy to

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the end of the skeleton maturation, and the genetic and environmental factors influencing bone mass accrual, hence PBM.

Characteristics of Peak Bone Mass Acquisition

Measurement of Bone Mass Development

Most of the information on the characteristics of skeletal growth during childhood and adolescence has been obtained through noninvasive techniques allowing one to quantify bone mineral mass at various sites of the skeleton [5, 6]. The bone mass of a part of the skeleton is directly dependent upon both its volume or size, and the density of the mineralized tissue contained within its periosteal envelope. The mean volumetric mineral density of bony tissue (BMD in g of hydroxyapatite per cm^3) can be determined noninvasively by quantitative computed tomography (QCT). The technique of either single or dual X-ray (SXA, DXA) absorptiometry provides measurement of the so-called "areal" bone mineral density (aBMD in g of hydroxyapatite per cm^2). The values generated by this technique are directly dependent upon both the size and the integrated mineral density of the scanned skeletal site. The latter variable is made of several components including the cortical thickness, the number, and thickness of the trabeculae and the "true" mineral density corresponding to the amount of hydroxyapatite per unit volume of the bone organic matrix. The term bone mineral *density* without the additional "areal" qualification has been widely used with the general understanding that neither SXA nor DXA techniques provide a measurement of volumetric density. Therefore, aBMD is the summation of several structural components which may evolve differently in response to genetic and environmental factors. Nevertheless, aBMD remains of clinical relevance in the context of osteoporosis [7]. Indeed, aBMD has been shown to be directly related to bone strength, that is, to the resistance of the skeleton to mechanical stress both in vivo and in vitro [8–10]. There is an inverse relationship between aBMD values and the incidence of osteoporotic fractures [3].

In the spine, the total mineral content (BMC in g of hydroxyapatite) of the vertebrae, including the posterior arch, can be measured using the classical antero-posterior projection. BMC and aBMD of the vertebral body "isolated" from the vertebral posterior arch can also be obtained by using DXA in the lateral projection [11]. Low accuracy and precision preclude this measurement to be performed in routine clinical practice. The so-called bone mineral "apparent" density (BMAD in g/cm^3) is an indirect and rather imprecise estimate of the volumetric skeletal density [12]. This extrapolated variable can be expected to be less related to bone strength than aBMD, since it does not take into account the important geometry component that influences the mechanical resistance [8].

Therefore, in terms of overall bone strength prediction, aBMD/BMC values are more informative than the isolated measurement of volumetric trabecular density, since the former variable includes both bone geometry, cortical thickness, and its integrated volumetric density. This statement does not mean that other variables, which are more difficult to accurately assess, such as the microstructure of the trabecular network [13] and/or the material level properties of the mineralized tissue, do not contribute to the resistance to mechanical force. Furthermore, it is obvious that a full understanding of the fundamental mechanisms that underlie the marked interindividual variability observed in bone mass gain will require separate analysis of how bone size, cortical thickness, volumetric trabecular density, and microstructure evolve during growth and to identify which are the main respective genetic and environmental factors that determine the development of each of these three important contributors to bone strength in adulthood.

Bone Mass Development

There is no evidence for a gender difference in bone mass at birth. Likewise, the volumetric bone mineral density appears to be also similar

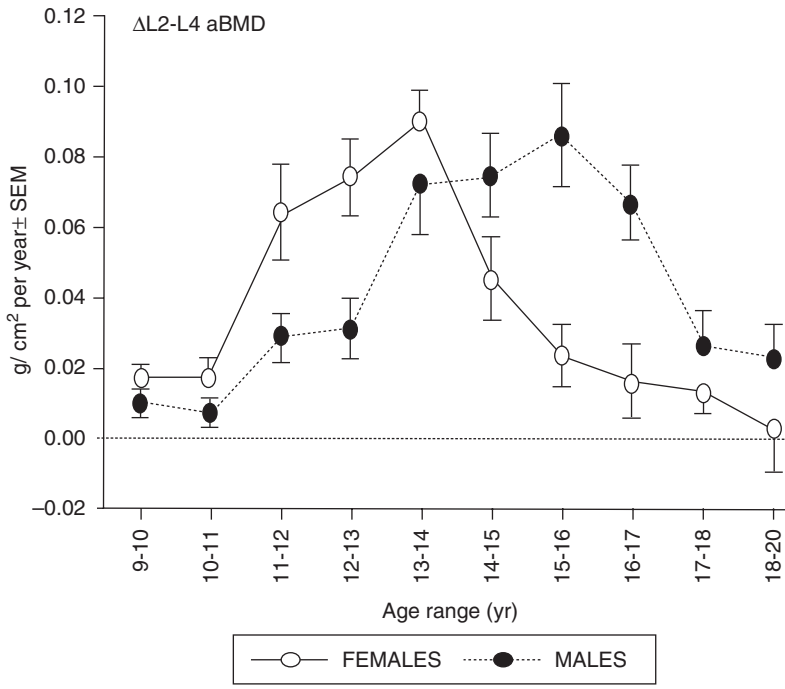

Fig. 6.1 Yearly increase in L2–L4 aBMD during puberty in females and males. High bone accrual rate lasts between 11 and 14, and between 13 and 17 years, in girls and boys, respectively. (Reprinted from Theintz et al. [18]. With permission from Oxford University Press)

between female and male newborns [14]. This absence of substantial sex difference in bone mass is maintained until the onset of pubertal maturation [15, 16]. During puberty, the gender difference in bone mass becomes apparent [17]. This difference appears to be mainly due to a prolonged period of bone maturation in males versus females (Fig. 6.1), with a larger increase in bone size and cortical thickness [18]. Puberty affects much more the bone size than the volumetric mineral density [19]. There is no significant sex difference in the volumetric trabecular density at the end of pubertal maturation [16]. During puberty, aBMD changes at both the lumbar spine and femoral neck levels and increases four- to sixfold over 3- and 4-year periods in females and males, respectively [18]. The change in bone mass accumulation rate is less marked in long bone diaphysis [18]. During pubertal maturation, cortical thickness increases by periosteal apposition in males and by inhibition of endosteal resorption in females [17]. There is an asynchrony between the gain in standing height and the accumulation of bone mineral mass during pubertal maturation [15, 18, 20]. This phenomenon may be responsible for the occurrence of a transient fragility that may contribute to the higher incidence of fracture known to occur when the dissociation between the rate of statural growth and mineral mass accrual is maximal [21–23]. Another mechanism involves a transient period during puberty of higher cortical porosity, particularly detectable in males [24, 25, 26].

Time of Peak Bone Mass Attainment

In adolescent females, bone mass gains decline rapidly after menarche [18] such that bone mass accrual essentially stops by approximately 2 years after menarche (Fig. 6.1) [18]. In adolescent males, the gain in BMD/BMC which is particularly high from 13 to 17 years markedly declines thereafter, although it remains significant between 17 and 20 years in both L2–L4 BMD/BMC and midfemoral shaft BMD [18]. In contrast, no significant increase is observed for femoral neck BMD. In subjects having reached pubertal stage P5 and growing less than 1 cm/year, a significant bone mass gain is still present in male but not in female individuals.

This suggests an important sex difference in the magnitude and/or duration of the so-called "consolidation" phenomenon that contributes to PBM level.

Observations made with QCT technology also indicate that the maximal volumetric bone mineral density of the lumbar vertebral body is achieved soon after menarche since no difference is observed between the mean values of 16-year-old and 30-year-old subjects [27, 28]. This is in agreement with numerous observations indicating that bone mass does not increase from the third to the fifth decades. All available data do not sustain the concept that bone mass at any skeletal site, in both genders, in all races and in any geographical area around the world continues to substantially accumulate until the fourth decade. On the contrary, numerous cross-sectional studies suggest that proximal femur areal BMD begins to decline already early in the third decade [29].

Bone outer dimensions can become larger during the adult life. This phenomenon has been documented by measuring the external diameter of several bones by radiogrammetry [17, 30, 31]. It may be the consequence of an increased endosteal bone resorption with enlargement in the internal diameter. Such a modeling phenomenon would be a response to bone loss, tending to compensate the reduction in the mechanical resistance [32].

Peak Bone Mass Variance

At the beginning of the third decade of life, there is a large variability in the normal values of aBMD in axial and appendicular skeleton [19]. This large variance is barely reduced after correction for standing height, and it does not appear to substantially increase during adult life. The height-independent broad variance in bone mass which is already present before puberty appears to increase further during pubertal maturation at sites such as lumbar spine and femoral neck [15, 18]. In young healthy adults, the biological variance in lumbar spine BMC is – four to five times larger than that of standing height; the latter does not increase during puberty [20].

Calcium–Phosphate Metabolism during Growth

Two adaptive mechanisms affecting calcium-phosphate metabolism during growth appear to be particularly important, namely the increase in the plasma concentration of 1,25-dihydroxyvitamin D_3 (calcitriol), and the stimulation of the renal tubular reabsorption of inorganic phosphate (Pi) (Fig. 6.2). The increased production and higher plasma level of calcitriol enhance the capacity of the intestinal epithelium to absorb both calcium and Pi. The increase in the tubular reabsorption of Pi results in a rise in its extracellular concentration. These two concerted adaptive responses contribute to optimal growth and mineralization. The increase in tubular Pi reabsorption is not mediated by a rise in the renal production or in the plasma level of calcitriol, but it appears to be a response

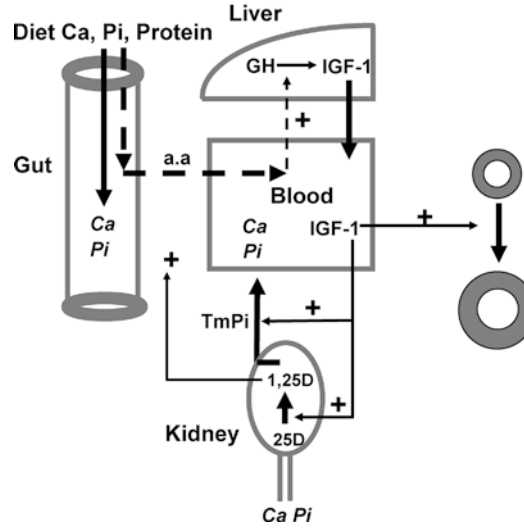

Fig. 6.2 Role of insulin-like growth factor-I (IGF-I) in calcium phosphate metabolism during pubertal maturation in relation with essential nutrients for bone growth. During the pubertal bone growth spurt, there is a rise in circulating IGF-I. The hepatic production of IGF-I is under the positive influence of growth hormone (GH) and essential amino acids (a.a.). IGF-I stimulates bone growth. At the kidney level, IGF-I increases both the 1,25-dihydroxyvitamin D (1,25 D) synthesis from 25-hydroxyvitamin D (25D) and the maximal tubular reabsorption of Pi (TmPi). By this dual renal action IGF-I favors a positive calcium and phosphate balance as required by the increased bone mineral accrual.

to higher insulin-like growth factor-I (IGF-I) levels [33].

These two adaptive mechanisms could be essential to cope with the increased bone mineral demand during the pubertal growth spurt. An increase in plasma calcitriol concentrations has been reported during pubertal maturation [34]. A tight relationship exists between tubular reabsorption of Pi, plasma Pi level, and growth velocity in children [35]. A rise in plasma Pi occurs during puberty [36, 37].

The mechanism underlying the parallel rise in calcitriol and the tubular reabsorption of Pi involves insulin-like growth factor-I (IGF-I), which could be responsible for the stimulation of both calcitriol production and tubular Pi reabsorption (TmPi/GFR) in relation to the increased calcium and Pi demand associated with bone growth [38]. In humans, IGF-I plasma level transiently rises during pubertal maturation, to reach a peak during mid-puberty. Its maximal level thus occurs at an earlier chronological age in females than in males [39]. IGF-I, whose growth hormone-dependent production is influenced by dietary protein intakes [40], enhances longitudinal and radial bone growth, increases renal tubular reabsorption of phosphate and stimulates renal calcitriol synthesis. The rise in IGF-I, calcitriol, and Pi plasma levels are correlated with elevation in indices of the bone appositional rate such as alkaline phosphatase [41] and osteocalcin [42]. Plasma concentrations of gonadal sex hormones, as well as those of adrenal androgens (dehydroepiandrosterone and androstendione), which increase before and during pubertal maturation, do not seem to accord with the accelerated bone mass gain [43]. Whether differences in the adaptive responses which control calcium and phosphate homeostasis could play a role in the increased variance in lumbar spine or femoral neck BMD/BMC remains to be explored. The interaction between the growth hormone-IGF-I axis and sex steroids is quite complex [42]. A bone-derived factor, FGF23, has been suggested to contribute to the bone-kidney link [44]. In young adults, serum FGF23 concentrations are influenced by dietary phosphorus intakes [45].

Bone Biochemical Markers DuringPuberty

The interpretation of the changes in bone biochemical markers during growth is more complex than in adulthood (see for review [42]). The plasma concentrations of the bone formation markers are the highest when the velocity of bone mineral accrual is maximal. This suggests that the two phenomena are related. The high urinary excretion of bone resorption markers, such as collagen pyridinium cross-links, observed during childhood, decreases after the growth spurt and reaches adult values at the end of pubertal maturation, that is, at 15–16 and 17–18 years of age in girls and boys, respectively (see for review [42]). In a longitudinal study in pubertal girls, bone turnover markers (osteolcalcin, bone specific alkaline phosphatase, and collagen pyridium cross-links) were modestly related to statural height gain, but not predictive of gains in either total bone mineral content or density as assessed by DXA [46].

Determinants of Bone Mass Gain

The factors influencing bone mass accumulation during growth include heredity, sex, dietary intakes (calcium and proteins), endocrine factors (sex steroids, calcitriol, and IGF-I), mechanical forces (physical activity and body weight), and exposure to other risk factors [1, 47, 48]. Quantitatively, the most prominent determinant appears to be genetically related.

Genetic Determinants

As mentioned earlier, the statural height-independent variability in lumbar spine and proximal femur BMD/BMC increases during pubertal maturation. The contribution of heredity, compared to that of the environment, to this increased bone mass variability is not clearly elucidated. Genetic factors account for a large percentage of the population variability in BMD among age- and sex-matched normal individuals [47, 48].

Daughters of osteoporotic women have a low BMD [49]. To investigate the proportion of the BMD variance across the population explained by genetic factors, known as its heritability, two human models have been mainly used. In the twin model, within-pairs correlations for BMD are compared between monozygotic (MZ) twins, who by essence share 100% of their genes, and dizygotic twins, who have 50% of their genes in common. Stronger correlation coefficients among adult MZ as compared to DZ twins are indicative of the genetic influence on PBM. Genetic factors could explain as much as 80% of lumbar spine and proximal femur BMD variance. Lean and fat mass are also genetically determined [50], since it appears that 80% and 65% of variance of lean and fat mass, respectively, are attributable to genetic factors.

Parents–offspring comparisons have also shown significant relationships for BMD, albeit heritability estimates have been somewhat lower (in the range of 60%) than in the twin model. Actually, the magnitude of direct genetic effects on PBM as evaluated in both human models may be overestimated by similarities in environmental covariates [51]. BMC, areal and volumetric BMD and scanned bone area in the lumbar spine and femur (neck, trochanter, and diaphysis) were compared in premenopausal women and in their prepubertal daughters [52]. Regressions were adjusted for height, weight, and calcium intake, to minimize the impact of indirect genetic effects as well as of dietary influences on bone mineral mass resemblance among relatives. Despite great disparities in the various constituents of bone mass before puberty with respect to peak adult values, heredity by maternal descent is detectable at all skeletal sites and affects virtually all bone mass constituents, including bone size and volumetric BMD. Moreover, when daughters' bone values were reevaluated 2 years later, while puberty had begun and BMC/BMD had considerably increased, measurements were highly correlated with prepubertal values and mother–daughter correlations remained unchanged. Thus, a major proportion of this variance is due to genetic factors which are already expressed before puberty with subsequent tracking of bone mass constituents through the phase of rapid pubertal growth until PBM is achieved. Applying high resolution peripheral QCT to mother–daughter and mother–son pairs, volumetric bone density and microstructure are highly and similarly inherited between and within sexes, even after various adjustments including age, weight, height, gonadal status, and aBMD [53]. In contrast to the clear heritability of PBM, the proportion of the variance of bone turnover markers that depends on genetic factors appears to be small [54]. Hence, PBM is determined by numerous gene products implicated in both bone modeling and remodeling [48].

To determine the genes implicated in PBM acquisition, two different approaches have been applied. The first involves investigating for association between allelic variants or polymorphisms of genes known to regulate bone metabolism. A series of associations with genes coding for hormone receptors, bone metabolism regulating enzymes, and matrix proteins have been reported. However, polymorphisms in the most studied genes like those coding for vitamin D receptor (VDR), estrogen receptor alpha (ESR1) or type one collagen A1 chain (CollA1) account for at most 1–3% of PBM variance [55, 56]. Age, sex, gene–environment, and gene–gene interactions are recognized as explaining the inconsistent relationship between BMC/BMD and these genotypes. For instance, significant BMD differences between VDR-3′ *Bsm*I genotypes were detected in children [57, 58], but were absent in premenopausal women from the same genetic background [57]. Moreover, the latter study found that BMD gain in prepubertal girls was increased at several skeletal sites in Bb and BB subjects in response to calcium supplements, whereas it remained apparently unaffected in bb girls, who had a trend for spontaneously higher BMD accumulation on their usual calcium diet [57, 59]. Polymorphisms in the LRP5 gene appear to contribute to bone mass variance in the general population. Indeed, in a cross-sectional cohort of 889 healthy Caucasian subjects of both sexes, significant associations were found for a missense substitution in exon 9 (c.2047G-- > A) with lumbar spine BMC, with bone scanned projected area, and with stature [60]. The associations were observed mainly in adult

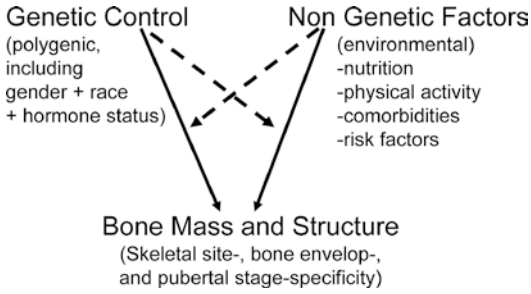

Fig. 6.3 Interaction between genetic and nongenetic factors on bone mineral mass and structure changes during puberty. Genetic factors are either acting directly on bone or indirectly by modulating the sensitivity to environmental factors. Similarly, environmental factors are acting either directly on bone or indirectly by modulating the genetic potential. Several influences varies according to the skeletal site, even the bone envelop at a given skeletal site, and according to pubertal stage

men, in whom LRP5 polymorphisms accounted for close to 15% of the traits' variances. LRP5 haplotypes were also associated with 1-year gain in vertebral bone mass and size in 386 prepubertal children. Again, significant associations were observed for changes in BMD and in scanned bone area in relation to LRP5 gene polymorphisms in males but not females. Altogether, these gene polymorphisms alone do not appear to be clinically useful as genetic markers for PBM.

Using Genome-Wide Associations Studies, a meta-analysis has revealed nine loci associated with aBMD at lumbar and proximal femur sites [61]. Like for polymorphism analysis, the contribution of these genes to aBMD variance is up to 3% only. Complex and gene–environment interaction models should be constructed to better appreciate the specific genes' roles in determining PBM and bone strength (Fig. 6.3). See Chap. 25 for a more detailed discussion on recent studies investigating the influence of genetics on aBMD and fracture risk.

Racial differences also provide an additional variable that contributes to bone mass acquisition. Indeed, in early adulthood, African-Americans have a higher aBMD than Caucasian controls [62]. While there is no difference in whole-body aBMD between black and white infants during the first 18 months of life [63], aBMD at several skeletal sites is higher in blacks than in whites by 10 years of age [64], suggesting a bone accrual rate higher in black children mainly during prepuberty. This, together with a slightly earlier onset of puberty [65], could explain the higher PBM in blacks compared to white individuals. This racial difference in PBM is related to differences in bone size and a slightly greater increase in vBMD at the vertebral level during puberty [66].

Physical Activity

The responsiveness to either an increase or a decrease in mechanical strain is probably greater in growing than in adult bones [1]. Hence, public health programs aim at increasing physical activity among healthy children and adolescents in order to maximize PBM. In children or adolescents involved in competitive sport or ballet dancing, intense exercise is associated with an increase in bone mass accrual in weight-bearing skeletal sites [67–69]. The question arises whether this increase in BMD/BMC resulting from intense exercise is translated into greater bone strength. In a cross-sectional study in male elite tennis players using peripheral QCT and side-to-side arm comparison, higher BMC reflected an increased bone size which was associated with an augmentation in a bone strength index. By contrast, no change in either cortical or trabecular vBMD was observed [70]. In terms of public health, observations made in elite athletes cannot be the basis of recommendations for the general population, since intense exercise is beyond the reach of most individuals. Much more relevant is information on the effect of *moderate* exercise on bone mass acquisition. Some, but not all, cross-sectional studies have found a slightly positive association between physical activity and bone mass values in children and adolescents. Measurements of the duration, intensity, and type of physical activity that are based on recall are not accurate, particularly in children. Controlled prospective studies carried out in prepubertal girls [71] or boys [72] indicate that exercise programs undertaken in schools, and considered on the average as *moderate*, can

increase bone mineral mass acquisition [73, 74] (for review, see [75]). These indicate that the growing skeleton is certainly sensitive to exercise and suggest that prepuberty would be an opportune time for implementing physical education programs consisting in various moderate weight-bearing exercises. Nevertheless, it remains uncertain as to what extent the greater aBMD gain in response to moderate and readily accessible weight-bearing exercise is associated with a commensurate increase in bone strength [72]. The magnitude of benefit in terms of bone strength depends upon the nature of the structural change, and possibly on the gender. Indeed, increasing levels of physical activity were associated with higher response weight bearing BMD in boys than in girls before puberty [76]. An effect consisting primarily of an increased periosteal apposition and consecutive diameter confers greater mechanical resistance than a response limited to the endosteal apposition rate leading essentially to a reduction in the endocortical diameter. There is a need for further studies aimed at examining the effects of different types of mechanical loading, such as magnitude and frequency of various types of exercise on the mass and geometry of bones in children and adolescents [77].

Studies in adult elite athletes indicate that increased bone mass gains resulting from intense physical activity during childhood and adolescence are maintained after training attenuates or even completely ceases [67, 78, 79, 80]. Finally, the question whether the increased PBM induced by physical exercise is maintained in old age and lead to a reduction in fracture rate remains open. A cross-sectional study of retired Australian elite soccer players suggests that this may not be the case [81]. However, the lack of information on the PBM values of these men does not allow one to draw firm conclusion about this observation.

Nutritional Factors

Puberty is considered to be a period with major behavioral changes and alterations in lifestyle, including food intake habits [82]. To what extent variations in the intakes of some nutrients in healthy, apparently well-nourished, children and adolescents can affect bone mass accumulation, particularly at sites susceptible to osteoporotic fractures, has received considerable attention.

Role of Calcium

It is usually accepted that increasing the calcium intake during childhood and adolescence is associated with a greater bone mass gain and thereby a higher PBM [83, 84]. However, a survey of the literature on the relationship between dietary calcium and bone mass indicates that some [85–87], but not all studies [88, 89], have found a positive correlation between these two variables. As with physical activity, several sets of cross-sectional and longitudinal data are compatible with a "two threshold model." On one side of the normal range, one can conceive the existence of a "low" threshold, set at a total calcium intake of about 400–500 mg/day, below which a positive relationship can be found. Within this low range, the positive effect of calcium would be explained merely by its role as a necessary substrate for bone mass accrual. On the other side of the normal range, there would be a "high" threshold, set at about 1600 mg/day, above which the calcium intake through another mechanism could exert a slightly positive influence on bone mass accrual. In addition, the levels of the two thresholds could vary according to the stage of pubertal maturation. In our own cross-sectional and longitudinal study, a significant positive relationship between total calcium intakes as determined by two 5-day diaries was found in females in the pubertal subgroup P1–P4, but not in the P5 subgroup [15, 18].

Several intervention studies carried out in children and adolescents [90–93] indicate greater bone mineral mass gain in children and adolescents receiving calcium supplementation over periods varying from 12 to 36 months. The benefit of calcium supplementation was mostly detected in the appendicular rather than in the axial skeleton [90–95]. In prepubertal children, calcium supplementation is more effective on cortical appendicular bone (radial and femoral diaphysis) than on axial trabecular rich bone (lumbar spine) or on the hip (femoral neck and trochanter) (for review, see [96]). The skeleton

appears to be more responsive to calcium supplementation before the onset of pubertal maturation [93]. In 8-year-old prepubertal girls with a spontaneously low calcium intake, increasing the calcium intake from about 700 to 1400 mg augmented the mean gain in aBMD of six skeletal sites by 58% as compared to the placebo group, after 1 year of supplementation. This difference corresponds to a gain of +0.24 standard deviation (SD) [90]. If sustained over a period of 4 years, such an increase in the calcium intake could augment mean aBMD by 1 SD. Thus, milk calcium supplementation could modify the bone growth trajectory and thereby increase PBM. In this regard, it is interesting to note that an intervention influencing calcium–phosphate metabolism and limited to the first year of life may also modify the trajectory of bone mass accrual. A 400 IU/day vitamin D supplementation given to infants for an average of 1 year was associated with a higher aBMD measured at the age of 7–9 years [97]. The aBMD difference between the vitamin D-supplemented and nonsupplemented group was particularly significant at the femoral neck, trochanter, and radial metaphysis. These observations are compatible with the "programming" concept, according to which environmental stimuli during critical periods of early development can provoke long-lasting modifications in structure and function [98, 99].

The type of the supplemented calcium could modulate the bone response. Thus, the response to a calcium phosphate salt from milk extract appears to differ from those recorded with other calcium supplements. Indeed, the positive effect on aBMD was associated with an increase in the projected bone area at several sites of the skeleton [90]. Interestingly, this type of response was similar to the response to whole milk supplementation [100]. But in the latter study, the positive effect on bone size could be ascribed to other nutrients contained in whole milk, such as protein, whereas in the former study, the tested calcium-enriched foods had the same energy, lipid, and protein content as those given to the placebo-group [90].

It is important to consider whether or not the gain resulting from the intervention will be maintained after discontinuation of the calcium supplementation. One year and 3.5 years after discontinuing the intervention, differences in the gain in aBMD and in the size of some bones were still detectable, but at the limit of statistical significance [19, 90]. These results need additional confirmation by long-term follow-up of the cohort, ideally until PBM has been attained, as well as by other prospective studies. Bone mineral density was also measured 7.5 years after the end of calcium supplementation. In these young adult girls, it appeared that menarche occurred earlier in the calcium-supplemented group, and that persistent effects of calcium were mostly detectable in those subjects with an earlier puberty [92].

In a meta-analysis on 19 calcium intervention studies involving 2859 children [96], with doses of calcium supplementation varying between 300 mg and 1200 mg per day, from calcium citrate-malate, calcium carbonate, calcium phosphate, calcium lactate-gluconate, calcium phosphate milk extract, or milk minerals, calcium supplementation had a positive effect on total body BMC and upper limb aBMD, with standardized mean differences (effect size) of 0.14 for both. At the upper limb, the effect persisted 18 months after cessation of calcium supplementation. Analyzing 17 studies involving 2088 individuals, the same authors concluded that calcium supplementation has no significant effect on weight, height, or body fat.

Despite a positive effect on mean aBMD gain, there is still wide interindividual variability in the response to calcium supplementation. As discussed above, it is possible that part of the variability in the bone gain response to calcium supplementation could be related to genetic background [101].

Role of Gut Microbiota: Effects of Prebiotics and Probiotics

The largest numbers of cells within the human body are bacteriae, Archae, Eukaryae, as well as fungi and viruses located in the intestinal tract. These organisms are collectively called the gut microbiota (GM). GM is now considered as an organ modulating the expression of genes

involved in mucosal barrier function, immune system, food digestion, and energy metabolism as it is capable of fermenting undigested nutrients into short-chain fatty acids with local and systemic effects [102, 103]. GM collected from malnourished children and transferred to gnotobiotic mice impaired their growth [104]. When the malnourished subjects received a supplementation containing peanuts, sugar, milk, vitamins, and minerals, their microbiota transplanted into mice corrected the impaired growth. This demonstrates an important role of GM in controlling bone growth.

Prebiotics are nondigestible fiber compounds that pass undigested through the upper part of the gastrointestinal tract, and stimulate the growth and/or activity of bacteriae that colonize the large bowel by acting as substrate for them [105]. Prebiotics refer to galactooligosaccharides (GOS), inulin, resistant starch, polydextrose, fructooligosaccharides (FOS), xylooligosaccharides, and lactulose. Oligosaccharides are composed of three to ten sugar units. Their length influences the site of fermentation. Foods particularly rich in fibers are dandelion greens, leeks, onion, wheat bran, and flour. Some GOS can also be found in peas and beans. In male adolescents, the consumption of 15 g of oligofructose per day was shown to stimulate fractional calcium absorption [106]. Among healthy adolescent girls aged 10–13 years who consumed smoothie drinks twice daily with 0, 2.5, or 5 g GOS for 3-week periods, fractional calcium absorption increased with both 5 and 10 g/day doses of GOS compared with the control (0.444, 0.419, and 0.393, respectively), although a dose–response relationship was not observed [107]. The increase in calcium absorption was the greatest after 24 h, consistent with distal gut absorption. Using a similar stable calcium absorption method, the same authors detected a 12% higher intestinal calcium absorption in adolescent boys and girls exposed to maize and corn fibers [108]. Fecal bifidobacteria increased with GOS treatment, which suggests that calcium absorption may be mediated by the gut microbiota, specifically bifidobacteria [107]. Differences in calcium absorption were correlated with various bacteria genera at the end of the study [108]. In a randomized controlled trial conducted in adolescents, 8 g/day of FOS and inulin for 1 year increased whole body BMC [109]. In various populations of different age from adolescents to postmenopausal women, and with various treatment durations, from 9 days to 1 year, higher intestinal calcium absorption was consistently detected in response to prebiotics [106, 109–112].

The amount of prebiotics required to produce significant bone effects is limited by the tolerance. Indeed, undigested saccharides/fibers fermentation in the large intestine may be associated with flatulence and abdominal discomfort, precluding amounts of prebiotics ingestion sufficient to exert meaningful biological effects. However, in the studies by Whisner [107, 108, 113], the tolerance to prebiotics amounts associated with increased calcium absorption was reported as good in adolescent girls.

Probiotics are live microorganisms which, when administered in adequate amounts, confer a health benefit on the host [114]. By adequate, one means an amount able to trigger the targeted effect. It depends on strain specificity, process and matrix, and sought targeted effect. With concentration of around 10^e7 to 10^e8 probiotic bacteriae per gram, a serving size is around 100–200 mg. Various species are provided as probiotics, such as Lactobaccilli, Bifidobacteriae, Escherichia, Enterococcus, and *Bacillus subtilis*. Yeast like *Saccharomyces* has been used too. In humans, the main source of probiotics is fermented dairy products [115], which also provide calcium, protein phosphorus, and zinc. The problem is to provide a sufficient amount of bacteriae capable of reaching the distal part of the gastrointestinal tract. However, it has been reported that yogurt consumers had lower level of Enterobacteriaceae and higher beta-galactosidase activity, the latter and Bifidobacterium population being positively correlated to the amount of fermented products ingested [116]. In experimental animals, probiotics and/or probiotics-induced butyrate production in the gut are able to reduce bone resorption and stimulate bone formation [117]. Whether intakes of probiotics are able to influence PBM acquisition is not known.

Role of Protein

Among nutrients other than calcium, protein intake influences bone mass acquisition and loss [40, 118]. Available information suggests that either a deficient or an excessive protein supply could negatively affect calcium balance and the amount of bony tissue contained in the skeleton [119].

Low protein intake could be particularly detrimental for both the acquisition of bone mass and the conservation of bone integrity with aging. During growth, undernutrition, including inadequate supply of energy and protein, can severely impair bone development. Studies in experimental animals indicate that isolated protein deficiency leads to reduced bone mass and strength without histomorphometric evidence of osteomalacia [120]. Thus, inadequate supply of protein appears to play a central role in the pathogenesis of the delayed skeletal growth and reduced bone mass observed in undernourished children [121]. Low protein intake could be detrimental for skeletal integrity by lowering the production of IGF-I. Indeed, the hepatic production and plasma concentration of this growth factor, which exerts several positive effects on the skeleton, is under the influence of dietary protein [122–124]. Protein restriction has been shown to reduce circulating IGF-I by inducing resistance to the hepatic action of growth hormone. In addition, protein restriction appears to induce a resistance to the anabolic actions of IGF-I [124]. In this regard, it is important to note that growing rats maintained on a low protein diet failed to restore growth when IGF-I was administered at doses sufficient to normalize its plasma concentrations.

Variations in IGF-I production could explain some of the changes in bone and calcium–phosphate metabolism that have been observed in relation to intake of dietary protein. In humans, circulating IGF-I, of which the major source is the liver, progressively increases from 1 year of age to reach peak values during puberty. As described above, this factor appears to play a key role in calcium–phosphate metabolism during growth by stimulating two kidney processes, Pi transport and the production of calcitriol [33]. IGF-I is considered an essential factor for bone longitudinal growth, as it stimulates proliferation and differentiation of chondrocytes in the epiphyseal plate [125, 126]. It also plays a role on trabecular and cortical bone formation. In experimental animals, administration of IGF-I positively influences bone mass [127], by increasing the external diameter of long bone, and by enhancing the process of periosteal apposition. Therefore, during adolescence, a relative deficiency in IGF-I or a resistance to its action that could be due to an inadequate protein supply may result not only in a reduction in the skeletal longitudinal growth, but also in an impairment in cross-sectional bone development.

In well-nourished children and adolescents, the question arises of whether variations in the protein intake within the "normal" range can influence skeletal growth and, thereby, modulate the genetic potential in PBM attainment. There is a positive relationship between protein intake, as assessed by two 5-day dietary diary methods with weighing most food intakes [82, 120], and bone mass gain, particularly from pubertal stage P2 to P4. The correlation remained statistically significant even after adjusting for age or calcium intake. The association between bone mass gain and protein intake is observed in both sexes at the lumbar spine, the proximal femur, and the femoral midshaft.

In a prospective longitudinal study performed in healthy children and adolescents of both genders, between the age of 6 and 18, dietary intakes were recorded over 4 years, using an yearly administered 3-day diary [128]. Bone mass and size were measured at the radius diaphysis using peripheral computerized tomography. A positive association was found between long-term protein intakes, on one hand, and periosteal circumferences, cortical area, bone mineral content, and with a calculated strength strain index, on the other hand. The relatively high mean protein intakes in this cohort with a Western style diet should be highlighted. Indeed, protein intakes were around 2 g/kg body weight × day in prepubertal children, whereas they were around 1.5 g/kg × day in pubertal individuals. The minimal requirements for protein intakes in the corresponding age groups are 0.99 and 0.95, respectively [129]. There was no association between bone variables and intakes of

nutrients with high sulfur-containing amino acids, or intake of calcium. Overall, protein intakes accounted for 3–4% of the bone parameters variance [128]. However, even when they are prospective and longitudinal, observational studies do not allow one to draw conclusion on a causal relationship. Indeed, it is quite possible that protein intake could be to a large extent related to growth requirement during childhood and adolescence. For instance, rats treated with growth hormone are spontaneously selecting a high-protein diet [130]. Only intervention studies could reliably address this question. To our knowledge, there is no large randomized controlled trial having tested the effects of dietary protein supplements on bone mass accumulation, except for milk or dairy products.

In addition to calcium, phosphorus, calories, and vitamins, 1 l of milk provides 32–35 g of protein which is mostly casein, but also whey protein which contains numerous growth-promoting elements [131]. The correlation between dairy products intake and bone health has been investigated in both cross-sectional and longitudinal observational studies, and in intervention trials. In growing children, long-term milk avoidance is associated with smaller stature and lower bone mineral mass [132–140]. Low milk intake during childhood and/or adolescence increases the risk of fracture before puberty (+2.6-fold), and possibly later in life [141–143]. In a 7-year observational study, there was a positive association between dairy products consumption and a BMD at the spine, hip, and forearm in adolescents, leading thereby to a higher PBM [87]. In addition, higher dairy products intakes were associated with greater total and cortical proximal radius cross-sectional area. Based on these observations, it was suggested that whereas calcium supplements could influence volumetric BMD, thus the remodeling process, dairy products may have an additional effect on bone growth and periosteal bone expansion, that is, a modeling influence [87]. In agreement with this observation, milk consumption frequency and milk intake at age 5–12 and 13–17 years were significant predictors of the height of 12- to 18-year-old adolescents, studied in the NHANES 1999–2002 [144].

A variety of intervention trials have confirmed a favorable influence of dairy products on bone health during childhood and adolescence [100, 145–155]. In an open randomized intervention trial, Cadogan et al. studied the effects of 568 ml/day milk supplement for 18 months in 12-year-old girls [100]. With this milk supplement, the differences between the treated and control groups in calcium and protein intakes at the end of the study were around 420 mg/day and 14 g/day, respectively, taking into consideration the spontaneous consumption. In the milk-supplemented group, serum IGF-I levels were higher (+17%). Compared to the control group, the intervention group had greater increases of whole-body BMC/BMD.

In another study, cheese supplements appeared to be more beneficial for cortical bone accrual than a similar amount of calcium supplied under the form of tablets [146]. This positive influence of milk products on cortical bone thickness may be related to an effect on the modeling process, since metacarpal periosteal diameter was significantly increased in Chinese children receiving milk supplements [154].

As was the situation for other nutrients such as calcium, only prospective interventional studies will establish whether variations in protein intake within the range recorded in our Western "well-nourished" population can affect bone mass accumulation during growth. Such prospective intervention studies should delineate the crucial years during which modifications in nutrition would be particularly effective for bone mass accumulation in children and in adolescents. This kind of information is of importance in order to make credible and well-targeted recommendations for osteoporosis prevention programs aimed at maximizing PBM.

Conditions Impairing Peak Bone Mass Attainment

Various genetic and acquired disorders can impair optimal bone mass acquisition during childhood and adolescence [156, 157]. In some endocrine disorders, such as Turner's syndrome, Klinefelter's

syndrome, glucocorticoid excess, hyperthyroidism or growth hormone deficiency, low bone mass has been attributed to abnormalities in a single hormone system. In diseases such as anorexia nervosa and exercise-associated amenorrhea**,** malnutrition, sex steroid deficiency, and other factors combine to increase the risk of osteopenia or low bone mass. This is probably also the case of various chronic diseases, which in addition may require therapies that can affect bone metabolism. Impaired bone growth has been frequently observed in chronic rheumatoid arthritis, chronic renal failure, cystic fibrosis, inflammatory bowel diseases [158], childhood leukemia, and hemoglobinopathies such as thalassemia major.

Delayed Puberty

Epidemiological studies suggest that late menarche is a risk factor for osteoporosis through a negative effect on PBM (Fig. 6.4) (for review, see [159]). In a cohort of men with a history of delayed puberty, osteopenia has been reported [160]. Cortical and trabecular microstructure at PBM are influenced by menarcheal age. In a cohort of healthy females followed prospectively from the age of 7.9 to 20.4 years, an inverse relationship between forearm bone microstructure and menarcheal age has been found [161]. Subjects with later but still within the normal age range, menarche, had lower radius aBMD, cortical vBMD, and cortical thickness (Fig. 6.5). In males, later pubertal development is associated with lower PBM, alterations in bone microstructure and strength, together with higher fracture risk during childhood and adolescence [163].

The causes of delayed adolescence have been classified into permanent and temporary disorders [164]. The permanent ones can be due to either hypothalamo-pituitary or gonadal failure [164]. Heritable factors play a major role in the determination of menarcheal age. Thus, ages at which mothers and daughter experience their first menstruation are correlated, with heritability coefficients suggesting that 50% of the phenotypic variation in menarcheal age is genetically

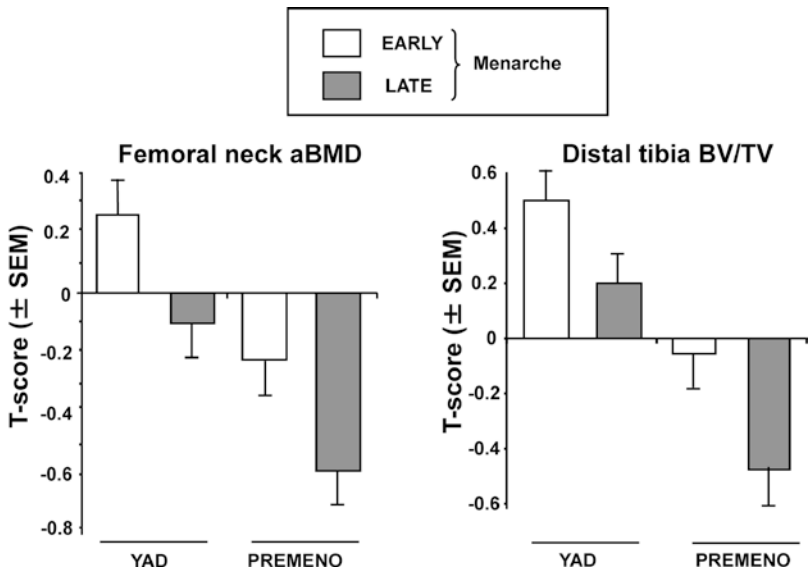

Fig. 6.4 T-score of femoral neck aBMD and trabecular bone volume fraction (BV/TV) of distal tibia in relation with menarcheal age in young (YAD) and middle-aged premenopausal (PREMENO) healthy women. The two cohorts of young adult (YAD, 20.4 years, n = 124) and middle-aged premenopausal (PREMENO, 45.8 years, n = 120) women were segregated by the median in EARLY and LATE menarcheal age. The mean menarcheal age (years±SD) were in: YAD EARLY: 12.1 ± 0.7; YAD LATE: 14.0 ± 0.7; PREMENO EARLY: 11.8 ± 1.0; PREMENO LATE: 14.4 ± 1.1. (Reprinted from Chevalley et al. [166]. With permission from John Wiley & Sons, Inc.)

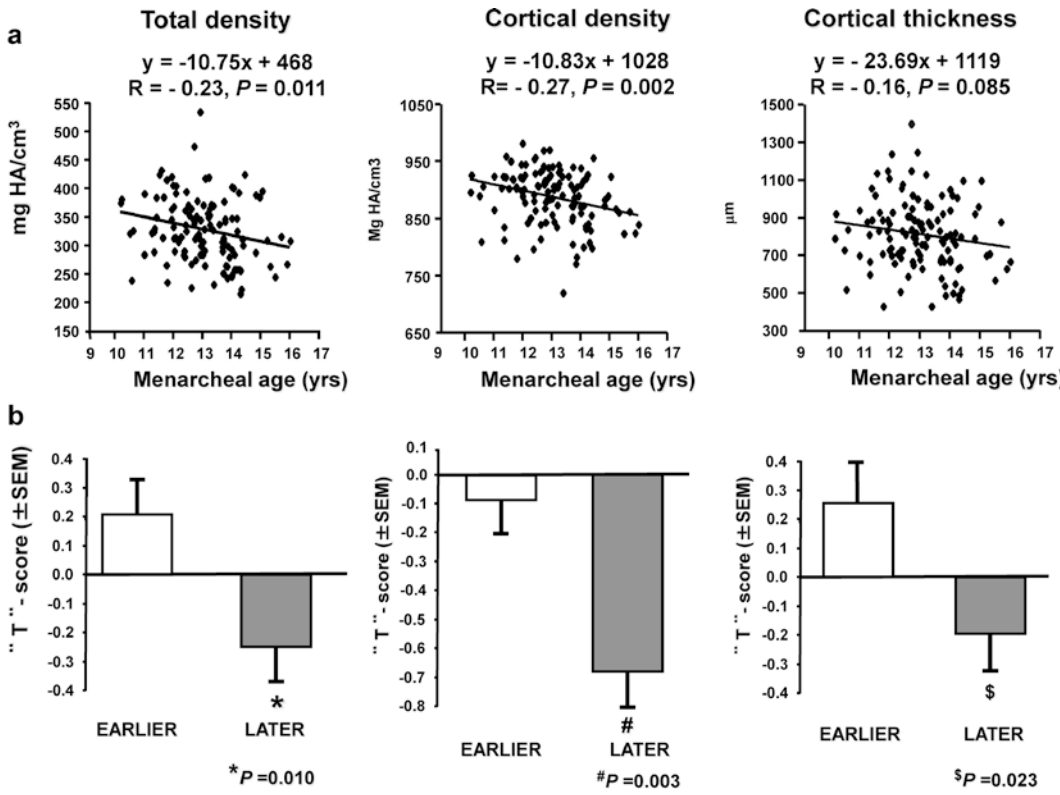

Fig. 6.5 Influence of menarcheal age on distal radius bone microstructure in healthy young adult women. Total density, cortical density, and cortical thickness of the distal radius were inversely related to menarcheal age. *P* values after adjustment for calcium intervention, standing height, and body weight were 0.018, 0.002, and 0.091 for total density, cortical density, and cortical thickness, respectively. The cohort of the 124 healthy women was segregated by the median of menarcheal age. "T" -score calculated from an external cohort of healthy French women with mean age of 34 ± 7 years [162] was significantly lower in LATER (N = 62) versus EARLIER (N = 62) group for total density, cortical density, and cortical thickness of the distal radius. (Reprinted from Chevalley et al. [161]. With permission from John Wiley & Sons, Inc.)

determined [165, 166]. Among the temporary disorders, some can be explained by the presence of chronic diseases, nutritional disorders, psychological stress, intensive competitive training, or hormonal disturbances such as hyposecretion of thyroid hormones or growth hormone, or hypercortisolism [164]. However, the most common cause of delayed adolescence is the so-called "constitutional delay of growth and puberty" (CDGP). It is a transient disorder with, in some cases, a familial history of late menarcheal age of the mother or sisters, or a delayed growth spurt in the father. This condition has been considered so far as an extreme form of the physiological variation of the timing of the onset of puberty for which the "normal" range is about 8–12 and 9–13 years of age in girls and boys, respectively. The onset of puberty is a complex process involving the activation of the hypothalamic-pituitary-gonadal axis and other endocrine systems such as the growth hormone–IGF axis which are influencing bone mineral balance and skeleton growth rate. Several mechanisms whereby CDGP may lead to a low PBM have been suggested [167].

In preburtal girls who have undergone a menarche later than the median of the cohort, a lower aBMD can be detected already before the onset of pubertal maturation (Fig. 6.6) [168]. This observation does not support the hypothesis that a lower PBM in subjects with later menarche would be the result from a shorter exposure duration to estrogen.

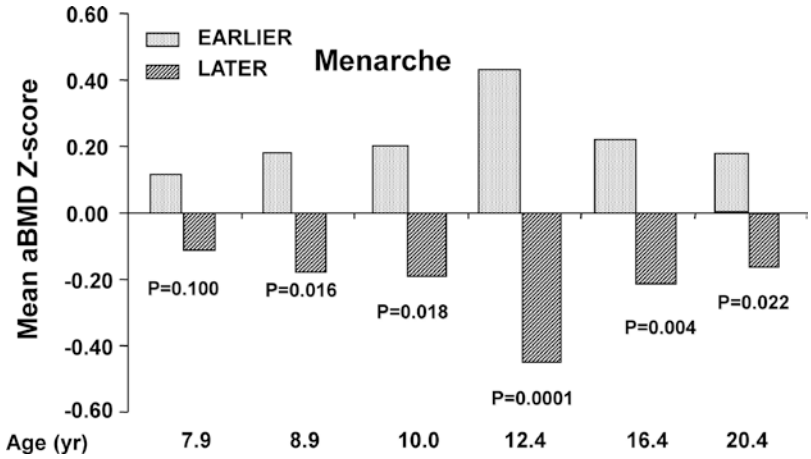

Fig. 6.6 Mean aBMD Z-score of six skeletal sites according to the median of menarcheal age from prepuberty to PBM attainment at 20.4 years of age. The pubertal stages were P1 at 7.9 and 8.9 years of age, P1–P2 at 10.0 years, P2–P5, and P1–P5 at 12.4 years in EARLIER and LATER, respectively. All the cohorts were postpubertal at 16.4 years of age. Between age 7.9 and 8.9 years, statistical analysis by two-way ANOVA indicated that the significant (P = 0.001) age-dependent aBMD increment did not interact with the influence (P = 0.0038) of future MENA. (Reprinted from Chevalley et al. [168]. With permission from Oxford University Press)

Anorexia Nervosa

Significant deficits in trabecular and cortical bones, which may result in osteoporotic fractures, have been observed in young adult women with chronic anorexia nervosa [169]. Several factors can contribute to the reduced bone mass acquisition, including low energy/protein intake resulting in a reduction in IGF-I production and, thereby, decreasing bone formation; estrogen deficiency and low calcium intake enhancing bone resorption; and glucocorticoid excess which interrupts normal acquisition of bone mineral and may contribute to increased bone loss [170, 171].

Exercise-Associated Amenorrhea

Impaired bone mass acquisition can occur when hypogonadism and low body mass accompany intensive physical activity [172, 173]. As in anorexia nervosa**,** both nutritional and hormonal factors contribute to this impairment. Intake of energy, protein, and calcium may be inadequate as athletes go on diets to maintain an idealized physique for their sport. Intensive training during childhood may contribute to a later onset and completion of puberty. Hypogonadism, as expressed by the occurrence of oligomenorrhea or amenorrhea, can lead to bone loss in females who begin training intensively after menarche [156]. Oligo-amenorrhea in long-distance runners was found to be associated with a decrease in a BMD affecting more the lumbar spine than the proximal and midshaft femur [174].

Fracture During Bone Acquisition

During growth, fractures, particularly at the forearm, are frequent, with an overall prevalence varying between 27% and 40% in females and between 42% and 52% in males [23, 175]. The highest incidence is observed between 11 and 12 years of age in girls, and between 13 and 14 years in boys [175]. The latter may be related to the dissociation between peak height velocity and peak bone mineral content velocity, the former preceding by about 1 year the latter [20]. In addition, a transient increase in peripheral bone cortical porosity has been reported [25, 26, 176]. However, lower BMC/BMD has been documented in children with fracture as compared

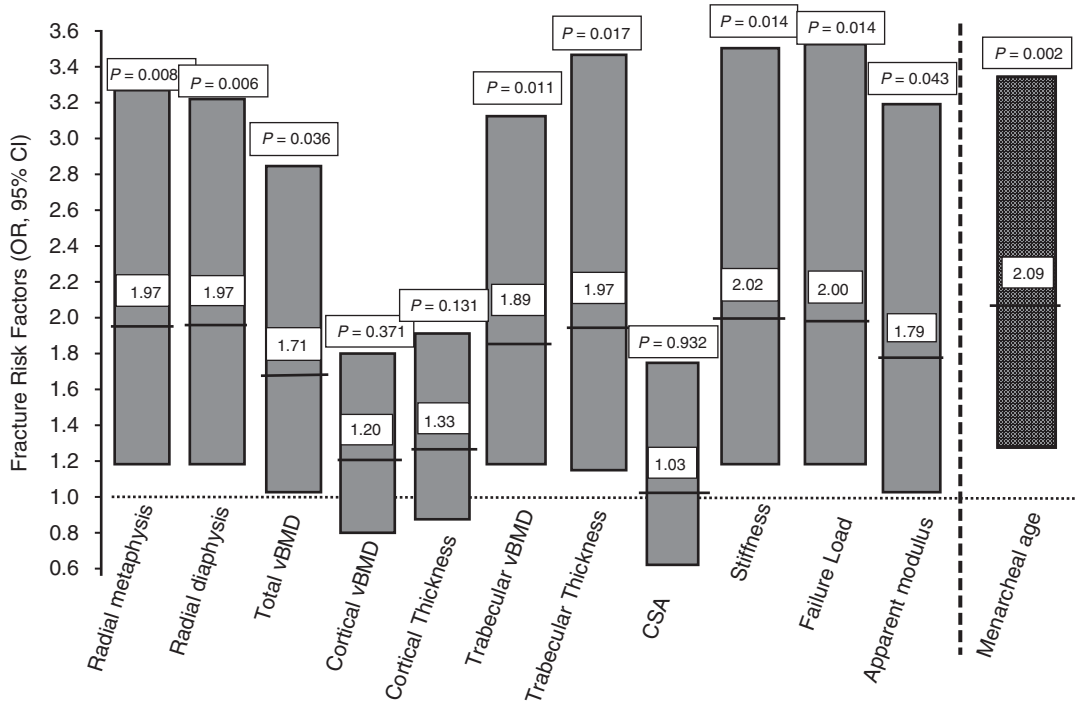

Fig. 6.7 Risk of fracture for 1 SD decrease in radial aBMD or in distal radius microstructure components and strength variables and for 1 SD increase in menarcheal age (MENA) in 124 girls. Bone densitometric values were measured at 20.4 years of age, once PBM was attained. *Columns* are OR ± 95% CI, as evaluated by logistic regression. Statistical significance (*P*) is indicated *above each column*. CSA, Cross-sectional area. (Reprinted from Chevalley et al. [179]. With permission from Oxford University Press)

with sex- and age-matched unfractured controls [23, 177, 178]. Furthermore, girls from a prospective cohort followed from 8 to 20 years, who have sustained a fracture, have a lower bone mass gain during puberty [22]. After puberty, these subjects with prevalent fracture had lower lumbar spine, ultradistal radius, and trochanter BMC [22]. At the age of 20 years, healthy young women with prevalent fracture had lower radius PBM, altered microstructure, and estimated bone strength as compared with unfractured women (Fig. 6.7) [179]. This suggests that a fracture during childhood and adolescence could be a marker of low PBM in females. In contrast, using a similar prospective study in healthy males, no difference in DXA-derived variables, in distal skeleton microstructure or estimated bone strength could be detected among 23-year-old male subjects with and without fracture during childhood and adolescence [180]. There appears thus a sex difference in prevalent fracture as a risk factor for low PBM, possibly related to the type of trauma in girls and boys.

Conclusion

Peak bone mass is an important determinant of osteoporotic fracture risk, hence, the interest of exploring ways of increasing PBM in osteoporosis primary prevention. Bone mineral mass accumulation from infancy to postpuberty is a complex process implicating interactions of genetic, endocrine, mechanical, and nutritional factors. From birth to PBM, which is attained in axial and in the proximal femur by the end of the second decade of life, the increase in mass and strength is essentially due to an increment in bone size, vBMD changing very little during growth. Therefore, the best simple clinical

estimate of bone strength is aBMD rather than vBMD which does not take into account the size of the bone. It can be estimated that in women, an increase of PBM by 10%, that is, by approximately 1 standard deviation (SD), could decrease the risk of fragility fracture by 50% or be equivalent to retarding menopause by 14 years [4]. Like standing height in any individual bone mineral mass during growth follows a trajectory corresponding to a given percentile or standard deviation from the mean. Nevertheless, this trajectory can be influenced by the environmental factors. On the negative side, various chronic diseases and their treatment can shift downward this trajectory. On the positive side and most important in the context of primary prevention of adult osteoporosis, prospective randomized controlled trials strongly suggest that increasing the calcium intake or mechanical loading can shift upward the age-bone mass trajectory. Prepuberty appears to be an opportune time for obtaining a substantial benefit of increasing physical activity with appropriate intakes of calcium and proteins. Further studies should demonstrate that changes observed remain substantial by the end of the second decade and, thus, are translated in a greater PBM. In this long-term evaluation of the consequence of modifying the environment, it will be of critical importance to assess whether any change in densitometric and morphometric bone variables observed at PBM confers a greater and sustain resistance to mechanical strain.

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Osteoporosis Screening and Diagnosis

7

Elaine W. Yu

Key Points

- Osteoporosis is defined by low bone mass and skeletal fragility and is highly predictive of future fractures.
- Fracture risk assessment takes into account clinical risk factors for osteoporosis and measurement of bone mineral density by dual-energy X-ray absorptiometry, if available.
- In North America, routine bone density screening is recommended for all women aged 65 years and older, as well as for postmenopausal women and men aged 50 years and older with clinical risk factors for osteoporosis.
- Pharmacologic osteoporosis treatment is recommended for adults with a history of fragility fracture, or bone density *T*-score ≤ -2.5. In the United States, treatment is also advised for osteopenic adults who have a $\geq 3\%$ risk of hip fracture or $\geq 20\%$ risk of major osteoporotic fracture over the next 10 years.

Introduction

Osteoporosis is a disease defined by low bone mass and skeletal fragility that leads to an increased risk of fractures. Osteoporosis affects an estimated 200 million women worldwide [1] and disproportionately affects older adults. It is expected that the prevalence of osteoporosis may further increase as the population of adults aged 65 years and older is expected to double between 2010 and 2040 [2]. "Osteoporotic" fractures, also known as "low-trauma" or "fragility" fractures, are interchangeable terms that refer to fractures resulting from an impact equivalent to a fall of less than or equal to standing height. Approximately nine million osteoporotic fractures occur every year worldwide [3]. One out of every two women and one out of five men over the age of 50 years will have a low-trauma fracture in their lifetime [4, 5]. Osteoporotic fractures often lead to rapid deterioration of health status and decreased quality of life [6, 7]. These fragility fractures are also associated with high rates of mortality compared to age- and sex-matched controls [8, 9], and hip fractures in particular lead to 37% and 17% excess mortality in men and women, respectively [10, 11]. There is also a significant economic burden from osteoporotic fractures. As of 2005, fractures cost $20 billion a year in the United States and €36 billion a year in Europe [2].

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Given these sobering statistics, screening for osteoporosis has been recommended to identify individuals at high risk of fracture who would benefit from treatments to minimize that risk. Osteoporosis screening typically involves assessments of clinical risk factors and measurement of bone mineral density (BMD).

Clinical Risk Factors

Numerous clinical factors have been identified that can aid in stratifying fracture risk for individual patients. Advancing age has been found to be an even stronger determinant of fracture risk than bone mass [12]. History of prior fragility fracture is also a robust risk factor for fracture, increasing future fracture probability by two to four times even after adjustment for age and BMD [13, 14]. Other validated risk factors that are independent of BMD include chronic glucocorticoid therapy, parental history of hip fracture, low body weight, current cigarette smoking, and excessive alcohol consumption [15].

Race and ethnicity are also clinical factors that influence fracture risk. In the United States, black and Asian-American women have lower rates of fracture than white, Hispanic, or Native American women [16, 17]. This relationship between race/ethnicity and fracture is not entirely mediated by bone density. For example, higher BMD among black women does not fully account for lower fracture rates [18], and Asian-American women experience fewer fractures despite having lower BMD than white women [19]. Worldwide rates of fracture also vary significantly by country, with the highest incidence of fractures occurring in Scandinavia [20].

As part of a thorough clinical assessment, it is important to evaluate for secondary causes of osteoporosis to inform prognosis and treatment decisions (Table 7.1). These disorders are associated with low bone density and increased fracture risk through varied mechanisms such as estrogen deficiency, vitamin D or calcium malabsorption, systemic inflammation, osteoblast/osteoclast toxicity, and/or high bone turnover states. Some of these secondary causes of osteoporosis are addressed in detail in other chapters of this volume.

Table 7.1 Secondary causes of osteoporosis

Endocrine disorders	*Gastrointestinal disorders*
Hyperparathyroidism	Celiac disease
Hyperthyroidism	Inflammatory bowel disease
Cushing's disease	Primary biliary cholangitis
Delayed puberty	Roux-en-Y gastric bypass surgery
Premature ovarian failure	Vitamin D and/or calcium deficiency
Hypothalamic amenorrhea	
Hypogonadism	
Anorexia nervosa	
Diabetes mellitus—Type 1 or type 2	
Medications	*Others: Liver disease, Human Immunodeficiency Virus (HIV)*
Glucocorticoids	Rheumatoid arthritis
Gonadotropin-releasing hormone (GnRH) agonists	Multiple myeloma
Aromatase inhibitors	Chronic kidney disease
Antiseizure medications	Cystic fibrosis
Immunosuppressive agents (cyclosporine, tacrolimus)	Multiple sclerosis
Heparin	Idiopathic hypercalciuria
Depot medroxyprogesterone acetate	Organ transplantation
Thiazolidinediones	Systemic mastocytosis

Bone Mineral Density

The most commonly used technique to assess BMD in clinical practice is dual-energy X-ray absorptiometry (DXA). DXA provides precise and accurate assessments of the density of bone mineral (i.e., calcium hydroxyapatite) at clinically relevant sites such as the lumbar spine, proximal femur, and distal radius [21]. This bone-imaging technique involves low doses of ionizing radiation that are equivalent to daily background radiation and are ten times lower than the radiation exposure of a chest X-ray film [22]. There is a robust correlation between skeletal biomechanical strength and BMD measured by DXA [23].

Multiple studies have verified that low bone density at axial and appendicular sites predicts future osteoporotic fractures [24–28]. BMD *T*-scores quantify the standard deviation difference between a patient and a reference population of healthy young adults. For every standard deviation decrease in age-adjusted BMD, there is a roughly twofold increase in the risk of fracture [28]. This leads to an exponential increase in fracture risk, such that a patient with a *T*-score of −3.0 will have an eightfold higher risk of fracture than a person of the same age with a *T*-score of 0. Although low BMD at any site can predict osteoporotic fractures at all sites, it has better predictive ability at the site of measurement. For example, hip BMD is superior to BMD measured at other skeletal sites for predicting hip fracture [24, 25].

A World Health Organization (WHO) working group developed a categorization of BMD based on *T*-scores, with *T*-scores of −1.0 or above classified as *normal*, *T*-scores between −1.0 and −2.5 classified as *osteopenia*, and *T*-scores of −2.5 or below classified as *osteoporosis* [29]. These classifications apply to postmenopausal women and men of age 50 years and older. Despite these discrete *T*-score categorizations, it is important to recognize that there is no clear threshold below which fracture risk is suddenly increased. On the contrary, the relationship between BMD and fracture risk is continuous [29]. Thus, while fracture rates are highest among women with osteoporotic *T*-scores of −2.5 or below, the majority of women who fracture have osteopenic or normal BMD. Studies estimate that between 55% and 80% of women who fracture have nonosteoporotic *T*-scores [30–32]. Using a threshold *T*-score of ≤−2.5 has a sensitivity of 46% and specificity of 84% for identifying adults who will sustain osteoporotic fractures [32].

In summary, DXA provides BMD measurements that are highly predictive of future fracture risk, but risk stratification based on *T*-score alone is insufficient to identify the majority of individuals who will fracture. Thus, an effective screening strategy should additionally incorporate other factors that are independent of BMD to identify those who are at high risk of fracture and would benefit from intervention.

Table 7.2 Clinical evaluation

Medical history	*Laboratory evaluation*
Prior fractures	Calcium
Age at puberty	Phosphorus
Age at menopause	Creatinine
Menstrual history	25-OH vitamin D
Parental fracture history	Parathyroid hormone (PTH)
Prior osteoporosis therapy	Alkaline phosphatase
Glucocorticoid use	Albumin
Calcium intake	Magnesium
Smoking	
Alcohol use	
Falls	
Secondary causes of osteoporosis	
Bone mineral density by dual-energy X-ray absorptiometry	*Additional laboratory tests in selected patients*
Lumbar spine	24-hour urine Ca/Cr
Proximal femur: Femoral neck and total hip	Thyroid stimulating hormone (TSH)
1/3 radius (selected cases)[a]	Liver function tests
	Serum protein electrophoresis (SPEP)/ Urine protein electrophroesis (UPEP)
	Tissue transglutaminase antibody
	24-hour urinary free cortisol
	Testosterone

[a]Bone mineral density (BMD) of 1/3 radius is suggested if spine/femur BMD is unobtainable, or in setting of primary hyperparathyroidism, hyperthyroidism, or androgen deprivation therapy for prostate cancer

Osteoporosis Evaluation: Putting It All Together

A comprehensive evaluation for osteoporosis involves assessment of both clinical risk factors and bone mineral density by DXA. Suggested components of a clinical osteoporosis evaluation are presented in Table 7.2. A complete medical history, basic laboratory evaluation, and DXA scan will provide useful information to risk stratify patients. Selected patients with medical history suggestive of secondary causes may further benefit from additional targeted laboratory testing.

Fracture Risk Calculators

One commonly proposed osteoporosis screening strategy involves identification of high-risk individuals based on assessment of their absolute fracture risk after taking into account clinical risk factors and/or BMD. Fracture risk calculators have been developed to integrate clinical variables with or without BMD into a model that will predict an individual's future fracture risk.

FRAX Calculator

The most studied calculator is the fracture risk assessment tool (FRAX) algorithm [33], which provides estimates of an individual's 10-year probability of hip fracture or major osteoporotic (combined clinical spine, hip, shoulder, and wrist) fracture. Fracture risk assessment tool (FRAX) was derived using data from 9 international cohorts and has been validated in more than 26 external cohorts [34]. This algorithm incorporates 11 patient factors (i.e., age, sex, height, weight, prior fracture, parental hip fracture, smoking, alcohol, glucocorticoids, rheumatoid arthritis, and either secondary osteoporosis or BMD) to calculate an individual's fracture risk.

Unique strengths of the FRAX algorithm are that it takes into account country-specific epidemiology and also incorporates competing mortality risk, thus potentially providing more accurate assessments among elderly patients [35]. An important caveat is that FRAX has only been evaluated in treatment-naive populations, and therefore should not be used to predict fractures in patients who are currently taking or have previously received pharmacologic osteoporosis treatments. In addition, FRAX has been shown to systematically underestimate fracture risk among adults with type 2 diabetes [36, 37]. Correction factors have been created to modify FRAX estimates based on discordant spine/hip *T*-scores [38], glucocorticoid dose [39], and vertebral textural assessment by trabecular bone score (TBS) [40].

Other Fracture Calculators

Many other fracture risk calculators have been developed that vary in scope and complexity. The most complex of these, the updated QFracture algorithm, encompasses 31 risk factors [41]. On the other end of the spectrum, Garvan involves five risk factors and takes into account dose–response relationships with prior fractures and falls to predict 5- or 10-year fracture risk [42, 43]. These calculators have been externally validated and have roughly similar discriminative power as FRAX to predict fractures [34, 44]. To date, however, only FRAX has been incorporated into national osteoporosis guidelines for screening and intervention (Table 7.3).

Table 7.3 Comparison of fracture risk calculators: FRAX, Garvan, and QFracture

	FRAX	Garvan	QFracture
Derivation cohort	Nine international cohorts	Dubbo cohort (Australia)	United Kingdom General Practice (UK GP) databases
No. of subjects in derivation cohort	46,340	2216	>two million
No. of risk factors in calculator	11 (BMD optional)	5 (including BMD)	18 (not including BMD)[a]
Fracture risk estimates	10 years	5–10 years	1–10 years
External validation studies	27	7	4
Range of area under the curve (AUC) in validation studies (i.e., discriminatory power)	0.54–0.82	0.69–0.84	0.64–0.89
Other notes	Population-specific calibration, competing mortality risk	Includes dose–response for prior fx and falls	Younger population, includes diabetes mellitus (DM), dose–response for smoking, alcohol (EtOH)

Based on data from Refs. [34, 45]
BMD bone mineral density
[a]Updated QFracture 2012 algorithm has 13 additional variables [41]

Screening Guidelines

North American Recommendations

In the United States and Canada, routine BMD screening with DXA is recommended in all women aged 65 years and older. This recommendation is endorsed by the US Preventative Services Task Force (USPSTF, grade B recommendation) [46] as well as multiple expert groups (Canadian Osteoporosis Society [47], American Association of Clinical Endocrinology (AACE) [48], American Association of Family Practice (AAFP) [49], National Osteoporosis Foundation (NOF) [50], International Society for Clinical Densitometry (ISCD) [51], North American Menopause Society (NAMS) [52], American College of Preventative Medicine (ACPM) [53], and American College of Obstetrics and Gynecology (ACOG) [54]).

There is less consensus about the utility of routine osteoporosis screening in older men. The USPSTF did not find sufficient evidence to recommend routine screening for men [46]. The Canadian Osteoporosis Society recommends BMD testing for all men older than 65 years [47], while the Endocrine Society, NOF, ACPM, and ISCD suggest that all men aged 70 years and older should be screened for low BMD [50, 51, 53, 55]. The American College of Physicians (ACP) recommends DXA tests in men who are at increased risk for osteoporosis (including men aged >70 years) and are candidates for drug therapy (strong recommendation, moderate quality evidence [56]).

North American guidelines also call for BMD screening of selected younger postmenopausal women and men who have clinical risk factors for osteoporosis. For example, the USPSTF suggests measuring BMD in women aged between 50 and 64 years whose FRAX-calculated 10-year risk of major osteoporotic fracture is ≥8.4%, which is the equivalent 10-year fracture risk of a 65-year-old white woman with no other fracture risk factors (grade B recommendation) [46]. While this approach to BMD screening has practical merit, the proposed FRAX threshold has neither undergone cost-effectiveness analysis nor has it been validated in any patient population. Other expert groups recommend DXA tests in men and women older than 50 years with clinical risk factors for fracture [47, 48, 50–53, 55].

Screening Recommendations from Around the World

Screening guidelines differ in different regions of the world where different cost–benefit models are employed [57]. In Japan, routine BMD screening in women starts as early as age 40 years [58], whereas Australian guidelines recommend DXA tests in all men and women aged 70 years and older [59].

In most of Europe, a case-finding approach is taken for osteoporosis screening, with recommendations for BMD testing based on risk stratification [60]. In particular, the decision to obtain a DXA test for an individual is based upon age-specific fracture probability thresholds calculated using FRAX (without BMD information). Low-risk individuals are recommended not to have a DXA test, given the lower likelihood of finding a low BMD that would necessitate intervention. Importantly, the fracture probability thresholds vary considerably by age, with older women needing to surpass a higher fracture threshold before BMD testing is recommended (see Fig. 7.1). Certain European countries, such as the United Kingdom, take a further parsimonious approach by applying an upper threshold to BMD testing, whereby adults with the highest probability of fracture are recommended to start osteoporosis treatment without requiring a screening BMD test, although BMD measurements might be obtained to monitor treatment. In this scenario, only adults with an intermediate fracture probability, in whom the addition of BMD results might change the decision for intervention, are referred for BMD testing [61].

Effectiveness of Osteoporosis Screening Approaches

Several cohort studies have suggested that BMD screening can improve osteoporosis treatment rates and potentially decrease fractures [62, 63].

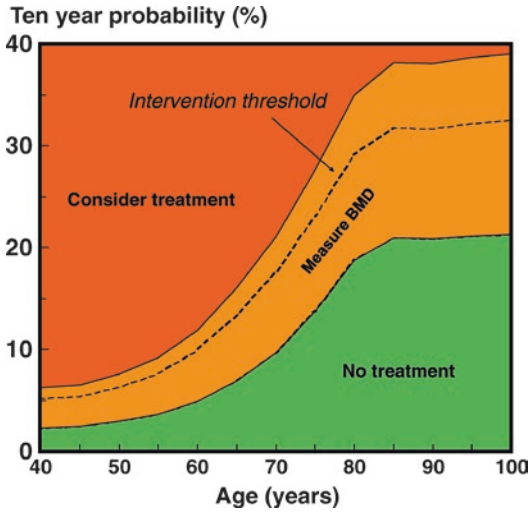

Fig. 7.1 Osteoporosis assessment guidelines based on the 10-year probability of a major fracture. The *dotted line* signifies the treatment intervention threshold. A bone mineral density test is recommended for individuals where the probability assessment lies in the *orange region* [60]. (With permission from Springer Nature)

However, confounding in these observational studies may be difficult to characterize and may hamper broad interpretation. A US-based modeling study found that screening strategies that initiate BMD screening in women as young as 55 years old were more effective and less expensive than not implementing any screening strategy [64].

Only three randomized controlled trials have been performed to study the effectiveness of osteoporosis screening. In one study of women aged 45–54 years who were randomized to receive screening BMD or usual care, the screened group had greater utilization of osteoporosis medications over a 9-year follow-up period [65]. As this study was initiated prior to the widespread introduction of *T*-scores, women were recommended to start on treatment if their bone density was in the lowest quartile of the population. In intention-to-treat analyses, no significant difference in fractures rates was found in the screened and control groups, although per-protocol analyses restricted to women who received DXA scans demonstrated a reduction in risk of fractures as compared to controls (hazard ratio (HR) = 0.741, 95% confidence interval [CI]: 0.551–0.998).

Another large randomized screening trial evaluated the effectiveness of a FRAX-based community screening program on 12,495 women aged 70–85 years in the United Kingdom screening in the community to reduce fractures in older women (SCOOP) trial [66]. Women were randomized to usual care versus risk stratification based on the FRAX algorithm, with the selected use of BMD testing in women who exceeded an age-specific FRAX threshold. Among the 14% of the study population that was deemed to be at high fracture risk, 78% initiated treatment. Over 5 years of follow-up, screening did not reduce the primary endpoint of all osteoporosis-related fractures but did lead to a 28% reduction in hip fractures (HR = 0.72, 95% CI: 0.59–0.89), a prespecified secondary outcome. Furthermore, this systematic community-based screening program was highly cost-effective [67].

The largest randomized trial of osteoporosis screening involved 34,229 Danish women aged 65–80 years (ROSE trial) [68]. Similar to the design of SCOOP, this study also involved a case-finding strategy using FRAX-based thresholds to guide DXA scan recommendations. Unlike SCOOP, treatment recommendations were based solely on BMD results and did not take into account FRAX intervention thresholds. After a median follow-up of 5 years, there was a small increase in osteoporosis medication use in the screening group (23% versus 18%, $p < 0.001$) but no significant differences in fracture rates were observed compared to controls in intention-to-treat analyses. Interpretation of this study is limited given high drop-out rates of high-risk women before DXA scan. Per-protocol analyses among women who received DXA scans did demonstrate a reduction in major osteoporotic fractures (HR = 0.87, 95% CI: 0.77–0.99) and hip fractures (HR = 0.74, 95% CI: 0.58–0.95) in comparison with controls.

Steps After Screening

Intervention Thresholds

Individuals are diagnosed with osteoporosis if they have sustained a fragility fracture, or if their

T-score is ≤−2.5 at the posterior–anterior lumbar spine, total hip, femoral neck, or 1/3 radius [51]. Postmenopausal women and men of age 50 years and older who have osteoporosis by this definition are recommended to start on pharmacologic therapy to reduce their fracture risk. Numerous randomized controlled trials have demonstrated antifracture efficacy of osteoporosis medications among individuals with osteoporotic *T*-scores [46].

Given the poor sensitivity of using osteoporotic *T*-score thresholds as a case-finding strategy, US guidelines also recommend providing pharmacologic intervention for high-risk osteopenic patients, as defined as adults whose 10-year fracture risk exceeds 3% at the hip or 20% for major osteoporotic fracture, as calculated by FRAX [48, 50, 52, 54, 69]. These criteria were developed from US-based cost-effectiveness analyses assuming osteoporosis pharmacotherapy costs of $600/year [70], and may not apply to other countries. The subsequent availability of both cheaper generic drugs (<$100/year) as well as newer more expensive therapies could conceivably alter the US cost-effectiveness thresholds. It is important to note that there have been no trials that have studied fracture prevention within the specific high-risk osteopenic population identified by these FRAX criteria. Nevertheless, a 6-year randomized placebo-controlled trial of 2000 women with osteopenic bone density found significant reductions in vertebral and nonvertebral fractures with zoledronic acid treatment [71], demonstrating proof of concept that pharmacotherapy in an osteopenic population can be beneficial. Furthermore, many Food and Drug Administration (FDA)-approved osteoporosis therapies have demonstrated no statistical interaction between fracture efficacy and baseline fracture risk as assessed by FRAX, which suggests that these medications have similar ability to reduce fractures across many baseline probabilities of fracture [72–75].

In Europe, osteoporosis treatment is recommended for women with a history of prior fragility fracture, and among men and women who surpass age-dependent intervention thresholds based on FRAX-calculated 10-year risk of major osteoporotic fracture (see Fig. 7.1) [61].Many other countries have instituted a tailored combination of fixed and/or age-dependent intervention thresholds based on FRAX [76].

Screening Intervals

The optimal BMD screening interval remains unclear for individuals who do not meet initial intervention thresholds. Several screening guidelines suggest a minimum of 2 years between repeated BMD tests based on limitations in the precision of DXA testing [46, 50]. However, there are conflicting data as to whether follow-up testing and/or rate of bone loss enhances population-based fracture risk prediction [77–82].

Two studies have investigated the length of time for individuals with normal or osteopenic bone densities to develop osteoporosis [83, 84]. As might be expected, both studies reported that the time intervals were highly dependent on baseline *T*-scores, with osteopenic baseline BMD predicting a shorter time to osteoporosis transition as compared to normal baseline BMD. One study in men and women over the age of 60 years found that this timing interval was also highly dependent on age, such that older adults with normal BMD transition to osteoporosis more quickly than younger adults [84]. Of note, osteoporosis diagnostic thresholds (based on *T*-score of −2.5 or lower) differ from treatment thresholds (based partly on absolute fracture risk), and therefore individuals with moderate or high absolute fracture risk may benefit from more frequent bone density testing to identify the proper timing for intervention [85]. Thus, while younger adults with normal BMD may not require repeat testing for 10–15 years, older patients and those with moderate or advanced osteopenia might benefit from testing in 2–5 years. Ultimately, repeat screening BMD testing is most likely to be valuable for individuals with risk factors or comorbidities that might lead to accelerated bone loss.

Screening and Diagnosis in Current Clinical Practice

Despite the significant burden of osteoporosis and the availability of screening tools and treatments, osteoporosis continues to be underdiagnosed. In the United States, less than half of women recommended for bone density screening received a bone density test [86]. There has been a decline in BMD testing for younger postmenopausal women since 2006 [87]. Only 34–42% of adults who have sustained fragility fractures get evaluation or osteoporosis treatment [88]. Given the large public health impact of osteoporosis, there is more work to be done to improve rates of osteoporosis screening to address these important diagnostic and treatment gaps.

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New Imaging Techniques for Bone

8

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Key Points

- Bone mineral density by dual-energy X-ray absorptiometry (DXA) is currently the gold standard measurement for assessing skeletal health and fracture risk but has limitations.
- Newer imaging modalities and techniques allow assessment of bone quality beyond bone mass and size.
- Trabecular bone score is a gray-scale textural analysis that may be applied to spine DXA images to enhance fracture prediction.
- High-resolution peripheral quantitative computed tomography (HR-pQCT) allows assessment of cortical and trabecular volumetric densities, structure, and geometry. Bone strength can be estimated from HR-pQCT-derived images using microfinite element analysis.
- Improvements in magnetic resonance imaging technology have made it possible to assess cortical and trabecular microarchitecture at numerous sites.

Introduction

Bone mineral density (BMD) assessment by dual-energy X-ray absorptiometry (DXA) is an important surrogate marker for fracture risk, used widely in both clinical and research settings, but fails to capture all factors contributing to bone strength and fracture risk. In addition to bone quantity, bone strength is also determined by qualities of bone such as its geometry, macro-, micro-, and nanostructure, and material composition. This is supported by the observation that most fragility fractures (60–80%) occur in women with higher bone mineral density than the threshold for defining osteoporosis (*T*-score < −2.5 SD) [1–4].

Over the last few decades, considerable progress has been made in developing noninvasive imaging techniques that allow assessment of cortical and trabecular microarchitecture in vivo. A novel application, trabecular bone score (TBS), is

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a relatively affordable and widely accessible technology that utilizes spine DXA images to provide measurements that correlate with skeletal microstructure and is a determinant of fracture risk independent of BMD. High-resolution peripheral quantitative computed tomography (HR-pQCT) allows noninvasive, in vivo, three-dimensional (3D) evaluation of volumetric bone density, macroarchitecture, and cortical and trabecular bone microarchitecture. Finite element (FE) analysis is a technique that uses HR-pQCT-derived images to noninvasively estimate bone strength. Magnetic resonance imaging (MRI) has advanced to enable greater detailed assessment of cortical and trabecular bone at multiple anatomical sites. Image acquisition and analysis, clinical applications, and limitations of these existing imaging techniques will be the focus of this chapter.

Trabecular Bone Score

As previously discussed, while DXA has many limitations in terms of its ability to predict fracture, it remains the primary tool for skeletal assessment and is widely utilized in patient care. A novel application called TBS has been developed for use with DXA images. Trabecular bone score is a gray-level textural assessment that is an integrated estimate of skeletal microarchitecture [5–7]. The TBS software (TBS Insight, Med-Imaps, Meriganc, France) applies an algorithm to a spine DXA image, which creates a gray-scale variogram to evaluate and sum the differences in the brightness of neighboring pixels. This application allows for assessment of the spatial variability of the pixels created from the DXA image, rather than sum of the brightness of the pixels reflecting overall bone mass. This method has been validated with the use of micro-computed tomography (micro-CT), which showed that TBS values of ex vivo samples correlated with trabecular microarchitecture parameters of trabecular bone number, trabecular separation, and connectivity density [5]. In a study of lumbar vertebrae from 16 donors, TBS correlated to trabecular bone volume and bone stiffness, although in this small study, TBS did not improve prediction of vertebral biomechanical properties beyond areal BMD (aBMD) [8].

The clinical utility of TBS has been demonstrated in both cross-sectional and prospective studies that show that low TBS predicts fracture risk independent of aBMD [9–24]. In the Manitoba cohort of more than 29,000 women, spine TBS predicted fractures as well as spine aBMD [9]. In addition, when divided into tertiles, there was a threefold increase in risk of fracture in the lowest TBS tertile compared with the highest TBS tertile. As established in a subsequent meta-analysis showing that TBS is a predictor of fracture independent of fracture risk assessment tool (FRAX), TBS may be divided into three tertiles with TBS values $\geq$1.31 considered lowest risk, between 1.23 and 1.31 considered intermediate risk, and $\leq$1.23 consistent with highest risk [25].

However, rather than relying on absolute values of TBS alone to predict fracture, the most useful application of TBS may be its incorporation into the FRAX fracture prediction model, as the addition of TBS-adjusted FRAX calculation may change the predicted fracture risk in either direction [5, 25, 26]. In addition, the use of TBS with FRAX (but not TBS alone) is endorsed by various professional societies for clinical use such as the International Society of Clinical Densitometry and is approved by the U.S. Food and Drug Administration [27, 28].

The utility of TBS measurements in longitudinal clinical trials is less well established. While TBS changes in response to treatment, [29–35] it is currently not known if these changes predict fracture risk reduction. In the Manitoba study of 534 women, the change in TBS did not predict fracture risk [36]. Notably, the treatment-induced TBS changes in this study were relatively small (less than 1% per year). In addition, changes in TBS with bisphosphonate treatments are also quite modest (less than 1% per year) [30, 31]. The effect of anabolic agents on TBS tends to be greater than those with bisphosphonates with increases of approximately 1–2% per year [27, 33]. In addition, in the DATA-Switch trial in which postmenopausal women received 2 years of denosumab followed by 2 years of teripara-

tide, there was a transient decrease in TBS of uncertain clinical significance [37]. Due to these relatively small changes in TBS with treatment, the use of TBS to monitor treatment cannot currently be recommended, particularly with antiresorptive medication.

Interestingly, there is a lack of consistent correlations between changes in TBS and changes in aBMD, which suggests that TBS may provide information distinct from DXA-derived aBMD [30, 32, 33]. In the large Manitoba study of 534 women of whom 86% received a bisphosphonate, 10% received raloxifene, and 4% received calcitonin over a mean duration of 3.7 years, changes in TBS and changes in aBMD weakly correlated weakly (R = 0.20) [30]. Similarly weak correlations were observed in the Swiss Horizon trial of 54 postmenopausal women who received zoledronic acid and 53 women who received placebo for 36 months [32].

Application of TBS for fracture prediction in specific clinical circumstances such as type 2 diabetes or glucocorticoid-induced osteoporosis may be particularly helpful. In a Canadian cohort of 29,407 women over age 50 years, women with diabetes were found to be more likely in the lowest tertile of TBS but not the lowest tertile of aBMD at the spine, total hip, or femoral neck [38]. In addition, in a cohort of 2758 people including 325 men and 370 women with type 2 diabetes, those with diabetes had lower spine TBS and TBS negatively correlated with hemoglobin A1c [39]. In a study comparing glucocorticoid-treated women who had taken prednisolone ≥5 mg/day for over 3 months (n = 64), women who sustained a recent fracture (n = 141), and healthy women (n = 279), TBS was lower in those treated with glucocorticoid compared with healthy controls despite similar BMD by DXA [40].

TBS has also been used to evaluate bone health in cross-sectional and prospective studies of patients with chronic kidney disease [41–48]. In patients with reduced kidney function (stages 3a–4), adults over age 40 years (n = 199) had lower TBS than those with normal kidney function in the Canadian Multicenter Osteoporosis Study. In addition, those with reduced kidney function and TBS value below the median (<1.277) had a higher 5-year probability of having a fracture than those above the median (18.1% versus 6.2%, p = 0.010) [41]. Use of TBS in patients on hemodialysis or who received a kidney transplant also appears to be clinically promising [42–48]. In a study of 327 kidney transplant adult recipients, 31 (9%) kidney transplant recipients sustained an incident fragility fracture over a mean follow-up interval of 6.6 years [42]. Those who sustained a fracture had significantly lower TBS than controls from a general population (matched on age, sex, and DXA date) (p = 0.003) and lower TBS was associated with fracture independent of FRAX.

TBS is not without limitations. Due to application of the software to two-dimensional (2D) DXA images, TBS is susceptible to some of the same technical limitations as DXA. Assessment of aBMD may be affected by body habitus, as previously described, [49, 50] and the use of TBS is limited to patients with body mass index (BMI) of 15–37 kg/m^2. In addition, TBS interpretation may be influenced by the presence of artifacts on DXA such as focal sclerosis. As such, vertebrae that are excluded from BMD analysis are excluded from TBS. Finally, reference databases for TBS are available for only certain region- and ethnic-specific patient populations. Given report of differing associations between TBS and fracture rates among various race/ethnicity and gender groups in National Health and Nutrition Examination Survey (NHANES) 2005–2008, the use of race/ethnicity-specific databases may be necessary for accurate clinical interpretation of TBS [51].

High-Resolution Peripheral Quantitative Computed Tomography

High-resolution peripheral quantitative computed tomography (HR-pQCT), a relatively new technology, provides the highest resolution in vivo to assess bone density and microarchitecture and may overcome some of the limitations of BMD assessment by DXA. HR-pQCT allows three-dimensional in vivo assessment of volu-

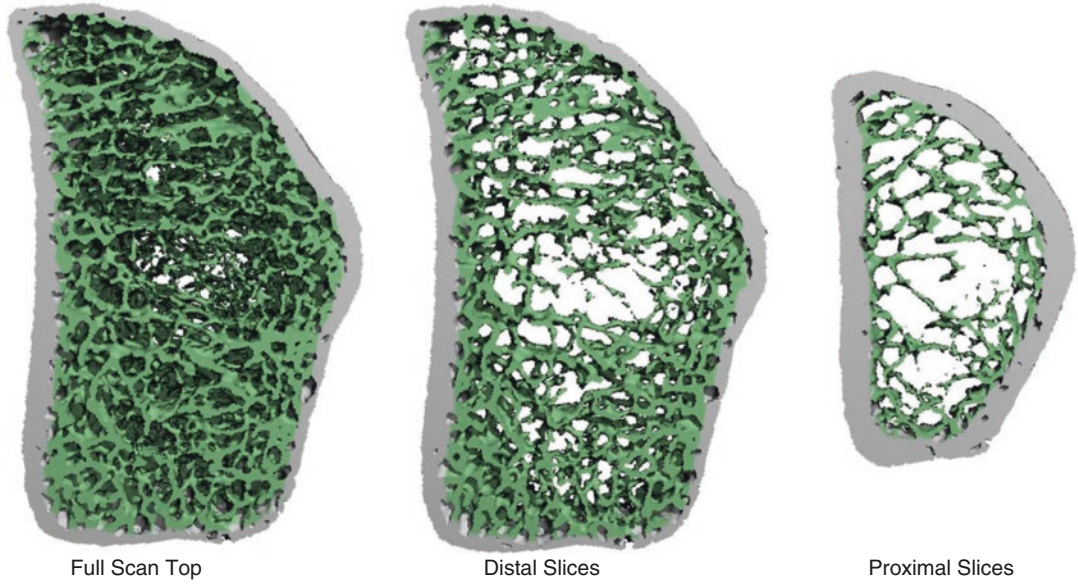

Fig. 8.1 Second-generation HR-pQCT (XCT II) images of the distal radius

metric bone mineral density (vBMD), geometry (cortical and trabecular area), and microarchitecture (cortical thickness and trabecular number, thickness, and separation) by noninvasive acquisition of high-resolution images of the distal tibia and distal radius, at a relatively low level of radiation exposure (Fig. 8.1). Bone can be characterized as a whole or separately as cortical and trabecular compartments. The distinction between bone loss from cortical or trabecular compartments may help to better understand the underlying pathophysiology of various disease states and responses to treatment. HR-pQCT also provides a novel method of noninvasively estimating integral bone strength by use of microfinite element analysis modeling of the acquired three-dimensional images.

HR-pQCT has been commercially available since the mid-2000s. Currently, two models are available (Xtreme CT I (XCT I) and Xtreme CT II (XCT II)), which both belong to the same company, SCANCO Medical AG, Brüttisellen, Switzerland. The second-generation scanner (XCT II), available since 2014, addresses many of the challenges of the first-generation scanner (XCT I) [52]. As listed in Table 8.1, XCT II has a higher spatial resolution (voxel size 61 μm versus 82 μm), faster average scanning time (2.0 versus 2.8 min per site), and permits scanning of a larger axial length (10.2 versus 9.0 mm) [52].

Table 8.1 Comparison of first-generation (XCT I) and second-generation (XCT II) HR-pQCT using standard patient mode

Parameter	XCT I	XCT II
Resolution		
Voxel size (μm)	82.0	60.7
Minimum voxel size (μm)	45.0	30.3
Scan time (min)	2.8	2.0
Single-effective radiation dose (μSv)	<3	<5
Scan region (mm)	9.02	10.24
Field of view, diameter (mm)	126	140
Number of slices	110	168

Since the availability of HR-pQCT over a decade ago, there has been an exponential increase in its use in clinical research, providing new insights into age, sex, and racial differences in bone microarchitecture; the effects of a wide range of pathological processes on bone structure; fracture risk determination; and the effects of various drug therapies that either improve or impair bone structure and strength. The majority

of these studies have been conducted using XCT I. Although this method confers advantages over standard imaging tools, the use of this technology is limited to research only.

Image Acquisition

A detailed description of the methods used for image acquisition and analysis are described elsewhere [52–56]. In brief, sectional high-resolution images of the nondominant distal radius and the distal tibia are acquired by X-ray attenuation. These sectional slices are then used to produce a three-dimensional model. At each site, 110 (XCT I) or 168 (XCT II), computerized tomography slices are obtained and used to reproduce 9.02 mm (XCT I) or 10.24 mm (XCT II) 3D images of either the distal radius or the distal tibia. The single-scan effective radiation dose is <3 μSv for XCT I and <5 μSv for XCT II [55].

The first image acquired is known as the "scout view image." This is a 2D anterior–posterior projection of the proximal limb acquired by the operator in order to define the anatomic region to be scanned (region of interest, ROI). The anatomic landmark, from which the horizontal reference line is drawn, is visually identified by the operator, and then the ROI is offset by a standard distance from this reference line. It has been demonstrated that in vivo precision errors were up to threefold greater when variability in scan positioning was included, but were significantly reduced with the use of a systematic training platform [57]. Precision errors were also greater with the use of multioperator data sets [57].

Given the marked heterogeneity in bone geometry, microstructure and material composition from slice to slice within a region of a few millimeters, it is essential that anatomically equivalent regions are measured, between and within individuals, in order to avoid misrepresentation of results. Currently a fixed ROI starting 9.5 mm proximal to the mid radiocarpal joint of the radius is used regardless of an individual's age, sex, race, height, or bone length, which may lead to errors in measurement. For example, men are taller and have longer forearms than women [58]. A fixed ROI in a taller person is more distal and would overestimate cortical porosity and trabecular density and underestimate matrix mineral density, whereas a fixed ROI in a shorter person would be more proximal and have the opposite effect. The net result of these differences would be an overestimation of sex trait dimorphisms. One proposed way to overcome this error and attempt to measure the same anatomical location in the forearm might be to position the ROI based on a percentage of forearm length, as proposed to be standardized in adults as 4.0% at the radius and 7.3% at the tibia [59, 60].

Attempting to measure the same anatomical region becomes more complex and challenging in a growing bone. For example, during skeletal development the radius grows predominantly at its distal growth plate while the tibia grows at its proximal growth plate. In this situation, it is uncertain whether the use of an ROI based on a percentage of the total bone length is more appropriate than a fixed ROI. Nonetheless, the possibility of a positioning error needs to be taken into consideration as failure to do so may lead to misrepresentation of the pathogenesis and structural basis of bone fragility and fracture risk. In the mature skeleton, longitudinal assessment of the same ROI is made possible by the default image registration on HR-pQCT scanners, which permits consecutive assessment of the same region by matching similar-sized slices of the baseline and repeat scans. Three-dimensional registration, though not commonly used, is another method that has shown short-term improvements in precision [61, 62].

Motion artifact can significantly impact the quality of images obtained and may render an image unusable. Microarchitecture parameters appear to be more susceptible to motion artifact compared to densitometric or geometric parameters [63–66]. The limb being scanned is fixed in a carbon fiber shell to limit motion artifact [66]. Although techniques have been developed to quantify and correct for motion artifact, rescanning is still the optimal method [65–67]. It has been recommended that rescanning be restricted to three scans per site as the International Commission on Radiological Protection recommends limiting the yearly radiation dose to

50 μSv per year. Improved immobilization and shorter scanning time with the XCT II will reduce motion artifact and improve the quality of the scans produced.

Image Analysis

Upon acquisition of the 3D images, semiautomatic or automatic methods are used to segment the whole bone into its cortical and trabecular compartments [56, 68–70]. In addition to standard microstructural and volumetric density parameters, novel techniques have been developed to quantify the plate and rod-like structure of trabecular bone [71]. Most information regarding the accuracy of these measurements are based on several ex vivo studies comparing XCT I with the gold standard micro-CT [72–76]. Compared with micro-CT, XCT I provides moderate to good accuracy in evaluating bone density and structure of the radius and tibia. Only one ex vivo study has compared the measurements derived from XCT II with micro-CT [52]. Precision errors, based on repeated-measures analyses from in vivo scanning, are greater in vivo than ex vivo [77, 78] and at the radius (<6.5%) than at the tibia (<5.2%) [63, 70]. As expected, precision errors are much greater for structural (<4.5%) than densitometric (<1.5%) parameters [56, 63, 77, 79, 80].

The first-generation scanners (XCT I) have a voxel size of 82 μm, which is near the lower limit of resolving individual trabeculae [56, 74, 75]. Hence, average trabecular thickness (Tb.Th) and separation (Tb.Sp) are derived measures from trabecular bone volume fraction (BV/TV) and trabecular number (Tb. N) [72, 80, 81]. The second-generation scanners (XCT II) have improved spatial resolution with a voxel size of 61 μm, which permits direct measurement of trabecular microstructure parameters [52]. This is important because derived measures are dependent on the accuracy of other measures and model assumptions [82, 83]. Indeed, two recent in vivo studies that compared bone microarchitecture parameters derived from XCT I with those derived from XCT II demonstrated strong agreement between most microarchitecture parameters except trabecular thickness [55, 84].

In addition to the standard analysis, which provides quantification of cortical vBMD, area and thickness, the extended cortical analysis allows assessment of cortical porosity. This technique involves generation of periosteal and endosteal contours and then segmentation of cortical porosity by identification of resolved Haversian canals within the cortical compartment. XCT I has a voxel size of 82 μm and a spatial resolution of approximately 130–160 μm, [78] which prevents accurate quantification of most pores, given that 60% of pores are less than 100 μm in diameter [85]. Improved resolution of XCT II should allow more accurate assessment of cortical porosity compared to XCT I.

Distinguishing between the cortical and trabecular compartments is difficult because there is lack of a clear border delineating these two compartments [86]. Defining this border becomes especially challenging in advanced age or pathological states where unbalanced remodeling fragments the cortex adjacent to the medullary canal, resulting in thinning of the cortex and increased cortical porosity. This "trabecularization" of the cortex is then erroneously "seen" as being part of the medullary canal, thereby underestimating cortical porosity and overestimating trabecular density [86]. Methods attempting to improve the segmentation of the cortical and trabecular compartments have been developed [69, 87, 88]. A dual-threshold approach is currently available as part of the HR-pQCT software package and has shown excellent agreement when compared to hand-contouring of micro-CT images of the same bones (r = 0.9–1.0) [70]. Alternative segmentation methods are also available as external software. One such method uses a non-threshold-based automated technique to separate bone from background and into its compact appearing cortex, corticotrabecular compartment (transitional zone), and trabecular compartment [88] It should be noted that this method uses only the 49 most proximal HR-pQCT slices (SCANCO Medical AG, Brüttisellen, Switzerland), where the cortex is thicker and is thought to enable more accurate assessment of cortical porosity.

Finite Element Analysis

Microfinite element (FE) models of the radius and tibia can be created directly from the segmented HR-pQCT images and used to noninvasively determine local mechanical properties, such as failure load and stiffness. The segmented images are converted to finite element models using the voxel conversion approach [89]. The images are binarized to bone and background and then directly converted into linear hexahedral elements that are small enough to assume local material isotropy and material homogeneity. Under simulated loading conditions, micro-finite element analysis (μFEA) outcomes are mechanical properties such as deformations and stress maps with one data point per element. In addition to this, estimates of bone strength such as integral stiffness and failure load can also be derived from linear and nonlinear analyses. Some limitations of μFEA are that it intrinsically incorporates a composite of BMD and structural data and is therefore susceptible to errors in measurement of these parameters.

Fracture Risk Prediction

The population burden of fractures occurs in women with osteopenia, not osteoporosis [1–4]. Use of algorithms, such as FRAX, that combine femoral neck aBMD with clinical risk factors for osteoporosis still fails to capture a large proportion of women at risk for fracture. Improved identification of women at risk for fracture, and thus treatment of these women before sustaining a fracture, remains an ongoing challenge.

Measurement of bone microstructure and strength by HR-pQCT appears to provide improved discrimination, compared to aBMD, between women with and without a history of prevalent fracture [56, 90–97]. In a cross-sectional analysis of pooled data from 1379 Caucasian women from five study centers, consistent and significant deficits in both cortical and trabecular traits at the distal radius and tibia, independent of total hip *T*-score, were observed in postmenopausal women with prevalent fracture(s) compared with those without fracture(s) [95].

This study and others have also demonstrated that in women with similar BMD, those with prevalent fractures have poorer bone microarchitecture and decreased bone strength compared with nonfracture controls [56, 95, 97, 98]. In the subgroup of women with osteopenia in the above study, the risk of major fragility fractures was increased significantly (55–88%) per standard deviation decrease in total and trabecular vBMD [95]. These observations have also extended to younger women. In a study of 40 premenopausal women with a recent distal radial fracture compared with age-, race-, and BMI-matched control subjects, those who had sustained a fracture had poorer cortical and trabecular bone microarchitecture at both the radius and tibia compared with nonfracture controls, despite both groups having similar BMD [97].

In a few studies, the additional value of microarchitectural parameters in identifying women with prevalent fractures occurred only in the subgroup of women without osteoporosis. In a case–control study of 68 postmenopausal women with forearm fractures and 70 controls, measurement of cortical porosity improved the detection rate among women with osteopenia but not osteoporosis [99]. Similar observations were made in another study of 211 postmenopausal women with nonvertebral fracture and 232 controls [100]. Cortical porosity was associated with fracture independent of FRAX score in women with normal femoral neck BMD (odds ratio [OR] = 1.88; 95% confidence interval [CI]: 1.21–2.96) or osteopenia (OR = 1.40; 95% CI: 1.06–1.85) but not in women with osteoporosis (OR = 1.48; 95% CI: 0.68–3.23) [100].

A limitation of the majority of current trials evaluating the utility of HR-pQCT parameters in fracture prediction is their cross-sectional design. Although there is improved detection of prevalent fractures with HR-pQCT parameters independent of aBMD, it was not known if the same would apply for incident fractures. Only in the last 1–2 years have data from longitudinal studies

[101–104] addressed this question. The most recent and largest trial conducted to date by the Bone Microarchitecture International Consortium (BoMIC) provides the strongest evidence that HR-pQCT indices enhance fracture prediction independently of femoral neck aBMD and FRAX [104]. In this study, individual patient data were pooled from eight international cohorts to evaluate the association between HR-pQCT bone indices and incident fracture, after adjustment for age, sex, height, weight, and cohort. Additional adjustments were also made for femoral neck DXA BMD or FRAX. A total of 7254 participants (66% women and 34% men), among whom 92% did not have baseline osteoporosis based on femoral neck BMD, were evaluated. Fractures occurred in 765 (11%) participants over a mean follow-up of 5 years. Eighty-six percent of those who fractured had femoral neck *T*-scores > −2.5 standard deviations. Cortical vBMD and trabecular number and thickness at the radius and cortical vBMD and area and trabecular number and thickness at the tibia best predicted fracture and were noncollinear. Failure load, determined by μFEA, was most strongly associated with incident fracture with a hazard ratio (HR) of 2.13 (95% CI: 1.77–2.56) for the distal radius and HR = 2.40 (95% CI: 1.98–2.91) for the distal tibia for every 1 SD decrease in failure load, after adjustment for age, sex, cohort, height, and weight. This risk remained similar even after adjustment for femoral neck aBMD (HR = 1.76, 95% CI: 1.48–2.09, for the distal radius, and HR = 1.78, 95% CI: 1.50–2.12, for the distal tibia) or for cohort and FRAX (HR = 1.76, 95% CI: 1.48–2.09, for the distal radius, and HR = 1.78, 95% CI: 1.50–2.12, for the distal tibia) [104]. Finite element analysis may be a better predictor of fracture risk as it combines both BMD and microarchitectural parameters, which could provide a more comprehensive assessment of bone strength.

Of interest, after adjustment for ultra-distal radius aBMD, only radius trabecular vBMD and number remained significantly associated with incident fracture risk. Furthermore, neither radius microarchitecture parameters nor radius failure load was able to significantly improve the area under the curve for ultra-distal radius aBMD [104]. Similar observations were also noted in the recent study conducted by Biver et al. [101] and may support a potential role for ultra-distal radius aBMD as a predictor of incident fracture risk. However, this was a secondary analysis and further confirmatory studies are required.

Monitoring Therapy-Induced Skeletal Changes

Treatment-induced changes in bone parameters, other than BMD, may contribute to fracture risk reduction. HR-pQCT allows assessment of treatment-related changes in bone microarchitecture, which previously could only be measured invasively by histomorphometric analyses of iliac crest bone biopsies. Changes to matrix mineral density and estimated bone strength can also be assessed by HR-pQCT.

The effects of antiresorptive and anabolic therapies, alone or in combination, on HR-pQCT parameters have recently been summarized [105, 106] and are presented in Tables 8.2 and 8.3 [107–117]. Antiresorptive and anabolic therapies produced different effects on bone microstructure and bone strength, as assessed by μFEA. In general, current anabolic agents tend to increase cortical porosity with an increase or preservation of bone strength whereas antiresorptive agents reduce cortical porosity and increase bone strength.

These studies were predominantly conducted in postmenopausal women and varied significantly in study design, duration, and sample size. In addition, none of these studies were designed to assess fracture as a primary outcome; hence, the effects of treatment-induced changes in bone density, microarchitecture, and strength on antifracture efficacy are not known. Validation of this is important in determining the potential role for HR-pQCT as a treatment endpoint in future regulatory clinic trials.

In the majority of these studies, greater treatment responses, in both cortical and trabecular compartments, were seen at the distal tibia compared with the distal radius [107, 108, 110–112,

Table 8.2 Summary of within-group changes from baseline of trabecular density (trabecular vBMD or calculated BV/TV), cortical vBMD, and cortical thickness (Ct.Th) at the distal radius in HR-pQCT studies evaluating antiresorptive and anabolic treatment

Effect of antiresorptive and anabolic therapy on trabecular and cortical density and cortical thickness at the distal radius, as assessed in vivo by HR-pQCT							
Study	Drug	Duration (months)	*N*	Age (years)	Distal radius Tb.vBMD or BV/TV	Distal radius Ct.vBMD	Distal radius Ct.Th
Burghardt et al. (2010) [107]	Alendronate	24	13	56 ± 4	NS	NS	NS
	Placebo	24	20	56 ± 2	NS	NS	NS
Rizzoli et al. (2012) [108]	Alendronate	24	42	64 ± 8	NS	NS	NS
	Strontium	24	41	64 ± 8	NS	NS	NS
Seeman et al. (2010) [109]	Alendronate	12	82	61 ± 5	NS	NS	~+2 to 3%[a]
	Denosumab	12	83	60 ± 6	~0 to +1%[a]	~ 0 to + 0.5%[a]	~+3 to 4%[a]
	Placebo	12	82	61 ± 5	~−2%[a]	~ −1.5%[a]	~0 to −1%[a]
Chapurlat et al. (2013) [110][b]	Ibandronate	24	72	63 ± 5	No difference between groups	No difference between groups	No difference between groups
	Placebo	24	76	63 ± 5			
Bala et al. (2014) [111]	Risedronate (<55 years)	12	112	53 ± 2	−1.60 ± 4.49%	NS	Not reported
	Placebo (<55 years)	12	49	53 ± 2	−3.61 ± 8.21%	NS	Not reported
	Risedronate (>55 years)	12	109	62 ± 6	NS	NS	Not reported
	Risedronate (>55 years)	12	54	61 ± 4	NS	NS	Not reported
Hansen et al. (2013) [112]	Zoledronic acid	18	33	70 (54–86)	+2.5 ± 5.1%	NS	NS
	PTH (1-34)	18	18	72 (59–80)	NS	−2.4 ± 4.5%	+ 2.0 ± 3.8%
	PTH (1-84)	18	20	70 (61–86)	NS	−3.5 ± 3.3%	NS
Cheung et al. (2014) [113]	Odanacatib	24	72	64 ± 7	+2.57%[a]	+0.78%[a]	+1.57%[a]
	Placebo	24	74	64 ± 6	NS	−1.65%[a]	−5.28%[a]
Schafer et al. (2013) [114]	Ibandronate and PTH (1-84)[c]	24	43	62 ± 4	+2.26% (1.37, 3.14)[a]	−0.76 (−1.33, −0.20)[a]	−1.90 (−2.61, −1.18)[a]
Tsai et al. (2016) [115]	PTH (1-34)	24	28	66 ± 8	NS	−3.1 ± 3.7%	NS
	Denosumab	24	31	66 ± 8	+1.9 ± 4.1%	+0.7 ± 1.5%	+5.1 ± 3.1%
	Denosumab and PTH (1-34)	24	24	66 ± 9	+4.0 ± 3.4%	+0.9 ± 1.6%	+4.7 ± 5.3%

BV/TV trabecular bone volume fraction, *HR-pQCT* high-resolution peripheral quantitative computed tomography, *NS* not significant, *PTH* parathyroid hormone, *vBMD* volumetric bone mineral density

[a]Values are means ± SD unless otherwise noted as least-squares means, and if values reported, with 95% confidence intervals

[b]For the Chapurlat et al. [110] study, significance of within-group changes was not reported

[c]For the Schafer et al. 2012 study, subjects were treated within 6 months of PTH (1-84), either as one 6- or two 3-month courses, in combination with ibandronate over 2 years

Table 8.3 Summary of within-group changes from baseline for trabecular density (trabecular vBMD or calculated BV/TV), cortical vBMD, and cortical thickness (Ct.Th) at the distal tibia in HR-pQCT studies evaluating antiresorptive and anabolic treatment

Effect of antiresorptive and anabolic therapy on trabecular and cortical density and cortical thickness at the distal tibia, as assessed in vivo by HR-pQCT							
Study	Drug	Duration (months)	*N*	Age (years)	Distal tibia Tb. vBMD or BV/TV	Distal tibia Ct. vBMD	Distal tibia Ct.Th
Burghardt et al. (2010) [107]	Alendronate	24	13	56 ± 4	~ +1 to 2%	NS	~ +3 to 4%
	Placebo	24	20	56 ± 2	NS	NS	NS
Rizzoli et al. (2012) [108]	Alendronate	24	42	64 ± 8	NS	NS	NS
	Strontium	24	41	64 ± 8	+2.5 ± 5.1%	+1.4 ± 2.8%	+6.3 ± 9.5%
Seeman et al. (2010) [109]	Alendronate	12	82	61 ± 5	+0.5 to 1%[a]	NS	+4 to 5%[a]
	Denosumab	12	83	60 ± 6	+1 to 1.5%[a]	+0.5 to 1%[a]	+5 to 6%[a]
	Placebo	12	82	61 ± 5	−0.5 to −1%[a]	−0.5 to −1%[a]	+1 to 2%[a]
Chapurlat et al. (2013) [110][b]	Ibandronate	24	72	63 ± 5	No difference between groups	Greater increase in ibandronate group	Greater increase in ibandronate
	Placebo	24	76	63 ± 5			
Bala et al. (2014) [111]	Risedronate (<55 years)	12	112	53 ± 2	NS	−1.09 ± 2.41%	Not reported
	Placebo (<55 years)	12	49	53 ± 2	NS	NS	Not reported
	Risedronate (>55 years)	12	109	62 ± 6	NS	NS	Not reported
	Risedronate (>55 years)	12	54	61 ± 4	+0.40 ± 1.51%	+0.50 ± 1.68%	Not reported
Hansen et al. (2013) [112]	Zoledronic acid	18	33	70 (54–86)	+2.2 ± 2.2%	+1.5 ± 2.0%	+3.0 ± 3.5%
	PTH (1-34)	18	18	72 (59–80)	+ 3.3 ± 5.7%	-1.6 ± 4.4%	+3.8 ± 10.4%
	PTH (1-84)	18	20	70 (61–86)	NS	−4.7 ± 4.5%	−2.8 ± 4.7%
Cheung et al. (2014) [113]	Odanacatib	24	72	64 ± 7	2.27%[a]	NS	+ 2.15%[a]
	Placebo	24	74	64 ± 6	+0.84%[a]	−1.05%[a]	−3.03%[a]
Schafer et al. (2013) [114]	Ibandronate and PTH (1-84)[c]	24	43	62 ± 4	+ 3.22% (2.35, 4.10)[a]	NS	NS
Tsai et al. (2016) [115]	PTH (1-34)	24	28	66 ± 8	NS	−3.2 ± 2.7%	NS
	Denosumab	24	31	66 ± 8	+1.5 ± 3.1%	NS	+6.0 ± 4.5%
	Denosumab and PTH (1-34)	24	24	66 ± 9	+2.0 ± 2.8%	+1.2 ± 1.6%	+8.1 ± 4.3%

BV/TV trabecular bone volume fraction, *HR-pQCT* high-resolution peripheral quantitative computed tomography, *NS* not significant, *PTH* parathyroid hormone, *vBMD* volumetric bone mineral density

[a]Values are means ± SD unless otherwise noted as least-squares means, and if values reported, with 95% confidence intervals

[b]For the Chapurlat et al. 2013 [110] study, significance of within-group changes was not reported

[c]For the Schafer et al. 2012 study, subjects were treated within 6 months of PTH (1-84), either as one 6- or two 3-month courses, in combination with ibandronate over 2 years

114, 118]. A possible reason for the discrepancy between these two peripheral sites may be reflective of technical limitations, as movement artifacts may compromise the quality of radius scans and in some instances render these images unusable [64]. In one trial, 28% of images from the radius were of poor quality and may have biased the results of the study [108].

As discussed earlier, issues with segmentation and matching an ROI in longitudinal studies are an ongoing challenge that may also affect assessment of treatment effects on bone microarchitecture. For example, reports of changes in cortical microarchitecture following alendronate were inconsistent across three studies that used different methods of segmentation [107, 109, 118]. In addition, issues with limited resolution with XCT I may account for the lack of response observed in trabecular parameters in some of these studies. In order to permit direct comparison of results, improved accuracy and standardization of bone microarchitecture estimates are required.

Finally, μFEA provides a method of biomechanical estimation of bone strength and can be used to provide valuable information regarding how therapy-induced changes in bone microarchitecture may affect bone strength. As an example, there is a greater increase in bending or torsional strength with deposition of newly formed bone in the cortical compared to the trabecular compartment.

Limitations

Although HR-pQCT has several advantages over conventional imaging methods, there are still some limitations to its use. At present, HR-pQCT can only be applied to the peripheral skeleton and it is unclear if these two peripheral sites are reflective of axial bone strength at the hip and spine. Ongoing issues with image acquisition and analysis exist. Images are susceptible to motion artifact, beam hardening, and scatter artifacts. The heterogeneity in bone morphology from slice to slice within a few millimeters of an ROI may produce differences due to errors in positioning rather than differences in the biology of growth, aging, race, sex, or the effects of therapy. Segmentation is an ongoing challenge. The XCT II scanner, due to its improved resolution (61 μm versus 82 μm), holds promise for improved quantification of trabecular parameters and cortical porosity but issues with correctly apportioning the cortical and trabecular compartments remain. Errors in appropriately defining the endocortical perimeter may overestimate trabecular density and underestimate cortical porosity and may impact our assessment of the effects of aging, disease states, and therapy on the skeleton. Further studies evaluating the utility of HR-pQCT parameters, including μFEA, in fracture prediction are warranted. Ongoing generation of normative data from different populations such as the work by Burt et al. may improve its utility in fracture prediction by determination of a fracture risk gradient akin to the use of *T*-scores for DXA BMD [119]. At present, official endorsements do not exist for the use of HR-pQCT in fracture risk prediction. Finally, whether assessment of bone microstructure can assist in identifying and targeting therapy more effectively remains an unmet challenge.

Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) is a noninvasive, nonionizing method of assessing bone structure and microarchitecture with several measurement techniques currently available [120–128]. Magnetic resonance imaging is used to quantify both cortical and trabecular structures, although until recently, most work has involved measurement of trabecular bone.

Advances in image acquisition time, signal-to-noise ratio (SNR), and spatial resolution have improved the accuracy and precision of trabecular microarchitecture measurements and made it possible to scan more proximal sites, such as the distal and proximal femur and hip [121, 122, 129–132]. Relatively novel technical advances in measurement of cortical bone, such as the use of ultrashort echo tine, [133] have enabled quantitative assessment of cortical bone porosity and collagen bound water in vivo. Further work is required to determine how these properties affect bone strength and fracture risk. The application of finite element analysis to magnetic resonance images of bone microarchitecture is another recent development in image processing.

A limited number of relatively small studies have provided some evidence for the use of MRI-derived bone parameters as potential surrogate

markers for fracture risk beyond DXA-derived BMD. In one study, postmenopausal women with fragility fractures ($n = 18$) had inferior trabecular microarchitecture at the distal radius (lower surface curve ratio, lower trabecular bone volume fraction, and higher erosion index) compared to the age- and BMI-matched women ($n = 18$) without fracture [134]. Notably, there were no differences in hip, spine, or distal radius BMD. Similar results were observed in another study of postmenopausal women with fragility fractures ($n = 22$) who were shown to have lower MRI-computed elastic moduli of the proximal femur, compared to age- and BMI-matched controls without fracture ($n = 22$), despite no differences in femoral neck, total hip, or spine BMD between the groups [122]. These studies have all been cross-sectional in design, and larger, longitudinal trials are required to adequately assess the value of MRI-derived bone parameters in incident fracture risk prediction.

The use of MRI to monitor changes in bone microarchitecture in response to oral bisphosphonates, parathyroid hormone (PTH 1-84), and estradiol therapy has also been conducted [128, 135–137]. Although these studies indicated that MRI is a more sensitive measure of treatment-induced changes than aBMD, these studies have been relatively small and of short duration (12–24 months), and the antifracture efficacy of these treatment-induced changes in bone structure, measured by MRI, has not been determined. Furthermore, the additional clinical utility of measuring these changes in microarchitectural parameters by MRI, beyond that provided by aBMD or other methods and existing imaging modalities such as HR-pQCT, needs to be established.

Summary

The advent of newer imaging modalities has allowed for detailed assessment of both cortical and trabecular compartments and provided novel insights in our understanding of skeletal pathophysiology and treatment effects. Trabecular bone score is clinically available for use with FRAX to improve fracture risk prediction. Improvements in HR-pQCT and MRI may enhance fracture prediction independent of DXA BMD, particularly in secondary osteoporosis or osteopenia, where the use of DXA is limited. While these imaging techniques are generally sensitive to treatment effects, antifracture efficacy based on these changes is not known. Further work using finite element analysis to estimate bone strength may improve fracture risk prediction and allow better assessment of treatment efficacy.

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Biochemical Markers of Bone Turnover

9

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Key Points

- Many bone turnover markers (BTMs) are themselves the product of secretion or resorption of the organic bone matrix. In addition to reflecting alterations in bone metabolism, changes in the levels of a number of BTMs can be associated with other processes, thus interpretation in a clinical context is critical.
- Pre-analytic variation is a major challenge to effective clinical implementation of BTMs, but its effects can be minimized through careful patient selection and standardization of phlebotomy practice.
- The clinical utility of BTMs to predict bone loss or fracture remains limited.
- The pattern of BTM changes in response to osteoporosis treatment is well characterized and serves as a useful surrogate to understand skeletal physiology in a research setting.
- While monitoring BTM levels is not standard clinical practice, bone formation markers may be used to predict antiresorptive antifracture efficacy in future trials as based on the recent Foundation for National Institutes of Health report.

Introduction

While radiographic approaches such as Dual-energy X-ray absorptiometry (DXA) remain a primary diagnostic modality to assess for osteoporosis and fracture risk, radiographic methods have several limitations. Radiographic methods tend to respond relatively slowly to disease processes or therapeutics that influence bone metabolism, and there is commonly an interest in assessing therapeutic or disease-mediated impact on bone before radiographic changes are detectable [1]. Moreover, it has become apparent that radiographic measures of total bone mass only capture a portion of fracture risk, thus spurring interest in complimentary alternative approaches

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such as serum or urine biomarkers that reflect the dynamics of bone turnover (hereafter, bone turnover markers, BTMs) [2, 3].

Ultimately, bone mass reflects the balance in activity between bone formation by osteoblasts and bone resorption by osteoclasts. Accordingly, BTMs can also be mapped to these cell types. Generally, anabolic markers reflect either characteristic proteins secreted by osteoblasts, such as bone-specific alkaline phosphatase (BSAP), or matrix protein fragments thereof that are released into circulation when the organic matrix of bone is secreted. The most widely utilized markers of bone resorption all tend to be fragments of matrix proteins that are released into circulation during the course of controlled proteolysis that accompanies bone resorption. Here we will first profile the bone turnover markers with the widest clinical utilization, reviewing the composition of each of these markers and how they relate to bone physiology. Next we will consider how both pre-analytic variation and differences in analytic methods pose challenges to the clinical use of BTMs. Lastly, we will review evidence supporting the use of BTMs for a variety of clinical applications.

C- and N-Terminal Telopeptides of Type I Collagen

Type 1 collagen is the major organic component of bone, and, accordingly, many BTMs represent type 1 collagen fragments generated during matrix secretion or resorption. After secretion and processing of the propeptides, triple-helical collagen is flanked by non-helical regions near the N- and C-terminal regions termed the C-terminal and N-terminal telopeptides (CTX and NTX). These telopeptides are cleaved when osteoclasts resorb bone, with CTX resulting from cathepsin K-mediated as opposed to matrix metalloproteinase-mediated cleavage [1, 2]. Matrix metalloproteinase or trypsin-mediated digestion releases an alternative peptide fragment, termed the C-terminal cross-linked telopeptide of type I collagen (ICTP). As MMPs are implicated in certain forms of pathologic inflammatory or tumor-mediated bone destruction, ICTP may be conceptually well suited to monitoring these processes [1, 3]. However, ICTP assays are currently not widely available for clinical use.

The two most widely used automated CTX assays recognize an octapeptide sequence within the α1 chain telopeptide. Notably, the CTX octapeptide also contains an aspartic acid that undergoes isomerization over time, converting from the newly synthesized form (αCTX) to an isomerized form (βCTX). Due to the potential presence of lysine crosslinks between α1 chains in the CTX peptide, both monomeric (αCTX and βCTX) and dimeric (α-αCTX, α-βCTX and β-βCTX) forms of CTX occur. Due to the gradual conversion of αCTX to βCTX, the ratio of αCTX to βCTX has been proposed to provide information on the duration of time between collagen deposition and resorption, though further study is needed to determine the potential clinical utility of this ratio. CTX assays can show a relative preference for any of these forms of CTX, with most of the "Crosslaps" assays preferentially recognizing β-βCTX, and the "Alpha CTX" assays preferentially recognizing α-αCTX. In addition to recognizing the isomerized forms of CTX, assays have also been developed to recognize racemized forms of the CTX peptide, allowing discrimination of native, isomerized, racemized, and isomerized and racemized forms of CTX [4, 5]. NTX is the N-terminal telopeptide of the α2 collagen chain that participates in crosslinks with either α1 or α2 chains.

CTX and NTX assays generally show only modest differences in analytic and clinical performance [6]. As both CTX and NTX are renally cleared, these assays can be conducted on either serum, plasma, or urine. However, NTX is often run on urine, and CTX is often run on serum. This preference is due in part to serum NTX showing a relatively blunted response to antiresorptive therapy [7].

N-Terminal and C-Terminal Propeptide of Type 1 Procollagen (PINP, PICP)

Type 1 collagen is initially synthesized by osteoblasts as an intact procollagen molecule contain-

ing globular propeptide sequences on the N- and C-termini (PINP and PICP, respectively). These propeptides are cleaved shortly after collagen secretion, and accordingly, PINP and PICP are anabolic markers reflecting rates of collagen production by osteoblasts. Immediately after processing, PINP is present as a trimer of the three type 1 collagen propeptides, and this trimer can subsequently disassociate into monomers. Assays recognize either both monomeric and trimeric PINP (termed total PINP) or just trimeric PINP (termed intact PINP). As monomeric but not trimeric PINP undergoes renal clearance, total PINP levels are elevated in renal failure, implying that intact PINP assays are preferred for patients with renal insufficiency [8]. Trimeric PINP undergoes hepatic clearance through the scavenger receptor [9].

Interestingly, PICP does not undergo the same disassociation to monomeric forms described for PINP, likely due to stabilization by intrachain and interchain disulfide bonds. Accordingly, PICP is not cleared renally, but instead undergoes uptake through hepatic mannose receptors [10]. As the expression of mannose receptors is regulated by differing progesterone levels during the course of the menstrual cycle (or during pregnancy), this has led to concern that this or other factors regulating mannose receptor expression may have a confounding effect on PICP levels [11].

Osteocalcin

After collagens, Osteocalcin (OC, gene symbol BGLAP) is the most abundant protein component of bone matrix. OC is a relatively small calcium-binding soluble protein that is produced by osteoblasts and is incorporated into the bone matrix at the time of synthesis. While the majority of OC produced by osteoblasts is incorporated into the bone matrix, a fraction escapes into the systemic circulation at the time of synthesis. Thus, OC is largely utilized as a marker of anabolic bone formation, and, accordingly, OC levels correlate with bone formation parameters as measured by histomorphometry [12]. However, OC embedded in the bone matrix can be released into serum during bone resorption, raising the possibility that in some conditions increased catabolic activity may also contribute to OC levels.

The functional role of circulating osteocalcin has been an area of recent interest. Osteocalcin has been nominated as the secreted mediator of osteoblast effects on muscle function, insulin secretion, and male fertility [13–16]. There is interest in clinical assays that reflect this biology by measuring osteocalcin levels [17]. Relevant to this, osteocalcin is subject to vitamin K-dependent γ-carboxylated on three glutamic acids at the time of synthesis in osteoblasts (amino acids 13, 17, and 20). A portion of osteocalcin undergoes decarboxylation subsequent to synthesis, and only this fraction of osteocalcin may mediate systemic effects, especially with regard to the metabolic effects of OC [18]. Accordingly, treatment with vitamin K antagonists lowers both levels of OC carboxylation and total serum OC levels [19]. In mediating systemic effects, OC acts through a family of orphan G protein-coupled receptors, with Gprc6a identified as important for the effects of osteocalcin on fertility, and a recent report suggests that osteocalcin may have effects on the central nervous system through Gpr158 [16, 20–22].

When considering OC assays, it is important to note that OC undergoes proteolytic fragmentation by plasmin and other proteases [23]. Accordingly, OC is present in variety of N- and C-terminal truncated forms in both urine and serum [21, 24]. These truncated fragments contain amino acid isomerization forms suggestive of proteins that have been maintained for extended periods after synthesis, suggesting that these OC fragments are enriched for the fraction of OC that is liberated by osteoclastic bone resorption [24]. Accordingly, ELISA using a reagent antibody targeting an epitope at amino acids 21–29 shared by many of these fragments shows rapid responses to anti-resorptive therapy [24]. OC assays show differing reactivity for these OC fragments, with some assays solely reacting with the full-length "intact" OC [25, 26].

Due to the rapid proteolytic processing of OC, analyte stability has been a major barrier to widespread, routine adoption of clinical OC measurement. Specimens collected for OC assay have

special requirements, needing to be kept at 4 °C and assayed within 4 h of collection. Hemolysis also lowers OC levels by promoting OC proteolysis [27]. Oxalate, fluoride, and citrate tubes were found to significantly decrease OC levels and are not recommended for OC measurement [27].

Pyridinoline (PYD) and Deoxypyridinoline (DPD)

The fibrillar collagen network of bone is stabilized by crosslinks formed both within collagen fibrils and between collagen fibrils, and these crosslinks are critical for the overall biomechanical strength of bone [28, 29]. Pyridinoline (PYD) and deoxypyridinoline (DPD) are PYD-specific forms of crosslinks occurring between lysine or hydroxylysine residues in the collagen telopeptides pairing with residues within the triple-helical region. Different forms of PYDs are present depending on which three amino acids are participating in the crosslink. PYD (also termed Hydroxylysl PYD) is formed from three hydroxylysine residues, whereas DPD (also termed lysyl PYD) is formed from one lysine and two hydroxylysine residues. PYD crosslinks are found in a variety of tissues, including cartilage, bone, ligaments, and blood vessel adventitia. DPD crosslinks are specific to bone and dentin and thus may be less subject to confounding under conditions influencing cartilage or ligament matrix turnover. The ratio between urinary DPD/PYD has been found to be fairly invariant at approximately 0.2 in both controls and patients with metabolic bone disease, though deviations from this ratio may be found in specific subsets of osteogenesis imperfecta or in patients with type IV Ehlers-Danlos syndrome due to mutations in the PLOD1 enzyme responsible for formation of PYD [30–33].

PYD crosslinks are relatively stable, persisting after bone resorption and collagen degradation until they are cleared by the kidney. For this reason, PYD and DPD are considered resorption markers. In the urine, approximately 50–70% of PYDs are present in protein-bound complexes and the rest are soluble-free species. PYDs are measured either by HPLC-based fractionation and quantitation of their native fluorescence or by an immunoassay [33–35]. Total urinary PYDs can be measured through an acid hydrolysis step to liberate protein-bound PYDs or can alternatively only measure free PYDs by omitting this step [34].

Bone-Specific Alkaline Phosphatase (BSAP)

Osteoblasts characteristically express a bone-specific isoform of the ALPL gene (BSAP, unique due to a distinct glycosylation pattern) early in their process of differentiation into mature bone-forming osteoblasts. ALP activity is a classic osteoblast marker used for in vitro studies, and in vivo osteoblasts release BSAP into circulation in a manner proportional to their number and activity [36]. BSAP activity is functionally important during skeletal mineralization to cleave local pyrophosphate, an endogenous inhibitor of mineralization. Indeed, patients or mice with hypophosphatasia due to mutations in ALPL show a potentially severe rickets-like phenotype (see Chap. 5) [37, 38]. Accordingly, total ALP enzymatic activity levels can correlate with bone remodeling, especially in disorders of markedly high bone turnover, such as Paget's disease of bone. However, total unfractionated alkaline phosphatase activity measured in serum or plasma is the sum of the activity of four different alkaline phosphatase genes (ALPI, ALPL, ALPP, ALPP2) and an even greater number of isoforms encoded by these genes. Thus, the utility of total ALP in the evaluation of bone pathology is limited under most conditions. To address this, a number of approaches have been used to fractionate total ALP activity to selectively measure BSAP. These include heat fractionation approaches that build upon observations that BSAP is more heat labile than liver or placental forms of alkaline phosphatase, though heat fractionation has a relatively poor ability to reliably distinguish between ALP isoforms [39]. Zone electrophoresis followed by ALP enzymatic activity visualization with α-napathyl phosphate has also been used for clinical fractionation of

ALP isoforms, but its application is relatively labor and skill intensive, rendering this approach not suitable for high-volume or automated application. Moreover, zone electrophoresis in some instances shows a suboptimal resolution of liver and bone isoforms of ALP, though wheat germ lectin-based selective subtraction of sialic acid-rich BSAP has been used as a solution to this limitation [40–42]. Due to these limitations of these other ALP fractionation methods, isoform-specific immunoassays are currently the most commonly utilized method to selectively measure BSAP in routine practice. However, the current BSAP immunoassays do display some degree of cross-reactivity with liver alkaline phosphatase, and a proportional bias is observed in comparing BSAP immunoassays with electrophoresis methods [42, 43]. Thus, elevated BSAP levels must be interpreted in caution in patients with liver disease.

Sources of Pre-analytic Variation and Bias in Measurements of BTMs

For many biomarkers, the greatest source of bias and imprecision in measurement comes not during the assay itself (analytic factors) but is rather a consequence of factors that occur prior to assay (pre-analytic factors), including patient demographics, comorbid conditions or substances interfering with the assay present in the patient, or issues relating to how and when the analytic specimen is obtained, transported, and stored prior to assay. Some of these factors, especially those related to specimen collection, can be controlled through rigorous application of clinical protocols designed to standardize specimen draw. Others, such as patient demographics, are inherently uncontrollable for a given patient.

Bone resorption displays stereotypic circadian variation, with a peak in levels of resorptive markers between midnight and 8:00 AM and a corresponding nadir in the afternoon [44]. While most BTMs are subject to circadian rhythms, CTX has been suggested to have particularly large circadian variations. Seasonal variation in BTM levels is also observed, as bone turnover peaks during winter months. Premenopausal women appear to be the most subject to seasonal influence on BTM levels [45]. Bone formation markers appear to be less affected than resorption markers by these factors [46]. Due to these issues, it is recommended that BTM levels be consistently drawn in the morning after an overnight fast.

BTMs also display a postprandial decrease, which is thought to contribute to the early morning peak in CTX levels. This effect is due to the effects of gastrointestinal hormones, such as glucagon-like peptide 2 on bone resorption [47]. Exercise can acutely change BTM levels, and it is recommended that exercise be avoided for 48 h prior to obtaining a specimen for BTM measurement. Bone turnover also displays variation across the menstrual cycle, with increased levels during the follicular phase and decreased levels during luteal phase. In premenopausal women, it is recommended that sampling ideally occur during the follicular phase [48].

Demographic factors also impact BTM levels. The high levels of bone turnover that accompany bone modeling in children lead to greatly increased baseline BTM levels for bone anabolic and catabolic markers. These levels correlate with the rate of increase in height, peaking during puberty [49]. BTM levels tend to be higher in young men than in young women, however in postmenopausal women, the relative increase in bone resorption reverses this difference [50].

Another source of confounding in the measurement of BTMs is that some comorbid conditions may cause BTM elevations. This can occur via three distinct mechanisms, each with different clinical implications. An important example of this is that BTMs are elevated during pregnancy, increasing over the course of gestation and continuing to increase postpartum during lactation [51]. Increased BTM levels in pregnancy may in large part be due to underlying changes in bone metabolism, though it is important to note additional contributing physiologic changes in renal function and plasma volume. While resorptive BTMs may be truly elevated by each of these conditions, it is unclear if elevations occurring through these comorbid conditions impart an equivalent fracture risk as if the same BTM levels

were seen as baseline values. Other examples include osteomyelitis and systemic infectious or inflammatory disorders promoting bone resorption [52]. BTMs are also elevated after fracture due to remodeling at the fracture site [53]. A second mechanism is that comorbid conditions can cause a true rise in the BTM being measured due to the remodeling of the extracellular matrix present in a non-bone organ. For instance, cutaneous and pulmonary involvement in systemic sclerosis are associated with increases in BTM levels [54, 55]. Congestive heart failure and dilated cardiomyopathy are also associated with changes in BTM levels [56, 57]. Lastly, comorbid conditions can influence the levels of BTM analyzed by altering their clearance. Chief among this category of effect is the impact of renal insufficiency on levels of many BTMs, including CTX, NTX, and monomeric forms of PINP [58]. Assays that measure an osteoclast-derived form of tartrate-resistant acid phosphatase (TRAP5b) have been proposed as resorption markers suitable for use in renal insufficiency due to avoiding renal clearance [59]. However, outside of this context, TRAP5b appears to be inferior to CTX or NTX in its ability to predict fracture risk [60]. Among bone anabolic markers, BSAP is not renally cleared and may have utility in the setting of renal insufficiency [61].

Harmonization of BTM Measurement

Harmonization is the process of ensuring that a series of assays measuring the same analyte provide comparable results in the absence of a gold standard method [62]. This is usually contrasted with standardization, which is the process of ensuring that a series of assays provide comparable results to an established gold-standard reference method or a reference calibrator. As most BTMs lack an established gold-standard reference method or traceable calibrators suitable for formal assay standardization, harmonization efforts are the most relevant to BTM measurement. Given that many of the BTMs discussed above represent complex analytes with multiple isoforms, cleavage forms, or other posttranslational modifications, harmonization is critical to allow BTM values to be compared among different analytic methods. This in turn is critical for allowing BTM data to be compared between institutions, which is important for the ability to (1) pool testing results performed at multiple laboratory sites as part of a multicenter clinical trial, (2) conduct meta-analyses utilizing BTM data, or (3) utilize published literature on BTMs to guide local practice at a given institution. A proposed strategy to address the lack of harmonization in BTM measurement is to focus efforts on one reference resorption marker, serum CTX, and one reference anabolic marker, serum PINP [63]. Progress in these harmonization efforts includes the adoption of consistent reporting units for serum/plasma CTX to ng/L and for PINP to μg/L and ongoing efforts to provide assay harmonization guidelines [64].

Reference Intervals in BTM Measurement

While reference intervals are typically ideally generated from age- and gender-matched healthy controls, in older patients, the prevalence of metabolic bone disease may be high enough to preclude the use of age-matched reference intervals as values reflective of desirable levels of bone turnover. Thus, for some uses of BTMs, it is necessary to apply an approach similar to that used for the reporting the T-score DXA scanning, for much the same reason, where patient results are compared to an age-invariant relatively young cohort representative of adult bone health prior to the engagement of aging-associated forms of bone loss [65]. It is also recommended that reference intervals be matched to ethnicity and nationality. Reference values in pre-menopausal women have been generated for a variety of nationalities for CTX and PINP [64]. Additionally, given the demographic effects on baseline BTM levels, it is important that these be taken into consideration when choosing a reference range for a given patient. Importantly, as research into BTMs has

focused on postmenopausal women, little data is available for establishing reference ranges in children. There have also been fewer reference data for men given the female predominance of osteoporosis. Recent work has, however, aimed to establish reference ranges for older men [66, 67].

The Least Significant Change

The substantial, largely pre-analytic, variance in BTM levels poses a practical challenge for the clinical interpretation of serial BTM levels as it can be difficult to know whether a change in BTM levels represents a meaningful alteration or is within the expected range of biologic and analytic variance. A concept that has been applied to BTMs to address this challenge is the least significant change (LSC), the minimum alteration in the levels of a BTM that is statistically unlikely to be due to biologic or analytic variability. While the LSC would ideally be defined with reference to both local patient populations and analytic procedures at a given institution, literature values provide practically useful points of reference, with many serum BTM analytes having LSC values in the 20–30% range and many urinary BTMs having much higher LSCs >70% [7, 68]. If serial monitoring of BTM levels is used to assess therapeutic response rates, the difference in LSC among BTMs can result in very different assessments of response rates depending on the analyte monitored [68]. Notably, in addition to the LSC, others have suggested that both absolute levels and percent change should be taken into account in assessing treatment response [69].

Potential Clinical Applications for BTMs

Use of BTMs in Osteoporosis

Prediction of Bone Loss

Since total bone mass is a result of osteoclast and osteoblast activity, clinical use of BTMs remains an active area of investigation. During the perimenopausal transition, bone resorption rapidly increases followed by increases in bone formation, presumably due to coupling between osteoclastic and osteoblastic activity [70]. While this pattern of change in bone turnover marker levels during the perimenopausal transition is well documented, the relationship between bone turnover marker levels and subsequent bone loss in older, "late" menopausal women is less clear [71]. Overall, the predictive value of BTMs for bone loss in older Caucasian women is modest, and without clear thresholds, screening for change in bone turnover markers is not recommended [71].

Of note, rather than assessing single individual BTMs, a creative idea is to combine both bone formation and resorption markers in models together in order to predict bone loss based on the premise that the net bone balance may predict bone loss. Various models include a bone balance index or an estimate of bone balance using T-scores of BTM values. The bone balance index (BBI) is a model that is based on regression to determine the relative amounts of OC versus urine NTX in a patient cohort with stable bone mass [72]. Alternatively, T-scores may be calculated based on a reference database of premenopausal women and "bone balance" is estimated by subtracting the bone resorption T-score from the bone formation T-score [73]. At this time, these models are used for research only.

Prediction of Fracture Risk

Since measurement of bone mineral density may not fully capture patients at high fracture risk, use of other surrogate markers such as BTMs has been assessed to predict fracture risk. While bone formation markers do not have clear utility in fracture prediction, multiple studies have demonstrated that elevated bone resorption markers are predictive of fragility fracture [74]. This association of increased bone resorption markers was predictive for short-term fracture risk (in the immediate 5 years) but did not remain predictive in longer-term follow-up [75]. As such, use of BTMs alone to predict fracture risk is not standard clinical practice.

Of note, BTM levels are known to be altered in many conditions that may cause secondary osteo-

porosis. For example, bone mineral density (BMD) measurements underestimate fracture risk for patients with diabetes, and characterization of BTM levels in individuals with type 1 or type 2 diabetes is ongoing [76, 77]. The utility of BTM levels to predict fracture risk in these populations with secondary osteoporosis remains an area of active investigation.

Use of BTMs in Monitoring Osteoporosis Treatment

In contrast to the use of BTMs for prediction of bone loss and fracture, the clinical utility of BTMs to monitor osteoporosis therapy has been more promising, as discussed below.

Pattern of BTMs with Treatment

The change in BTMs with various osteoporosis treatments is well characterized. Antiresorptive medications suppress bone resorption marker levels, as well as bone formation markers. The nadir (typically occurring at months 1–3) and duration of effect on BTMs vary with the potency of each antiresorptive with the greatest antiresorptive effect observed with parental treatments such as zoledronic acid or denosumab [78–82].

In contrast to antiresorptives, parathyroid hormone (PTH) analogs such as teriparatide (PTH 1-34) and abaloparatide (a synthetic variant of PTH-related protein 1-34) increase bone formation markers within 1 month of treatment followed by more modest increases in bone resorption with a net overall result of an increase in bone mass [83, 84]. The greatest increases of BTMs are in the first year, followed by a plateau of both formation and resorption markers after 1 year.

The pattern of BTM changes with romosozumab, an anti-sclerostin antibody, contrasts with other anabolic medications [85]. Romosozumab results in a simultaneous transient increase in bone formation as well as a decrease in bone resorption markers during the first 3 months. While there is a persistent antiresorptive effect, there is a gradual decrease in bone formation markers back to baseline by the end of 1 year of treatment. The mechanism(s) that underlie why bone formation marker levels are not sustained with prolonged romosozumab treatment are not yet well understood.

Additionally, combination anabolic and antiresorptive therapy has been studied in postmenopausal women with osteoporosis. The combination of denosumab and teriparatide suppressed serum CTX similarly to denosumab monotherapy over 24 months [86]. This unique combination contrasts with bisphosphonate-containing combinations in which combined zoledronic acid and teriparatide suppressed CTX transiently and combined alendronate with PTH-analogs suppressed bone resorption less than alendronate alone [87–90]. While these studies may not be directly comparable, differences in the pattern of bone resorption may account for the differential effect of the denosumab and teriparatide combination which results in the largest increases in BMD compared to bisphosphonate-containing combinations.

Given these known changes in BTMs with treatment, the use of BTMs to confirm compliance with medications and/or adequate absorption of medications remains an attractive strategy. However, due to cost and limitations in marker variability in a clinical setting as previously discussed, assessing BTM levels is not standard clinical practice to monitor absorption and compliance of therapy.

Use of BTMs to Predict Clinical Outcomes

Both baseline values and early changes in BTMs induced by treatment predict BMD changes. Baseline values of PINP correlate positively with teriparatide-induced 18- and 24-month increases in spine and hip BMD [65, 91]. Additionally, early 1- and 3-month increases in PINP were predictors of 1- to 2-year increases in spine BMD in those receiving teriparatide [65, 92]. Similarly, early decreases in BTM levels induced by bisphosphonates and denosumab correlated with 2–3-year increases in BMD [93, 94].

Despite these predictive relationships between BTMs and the increase in BMD, the ability of BTMs to predict fracture risk with treatment had

been inconsistent among individual studies [95]. These inconsistencies may be due to the wide variety of markers measured in each study. In general, many studies showed at least one BTM with positive predictive ability independent of BMD. For example, in the posthoc analysis of the Fracture Intervention Trial (FIT), greater decreases in serum PINP, BSAP, and CTX with alendronate treatment were associated with a greater reduction in spine and hip fractures [96]. Similar relationships were observed with PINP and zoledronic acid in the Health Outcomes and Reduced Incidence with Zoledronic Acid Once Yearly-Pivotal Fracture Trial (HORIZON-PFT) study [97]. More recently, results from the Foundation for National institutes of Health (FNIH) Consortium to assess change in BTMs in antiresorptive trials as a surrogate for fracture outcomes were promising [98]. In this study, individual-level analysis of pooled change in bone ALP, PINP, and N-terminal and C-terminal telopeptide of type I collagen of 28,000 participants who received bisphosphonates or selective estrogen receptor modulators were assessed. The change in bone ALP and PINP showed the strongest relationship for vertebral fracture risk reduction ($r^2 = 0.82$, $p < 0.001$ and $r^2 = 0.75$, $p = 0.011$, respectively) and non-statistically significant relationships with nonvertebral and hip fracture outcomes. To extrapolate from those results, for example, a 12% net reduction in bone ALP would predict a 33% reduction in vertebral fracture risk and a 22% net reduction in PINP would predict a 30% reduction in vertebral fracture risk. For bone resorption markers, all relationships were weaker and not significant for each fracture type. Based on these results, bone formation markers may be useful to predict vertebral fracture efficacy of new antiresorptive drugs or new dosing regimens with currently approved antiresorptive drugs.

Lastly, monitoring BTM levels after discontinuing therapy, commonly called a "drug holiday," in order to assess fracture risk and guide timing of re-treatment remains an attractive idea. However, in the Fracture Intervention Trial Long-Term Extension (FLEX), 1-year changes in bone ALP and urine NTX after treatment discontinuation did not predict fracture rates [99]. Additionally, in the 3-year HORIZON extension, PINP at the entry of the extension did not predict fracture in those who are receiving 3 years of zoledronic acid followed by 3 years of placebo [88].

In summary, the use of BTMs to predict fracture risk and to monitor treatment efficacy remains helpful in the research area only. Potential clinical applications may be the use of BTMs to support approval of new antiresorptive regimens for fracture prediction and the use of BTMs to determine optimal re-treatment strategies.

Use of BTMs in Renal Disease

Osteoporosis and/or renal osteodystrophy are common problems for patients with chronic kidney disease. Differentiating between adynamic bone disease, osteomalacia, and hyperparathyroid renal bone disease is important to make appropriate treatment decisions. Although biopsy-based bone histomorphometry remains the gold standard for diagnosing renal osteodystrophy and chronic kidney disease-mineral bone disorder (CKD-MBD), this is not commonly performed because it is labor- and skill-intensive, in addition to having a slow turnaround. As discussed above, TRAP and BSAP are not renally cleared, and therefore may have utility in the setting of CKD. In a large sample size of subjects with bone histomorphometry, extreme high and low values of PTH correlated with bone formation rate. The use of BSAP in conjunction with PTH was suggested to be helpful as low levels of BSAP are associated with adynamic bone disease [100]. Therefore, while bone histomorphometry remains the gold standard for diagnosing adynamic bone disease in the setting of chronic kidney disease, extreme values of BSAP may provide a useful proxy measure [100].

Role of BTMs in Oncology

Many solid tumors, such as breast and prostate carcinoma, metastasize to bone, and primary skeletal involvement is nearly synonymous in a number of hematopoietic malignancies, particular

multiple myeloma. Metastatic bone involvement is characterized by alterations in bone remodeling, which ultimately increases the risk of local pathologic fractures. Indeed, preclinical data suggests that these alterations in bone metabolism are mechanistically critical for sustaining bone metastases. In addition to local changes in bone metabolism, many tumors also produce systemic effects leading to bone loss even at uninvolved sites. Measurement of BTMs can provide prognostic information, although the added value of BTMs beyond a radiographic assessment of skeletal metastases remains to be determined. In patients with castration-resistant prostate cancer, lung cancer, or other solid tumors, elevated levels of NTX predicted negative outcomes, including skeletal-related events, disease progression, and death [101]. This topic has been considered in depth in recent reviews [102].

Bone metastasis produces a local increase in bone turnover; thus BTMs have been studied to identify subclinical bone metastases. A major challenge to this approach comes from the many pre-analytic sources of variability in BTM levels (reviewed above) combined with the potentially confounding effect of active chemotherapeutic and endocrine therapies on bone turnover. For instance, in a cohort of patients with a mixed group of solid tumors, screening BTMs showed significant elevation of NTX and DPD in patients with skeletal metastases [103]. However, the sensitivity of even the best-performing BTM in this study, NTX, was below the limit of practical clinical utility (below 50%). In another study comparing BTM levels with radionuclide bone scintigraphy, several BTMs were elevated in patients with lung carcinoma metastasis to bone, but each of these markers displayed low sensitivity [104]. Thus, the low sensitivity of elevated BTM levels for detecting skeletal metastases, in part due to the high pre-analytic variability of BTMs, precludes the use of BTMs as a standalone screening test for this indication. However, approaches to account for this variability, such as analyzing the serial change in BTM levels over time, are currently under exploration. For instance, prospective changes in the CTX/BSAP ratio in individual patients may predict the appearance of osteolytic lesions in multiple myeloma [105].

BTMs have also been considered as prognostic markers in patients with known skeletal metastases. In patients with bone metastases from solid tumors, elevations in BSAP or NTX predicted increased risk of skeletal-related events, such as fracture, disease progression, or death [101]. However, the relative risk associated with elevated BTM levels was only moderate (ranging from 1.5 to 3.5 for any skeletal-related event), suggesting that BTMs should be considered part of a comprehensive risk model that includes other prognostic factors. Similar findings were obtained in a cohort of patients with non-small cell lung cancer [106]. Normalization of NTX levels after treatment was correlated with prolonged event-free and overall survival in multiple solid tumors, suggesting that BTMs may have utility in monitoring therapy in this setting [106, 107].

In a subset of primary bone neoplasms, BTMs may themselves function as tumor markers directly secreted by tumor cells. Osteoid osteoma has been proposed to secrete OC [108]. BSAP can similarly be secreted from osteosarcomas [109]. Due to the lower baseline variability and reference range stability of BSAP levels in adults as compared with adolescents, BSAP has been proposed to have greater diagnostic utility for the detection of osteosarcoma in adult versus adolescent onset osteosarcoma. Taken together, while it is clear that BTMs may be elevated in malignancies that metastasize to bone, the utility of routine measurements in clinical practice appears limited. Future studies are needed to define the relative roles of BTMs versus standard radiographic approaches for following skeletal metastatic burden.

BTMs in Rheumatologic Disorders

Bone resorption markers are often increased in rheumatologic disorders (such as rheumatoid arthritis) for three distinct reasons. First, active bone erosion in RA near the affected joints can lead to systemic increases in resorption markers. Second, glucocorticoid therapy increases bone resorption and reduces bone formation. Finally, the inflammatory milieu of the disorder commonly causes systemic bone loss due to enhanced bone resorption and reduced bone formation. This last factor is not specific to rheumatoid

arthritis, and is seen across a wide range of chronic inflammatory, autoimmune, or infectious conditions [110].

Accordingly, the presence of rheumatoid arthritis is associated with high resorption and low bone formation markers [111]. Resorptive markers, especially CTX and PYD are associated with disease activity, correlating with the risk of radiologic progression of bone erosion [112, 113]. Furthermore, a therapy with disease-modifying biologics, such as anti-TNF antibodies, promotes a relative normalization of the BTM levels [114]. However, a normalization of bone turnover markers may not necessarily reflect an underlying clinical response to therapy in terms of the primary autoimmune disease process [114]. BSAP levels have also been observed to correlate with osteophyte formation [115].

Additionally, other rheumatologic disorders display high levels of resorptive BTMs. Polymyalgia rheumatica has been associated with increases in the levels of resorptive markers [116]. Both DPD and CTX levels are often elevated in psoriatic arthritis, ankylosing spondylitis, and reactive arthritis, and the degree of elevation correlates with the inflammatory markers of disease activity such as ESR and CRP [117]. As is the case with BTMs in cancer, the role of following these levels in standard clinical care in rheumatologic diseases such as RA, PMR, psoriatic arthritis, and ankylosing spondylitis remains to be determined.

BTMs in Other Conditions

Paget's disease of bone is another common metabolic bone disorder. Patients with Paget's show a signature of markedly high bone turnover, leading to expansion of affected bones. In affected patients, this can result in symptoms, most commonly due to osteoarthritis, nerve entrapment or fracture secondary to either expansion or fragility of the involved bones. Both Paget's and a series of Paget's-like skeletal disorders including expansile skeletal hyperphosphatasia, familial expansile osteolysis, juvenile Paget's disease, and fibrous dysplasia are characterized by a substantial increase in the levels of essentially all the BTMs [118]. Furthermore, BTM levels, particularly P1NP levels, correlate with measures of disease activity and respond to antiresorptive therapy, suggesting that BTMs may have a role in both diagnosis and disease monitoring in Paget's disease of bone [119]. An important open question in this clinical setting is the added value of tracking P1NP levels for patients with Paget's versus total alkaline phosphatase, which remains the clinical standard of care.

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Biomechanics of Bone

10

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Key Points

- The ability of the skeleton to bear loads without fracture depends on both the applied loading conditions and the structural properties of bone.
- Many factors can alter the structural properties of bone, including aging, trauma, and disease, as well an individual's loading history and mechanobiological response.
- Combined imaging-modeling approaches that include contributions of bone mass, architecture, and material properties can help elucidate mechanisms of skeletal fragility.
- More realistic material mapping and mimicking of in vivo loading conditions are needed to calculate bone strength more accurately and predict fracture risk reliably for individuals.

Bone Strength and Fracture

The skeletal system has important metabolic, physiologic, and mechanical functions, including storing minerals, protecting vital organs, and bearing functional loads. Individuals constantly impose dynamic mechanical stimuli on their bones during daily activities. A healthy skeleton generally has sufficient bone strength to support these loads without fracture, but trauma, aging, and disease can compromise its structural function. With trauma, loading may exceed the load-bearing capacity of the skeleton, either healthy or otherwise, and produce fracture. Aging and many skeletal diseases reduce bone strength, thereby producing skeletal failure even under normal or non-traumatic loading conditions. Fractures result not only in individual morbidity and mortality but also in high healthcare and societal costs [1–3]. Therefore, an understanding of the factors that contribute to bone strength is critical for the prevention and treatment of skeletal fractures.

Failure of any load-bearing structure can stem from a single traumatic overload or from the accumulation of damage with repetitive loading. Here we will focus on the former: what determines whether a given load applied to a bone will result in fracture? The interaction between applied loading and the ability of a bone to bear the applied loads can be summarized in a term called *factor of risk* [4]. The factor of risk is the ratio between the load applied to a bone and the load required to

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fracture that bone, or *failure load*. If the applied load exceeds the failure load for any given bone, then the factor of risk is greater than one, and fracture will occur. To predict fracture accurately for a particular skeletal site, characteristics of both the applied and failure loads must be considered. The load applied to the bone is influenced by the type of activity or trauma, the impact location and direction, and any protection imparted by overlying soft tissues. The failure load for that bone is determined by the quantity, distribution, and structural arrangement, and characteristics of the constituent components of the bone tissue [5]. The ability of the skeleton to resist fracture under applied loading varies with aging and disease, primarily through changes in these components of failure load. Our focus here will be on the determinants of whole bone strength and factors that affect whole bone behavior when loaded.

The mechanical function of bone is strongly shaped by the in vivo loading experienced by the skeleton. Bone tissue is exquisitely mechanosensitive, and bone cells respond to mechanical stimuli by altering turnover to increase or decrease the amount of tissue present, which in turn alters the tissue architecture and material properties [6, 7]. Therefore, the loading history experienced throughout an individual's lifetime contributes to these bone properties and greatly impacts skeletal structure and the failure load of bone [8]. This process of mechanoregulation, whereby physical forces influence cell behavior and bone (re)modeling, is an active field of research called *mechanobiology*. This concept of functional adaptation in response to mechanical stimuli has been around since the late 1800s, pioneered by the work of Roux [9] and Wolff [10]. It has been studied extensively in many in vivo, in vitro, and in silico models, and more recent studies have combined these mechanobiology models with new "omics" technologies (e.g., genomics, proteomics) to probe the effects at the molecular level in an emerging area of *mechanomics* (reviewed by [11]).

Factors Contributing to Whole Bone Strength

Measurements of whole bone strength and other structural properties are different for different types of loading. In vivo the skeleton withstands a complex array of different types of applied loads during the course of its various activities, such as walking, stair climbing, and lifting objects [12, 13]. To characterize the mechanical behavior of a whole bone, more simple loading modes – axial (tension or compression), bending, or torsion (twisting) – are often applied during mechanical testing in the laboratory. Because bone is metabolically active and capable of dynamic adaptation in response to loading, its properties will vary over time, a factor that must be considered when comparing bone properties or making fracture predictions.

True structural properties of bone can only be measured with ex vivo mechanical testing, so our understanding about bone properties comes primarily from studies using whole cadaver or animal bones or bone biopsies. When a force is applied to a whole bone, the structure experiences measurable displacement, or deformation (Fig. 10.1a). When the load is examined as a function of the displacement, the resulting curve has several distinct characteristics: an initial linear or *elastic* region, a nonlinear region with a maximum defined as the ultimate point, and a failure point at which the bone fractures and can no longer withstand the applied load. Applied loads that fall within the initial linear range can be resisted without permanently deforming the bone or causing failure.

The two most critical measures obtained from load-displacement data are structural stiffness and strength. The stiffness of a whole bone is the resistance to deformation for a given applied load and is the slope of the linear portion of the load-displacement curve. For a whole bone, the structural strength is the maximum or *ultimate* load that the bone can withstand. Whole bone stiffness and strength will have different values for different loading modes, such as compression, bending, and torsion, and these values depend on the intrinsic properties of the bone tissue, how much tissue is present, and the geometric arrangement of the tissue. For example, the failure strength of a vertebral body will be different when loaded in compression than in bending. Stiffness and strength are distinct parameters but are often correlated. Other parameters of interest include the yield point (the transi-

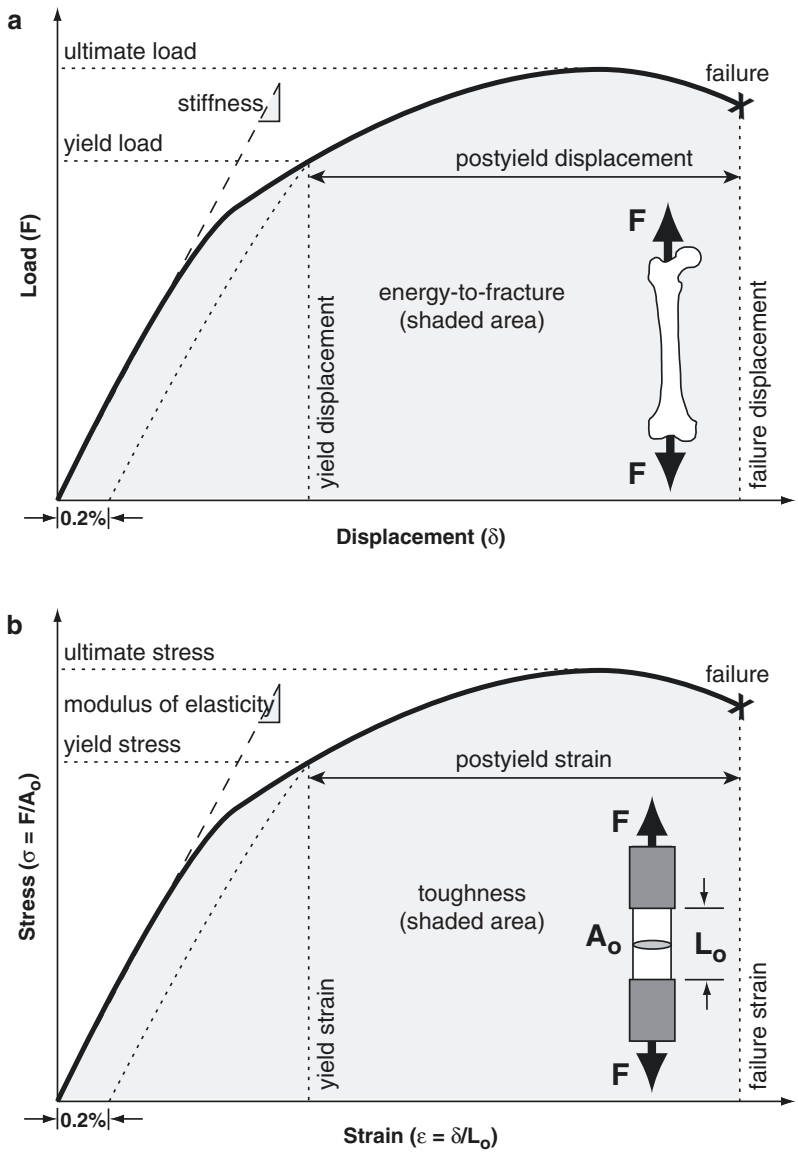


Fig. 10.1 (**a**) Load-displacement behavior for a structural test such as a whole bone. The structural stiffness is determined from the initial linear region. Yield is the transition point from linear to nonlinear behavior. Structural strength is the load required to fail the whole bone. Energy-to-fracture is the area under the entire curve (shaded). (**b**) Stress-strain behavior for a tissue materials test. These measurements are independent of specimen size and shape. The modulus of elasticity, or tissue stiffness, is determined from the initial linear region; tissue strength is the maximum or ultimate stress; and toughness is the shaded area. Both structural and material parameters depend on loading mode (tension, compression, bending, or torsion)

tion between the linear and nonlinear regions), post-yield displacement (the amount of deformation between the yield and failure points), and energy-to-fracture or work-to-fracture (the area under the entire load-displacement curve), which represents the amount of energy the bone dissipates up until failure or, equivalently, the amount of work the applied load performs to deform and break the bone.

Structural properties, such as whole bone stiffness and strength, are *extrinsic properties* that vary with the size and shape of the bone being tested. The forces and deformations of the whole bone also create internal forces and deformations within the bone tissue that are known as stresses and strains. Material characteristics, such as stresses and strains, are *intrinsic properties* that are independent of bone size and shape. These material properties can be measured on small, homogeneous tissue samples, such as a machined microbeam. Similar to a whole bone test, a bone materials test examines deformation in response to an applied load, and the resulting stress-strain curve can be examined for properties analogous to the ones for a whole bone test (Fig. 10.1b), such as modulus of elasticity (tissue stiffness), ultimate

stress (tissue strength), post-yield strain, and toughness (energy dissipated per unit of tissue up until failure). Similar to structural properties, material properties depend on the direction or mode of loading. More details about bone material properties will be discussed in a later section.

Whole bone behavior depends on the behavior of the constituent tissues, cortical and cancellous bone. During whole bone bending, for example, the behavior is dominated by cortical bone geometry and material properties in the diaphysis. Cortical and cancellous bone are both complex structures, and their behavior depends on similar factors as those for whole bone strength, as discussed below. The continuum properties of these bulk tissues are referred to as *apparent* properties, which is at a length scale below the whole bone properties but above the tissue material properties. These properties can be determined using mechanical tests on specimens in this range, such as a cancellous bone core from a vertebra. The porous structure of cancellous bone and its location in vertebral bodies and in the ends of long bones are important for distributing joint contact forces during daily activities, but they also make the tissue more susceptible to the surface-focused resorption that occurs with aging and skeletal disease. The structural behavior of cortical and cancellous bone is governed chiefly by the quantity of bone tissue present (bone mass or density), the size and spatial arrangement of that tissue (cortical geometry and cancellous architecture), and the intrinsic tissue material properties [14–18]. Alterations in any of these components could compromise the integrity of the overall bone structure and its ability to bear loads. Although most in vivo imaging tools measure bone mass or apparent bone mineral density (apparent BMD), these measures alone do not fully explain variations in mechanical properties observed experimentally. In the following sections, the contribution of bone mass, architecture, and material properties to the structural behavior of cancellous bone will be described, as well as the clinical and laboratory tools used to characterize them. The role of bone quantity (bone mass or density) has been studied most extensively, although the effects of architecture and tissue material properties have been examined more in recent years through technological advancements in imaging and image-based computer models.

Bone Quantity

The most-studied determinant of bone structural behavior is the overall quantity of bone at a given skeletal site. Bone mass and bone mineral density (BMD) are most commonly assessed in vivo using dual-energy X-ray absorptiometry (DXA, see Chap. 7), which evaluates the inorganic mineral phase of bone with minimal radiation exposure to patients. DXA scans can be performed for large regions, such as the lumbar spine, proximal femur, forearm, or even the whole body, thereby providing a noninvasive global measure of bone mass. However, DXA-based BMD alone cannot account for differences in mineral distribution and bone structure and only partially discriminates individuals who will fracture from those who will not [19, 20]. This is not surprising: DXA scans are two-dimensional and provide projected areal measurements of BMD (aBMD), which integrate geometric and material contributions into BMD values and create a size bias that overestimates the volumetric mineral density for larger individuals [21]. Because the resolution of DXA is relatively low (on the order of 1 mm), cortical bone tissue cannot be distinguished from cancellous tissue, architectural features of cancellous bone (on the order of 0.1 mm) cannot be captured, and the mineral distribution within the bone tissue cannot be measured. Because unmineralized tissues do not inherently attenuate X-rays, DXA scans cannot evaluate the organic phase of bone or the soft tissues surrounding bone. DXA aBMD correlates well with in vitro vertebral failure load in compression [22].

Quantitative computed tomography (QCT) is a true three-dimensional method based on X-ray imaging that overcomes many of the limitations of DXA, though with a slightly higher radiation exposure for the patient. The resolution of this technique is typically better in the scan plane (~0.5 mm) than axially between slices (~1 mm). QCT provides volumetric measures of BMD

(vBMD) and can distinguish between cortical and cancellous bone, but it cannot accurately capture cancellous architecture or mineral distribution. Due to recent advancements in clinical imaging technology, high-resolution peripheral QCT (HR-pQCT) can resolve bone features much more accurately than DXA or QCT [23], with isotropic voxel sizes of 82 μm (spatial resolution of about 130–150 μm) [24] or 61 μm in second-generation scanners. HR-pQCT also measures volumetric BMD, but it can visualize trabecular bone much better than QCT, especially in newer scanners, enabling some quantification of trabecular architecture [25]. However, it can currently only examine peripheral sites, such as the distal forearm and tibia. Because the spatial resolution is similar to the thickness of a trabecula, several of the architectural parameters cannot be directly measured (trabecular thickness and separation) but are derived from bone volume fraction (BV/TV) and trabecular number, assuming a plate model [26, 27].

For cancellous bone, quantity is typically measured either by BV/TV, which is the volume of bone tissue present within the total volume of interest, or by apparent BMD, which is the mass of bone tissue present within the total volume. Additionally, tissue mineral density, or TMD, which is the mass of bone tissue within only the volume containing bone, can be computed as the product of BV/TV and apparent BMD. Variations in bone mass can produce 100-fold differences in the cancellous bone stiffness within an individual's tibial metaphysis, ranging from 4 to 433 MPa [28].

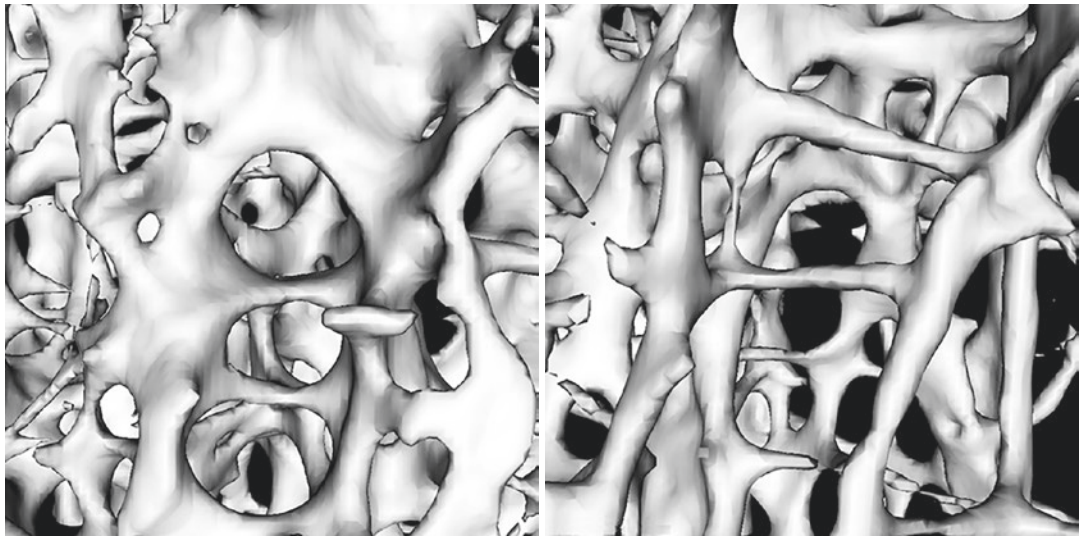
In the laboratory, empirical formulations have been developed to predict bone tissue strength and apparent tissue stiffness from apparent BMD [14, 29–33]. These relationships are often expressed in power law form, with the exponent (b) relating apparent BMD (ρ) to cancellous stiffness or strength (S) and ranging from 1 to 3:

$$\rho = aS^b$$

The coefficient a is a constant that scales the ρ–S relationship and is based on experimental data in bone specimens from various anatomic sites. As a result, for a relationship with an exponent greater than 1, a decrease in apparent BMD (or BV/TV) will result in a substantially greater decrease in stiffness and strength. For example, a 21% reduction in bone mass would predict a 38% reduction in cancellous stiffness and strength for a squared relationship and a 51% reduction for a cubic relationship (Fig. 10.2). Regardless of the relationship used, apparent BMD and BV/TV obtained experimentally or from micro-computed tomography (micro-CT) can explain 60–85% of the variability in compressive apparent stiffness and strength for human cancellous bone [34–38]. Although bone mass measurements generally have a high explanatory power for bone mechanical properties, these surrogate measures only capture one aspect of bone strength and cannot capture differences in how this mass is distributed. While mass is critical to bone integrity, additional factors are clearly needed to determine whether an individual will or will not fracture.

Several adjunct geometric parameters have been derived from DXA to try to improve fracture risk assessments beyond aBMD, including hip geometry metrics (e.g., hip structural analysis, hip axis length, neck-shaft angle) and a spine texture parameter. The only hip measure approved by the International Society for Clinical Densitometry (ISCD) for clinical hip fracture risk assessments is hip axis length (HAL), the distance through the femoral neck from the base of the greater trochanter to the inner pelvic rim [39, 40]. HAL is associated with hip fracture risk in women [39, 41, 42] and perhaps also in men [43], independent of aBMD and FRAX®, which is a 10-year fracture probability assessment using clinical risk factors [44].

Trabecular bone score (TBS), a gray scale textural analysis of DXA lumbar spine scans, was more recently developed to provide some information about bone microstructure [45]. Since its approval by the Food and Drug Administration in 2012, TBS has been shown in several studies to predict fractures in both women and men independent of lumbar spine aBMD [46–52]. A meta-analysis of 14 international cohorts showed that TBS predicts major osteoporotic fracture in both women and men, with an overall 32% increased fracture risk per



Normal
74-year-old female
T-score = –0.8
BV/TV = 12.7%
Tb.Th= 117 µm
Modulus, E = 844 MPa
Strength, σ_u = 3.5 MPa

Osteoporotic
92-year-old female
T-score = –2.6
BV/TV = 10.0% (–21% vs. Normal)
Tb.Th= 90 µm
Modulus, E = 470 MPa (–44%)
Strength, σ_u = 2.1 MPa (–40%)

Fig. 10.2 Micro-CT images of two cancellous cores taken from the center of the L2 vertebra of two different females. Measured T-score, bone volume fraction (BV/TV), trabecular thickness (Tb.Th), and apparent modulus and strength are indicated, as well as percent differences for the osteoporotic female relative to the normal female. For this 21% bone loss, a squared power law relationship would predict a 38% reduction in modulus and strength, and a cubic power law would predict a 51% reduction, both of which are comparable to the 40–44% reductions found experimentally

standard deviation decrease in TBS after adjusting for age and FRAX® probability [53]. Therefore, TBS seems to be a promising tool to aid in fracture risk prediction, but is only weakly correlated with aBMD at the lumbar spine (r = 0.33) or femoral neck (r = 0.27) [40]. Furthermore, in ex vivo testing of 16 human cadaver lumbar vertebrae, while TBS was significantly correlated with compressive stiffness independent of DXA aBMD, it did not significantly improve prediction of vertebral bone strength over aBMD alone [54]. While not a direct measure of bone architecture, TBS does correlate moderately with some trabecular measures based on comparisons with micro-CT in ex vivo studies and with HR-pQCT in in vivo studies, which may explain its ability to aid fracture prediction.

Bone Geometry and Architecture

For cortical bone, geometric parameters – such as the periosteal diameter, cross-sectional area, cross-sectional moment of inertia, and a geometric indicator of failure strength called the section modulus – all influence the whole bone structural behavior [55]. For bones loaded in bending, the cross-sectional moment of inertia (I) is a geometric measure of the distribution of bone about a central or *neutral* plane indicative of the bone's resistance to bending deflection, computed as follows for a hollow circular cross section [56]:

$$I = \frac{\pi}{4}\left(R_p^4 - R_e^4\right)$$

R_p is the periosteal radius, and R_e is the endosteal radius, computed about the neutral plane.

For bones loaded in torsion, the polar moment of inertia (J) is the distribution about the longitudinal or *neutral* axis and represents the bone's resistance to angular deflection or twist, computed as follows for a hollow circular cross section [56]:

$$J = \frac{\pi}{2}\left(R_p^4 - R_e^4\right) = 2I$$

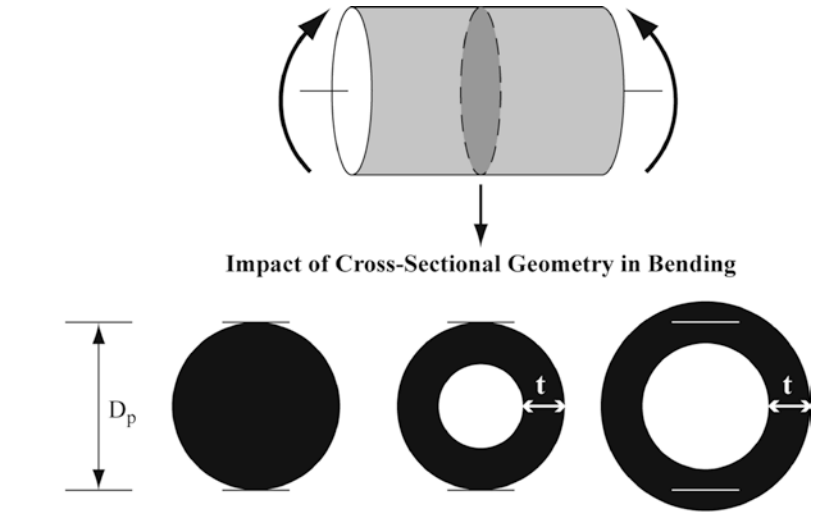
The section modulus (Z) represents a whole bone's resistance to bending or torsional loads and is computed as follows for a hollow circular cross section:

$$Z_{\text{Torsion}} = \frac{J}{R_p} = \frac{\pi}{2R_p}\left(R_p^4 - R_e^4\right) = 2Z_{\text{Bending}}$$

For a long bone loaded in bending, as seen in the proximal femur, both the size and geometric distribution of cortical bone relative to the loading axis contribute to the whole bone's resistance to applied loads and thus to fracture. To illustrate this concept, we will compare the properties of three "bones" that have a circular cross section, one solid and two hollow with cortical thickness equal to 20% of the periosteal diameter (Fig. 10.3). Comparing the solid "bone" to the first hollow one, which is comparable in size with the same periosteal diameter, the hollow one has a 25% smaller cortical area but only a 6% lower section modulus, which is proportional to the bending failure strength. If we compare the same solid "bone" to another hollow "bone" that has the same cortical area as the solid "bone" yet maintains the same cortical thickness as the first hollow "bone," then the new hollow one will have a 25% larger periosteal diameter, resulting in a 70% larger section modulus (and thus bending strength). Therefore, even small changes in overall bone size can compensate for losses in bone strength when the remaining bone is redistributed farther from the neutral plane or axis. Periosteal expansion is a common compensatory adaptation in aging bone that increases bending strength to help offset other losses.

Similarly for cancellous bone, the size and spatial arrangement of trabeculae that make up the cancellous architecture also play a key role in the structural competence of bone. As early as the mid-nineteenth century, increased fracture incidence was observed in older patients with thinning bone [57]. Two different sites of cancellous bone with similar apparent BMD can vary substantially in their stiffness and strength due to differences in tissue architecture [58, 59]. In addition, the architecture of cancellous bone often has a preferred orientation, creating substantially different modulus and strength values when bone from a given anatomic site is loaded in different directions, a characteristic called material anisotropy. In human vertebrae, for example, the primary trabecular orientation is superior–inferior, corresponding to the strongest direction when loaded [60]. Cancellous bone is nearly twice as strong when loaded along the superior–inferior direction of the spine than when loaded in the anterior–posterior or medial–lateral directions [58]. Therefore, characterizing the cancellous bone structure is important for understanding the relationship between architecture and mechanical properties.

Cancellous bone architecture cannot be directly measured with DXA, although as mentioned previously, TBS from DXA moderately correlates with some architectural parameters, in particular connectivity density, trabecular number, and trabecular separation [45, 54, 61–65]. Although QCT cannot accurately measure cancellous architecture, geometry-based metrics from QCT have been successful at predicting hip fracture [66–75] and spine fracture [71, 76, 77] in men and women, although most studies show limited or no improvement over DXA aBMD. HR-pQCT can measure both cortical and cancellous bone architecture with high reproducibility, with coefficients of variation (CV) reported at <5% for cortical thickness, BV/TV, and trabecular number, thickness, and separation [27, 78, 79]. Cortical porosity was less reproducible, with CV of 12–14% at the distal radius and 4–8% at the distal tibia, although the least significant change was <1% and deemed small enough to detect group differences and longitudinal changes [78]. HR-pQCT measures generally have good agreement with micro-CT measures in cadaver bone (r^2 = 0.59–0.98) [80], with stronger correlations for parameters of trabecular



Periosteal Diameter (D_p)	100%	100%	125%
Cortical Area (A)	100%	75%	100%
Section Modulus (Z)	100%	94%	170%

Fig. 10.3 Variations in the size and distribution of bone mass in a cortical bone cross section influence the section modulus, which is proportional to the bending failure strength of the whole bone. The resorption of bone on the endosteal surface or the apposition of bone on the periosteal surface may change the cortical thickness (t) or the distribution of bone about the loading axis, thereby altering the ability of the bone to resist fracture. For example, compared to the reference bone (left), a bone of the same girth but with less material (middle) will be slightly weaker, but a bone with the same amount of material distributed farther away from the neutral axis of the bone (right) will be much stronger

plates compared with trabecular rods [81]. Almost all of the studies assessing fracture prediction with HR-pQCT have been retrospective cross-sectional studies. Overall, HR-pQCT measures (vBMD + architecture) can better distinguish between subjects with and without fractures than DXA (aBMD only), particularly at the forearm (reviewed by [80]). The one prospective study to date assessed fracture prediction in French postmenopausal women from the OFELY cohort and showed that vBMD and architecture (especially trabecular number and connectivity density) at both the radius and tibia predicted the risk of all types of fractures [82].

Computational anatomy approaches provide information about the spatial distribution of mass and geometric features within QCT scans [83]. Anatomical structures are modeled as curves, surfaces, or volumes and, using statistical parametric mapping (SPM) [84], these features are examined across multiple subjects to determine changes associated with disease progression or treatment [85–90]. Various techniques fall into this category, including voxel-based morphometry (VBM) for mapping volumetric BMD [69] and tensor-based morphometry (TBM) for mapping volume (shape and size) changes via contraction-expansion maps [70]. Additional techniques combine both density and shape mapping, such as statistical shape and density modeling (SSDM) [68, 91], and cortical bone mapping (CBM), which includes volumetric distributions of cortical BMD, endocortical trabecular BMD, and cortical thickness [74, 92]. Of these techniques, only CBM and SSDM have been compared with DXA, showing only a modest improvement in fracture prediction compared to DXA aBMD.

Similar to bone mass measures, trabecular microarchitectural parameters have also been experimentally correlated with elastic mechanical properties using cadaver bone [93–98]. Independent of apparent BMD, bone regions with different architectures exhibited variable elastic mechanical properties that differed by over 50% [59]. Based on studies using two-dimensional serial sectioning techniques, trabecular orientation and connectivity correlated with cancellous bone strength [18, 95, 99]. In sheep femoral bone assessed with micro-CT, architecture indices explained 10–70% of the variation in compressive strength [100]. A study using static histomorphometry indicated that similar architecture–strength correlations also hold true in human vertebral bone [101].

Bone Tissue Material Properties

The intrinsic material properties of bone tissue are important contributors to bone strength and are independent of the quantity or geometric arrangement of the constituent material. Cortical and cancellous tissues are believed to be similar at the material level, both forming lamellar-based structures via surface-based processes, and apparent-level differences between the two are thought to result from contributions of mass and architecture. In bone material tests, the small, homogeneous tissue samples can be loaded perpendicular to the face of the material to determine the tensile and compressive properties or parallel to the face to measure the shear properties. From these tests, bone material properties are computed by normalizing the resulting load-displacement parameters by geometric measures representing the sample size and shape. For example, applied load is converted to tissue stress, and displacement is converted to tissue strain, as described below.

Tissue stress is defined as the ratio of the applied load (tension, compression, or shear) to the sample cross-sectional area (Fig. 10.4). For a tissue sample tested in tension or compression, the tissue stress is defined as the applied load divided by the cross-sectional area perpendicular to that load (i.e., the area of the sample face on which the load acts). For a tissue sample tested in shear, the applied load is parallel to the surface and again is normalized by the area the force acts across. Bone tissue strain is measured as the amount of deformation in the direction of loading normalized by the initial sample dimension. Tensile or compressive loads produce stretched or compacted deformations, respectively, along the direction of the applied load. The resulting strain is computed as the ratio of the change in length to initial length. Shear loads create distortions in the sample by inducing the sample surfaces on which the loads are applied to slide with respect to each other. For shear strain, the distortion ratio is related to the change in angle, which, for small angles, is approximated by the ratio of the horizontal sliding deformation to the initial length of that side (Fig. 10.4).

The material tests described thus far are for characterizing material behavior in response to a single load applied to failure. In vivo, however, bones continually experience cyclic loading during normal activities, and bone failure from such loading is more common than with a single overloading event [102]. The failure of a material under cyclic loads below the ultimate load is known as fatigue. In bone, fatigue loading produces microscale damage in the tissue, known as microdamage. Microdamage alters bone tissue properties and thus may inhibit the ability of the whole bone to withstand loads and avoid fracture.

Bone is a composite tissue comprised of an organic matrix made mostly of type I collagen that is reinforced by inorganic mineral crystals. The characteristics of these organic and inorganic constituents, as well as their interaction with each other, determine the tissue material properties of bone, properties that at least partially define the popular term *bone quality*. Little is known about the individual and collective contributions of the collagen matrix and mineral constituents to bone quality and bone strength. Indeed, the strength of the composite bone tissue is greater than that of other materials composed primarily of only one of the constituents, such as collagen-rich tendon or mineral samples of calcium phosphate [103].

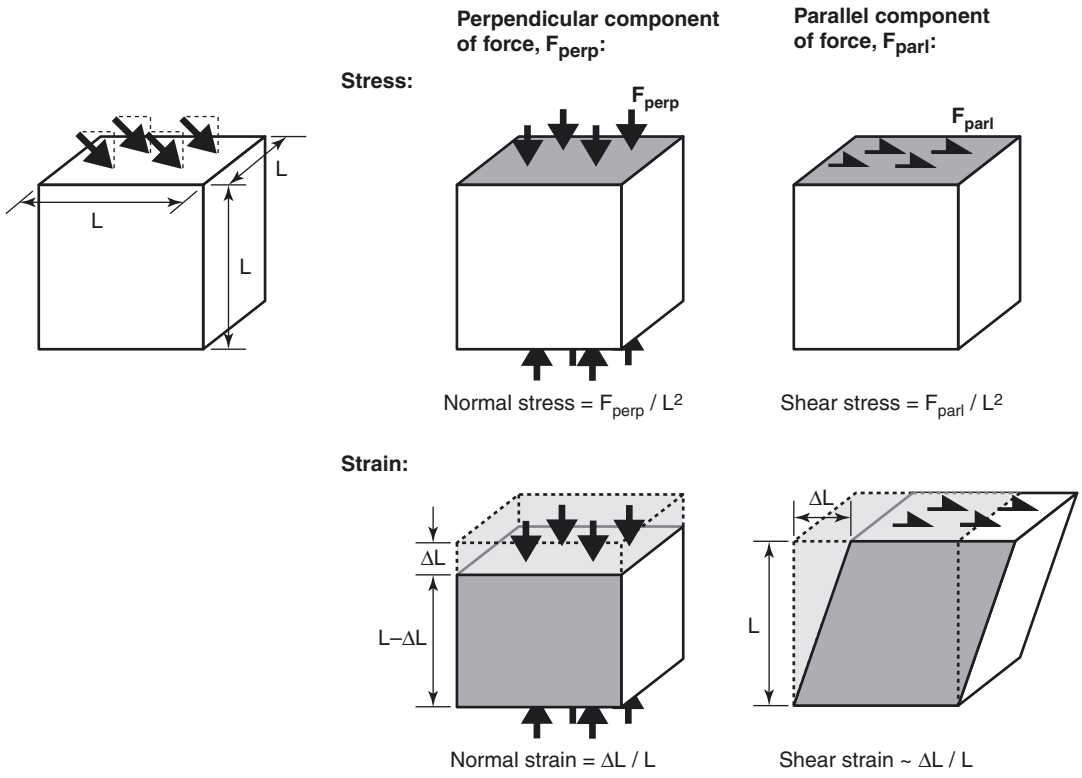


Fig. 10.4 Material stresses (local tissue forces) and strains (local tissue deformations) for bone tissue samples loaded in compression and shear. The applied loading is decomposed into components perpendicular (compression) and parallel (shear) to the cube face. The face for which the stress or strain is calculated is shaded. For strain, the original, undeformed volume is shaded

Studies of radiation in human bone and allograft specimens revealed that collagen damage compromises the toughness but not the stiffness of bone tissue [104, 105]. In rat bone, when the enzymatic crosslinking in collagen was disrupted through a lysyl oxidase inhibitor, bone strength was reduced without impacting mineralization [106]. Conditions with collagen defects, such as osteogenesis imperfecta, are associated with altered mineralization and bone fragility, as discussed in more detail below. In addition, numerous studies have shown a clear relationship between bone mineral content and material stiffness or strength [107–110]. These results suggest that the collagen and mineral phases of bone tissue contribute differently to its material behavior.

Characterizing the molecular structure of bone tissue is important for examining the relative contributions of the matrix and mineral constituents to the overall material behavior. Important compositional measures of bone matrix include collagen content, maturity, and orientation, as well as the molecular structure of various matrix proteins that aid in mineral crystal formation, binding, and maturation (e.g., osteopontin, osteocalcin, and bone sialoprotein). Important measures for bone mineral include the apatitic crystal size, orientation, and structure, as well as the degree of ion substitution, particularly the substitution of carbonate in the phosphate binding site, within the lattice or on the surface of the mineral crystals. These structural and compositional measures can be quantified using classic techniques such as gravimetry or more sophisticated techniques such as X-ray diffraction, backscattered electron imaging, and infrared (IR), Raman, and nuclear magnetic resonance (NMR) spectroscopies.

Healthy bone tissue properties show substantial variation both spatially [111–113] and temporally [114, 115] even for a given site and species. Materials testing techniques used to examine tissue properties include microbeam testing and nanoindentation. Using microbeam testing, the trabecular tissue modulus ranged from 3.8 to 20.7 GPa and varied depending on the loading mode [31, 116, 117]. The mean tissue modulus assessed by nanoindentation ranged from 7 to 26 GPa, depending on location within the tissue and type of lamellar tissue sampled; individual measurements varied by 17–62% [118–121]. This variation in modulus was true across individuals and for multiple anatomic sites. Even within a single trabecula, the indentation modulus ranged from 8 to 16 GPa [119]. As clearly evidenced by these studies, the variability in measurements of bone tissue properties can be quite large and depends on the technique used. Therefore, the effect of bone tissue composition and distribution on mechanical properties needs further exploration, particularly for cancellous bone. To date, almost all of the techniques used to measure bone material properties directly have been performed in vitro and require an invasive bone biopsy. Recent studies have explored the use of an in vivo Raman spectroscopic probe that can noninvasively measure bone matrix and mineral composition, although these devices are still in the developmental stages and have not yet been fully validated [122–124].

Other Influences on Bone Biomechanics

Many other factors influence the structural behavior of whole bones and the apparent behavior of cortical and cancellous tissue, including age, sex, and disease. These influences alter bone quantity, geometry/architecture, and tissue properties, all of which govern the mechanical performance of whole bones and bone tissue. For example, with aging, the compressive modulus of vertebral cancellous bone decreases 17% per decade [125]. Osteoporosis and aging are tightly coupled in women, and osteoporosis may in fact be the natural outcome of the aging process.

Aging

The factors described above (i.e., bone quantity, geometry/architecture, and material properties) vary independently with age. Age-related degradation of bone mass and architecture can seriously compromise bone integrity. Bone mass decreases with age after peak bone mass has been attained in both men and women [126–131], but especially in women due to peri-menopausal bone loss. By age 80, aBMD at the common fracture sites of the spine, hip, and forearm decreases by 13–18% in men [132] and 15–54% in women [133–136], thereby increasing the likelihood for developing osteoporosis [137–139]. As the life expectancy of the general population continues to increase, age-related declines will result in even lower bone mass, and the total incidence of skeletal fractures will rise, unless diagnosis and treatment of skeletal deficiencies can be significantly improved [140, 141].

While our understanding of the relationship between tissue composition and material behavior is limited, substantial progress has been made recently in characterizing tissue composition and variation with age. For example, osteons of cortical bone and individual trabeculae of iliac crest biopsies demonstrate spatially varying mineral crystallinity and collagen crosslinking by Fourier transform infrared microscopy [142]. The most crystalline (mature) bone mineral is located at the center of trabeculae, and newly deposited mineral is less crystalline than older mineral. Changes in mineral-to-matrix ratio and mineral maturity are documented with age and disease [143–148]. Femoral heads from patients with hip fractures undergoing total hip arthroplasty demonstrated a significantly increased mineral-to-matrix ratio compared to femoral heads of patients without fractures, suggesting that compositional changes may precede failure [149]. Tissue heterogeneity is known to change with age [150], but studies looking at the relationship between compositional heterogeneity and fracture risk are mixed. In femoral neck biopsies from female hip fracture cases, the compositional heterogeneity (mineral-to-matrix ratio, carbonate-to-phosphate ratio) was lower than in non-fractured controls [151].

However, in iliac crest biopsies from BMD-matched females, compositional heterogeneity (mineral-to-matrix ratio, carbonate-to-phosphate ratio, crystallinity, collagen maturity) was not significantly different between fracture and non-fracture cases [152].

The critical question is how these compositional changes relate to tissue and whole bone mechanical behavior. In rat bone, the mineral-to-matrix ratio, mineral crystallinity, and type-B carbonate substitution were all increased with aging, and these compositional changes were associated with reduced elastic deformation capacity (based on reduced resilience and bending modulus) [153]. Collagen content decreases with age and is associated with reduced post-yield energy dissipation [154]. Age-related accumulation of pentosidine, a marker of advanced glycation endproducts and increased collagen crosslinking, is associated with decreased bone toughness [155]. This accumulation has also been shown to increase matrix protein modifications [156], and advanced glycation end products can predict in vitro fracture properties in aged human bone [157]. Clinically, elevated pentosidine levels in urine have been associated with increased fracture incidence in postmenopausal women in the OFELY study [158].

Sex Effects

Given the relatively higher incidence of fragility fractures in women, understanding the sex-related differences in bone quantity, geometry/architecture, and material properties with aging is critical for improved diagnosis and treatment of osteoporosis. For both sexes, volume fraction in human cancellous bone declines steadily throughout life [159–162], as does ash density [125, 163]. However, histomorphometry studies indicated that sex appeared to have minimal or no impact on this relationship [159, 161, 162, 164–166]. Although volume fraction and ash density may change similarly with age for both sexes, similarly altering bone mechanical performance, the mechanisms of bone loss seem to be different and are at least partially related to sex-specific changes in the cancellous architecture. Regardless of sex, mean trabecular thickness as measured with traditional histomorphometry techniques decreased with age for vertebral bone [160–162, 167]. For men, decreased bone volume resulted more from progressive thinning of trabeculae while maintaining the trabecular network, but for women, bone volume reductions resulted mainly from a loss of trabeculae (and consequently an increase in trabecular separation), while the thickness of the remaining trabeculae was maintained [159].

Interestingly, these sex-specific changes in architecture with age alter the modulus and strength of cancellous bone very differently. When a 10% reduction in bone density was modeled in human vertebral cancellous bone, uniform thinning of trabeculae only reduced the bone strength by 20%, while the random removal of entire trabeculae reduced strength by 70%, and a reduction in both thickness and number reduced strength by 77% [168]. Even when normal bone density was restored by increasing the thickness of trabeculae to compensate for the bone loss, a strength deficit of 63% remained, which may help explain the higher fracture incidence observed clinically in women.

Disease

Although bone is a living tissue that adapts to its mechanical environment, disruptions in bone metabolism by diseases such as osteoporosis and osteogenesis imperfecta can seriously compromise structural integrity and the ability of bone to bear loads. Osteoporosis is a skeletal condition marked by reduced bone mass and a deteriorated architecture, which reduces bone strength and increases the likelihood of fracture [169, 170]. About 50% of white women and 20% of white men over 50 years of age will experience an osteoporotic fracture at the spine, hip, or forearm in their lifetime [170]. For white women, the lifetime risk of hip fracture (1 in 6) is greater than the risk of breast cancer (1 in 9). By 2030, the prevalence of osteoporosis and low bone mass are expected to increase by 30% (relative to 2010 levels) in the United States, increasing from 54

million to over 71 million, thereby increasing fracture rates. Osteoporosis is often asymptomatic prior to fracture, thus making prediction and possible prevention difficult.

In addition to reducing bone mass, osteoporosis also detrimentally affects architecture and material properties. Osteoporotic patients who sustain a vertebral fracture experience more trabecular thinning at the spine and iliac crest than normal, non-fractured aging subjects, resulting in a lower trabecular density, loss of trabecular connectivity, and the disappearance of load-bearing trabecular struts [171, 172]. This architectural disruption from osteoporosis is sometimes accompanied by a compensatory increase in trabecular thickness [171], although this adaptive mechanism does not necessarily prevent fracture. Similarly, at the proximal femur, female patients with hip fractures had a lower bone volume fraction, trabecular number, and connectivity than normal cadaveric controls, and the orientation of the trabecular structure was more aligned with the primary direction of loading, a characteristic known as *structural anisotropy* [173]. The architectural deficits in subjects with osteoporotic fractures were accompanied by reduced bone material stiffness and strength. In addition, bone biopsies of fracture patients revealed changes in tissue composition with osteoporosis, with fracture patients having a lower mineral content, higher crystallinity, and higher collagen maturity than age-matched controls [146, 174].

Often referred to as brittle bone disease, osteogenesis imperfecta (OI) literally means *imperfect bone formation* and is a group of hereditary genetic disorders that primarily affect bone and lead to increased bone fragility. Most commonly OI results from mutations in the genes that encode for type I collagen [175], but mutations in other genes can also result in OI, including those important for collagen modifications preceding crosslinking and fibril formation and those involved in osteoblast differentiation and mineralization (reviewed by [176]). Therefore, most patients with clinical OI (i.e., types I–IV) experience abnormalities in type I collagen, the primary component of the bone tissue matrix, which may alter the normal mineralization process. Bone strength is compromised in patients with OI, as evidenced by the degradation in bone mass and material properties. Cortical bone in the femora of adult mice with a moderate-to-severe phenotype of OI (*oim/oim*) was significantly weaker than in wild-type mice, and the bone tissue was less compliant and resistant to fracture, as evidenced by reduced moment of inertia, ultimate load, stiffness, energy to failure, ultimate stress, and toughness and increased brittleness [177]. In this mouse model, the mineral-to-matrix ratio was increased, likely due to a lower matrix collagen content [178]. In children and adults with OI types I–IV, bone mineral content and bone size were substantially reduced by 1.6–5.2 standard deviations as compared to normal controls [179, 180]. Matrix collagen defects will adversely affect bone mineral formation and likely compromise bone tissue properties. Therefore, the accurate evaluation of bone strength using surrogate predictions from routine clinical and laboratory assessment tools is essential, as is understanding the determinants of bone mechanical behavior.

Bone Strength Predictions from In Vivo Measurements

Clinical imaging techniques are routinely used to assess bone mass and geometry, and advancements in CT imaging have enabled analyzing cortical and cancellous compartments separately, as well as characterizing spatial distributions of bone mass and geometry and some measures of cancellous architecture. Direct measurements of tissue material properties cannot yet be made noninvasively, although two instruments can measure resistance to microindentation in cortical bone tissue in vivo: BioDent™ and OsteoProbe® (Active Life Scientific, Santa Barbara, CA) [181–183]. This microindentation technology has produced mixed results related to its diagnostic utility. In clinical populations, several studies have reported that the bone material strength index (BMSi) measured in vivo with impact microindentation (OsteoProbe®) at the tibial mid-diaphysis can distinguish between subjects with and without fragility fracture [184–187]. However, in one study,

BMSi was not associated with prevalent fracture in older women (75–80 years old) [188], and in another study, BMSi values were similar across postmenopausal women without fracture and with atypical femoral fracture (AFF) or hip fracture [189]. The BioDent cyclic reference point indentation (RPI) device more consistently discriminated between fracture and non-fracture cases, particularly using indentation distance increase (IDI) measured in vivo in the tibia (fragility fractures, AFF) [181, 190] and ex vivo in femoral neck tissue extracted from hip fracture patients during surgery [191–193].

Although metrics from both microindentation tools seem to be associated with bone fracture in some studies, they are generally only weakly correlated with a few specific cortical bone material properties, and these relationships have been inconsistent across studies. IDI measured by cyclic RPI was largely independent of age, aBMD by DXA, and cortical geometry by HR-pQCT [194], and it explained only 25–35% of the variation in apparent-level ultimate stress and toughness from bending tests in one study [195], and only 16% in fracture toughness and derived elastic modulus in another study [192]. However, a finite element model of impact microindentation suggested that BMSi is sensitive to changes in material properties, especially elastic modulus and a scalar damage parameter [196]. In terms of composition, one study reported that accumulation of advanced glycation endproducts in collagen and cortical porosity were both correlated positively with IDI and negatively with BMSi [197], although these relationships were also very weak. Collectively, these studies suggest that metrics from cyclic RPI and impact indentation may reflect aspects of both elastic and plastic properties of cortical bone tissue but are not definitely associated with any particular material property. In addition, when cadaveric bone samples were experimentally manipulated (e.g., drying and ashing to reduce toughness), RPI parameters responded differently than traditional material properties from bending tests, challenging the previous notion that IDI was inversely associated with bone toughness [198]. Furthermore, cyclic and impact measurements are only weakly correlated with each other and likely are related to contributions from different bone properties [194]. More extensive testing is needed to understand the clinical utility of these microindentation devices for specific patient populations and their ability to predict fracture in individual patients. These measures may be useful in assessing bone tissue quality locally during implant surgeries, thereby predicting mechanical competence at the interface [199].

Several analytical techniques can be used to extract structural properties from subject-specific images with varying degrees of simplifying assumptions. These structural properties can then be used to predict the strength and fracture risk of skeletal sites that commonly fracture, as well as provide insight into the etiology of fractures. The analytical approaches include structural analyses of densitometric data based on assumed geometric models, and engineering beam theory and finite element (FE) analyses based on CT data. The strength of these methods is that a mechanically meaningful mechanism can be determined to compare the structural performance of bones from different individuals, rather than representing the complex structure with a single bone density value.

The X-ray attenuation profile obtained from DXA can be used to determine geometric properties, including cross-sectional area and polar moment of inertia about a plane perpendicular to the scan direction, assuming that these measures are defined solely by the mineral phase [55, 200–202]. If structural changes in whole bone properties are assumed to arise only from geometric changes and not from alterations in tissue properties, then DXA-derived parameters can also be used to predict structural performance. This method has been applied extensively to the femoral neck and midshaft [200, 203–205], the distal radius [201], and more recently the disal femur [206]. Calculating the structural behavior with this method requires assumptions to determine the underlying geometry, mineral distribution and density, and relative cortical and cancellous fractions; therefore, the application of this technique may be most appropriate for cortical sites.

QCT scans can be analyzed slice-by-slice to examine bone strength indices at sites where most fractures occur clinically, the spine, hip, and forearm [207]. The axial, bending, and torsional rigidity can be calculated in each slice based on composite beam theory [208–210], and assuming bone tissue fails at a constant strain [211], whole bone failure load can be determined as proportional to the minimum structural rigidity in the cross sections. This approach combines appropriate geometric properties of the bone or bone segment (i.e., cross-sectional area for axial tension/compression, moments of inertia for bending and torsion) with the voxel-based values of material properties (i.e., elastic modulus), calculated based on the apparent-level tissue density and empirical equations noted earlier. Model-based estimates of bending and torsional rigidity together were better predictors of fracture than were traditional radiographic methods [212]. Axial rigidity correlated better with experimentally measured vertebral strength than did BMD-based structural measures and was equivalent to finite element strength predictions, at least for this simple compression loading scenario [213, 214]. Historically, CT-based strength indices were used in retrospective population-based studies to compare the mechanical competence of bone in the spine, hip, and wrist across ages and between sexes [215, 216]. More recently, these CT-based methods were applied prospectively to predict incident vertebral and hip fractures in cancer patients with skeletal lesions [217, 218]; however, these studies used the ratio of the affected bone to the contralateral bone to discriminate fracture vs. non-fracture cases, an approach not appropriate for osteoporosis or other conditions that affect both limbs similarly.

Finite element models of the spine and proximal femur take this QCT-based approach further and provide the opportunity to include subject-specific bone geometry, distribution of apparent properties, and more complex loading conditions in a fully three-dimensional analysis [219, 220]. As in the two-dimensional analysis, the bone geometry is modeled with high fidelity from the scan data, and apparent-level material properties can be included based on the CT-measured density. In contrast to the stiffness determined from the two-dimensional analyses, FE models can predict both stiffness and strength when nonlinear analyses are performed. When FE models of vertebral and femoral bone are compared to ex vivo mechanical testing data, the FE-predicted strength correlates well with the experimentally measured failure strength (explaining 50–95% of the variance) and explains 10–40% more variability in strength than does BMD from DXA or QCT [213, 221–232].

The ability of QCT-derived, specimen-specific FE models to predict vertebral and hip fractures independently of BMD has been examined in two prospective clinical studies, the multi-center osteoporotic fractures in men (MrOS) study with a cohort of ethnically diverse men aged 65 and older [73, 77] and the single-center Age, Gene/Environment Susceptibility-Reykjavik Study (AGES-Reykjavik) consisting of Icelandic men and women born between 1907 and 1935 [76, 233–235]. In the MrOS study, FE vertebral strength predicted fracture independent of lumbar spine (LS) aBMD; and after adjusting for age, race, body mass index (BMI), and clinical center, FE strength was a better predictor of vertebral fracture than LS aBMD but not QCT integral (cortical and cancellous) vBMD [77]. In the AGES-Reykjavik study, after adjusting for age, BMI, and prior fracture, FE vertebral strength was associated with fracture, independent of vBMD, for men but not women [76]. In the proximal femur, strength from the FE models predicted hip fracture in both cohorts [73, 76, 233, 234], although it was not independent of BMD for all cases. In MrOS, after adjusting for age, BMI, clinical center, and total hip aBMD, FE femoral strength was no longer significantly associated with hip fracture [73]. In AGES, after adjusting for age, BMI, and CT-based femoral neck aBMD, FE strength remained associated with hip fracture for women but not for men, and if CT-based total hip aBMD was used in the model instead, FE strength was associated with fracture for both men and women [76].

QCT-based FE models are a promising technique to predict bone strength noninvasively at sites that commonly experience fragility frac-

tures, although they do not yet reliably predict fracture better than BMD in all studies. The predictive ability of these models depends on the specific density-modulus relationships used [236, 237] and may be improved by the use of subject-specific relationships [238]. In addition, most models simulate quasi-static loading conditions and are validated with quasi-static mechanical testing, but recent studies indicate that dynamic FE models that include more sophisticated material mapping strategies, and are validated with more dynamic impact tests simulating falling, may improve model accuracy [239]. In particular, improving our understanding of both cortical and trabecular bone behavior at high strain rates, and the specific loading conditions that lead to fracture, may lead to improved FE models that are more consistently predictive of fracture. Furthermore, higher spatial resolution in CT scans would also improve these models, as spatial variation in geometric and material properties would be captured more accurately [240].

HR-pQCT scans have enabled the development of micro-FE models [241, 242], although this technique is still mostly limited to research studies and can only be done in the peripheral skeleton. Because HR-pQCT scans overestimate bone volume compared with micro-CT (regression slopes of 0.73–0.86 for ex vivo experiments) [26, 243–245], micro-FE models overestimate bone stiffness and strength, which are highly dependent on bone volume fraction. Nevertheless, results from micro-FE models based on HR-pQCT scans are highly correlated with those based on micro-CT scans, and their behavior can be adjusted by altering the tissue modulus or parameters in the failure criterion [241]. Based on ex vivo mechanical testing experiments, micro-FE models from HR-pQCT can accurately predict bone strength in the distal radius and tibia, but results are highly dependent on the modulus and study parameters, which vary across studies [246–250]. In vivo studies showed that FE-predicted properties (e.g., stiffness, strength) at the distal radius and tibia were associated with several types of fragility fractures in men and women [251, 252]. An ex vivo study similarly found that FE-predicted radius strength correlated with measured L4 vertebral strength and that FE-predicted tibial strength was strongly correlated with both vertebral and femoral strength [253]. However, more studies, and in particular prospective studies, are needed to determine the efficacy of micro-FE calculated strength at peripheral sites in predicting vertebral or hip fractures. In addition, although micro-FE models consistently predict bone strength better than BMD, they do not clearly provide better prediction of fracture risk even at the distal radius [241].

In summary, current clinical tools that assess fracture risk based primarily on bone mass and geometry do not reliably predict whether or not a patient will fracture. Based on the concepts of bone mechanics and laboratory studies presented here, we see that the structure and properties of bones are complex and depend on many factors. Future techniques should combine information regarding an individual's bone mass, geometry/architecture, and tissue material properties to provide a more precise measurement of bone strength and susceptibility to fracture, regardless of age, sex, or the presence of skeletal diseases (and perhaps even more so because of these). A combined imaging–modeling approach can include all of these factors and has the potential to elucidate skeletal structural performance mechanistically and improve our ability to predict skeletal fragility. Recent advances in QCT-based FE modeling and HR-pQCT-based micro-FE modeling show promise for fracture prediction, although more research is needed to improve the accuracy of these models, particularly in terms of more realistic material mapping and mimicking of in vivo loading conditions. Furthermore, fracture risk also depends on the loading environment and the propensity for falling. Improving the accuracy of model boundary conditions through better estimates for the nature and magnitude of mechanical forces experienced during a variety of tasks, and expanding to multi-scale representations that capture other important factors, such as muscle strength and balance, could substantially advance clinical fracture prediction.

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Exercise in the Prevention of Osteoporosis-Related Fractures

11

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Key Points

- Regular physical activity will likely reduce the risk of osteoporotic fracture across the life span by optimizing peak bone mass in childhood, consolidating or enhancing adult bone, and reducing falls in old age.
- Osteogenic exercise is site specific, requires overload, is reversible if discontinued, is most effective in those with the weakest bones, and is optimized by adequate calcium.
- The optimum exercise regime involves a minimum of twice-weekly, high-intensity, weight-bearing impact loading and resistance training; however, the precise exercise prescription remains to be determined.

Introduction

The utility of exercise as a strategy to optimize bone health is an important public health goal. It is well known that skeletal unloading that can occur following spinal cord injury, prolonged bed rest, limb immobilization, or microgravity leads to bone loss, particularly in weight-bearing skeletal sites [1–6] and may not be fully reversed with return to normal weight bearing [7]. By contrast, the effect of additional loading (exercise) on the skeleton is highly dependent on the loading features of a given activity and may be further influenced by age and diet. Recent work has considerably advanced the development of an optimal exercise prescription to enhance bone health across the life span; however, whether or not exercise can prevent osteoporotic fracture remains an active area of inquiry.

The practical goal of a bone-targeted exercise intervention is to optimize bone health in order to reduce the incidence of osteoporotic fracture, but the etiology of osteoporotic fractures includes both low bone mass *and* falls. Falls account for more than 90% of hip fractures, more than 50% of vertebral fractures, and nearly all wrist fractures. Thus, optimizing exercise interventions to reduce the risk of fracture should be aimed to improve bone health and to prevent falls.

This chapter provides a review of exercise as a strategy to reduce osteoporotic fracture by maximizing bone health and modifying additional

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factors of risk. The review will summarize the high-quality evidence on exercise interventions across the life span and other quality forms of evidence that supports exercise as a fracture prevention strategy. Guidelines for prescribing exercise to reduce factors of risk are proposed and directions for future research are identified.

Factor of Risk

The factor of risk, based on engineering principles, is defined as the ratio between the applied load and the load at which a bone fractures (ϕ = applied load/fracture load). If the applied load is greater than the fracture load, then fracture is likely. Conversely, if the applied load is less than the fracture load, fracture is unlikely to occur. In a 70-year-old individual with average hip bone mass, the factor of risk of hip fracture ranges from 1.25 to 3.0 for a fall from standing height [8–10].

For hip fractures, exercise is a potentially powerful prevention strategy because it can alter both the numerator and the denominator of the factor of risk. Exercise can affect the numerator by eliminating falls, because when the numerator becomes zero, fracture becomes highly unlikely. To raise the denominator, exercise can increase bone mass and reduce skeletal fragility, thus raising the force required to fracture.

Given the strong relationship between bone mineral density (BMD—a DXA-derived bone mass surrogate based on densitometry) and failure load, increasing BMD is an important strategy for reducing fractures [11–13]. Bone strength is also strongly influenced by its geometric proportions. Small increases in cross-sectional area, width and moment of inertia, that are independent of changes in BMD can convey disproportionately large improvements in the resistance of long bones to bending [14]. Effecting changes in cross-sectional geometry then is an important strategy to reduce the factor of risk. Advances in the noninvasive measurement of bone geometry with techniques such as quantitative computed tomography (QCT) [15], peripheral quantitative computed tomography (pQCT) [16–22], quantitative ultrasound [23], and magnetic resonance imaging (MRI) [24] have improved our understanding of exercise effects on geometric properties of bone; however, measurement issues of validity and reliability continue to limit translation of findings on bone geometry into exercise recommendations.

Exercise Study Design

The influence of study design and rigor on the ability to interpret research findings is particularly important when examining the effect of exercise on bone health. In general, cross-sectional data reveal that physically active individuals have higher bone mass than people who are less active. One key limitation of cross-sectional data, however, is that of self-selection bias, where individuals who choose to participate in a specific type of exercise may have predisposing skeletal attributes that favor their ability to successfully perform the activity or sport and to do so without injury. For example, while power lifters have higher than average bone mass, success in this sport entails repetitive lifting of very heavy weights that may depend upon the athlete having an initially strong skeleton before training ever starts. Ergo, cause and effect cannot be established in a cross-sectional comparison. Another limitation of most cross-sectional exercise studies is the use of standard physical activity questionnaires (PAQs) to define a dose of exercise of relevance to bone. Most PAQs are designed to measure energy expenditure and do not assess the magnitude of forces applied to the skeleton during a given activity. Attempts to correlate bone mass to total energy expenditure rather than loading patterns introduce validity error and, as a consequence, the likelihood of drawing inappropriate conclusions. While a bone-specific PAQ has been developed to overcome this measurement problem of validity [25], randomized, controlled, intervention trials (RCTs) remain the most rigorous approach to examine the effects of exercise on bone.

In this chapter, we have therefore chosen to emphasize observations derived from exercise

RCTs, with bone as the primary outcome measure. Cross-sectional observations are included, where appropriate, to illustrate consistency with experimental findings, or when RCT data are absent or equivocal.

General Principles of Effective Bone Loading

Fundamental Principles of Exercise Training for Bone

Early in the history of the field of exercise and bone, Drinkwater [26] emphasized the need to incorporate the following five principles of exercise training into the design of exercise interventions for bone health: specificity, overload, reversibility, initial values, and diminishing returns. While the principles are clearly interrelated, independent consideration will assist in the development of customized exercise training programs for bone health.

Specificity

According to the principle of specificity, an exercise protocol must load a bone directly in order to stimulate a response from it. That is, exercise does not have a generalized systemic effect on the skeleton. For example, lower extremity impact activities that prevent bone loss at the hip do not influence the bones of the forearm [27].

Overload

Exercise must overload bone in order to stimulate it. That is, loads experienced at the skeleton must be either sufficiently different from or greater in intensity than normal daily loading to stimulate bone accretion [28]. Lack of attention to overload has been a frequent shortcoming of published intervention studies, and one that is particularly challenging to address given the limited approaches to directly measure loads applied to skeletal sites during exercise. Existing techniques are highly invasive and impractical for many sites. Derived estimates of loading are the only reasonable option but are seldom quantified.

Although not definitive, it is possible to make inferences from studies examining high- versus low-intensity loading to illustrate the principle of overload. For example, high-intensity strength training (>80% of one repetition maximum) more effectively increases spine and hip bone mass than low- or moderate-intensity training [29–31]. Similarly, weight squatted over the course of a 1-year progressive strength training program positively predicted change in trochanteric BMD [32]. The relationship between exercise intensity measured by accelerometry and hip bone mass has been reported for a cohort of premenopausal women over the course of a year [33]. It was found that physical activities that created accelerations exceeding 3.6 g were positively related to bone mass at the hip, which suggests that an exercise intensity threshold might exist. While these indirect approaches are informative, they remain insufficient to determine a precise effective dose of loading that can translate into an exercise prescription. Advances in technology that will improve our ability to directly measure or indirectly model bone strain during exercise will facilitate quantification of the overload principle and precision dosing of exercise.

Reversibility

When exercise is an adaptive stimulus, reversibility should be demonstrable. By proof of principle, the cessation of an activity would reverse exercise-induced bone accretion. The principle of reversibility applies primarily to mature adult bone that has reached a point of continuous remodeling [34–36], rather than to the growing skeleton that is in the modeling phase of growth. Recent data indicate that gains achieved from exercise during the period of longitudinal bone growth are maintained in the medium (1–3 years) term [37–39], but longer term follow-up studies are required to determine whether or not these improvements persist into adulthood. Cross-sectional adult data suggest that there is a bone maintenance effect from increased bone loading during childhood, as individuals who exercised during their youth have significantly higher bone mass and/or more favorable bone geometry later

in life than those who were less active [40–42]; however, the previously mentioned self-selection bias could also apply to these data.

Initial Values

The principle of initial values refers to the concept that responses from bone to loading will be greatest in individuals with the lowest bone mass. For example, premenopausal women with the lowest initial bone mass demonstrated the greatest improvement at the hip following 12 months of impact plus resistance training [43]. Postmenopausal women with low bone mass experience exercise-induced gains in spine and/or hip bone mass that are over twice as high [44, 45] as reported gains in similar cohorts with average bone mass [29, 31, 34, 46–48].

The initial values effect is likely to reflect the principle of overload, as smaller, weaker bones will experience greater strain than larger, stronger ones exposed to the same load. When loading is extremely high (>10 body weights [BW]), skeletal improvements are observed regardless of initial values [49], suggesting that even very robust skeletons will be overloaded at such high load intensities because the applied load far exceeds habitual loading.

Diminishing Returns

The principle of diminishing returns is evident when a ceiling effect in bone adaptation is observed when a consistent dose of loading is delivered over time. Diminishing returns is similarly related to the principles of initial values and overload, as bone will be strained less by the same load once mass and geometric adaptations to an exercise stimulus have taken place. Indeed, it is the *raison d'être* of the adaptive response to mechanical loading.

Characteristics of Bone Response to Exercise Loading

A number of characteristics distinguish the exercise response of the skeletal system from other body systems. First, changes are typically modest (1–5%). Second, the time required to elicit a measurable response is considerable (at least 4–6 months), and third, exercise-induced improvements in bone strength can occur in the absence of changes in bone mass, through morphological adaptations.

The magnitude of BMD increases in response to exercise training appears small when compared to other systems, such as skeletal muscle or cardiac function. For example, the mean change in BMD in adult women in response to generic exercise training has typically averaged ~1% [50], whereas increases in muscle strength and/or maximal aerobic fitness can be orders of magnitude greater. Nevertheless, even small BMD improvements can be clinically meaningful. For example, it has been estimated that a 1% increase in BMD following antiresorption drug treatment should reduce the risk of vertebral fracture around 4% [51]. Whereas both the neuromuscular and cardiovascular systems typically respond to a training stimulus within 4–6 weeks, bone requires at least 6 months to reflect measurable adaptation, that is, complete a full remodeling cycle and achieve a modicum of mineralization in new osteoid.

As previously noted, substantial gains in the resistance of a long bone to bending and fracture can be achieved by the strategic addition of even small amounts of new bone around the circumference of the shaft [52]. While changes in bone mass are not always observed following exercise intervention, measurement of the cross-sectional geometry of a long bone before and after an intervention may reveal these subtle but critical structural improvements.

Although there are data to the contrary [53], there are some evidence that bone age will influence the skeletal response to loading in animal studies [54] and human trials [55], but few high-quality RCTs have been conducted with age as an independent variable and/or with sufficient statistical power to consider age as an effect modifier of adaptability. The difference in the bone response by age could merely reflect a difference in intensity of effort, and therefore loading, during an exercise bout between young and older individuals. In fact, such differences in loading intensity between individuals may also partly

explain the phenomenon of responders and non-responders to any exercise intervention [30].

Important Load Parameters

Animal data have clearly shown that bone responds preferentially to certain characteristics of mechanical loading. It has long been known that, when other factors remain constant, high magnitude loads that induce relatively large bone strains (deformations) are more osteogenic than low [56]. As the frequency (cycles per second) of loading increases, however, the magnitude of the load required to stimulate an adaptive response from bone decreases [57]. Strain rate (the speed at which a bone deforms under load) is also a highly influential adaptive stimulus [58]. And finally, strain gradient, or the pattern of strain experienced across a loaded bone, is known to direct the location of bone remodeling [59].

The Osteogenic Index—An Exercise Algorithm Derived from Animal Data

Turner and Robling translated the findings of a generation of basic animal research into a theory for practical exercise application [60, 61]. They developed the Osteogenic Index (OI), a method to predict the effectiveness of an exercise regime to improve parameters of bone strength based on the known response of bone cells and tissue to certain types of loading [61]. The OI requires dynamic (cyclical) loading and accounts for load magnitude, rate, and frequency [62, 31, 46, 63–65]. They noted that animal bone tissue becomes desensitized to prolonged loading and, in fact, loses the majority of its mechanosensitivity after 20 loading cycles [56, 66]. Adding rest periods between bouts of loading markedly improves the bone response to a cyclical stimulus [67]. Thus, they propose that a regime of frequent, short, intense bouts of exercise should be most beneficial to bone.

The validity of the Osteogenic Index for human application remains to be formally tested. Preliminary evidence suggests the human response may vary in subtle ways, such as the importance of cycle number. For example, while 300 jump repetitions per week for 7 months produced positive effects at the hip and spine in prepubescent children, reducing the jump number to 150 failed to reproduce the effect [68]. Collective findings of jumping studies in premenopausal women, however, support the OI theory as even low numbers of weekly jumps can produce a bone response with little added benefit from additional impacts [69–71]. Recently, it was reported that women who performed the greatest amount of impact activity, measured by accelerometry, above a threshold level of intensity had significantly greater improvements in hip BMD compared with women who performed lower amounts of activity [72]. These data suggest that the cycle number may be an important determinant of bone responsiveness to impact activity, but that the effect may follow more of a threshold rather than dose–response pattern. As previously described, effective dose has also been examined in a 6-month unilateral impact study design in premenopausal women [73], which indicated more hopping sessions per week is more osteogenic at the femoral neck than less.

Studies of Exercise and Bone Across the Life Span

Exercise and Peak Bone Mass

The National Institutes of Health (NIH) Consensus Conference on Osteoporosis [74] reported that optimizing peak bone mass should be a primary strategy to prevent osteoporosis. The recent National Osteoporosis Foundation's Position Statement on peak bone mass development and lifestyle factors reports that lifestyle choices influence 20–40% of peak bone mass and that grade A evidence indicates physical activity is highly beneficial [75].

Children, Exercise, and Bone—Cross-Sectional Observations

Studies of children and adolescents of various races/ethnicities generally support significant

associations between physical activity and total body, hip, spine, and forearm bone mass [76–84]. Exercise appears to have the greatest effect when undertaken during the early pubertal years [41].

Compared with less active children, highly active children have a greater rate of bone mineral accumulation for the two peripubertal years during which bone is most rapidly accruing (12.5 years for girls and 14.1 years for boys) [79]. This greater accrual translated into 9% and 17% higher total body bone mineral content 1 year after peak bone mineral content velocity for active boys and girls, respectively. Others have also observed that the differences in spine bone mass of athletic and control children are greater in the peripubertal years of Tanner stages IV and V (average ages 13.5 and 15.5, respectively) compared with earlier Tanner stages [85].

Variability in the skeletal response to different types of sports and/or comparisons of BMD across different types of athletes reflect the different loading patterns of a given activity and exemplify the principle of specificity [83, 86]. The effect is elegantly demonstrated by a comparison between limbs within a person. Dominant limbs have greater bone mass than nondominant limbs [87], and athletes whose sport preferentially loads their dominant limbs develop even greater bilateral disparity [88, 89]. Again, differences in bone mass between playing and nonplaying arms in female squash and tennis players are about two times greater if participation in the sport begins during puberty [40, 85].

In general then, the majority of cross-sectional studies suggest that exercise benefits to the pediatric skeleton are site-specific and optimal during the peripubertal years.

Pediatric Exercise Intervention Findings

After a 2001 NIH Consensus statement [74] recommended optimizing peak bone mass for the prevention of osteoporosis, the influence of exercise on growing bone became a focus of intense research.

Infants

The known principles of optimal bone loading notwithstanding, even very low-intensity exercise may be appropriate for and beneficial for children who may have comprised bone health. In a study of premature infants, five repetitions of range of motion, gentle compression, flexion and extension exercises five times a week induced greater acquisition of bone mass at 4 weeks in exercised babies than in controls [90]. A similar protocol initiated at 1 week of age prevented typical postnatal loss of tibial speed of sound (a marker of bone strength) in very low-birth-weight infants [91]. Others have observed, however, that calcium intake exerts a greater influence on bone mineral accrual than 18 months of either gross or fine motor activity in 6-month-old infants [92]; thus, the combined importance of diet plus exercise should be recognized.

Preschoolers

The only intervention to target bone health in preschoolers assessed the material and structural response of bone in children randomized to gross motor activities compared with fine motor activities 30 min/day, 5 day/week for 12 months, with or without 1000 mg/day calcium [93]. Exercise alone increased tibial periosteal and endosteal circumferences, but the addition of calcium improved leg bone mass, cortical thickness, and cortical area of the distal tibia most markedly. While the differences in periosteal circumference remained between the groups 12 months after cessation of the intervention, the investigators reported that persistently higher activity levels among those in the gross motor activity group might have accounted for the disparity [94].

Pre- and Peripuberty

Favorable responses to bone loading exercise that included resistance and/or jump training have been observed for both prepubertal [95–97] and early pubertal girls [95, 98, 99], with the predominance of the evidence suggesting that early puberty is a particularly sensitive stage [41]. In a randomized study of 89 prepubescent boys and girls (mean age = 7.1 years), jumping 100 times,

3 day/week at ground reaction forces of eight times body weight, increased femoral neck and lumbar spine bone mass 4.5% and 3.1%, respectively, in comparison with controls [100]. The effect was maintained 7 months after detraining [37], suggesting that the program had the potential to augment peak bone mass. Early pubertal boys and girls (mean age = 10.6 ± 0.6 years) exposed to 9 months of thrice-weekly 10 minutes of capoeira and jumping activities during school time improved indices of bone strength and metabolic outcomes, an effect that appeared to be sustained 1 year later [38, 101, 102]. Geometric changes have also been observed in response to exercise training in this age group. Femoral midshaft cortical thickness increased in prepubertal boys after 8 months of weight-bearing activity [103]. Similarly, 2 years of participation in a school-based, high-impact, weight-bearing exercise program that supplemented regular physical education led to improvements in the structural properties of the femoral neck in prepubescent boys (mean age = 10.2 years) compared with controls [104]. A mere 10 minutes of jumping activity twice weekly for 9 months improved both femoral neck geometry and spine bone mass compared with controls in a healthy cohort of peripubertal boys and girls (mean age = 13.7 years) [105]. There have been few high-quality head-to-head comparisons of the responses of pre- versus peripubertal children to bone-targeted exercise. The one exception reported improvements at the hip and spine only in the early (peri-)pubertal girls [106].

Postpuberty

Few exercise interventions have exclusively targeted postpubertal adolescents. One trial reported that 15 months of resistance training produced a significant increase in femoral neck bone mass in adolescent girls (~ 2.5 years postmenarche), despite major challenges with subject compliance [107]. A study comparing the effects of twice weekly step aerobics for 9 months in pre- versus postmenarcheal girls reported bone mass and geometric parameters of bone strength increased at the spine and hip for premenarcheal girls only [108]. It is important to note that premenarcheal is not the same as prepubertal; thus, the latter findings reflect a peri- rather than prepubertal effect.

Findings from intervention trials support those of cross-sectional studies and indicate that exercise has a positive effect on bone mass and geometry in children. The consensus suggests that the effect is most marked during early puberty and that benefits are sustained, at least in the medium term during childhood. It remains to be seen if those benefits translate to a reduced risk of osteoporosis and/or fracture in later life. Only large, very long-term follow-up investigations can determine if such an outcome can be achieved.

Exercise and Bone Mass in Adults

Although the response of the adult skeleton to exercise has been studied extensively, considerable diversity in study design exists. Coupled with logistical challenges of resource intensive exercise trials, methodological inconsistency, sample heterogeneity, and the influence of concurrent interventions (e.g., dietary) often limit the ability to make direct comparisons between them. Randomization is particularly problematic, as adults who volunteer for an exercise study do not wish to be allocated to a control group and exercise allocation cannot be blinded. Innumerable studies are casualties to poor compliance, given the necessarily protracted duration of interventions, and poor acceptance of exercise by those who often need it most. Furthermore, there are relatively few studies in men due to the common misperception that osteoporosis is a female condition.

Adults, Exercise, and Bone—Cross-Sectional Observations

Observational data indicate that adults engaged in weight-bearing exercise at intensities of >60% of aerobic capacity have consistently greater bone mass than nonexercisers or those exercising at low aerobic intensities. These differences have been observed for BMD at the whole body [109–117], spine, proximal femur [81, 109–111, 113, 115–125], pelvis [110, 114], distal femur [126],

tibia [110, 117, 121, 127, 128], humerus [110], calcaneus [129, 130], and forearm [121]. Broadband ultrasound attenuation and speed of sound transmission in the calcaneus are similarly higher in runners than in controls [113]. Consistent with the principle of specificity, high bone mass is typically confined to the loaded bone(s) [114, 131–133].

There is an abundance of evidence that certain activities do not sufficiently overload the skeleton to invoke an adaptive response [134]. Athletes participating in moderate- to high-intensity impact activities such as running, jumping (e.g., volleyball or basketball) and power lifting have greater bone mass than those performing low-intensity or non-weight-bearing activities [112, 125, 135, 136], whereas individuals who participate in non-weight-bearing activities such as swimming have similar bone mass to nonexercisers [126, 137], although some data to the contrary exist for men [138]. Muscle forces on the skeleton during elite-level swimming training do not appear to offset the substantially reduced daily weight-bearing activity associated with long periods of time spent in a weight-supported environment (water).

In studies of nonexercising adults, as in children, the dominant arm exhibits greater total and cortical bone mass than the nondominant arm [87, 139], and side-to-side differences are similarly exaggerated when the dominant limb is chronically overloaded, for example, during racquet sports [88, 89, 119, 126]. Some have found that the difference is accounted for by increased periosteal area and cortical thickness rather than by bone mass [139], while others have observed both expanded diaphyseal diameters and increased bone mass in the dominant limb of athletes. A 27% difference in cortical cross-sectional area has been observed between left and right humeri of adult tennis players compared to a nonsignificant 5% difference in controls [88]. Others have observed differences in diameter *and* length of playing arm ulnae of tennis players compared to the contralateral arms [140]. The second metacarpals of playing hands were also wider and longer than those of the contralateral hands, whereas no differences were observed between limbs of controls. The latter somewhat isolated observations suggest that exercise may potentiate long bone growth in length, a curious and largely unrecognized finding with implications for overall height. That side dominance is not evident in athletes who load both limbs equally in the course of their training (rowers and triathletes) [141] attests to the principle of site specificity. The effects of a functionally side-dominant sport can also be masked by the addition of high-intensity bilateral cross training [142].

Although controlled trials suggest that not all types of exercise training (e.g., swimming, cycling) are effective in the prevention of age-related bone loss [143], there is clear and consistent evidence that active people who have exercised for many years generally have higher bone mass than less active people [77, 111, 118, 144–148]. While an early cohort study suggested that there was no relationship between osteoporotic fracture and exercise history in older men (in spite of a linear trend between lifetime and current exercise and hip bone mass) [147], there is growing evidence from more recent large, longitudinal cohort studies in the United States and Europe that physical activity is indeed associated with a lower risk of hip and other fractures [149–152]. Furthermore, two large meta-analyses of prospective cohort studies have shown a clear association between moderate to vigorous physical activity and a reduction in hip fracture [153, 154].

Exercise Interventions in Young and Older Premenopausal Women

Meta-analyses of randomized, controlled trials suggest that exercise training programs enhance the bone mass of premenopausal women in a site-specific manner [155, 156]. Both resistance and weight-bearing endurance exercise programs have been reported to increase spine, hip, and calcaneal bone mass of young adult women [65, 69, 155, 157–159]. However, in contrast to the developing skeleton, the principle of reversibility applies, that is, osteogenic loading must be sustained to maintain bone gains. For example,

increases in trochanteric and femoral neck BMD observed after 12 months of resistance plus jump exercise declined to baseline values after only 6 months of detraining in premenopausal women [36] (Fig. 11.1). Two-year observations of college gymnasts indicate that bone at the hip, spine, and whole body consistently increased over the training seasons and decreased in the off-season [160] (Fig. 11.2). By contrast, the relatively lower magnitude loading associated with field hockey playing was not sufficient to stimulate seasonal changes in a similar aged cohort [142].

Recognizing the importance of load magnitude and loading rate for bone stimulation, experimental training protocols have frequently employed impact loading (jumping) as an exercise mode. While load magnitude is similar for jogging and jumping (two to five times body weight [BW]), the loading *rate* for jogging is roughly 75 BW/second while jumping is approximately 300 BW/second. Unsurprisingly, jumping has consistently been shown to increase femoral and sometimes lumbar spine bone mass in premenopausal women [55, 69–71, 161].

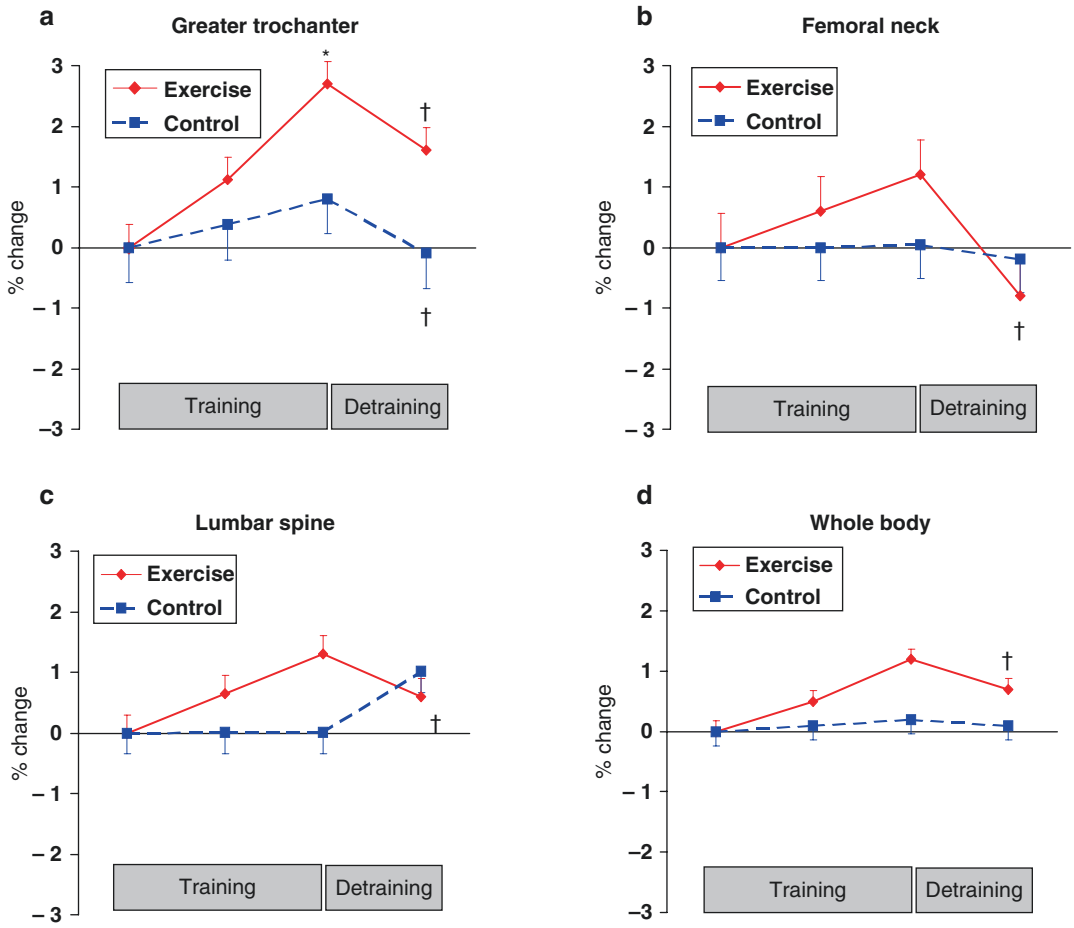

Fig. 11.1 Percent changes in BMD across training and detraining periods (mean ± SEM) at the (**a**) greater trochanter, (**b**) femoral neck, (**c**) lumbar spine, and (**d**) whole body. BMD, bone mineral density; SEM, standard error of the mean. ∗ = exercise group significantly different from controls, $p < 0.05$, † = change over detraining period significantly different from change over training period, within group, $p < 0.05$. (Reprinted from Winters and Snow [36]. With permission from John Wiley & Sons, Inc.)

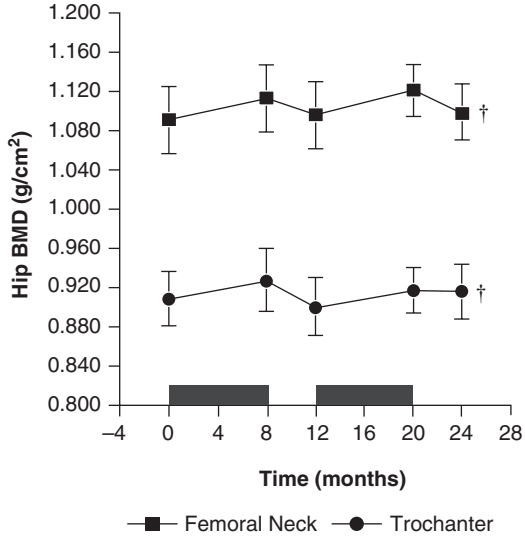

Fig. 11.2 Changes in hip BMD over 24 months in intercollegiate gymnasts ($n = 8$). The dagger represents significant quartic seasonal trends for repeated increases and decreases in hip BMD at the femoral neck and trochanter ($p = 0.03$). Black bars indicate the timing of the competitive training seasons. Data are expressed as mean ± SEM. BMD bone mineral density, SEM standard error of the mean. (Reprinted from Snow et al. [160]. With permission from Springer Nature)

The most recent meta-analyses involving premenopausal women concluded that impact-only exercise protocols are preferentially effective at the femoral neck [155], while resistance training protocols are most effective at the spine [162]. The number of impacts performed per session in the studies describing a bone effect ranges from 10 [70] to 100 [71], reinforcing observations from animal studies that magnitude rather than the number is the key loading characteristic. A 6-month unilateral impact study design in premenopausal women [73] revealed a subtle dose response at the femoral neck BMD of 50 hops per session that became significant at 7 days compared to 0 or 2 days, suggesting that increasing exposure to short bouts of impact loading will enhance the bone response.

Few studies have addressed the skeletal response to loading in the years just prior to menopause. The limited data suggest that perimenopausal women who exercise will maintain bone mass at loaded sites to a greater extent than women who remain inactive [163, 164].

Exercise Interventions in Postmenopausal Women

The reduction in circulating estrogen and associated acute and rapid bone loss that accompanies menopause represents a powerful confounding factor for the study of exercise effects in postmenopausal women. Furthermore, combining both early and late postmenopausal women in the target sample of exercise trials makes separating the competing effects of exercise and estrogen withdrawal difficult. In spite of this, encouraging findings have been reported to suggest that exercise of sufficient intensity can slow or stop the rapid bone loss that occurs in the years immediately after the onset of menopause. One study demonstrated that high-intensity resistance training was as effective as hormone therapy in preventing bone loss at the spine in early postmenopausal women [165]. A 4-year progressive strength training program found exercise frequency to be significantly positively associated with changes in bone mass at the hip and spine in women an average of 6 years postmenopause, regardless of hormone therapy status [166].

When exercise is applied five or more years postmenopause, the effects of estrogen withdrawal pose less of a confound because the skeleton has adapted to a lower level of circulating estrogen. Resistance training programs for a duration of 9–24 months in estrogen-depleted postmenopausal women have consistently been associated with a maintenance or small increase in bone mass compared to losses in controls at the whole body [46], lumbar spine [46, 48, 167–170], proximal femur [31, 48, 168, 169], calcaneus [169], and radius [31], although some exceptions exist [171–173]. Overall, meta-analyses of moderate-intensity resistance training interventions for bone have concluded that there is a positive but mild effect of exercise on BMD postmenopause [50, 174, 175]. A meta-analysis of high-intensity resistance training found a significant positive effect on spine bone mass of postmenopausal women, but inconsistent

effects at the femoral neck [176]. Significant changes at the hip were observed only in trials that excluded women on hormone therapy, and in these trials exercise effects were more pronounced with calcium supplementation and in women with low initial values. Weight-bearing aerobic or impact exercise interventions of 7–30 months' duration are also generally associated with increases or maintenance of bone mass compared to losses in controls at the whole body [46, 177], lumbar spine [34, 47, 177–179], proximal femur [46, 177, 180], radius [179], and calcaneus [181, 182].

As observed in other age groups, lower intensity activities typically do not promote bone gain or prevent loss in postmenopausal women. A 12-month, 5 day/week, 45-minute moderate-intensity aerobic exercise intervention did not provide sufficient overload to improve bone mass of obese postmenopausal women [183]. Similarly, 12 months of unloaded exercise in waist-deep water did not prevent spine bone loss or improve femoral bone mass in osteoporotic women, despite changes in other functional fitness parameters [184]. There is general agreement that walking alone is not an effective strategy for osteoporosis prevention in postmenopausal women [185], although walking remains the most popular recommended form of exercise in adults and is still mistakenly referred to as a form of weight-bearing exercise that prevents osteoporosis. One exception comes from a study that found that 7 months of walking 3 day/week at walking speeds equivalent to those reached in race walking (>4.5 mph) increased lumbar spine bone mass in postmenopausal women [47], which far exceeds typical walking speeds. The increased muscular forces associated with arm movements required for walking at high speeds combined with lower initial bone mass values might explain this isolated positive finding.

There has been an understandable reluctance from investigators to test the effect of high load magnitudes in patients with osteopenia or osteoporosis, despite their known osteogenic potential from animal studies, for fear of causing fracture. A recent novel study that combined supervised high-intensity resistance and impact training (HiRIT) in an 8-month, twice-weekly, 30-minute intervention for postmenopausal women with low to very low bone mass reported notable improvement at the spine and a maintenance effect at the femoral neck, with no injuries or vertebral deformities [30]. Notable improvements in functional performance outcomes related to risk of falls and fracture were also observed, suggesting that this relatively low-duration but high-intensity exercise program might be a potent exercise intervention for older women at risk of osteoporotic fracture (Fig. 11.3). Those findings suggest that previous recommendations for postmenopausal osteoporosis exercise prescription have been unnecessarily conservative.

As the skeletal sites most vulnerable to osteoporotic fracture are primarily composed of trabecular bone, cortical bone is often ignored in research trials. As long bone fractures do occur at cortical sites in osteoporotic individuals, an observation that both resistance and agility training increased cortical bone density in elderly osteopenic women is of clinical relevance [186]. Others have reported maintenance of tibial shaft bone strength index in the absence of substantial changes in femoral neck bone mass to a greater extent in elderly women performing 1 year of resistance and balance-jumping training than in controls [187].

Unfortunately, high-magnitude loading is not appropriate for all individuals who may have contraindications or physical limitations that preclude resistance, impact, or high-intensity weight-bearing aerobic training. Lower magnitude loading may be osteogenic if applied at high enough rate and/or frequency (roughly 30 Hz) and presents an alternative to higher intensity exercise. While the active sustained application of loads at frequencies higher than 2–3 Hz is not physically possible for most, whole-body vibration (WBV) devices have been developed, which can apply passive, low-magnitude loads at osteogenic frequencies. Preliminary findings of the effectiveness of WBV to enhance bone strength are mixed and warrant continued exploration [188–194]; however, as WBV is primarily a passive rather than active stimulus, it cannot strictly

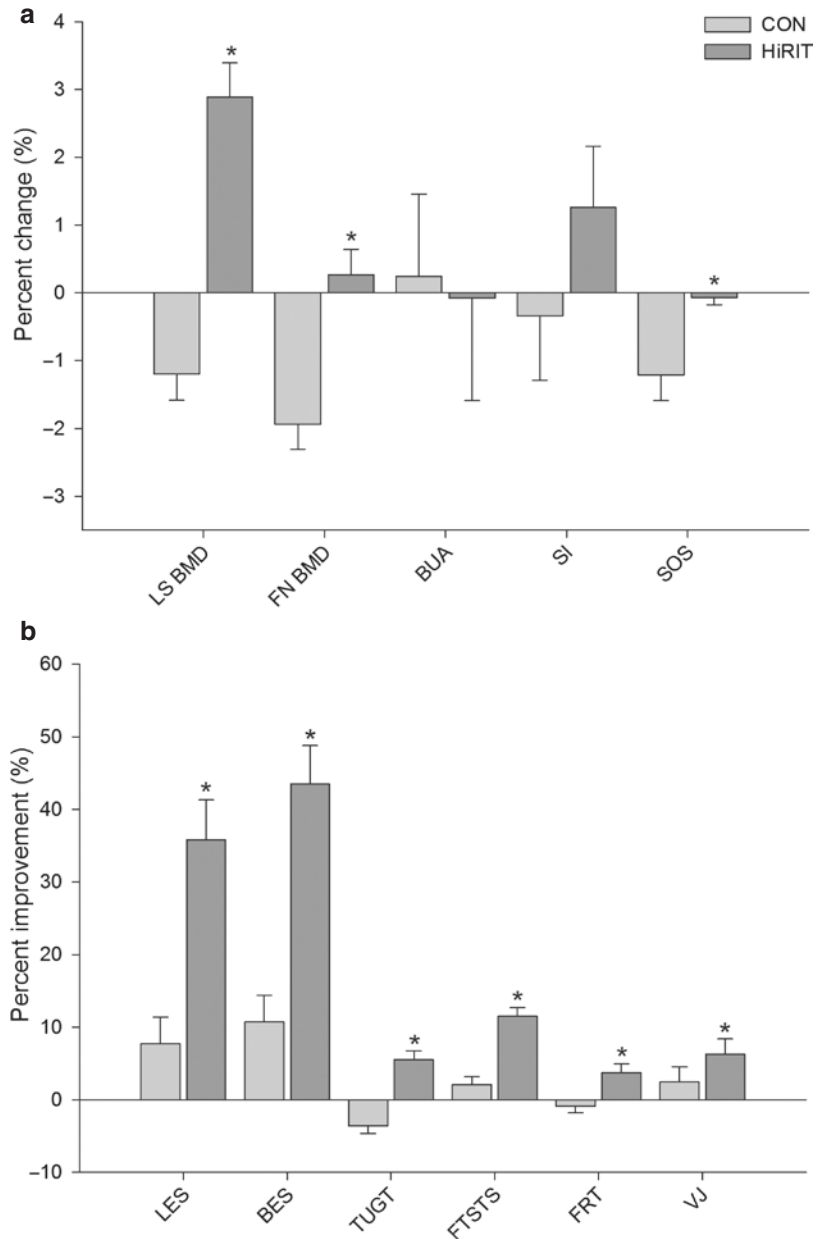

Fig. 11.3 Eight-month change (±SE) in (**a**) bone and (**b**) physical performance for HiRIT and CON (control) following an 8-month exercise intervention in postmenopausal women with low bone mass (n = 101). BES back extensor strength, BMD bone mineral density, BUA broadband ultrasound attenuation, FN femoral neck, FRT functional reach test, FTSTS five times sit-to-stand, LES leg extensor strength, LS lumbar spine, SI stiffness index, SOS speed of sound, TUGT Timed Up-and-Go Test, VJ vertical jump. Asterisk (∗) indicates between-group difference ($p < 0.05$). (Reprinted from Watson et al. [30]. With permission from John Wiley & Sons, Inc.)

be defined as a mode of exercise and will not be discussed further.

Frequency of exercise bouts per week may influence the bone response. A retrospective analysis of a 12-year study of exercise for bone concluded that at least two sessions per week were required to stimulate positive changes in spine and hip BMD in postmenopausal women with osteopenia [195]. The length of participation in weight-bearing exercise may be an important consideration for exercise programming in older adults. For example, although no change in femoral neck bone mass was observed in postmenopausal women following 9 months of jumping plus resistance exercise wearing weighted vests [196], 5 years of participation in the program prevented bone loss of more than 4% at the hip [35] (Fig. 11.4).

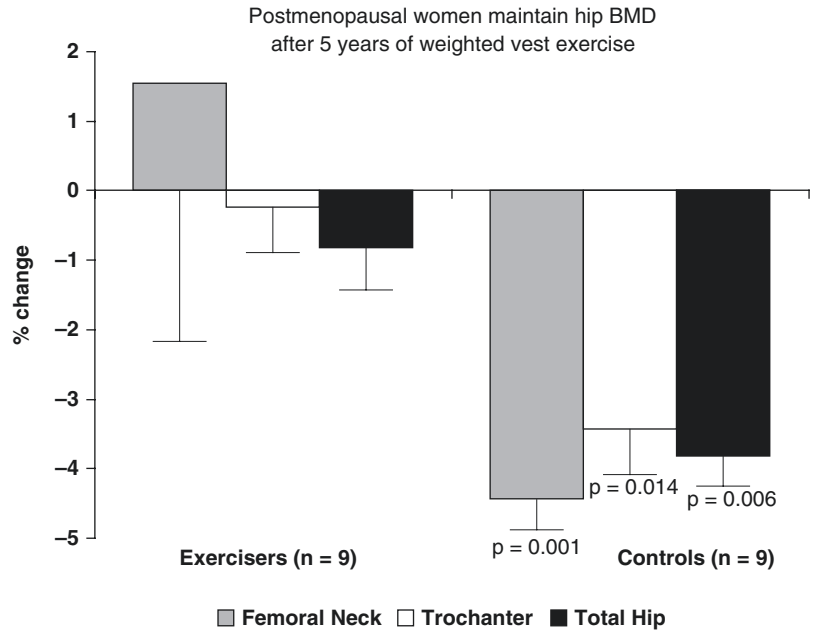

Fig. 11.4 Percent changes in BMD at the femoral neck, trochanter, and total hip in exercisers and controls after 5 years. Changes for exercisers were 1.54% + 2.37% (CI = −3.9% to 7.0%) at the femoral neck, −0.24% + 1.02% (CI = −2.6% to 2.1%) at the trochanter, and − 0.82% + 1.04% (CI = −3.2% to 1.6%) at the total hip, whereas controls decreased 4.43% + 0.93% (CI = −6.6% to −2.3%) at the femoral neck, 3.43% + 1.09% (CI = −5.9% to −0.92%) at the trochanter, and 3.80 + 1.03 (CI = −6.2% to −1.4%) at the total hip. Decreases in controls are significantly different from zero (unpaired *t* tests). Data are presented as means + SEM. BMD bone mineral density, CI confidence interval, SEM standard error of the mean. (Reprinted from Snow et al. [35]. With permission from Oxford University Press)

Given the importance of site specificity, it is not surprising that weight-bearing exercise does not increase forearm bone mass in postmenopausal women [46, 71]. In fact, some have suggested that upper body bone mass may suffer at the expense of lower body bone mass in female runners [197]. For those at risk of Colles' (distal forearm) fractures, however, it is encouraging to observe that upper extremity loading of high rate and magnitude stimulated higher forearm bone density in osteoporotic, postmenopausal women after only 5 months [198, 199].

Exercise Interventions in Young Adult Men

Although there have been few longitudinal studies involving young men, the response of the male skeleton to exercise appears to be similar to that of same-aged women.

Basic military training has served as an opportune model to observe the effect of brief, high-intensity, physical training interventions. After 14 weeks of basic training, male army recruits have been observed to improve calcaneal strength [200] and increase leg bone mass by around 12%, with those having the lowest initial bone mass gaining the greatest amount from training [127, 201]. Recruits who temporarily stopped training due to stress fracture also gained bone mass, but to a lesser degree (5%), suggesting that most recruits adapted to the high-intensity training stimulus to some extent; however, that adaptation was insufficient in some individuals to overcome the weakening effect of load-related bone tissue damage in the initial stages of training. Curiously, 10% of recruits lost bone mass. The latter effect not only may be related to measurement error but also may be a function of incomplete adaptive

remodeling owing to the short observation period (bone resorption not yet matched by formation).

The influence of training intensity on bone response becomes evident when findings from army trials are compared with those of recreational athletes. In contrast to recruits, men aged 25–52 years failed to gain bone at the spine, humerus, femur, calcaneus, or forearm following 3 months of either walking (3 km, 5 days a week) or running (5 km, 3 days a week) [135]. The disparity of findings likely reflects the novelty of loading and higher load magnitudes experienced during basic training, and the youth of the army recruits. The only other young male exercise intervention to have been reported involved 9 months of marathon training. The investigators observed significantly higher calcaneal bone mass in the runners than nonrunners with a positive association between average distance run and percent change in bone mass [182].

A 7.8-year longitudinal study of young Caucasian men (mean age = 17 years) measured change in bone mass over time according to the change in level of activity [182, 202]. The investigators reported those men who stayed active gained bone mass, while those who ceased activity lost bone mass at the hip but remained significantly higher than inactive controls at final follow-up. The study is an illustration of the ability of exercise to potentiate male peak bone mass even in the very final stages of skeletal growth.

Exercise Interventions in Older Adult Men

There are few reports of exercise interventions with older men, although more have been published in recent years and others are underway [203]. A 2013 systematic review identified eight relevant trials, of which five scored less than 50% on the Delphi quality rating scale. The authors concluded that methodological heterogeneity limited the strength of conclusions from their review but that, in general, six interventions produced positive effects and two had no effect on BMD [204].

The effects of 6 months of either a high-intensity, standing, free-weight program or a moderate-intensity, seated, resistance training program on bone mass have been examined in older men and women [29]. High-intensity training increased lumbar spine BMD by 2% in the men (mean age = 54.6 years), whereas moderate-intensity training induced no change. Relative to a control period, increased bone mass was observed at the greater trochanter regardless of training intensity. More recently, a 12-month, within-subject unilateral high impact exercise intervention (hopping) was utilized to control the confounding effect of lifestyle variables on bone in men aged 69.9 ± 4.0 years [205]. Femoral neck BMD and cross-sectional area increased in the exercise leg to a small degree (0.7% and 1.2%, respectively) compared to losses in the control leg (−0.9% and − 1.2%), but between limb differences were not significant. Section modulus, however, increased significantly in the exercise leg, suggesting a clinically meaningful effect of the impact activity on femoral neck strength [205]. Additional computed tomographic (CT) analysis of bone changes confirmed that the femoral neck underwent positive geometric adaptations to the hopping intervention [206]. A 9-month study of men aged 50–74 years found minimal effect of four sessions/week upper body resistance exercise and impact-loading on BMD, although a tendency for greater efficacy was observed in men completing 80 jumps per session versus 40 [207]. An RCT of physically active osteopenic men (44 ± 2 years) found that 6 months of resistance or jump training increased WB and LS BMD but that only resistance training increased total hip BMD [208].

Summary of Exercise Effects Across the Life Span

Evidence from exercise interventions longer than 6 months suggests that activities of high magnitude and rate of loading improve bone mass and geometry in children and adults of both sexes. While gains may be maintained if achieved during the growing years, exercise-induced bone gain in adulthood will likely be lost if exercise is discontinued. Effective activities include jumping and high-intensity weight training, with resistance training being more effective at the spine

and impact loading more beneficial for the hip. Although walking and other low-intensity exercises are unlikely to be substantially effective as an intervention strategy, a lifetime of walking may reduce the risk of fractures in later life [152].

Calcium and Exercise

The permissive action of calcium in enhancing the effect of exercise on bone mass is somewhat controversial. In a review of 17 trials, Specker [209] concluded that an intake of 1000 mg/day of calcium is necessary in order to observe a skeletal response to exercise. Specifically, the evidence suggests that the combination of calcium supplementation and exercise is more effective for a bone response in children [93, 210, 211], adolescents [212], and postmenopausal women than calcium supplementation alone [34, 45, 213]. Nevertheless, a recent cross-sectional study of 422 women found that even though high levels of physical activity and calcium intake were associated with a higher total body bone mass than low activity levels and low calcium intake, there was no significant interaction between exercise and calcium [115]. Furthermore, 2 years of combined aerobics and weight training increased bone mass in young women, but calcium supplementation neither enhanced the exercise benefit nor improved bone mass in the absence of exercise [157].

Although exercise likely provides a greater stimulus to bone than calcium, at least in older children and adults, adequate calcium intake is recommended, particularly in children, to avoid the negative impact of calcium insufficiency on bone health and to provide the building blocks for exercise-induced gains in bone mass.

Hormone Response to Intense Exercise

Women

Exercise-associated amenorrhea occurs in some premenopausal women who train at high exercise intensities. While low body fat was once thought to precipitate exercise-associated amenorrhea, it is now thought that reduced energy availability disrupts the hypothalamic–pituitary–thyroid axis [214] and ultimately circulating reproductive hormones. The effect of reduced energy availability on bone resorption and formation markers is well documented [215]. The reduction in estrogen provides the link between exercise-associated amenorrhea and bone loss [134, 216, 217]. The Female Athlete Triad describes the combined conditions of excessive dietary restraint, reproductive hormone disturbance, and bone loss in female athletes, that is more prevalent among athletes who perceive that their performance benefits from having a low body weight.

The question of whether the Triad should be considered a pathological or even psychopathalogical condition has recently become contentious [218–220]. What is generally accepted is that in all but cases of extreme (high magnitude) loading, the positive effect of exercise on bone rarely offsets the negative effects of inadequate energy availability during high-intensity, high-volume exercise training. To illustrate, gymnasts load their skeletons at very high magnitudes and rates, and thus, despite a high prevalence of menstrual disturbance, have bone mass well above normal [221]. Long distance runners, on the other hand, who load their skeletons at much lower magnitudes and rates are not protected from estrogen-related bone loss. Although there are individual differences, the loss of bone mass in amenorrheic distance runners increases their risk of stress fracture and premature osteoporosis compared with their eumenorrheic running counterparts [222]. Loucks and Heath suggest that exercise-associated amenorrhea may be prevented or reversed by increasing energy consumption, without alterations in training [223].

There is some suggestion that oral contraceptives (OCs) may offset bone loss in athletes with menstrual dysfunction, but there are insufficient data to fully corroborate the effect [224]. Keen and Drinkwater [225] reported that initiating OC use approximately 8 years after the onset of athletic oligo- or amenorrhea did not improve bone mass, concluding that intervention should begin at the onset of dysfunction in order to prevent

significant loss but there is no evidence to directly support that supposition. The effect of OCs, alone or in combination with exercise, on bone strength indices remains poorly understood. In fact, for women aged 18–31 years, exercise alone and OCs alone depressed normal age-related increases in femoral neck mass and size, although the combination of exercise and OCs was slightly less detrimental [226]. It is likely that a complex interaction of factors yet to be identified will account for these puzzling findings.

Men

Intense training is not associated with commensurately severe alterations in reproductive hormones in men. Male athletes exercising at a range of intensities have serum concentrations of testosterone that lie within the normal range [128, 130, 141, 227, 228], including adolescents [229]. In some athletes, however, a degree of subtle hormonal perturbation can occur. Smith and Rutherford [141] reported that, although in the normal range, serum total testosterone was significantly lower in triathletes than in controls, but not rowers. Furthermore, total serum testosterone, nonsex hormone-binding globulin (SHBG)-bound testosterone, and free testosterone concentrations in men running more than 64 km/week averaged 83%, 69.5%, and 68.1% that of controls, respectively [230]. Others have similarly observed that resting and free testosterone concentrations of trained athletes are 68.8% and 72.6% that of controls [231]. Age may influence the effect as elderly endurance athletes have significantly greater levels of SHBG than controls whereas younger athletes demonstrate no differences compared with controls [130, 232].

Whether hormones potentiate the effect of exercise on bone in men is relatively unexamined. Suominen and Rahkila [130] reported a negative correlation between bone mass and SHBG in older endurance athletes but no relationship of bone mass with testosterone. Furthermore, the addition of self-administered anabolic steroids (testosterone: 193.75 ± 147.82 mg/week) to high-intensity body-building training does not stimulate greater osteoblastic activity or bone formation than exercise alone [233]. Four months of progressive resistance exercise training 4 day/week, with or without growth hormone supplementation, did not significantly increase whole body, spine, or proximal femur bone mass in elderly men (mean age = 67 years) with normal bone mass [234]. Similarly, the addition of recombinant human growth hormone to 6 months of resistance exercise training induced no change in bone mass of older men [235, 236].

Osteoporotic Fracture and Falls

Fracture and Exercise

Fractures are a relatively uncommon event, to the extent that the sample size needed to adequately power a trial with fractures as a primary endpoint (almost 15,000 for a two-arm exercise trial of European women) has been suggested to far exceed the available funding and resources to support such an effort [153]. Those prohibitive figures notwithstanding, one 30-month randomized controlled trial of high-impact exercise in 160 elderly women with low bone mass reported a lower incidence of fall-related fractures among exercisers (6) compared to controls (16), despite minimal effects on hip bone mass [27]. While numbers were low, significance was reached in the between-group comparison. Very long-term follow-up of controlled trials is an alternative approach for tracking fracture incidence, but since many men and women at risk for fracture are prescribed antiresorptive therapy, even those types of investigations are problematic. For this reason, the definitive exercise and fracture trial are unlikely to ever be conducted. A number of surrogate analyses have been reported, however, and will be discussed.

In general, the literature supports a protective effect of physical activity on the risk of fracture, especially at the hip [237–240]. Two studies that tracked fractures over a prolonged period of exercise [241] or over a follow-up period after completion of an exercise intervention [242] suggest a

protective effect of exercise against fracture. The incidence of vertebral fractures was lower (1.6%) 8 years after a 2-year back extension exercise program compared to controls (4.3%) [242]. Original exercisers had better back extension strength at follow-up and a 2.7 lower relative risk of vertebral compression fracture than controls [243]. A 5-year follow-up of a 1-year exercise RCT of resistance and/or balance and jumping training reported 51% fewer injurious falls and 74% fewer fractures among persons assigned to the combined training group [244]. A 16-year follow-up of the Erlangen Fitness and Osteoporosis Prevention study in 105 early postmenopausal women reported an overall reduction in relative risk of low trauma fractures of 49% [245]. The results of a meta-analysis of 13 controlled exercise trials with fracture endpoints indicate a 51% reduction in overall fracture risk and a non-significant 44% reduction in vertebral fracture risk [243]. Therefore, despite something of a vacuum of direct RCT evidence for the ability of exercise to prevent osteoporotic fracture, there is an increasingly smoking gun.

Falls and Exercise

Falls are the cause of almost 90% of hip fractures [246–248]. As previously indicated, exercise can affect both the numerator and denominator of the factor of risk. Discussion thus far has focused on exercise as a means of altering the denominator of the factor of risk, that is, on increasing fracture load by improving parameters of bone strength. However, exercise can also reduce the numerator by preventing falls entirely.

Risk factors for falls are numerous, and some can be modified by exercise. Lateral instability, muscle weakness of the lower extremities, and poor gait have been found to independently predict hip fracture and falls [249–252]. Impaired balance is similarly related to incidence of vertebral fracture [253]. In the Study of Osteoporotic Fractures in Men, men in the upper quartile of leg power and grip strength had an 18–24% lower risk of falls compared with men in the lowest quartile [254]. Since exercise promotes and maintains muscle strength, balance, and mobility, it is an intuitive strategy for reducing osteoporosis-related fractures [255, 256]. The general findings and principles of fall prevention exercise will be summarized, but this broad topic is otherwise beyond the scope of this chapter.

Improvements in neuromuscular function resulting from low-intensity exercise, including water-based exercise [187, 257, 258], while not osteogenic, may likewise be efficacious for fall and fracture prevention but minimal direct evidence is available. Data from the FICSIT (Frailty and Injuries: Cooperative Studies of Intervention Techniques) trials indicate that activities that are most beneficial for reducing incidence of falls include those that result in muscle strength gains and dynamic balance improvements [259]. In fact, muscle strengthening and balance training have been demonstrated to reduce extra-skeletal risk factors for hip fracture in elderly men and women [260, 261] and overall risk of falling by as much as 75% [261, 262]. On the other hand, improvements in strength and balance have been reported in elderly women in the absence of change in incidence of falls after 12 months of exercise that included resistance [263]. A similar null effect on falls was observed after an individualized prevention program that included exercise [264]. Community-based trials report a reduction in falls among the elderly who participated in group exercise in both community-dwelling [265] and retirement home [266] settings. A multifactorial exercise intervention involving muscle building plus walking reduced injurious and noninjurious falls by 40% in elderly women [267]. The study required home visits by physical therapists and it was not clear which component of the program, muscle building, walking, or the two combined, was most potent for reducing falls, but this limitation may be irrelevant in practice.

A 2011 meta-analysis of 54 studies reporting the effect of exercise on falls in older adults concluded high-dose (minimum of 2 hours per week) exercise programs that included moderate to high challenge balance training most effectively reduced falls and that high-risk individuals should not be prescribed walking [268]. A second, more

selective, meta-analysis in 2013, including 12 studies specifically measuring the effect of exercise on falls, similarly reported a protective effect that was strongest when different forms of exercise were combined for at least 1 month, two to three times per week, but that the effect did not translate to a reduction in fractures [269]. Based on the findings of those works, highly specific exercise recommendations for the prevention of falls through exercise are now available [270].

Sadly, the fall-reducing benefit of exercise may not extend to the very frail elderly [271–274], despite improvements in fall risk factors and physical function [271, 273]. Trends toward lower falls among exercisers, however, were apparent in studies of longer duration [273, 274], suggesting that a longer period of adaptation might be required to detect protective effects in this population. In practice, other fall prevention approaches (e.g., environmental modification, polypharmacy, visual health) should be implemented when training starts.

Recommendations: Exercise Prescription

Position Statements—Recommendations Based on Human Data

Based on the best evidence to date, recently Exercise and Sports Science Australia (ESSA) published a Position Statement on exercise for osteoporosis [275] with the recommendations tailored to low-, moderate-, and high-risk individuals in terms of risk for osteoporotic fracture. All groups are recommended to engage in a variety of weight-bearing impact, progressive resistance, and balance training with the degree of intensity and supervision appropriate to capacity. Prolonged immobilization and bed rest should be avoided at all costs, given the very negative effect of unloading on bone mass and the limited ability to fully regain losses with remobilization.

High-magnitude (impact) activities, recommended for increasing bone mass of the young and/or uncompromised adult skeleton, may require modification for people who are frail and/or have comorbid conditions such as osteoarthritis. In the frail elderly population, particular care must be taken to maintain a balance between safety and efficacy, since an exercise intervention itself presents not only the potential for skeletal and neuromuscular benefit, but also an increased risk of fracture by virtue of increased opportunities for falling because of moving more. Osteoporotic individuals, with or without a history of vertebral compression fractures, should not engage in deep forward trunk flexion exercises such as rowing, toe touching, and full sit-ups. Before initiating a program of high intensity, elderly individuals should consider a bone density evaluation. Any individual undertaking a new program should begin slowly with careful attention to exercise form and appropriate progressions. Exercises that produce severe joint pain or muscle soreness of more than 3 days should be discontinued until exercise of lower intensity can be tolerated.

Future Research

The large body of research data notwithstanding, in fact it is not yet possible to unreservedly claim that exercise will prevent osteoporotic fracture. As Karlsson has stated, much of the research has been "hypothesis generating" rather than "hypothesis testing" [276]—a consequence of the confounding challenges associated with exercise RCTs and the protracted nature of follow-up required to compare real fracture rates between exercise and control groups. Indeed, while much has been achieved in our understanding of the use of exercise for the prevention of age-related fractures, many questions and challenges remain. For instance, we know little about the relative importance of endocrine and genetic factors and how they may moderate the bone response to exercise across the life span. It is likely that the complex interplay of genetics, nutrition, hormone status, and even a degree of central control [277] accounts for much that remains unexplained about the bone response to exercise. That mechanical loading cannot entirely prevent spi-

nal cord injury-related bone loss [4] is testament to the presence of influences yet to be explained.

Thus, while the power law model incorporating load magnitude and the number of repetitions of Whalen and colleagues [278], the Osteogenic Index of Turner and Robling [61], and the recent highly successful LIFTMOR (Lifting Intervention For Training Muscle and Osteoporosis Rehabilitation) trial [30] have moved us forward in our ability to test and/or form conclusions from exercise regimes for bone health, an optimal and precise exercise prescription for all remains elusive. The challenge remains to identify a means by which optimal overload can be determined in order to safely stimulate a positive bone response. The complex interplay of dose (load magnitude and rate), cycle number, and duration must be elucidated in human models. Only then can we customize exercise prescription for bone with confidence.

Finally, it is important to consider the issue of compliance. The commitment to regular exercise of any kind, much less the highly specific form required to effect change in bone, is challenging for most individuals. Compliance, even with study protocols when volunteers often have access to state-of-the-art facilities and personnel to encourage and support their efforts, is routinely disappointing. For example, compliance of a mere 17.8% was reported for an 18-month, home-based, exercise program for the prevention of postmenopausal osteoporosis, primarily due to lack of motivation [279]. A highly targeted multimodal exercise intervention in the community produced very modest gains in BMD, potentially a reflection of relatively poor compliance and a high rate of injuries [280]. Few maintain a lifelong exercise routine, and those who do are unlikely to vary their regime the extent required to stimulate ongoing bone adaptation. In reality, the greatest challenge for bone physiologists may not be the identification of the optimal exercise program, but the engagement of the community to adopt effective programs. The ultimate question then remains whether or not an efficacious intervention that cannot be widely implemented is the best "recommended" exercise prescription or if recommendations should be tailored to the audience. For example, exercise professionals who may train clients with goals to avoid osteoporosis need evidence-based programs and are more likely to successfully implement an effective program with a single client or small group. On the other hand, the general public may benefit and respond more to recommendations to avoid inactivity and engage in weight-bearing exercise to limit bone loss related to prolonged unloading from sitting and minimal effective loading from non-weight-bearing activity.

Conclusions

Regular physical activity has the potential to reduce the risk of osteoporosis and fragility fractures by (1) optimizing peak bone mass, (2) enhancing, slowing, or preventing loss in adult bone, and (3) reducing falls. As bone responds to the same stimuli throughout life, exercise prescription for the prevention of osteoporosis-related fractures is likely to differ across the life span only in terms of delivery. That is, as individuals get older, modest introductory loading with conservative progressions and increasing supervision are likely to lead to better outcomes while ensuring safety.

Exercise will only affect bones that are loaded during the activity. Bone requires substantial overload for prolonged durations for positive adaptations to be observed. With the possible exception of the pediatric population, bone gains will likely be lost if a stimulating exercise is discontinued. Individuals with the weakest bones can expect the greatest improvements from initiating exercise. Exercise is most efficacious when accompanied by adequate nutrition, particularly calcium.

Exercises that are most or least likely to substantially alter bone mass and prevent falls can be identified with relative certainty. Development of individualized and population-specific exercise prescription across the life span is more challenging. Issues such as determining actual bone strain exposure during activity, optimal dose response, safety, and the interaction of exercise with pharmacology remain opportunities for future

research. It will be important to determine the degree to which exercise-invoked improvements in bone strength and falls prevention will translate to a reduction in incidence of fracture.

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12 Effects of Estrogens and SERMs on Bone Metabolism: Clinical Aspects

Bart L. Clarke

Key Points

- The pathophysiology of early postmenopausal bone loss is largely caused by estrogen deficiency.
- Hormone or estrogen therapy prevents bone loss and reduces fracture risk, and gives other benefits, with associated adverse events dependent on age and other risk factors.
- Selective estrogen receptor modulators prevent bone loss and reduce fracture risk, while minimizing some of the risks of hormone or estrogen therapy, and giving other benefits.

Introduction

This chapter will focus on clinical aspects of estrogens and selective estrogen receptor modulators (SERMs) on bone metabolism. Estrogens and SERMs exert their actions on the skeleton by binding to estrogen receptors and causing downstream signaling activity, with variable tissue selectivity. Estrogens and SERMs, which interact with both ERα and ERβ in a tissue-specific manner to produce diverse outcomes in multiple tissues, continue to generate significant interest for clinical application in osteoporosis and other disorders.

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Estrogens

Estrogens were initially prescribed to prevent bone loss and treat osteoporosis based on observational trials of estrogen effects on the skeleton in healthy women. Many years of investigation led to an improved understanding of the normal female menstrual cycle and skeletal changes that occur after menopause with estrogen deficiency.

Normal Menstrual Cycle

The normal menstrual cycle occurs due to complex interplay between tissues in the multiorgan female reproductive system involving the hypothalamus, pituitary gland, ovaries, uterus, including the endometrium and cervix, and vagina. These various organs undergo a series of cyclic and closely regulated events once a month in healthy nonpregnant females starting at menarche and ending with menopause. Menarche usually begins at age 11–13 years with the onset of new circadian (24 hour) and ultradian (60–90 minute) pituitary secretion of gonadotropins,

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leading eventually to the maturation of a positive estrogen feedback loop that controls the monthly rhythm. Sleep-related increases in gonadotropins and gonadal steroids begin during puberty, persist during adult life, and then gradually decline and eventually stop over several years during menopause. Menopause may occur normally as early as 40 years, but typically occurs at an average age of 51–52 years in the United States.

The menstrual cycle is controlled by a tightly regulated sequence of hormonal events that occurs every 28–32 days. Normal menstrual cycles are driven by cyclic secretion of gonadotropin-releasing hormone (GnRH) by the hypothalamus. This leads to cyclic secretion of luteinizing hormone (LH) and follicle stimulating hormone (FSH) by gonadotropes in the anterior pituitary gland. Regular cyclic secretion of LH and FSH normally results in maturation of one ovarian follicle to a fully mature ovum each month, ovulation, and migration of the ovum to the uterine endometrium via the Fallopian tubes. Ovarian secretion of sex steroid hormones causes changes in the uterine endometrial lining that support implantation of the fertilized egg. If the ovum is not fertilized, ovarian secretion of estrogen and progesterone decreases over several days, and the endometrial lining breaks down, leading to the onset of menstruation.

The first day of vaginal bleeding is counted as the first day of the menstrual cycle, and the last day is counted as the day before the next menstrual cycle starts. The duration of the median menstrual cycle is 28 days, but normal cycles may vary from 21 to 40 days. Menstrual cycle duration varies fairly widely in the first several years after menarche and in the several years before menopause [1]. Menstrual blood flow typically lasts 5 ± 2 days, with typical blood loss with each cycle ranging from 30 to 80 mL [2].

The normal menstrual cycle is categorized based on ovarian function into the earlier follicular, or proliferative, phase, and the later luteal, or secretory, phase. The follicular phase is more variable in duration, whereas the luteal phase consistently lasts about 14 days in most healthy women. Mature ovarian follicles are ovulated at the end of the follicular phase, during the transition to the luteal phase, with the ovulatory phase beginning one day prior to the LH surge and continuing until ovulation, which typically occurs 16–32 hours after the surge in LH.

Serum FSH increases late in the luteal phase of each menstrual cycle, and remains increased into the early follicular phase of the next cycle, thereby stimulating the growth and development of several ovarian follicles with each cycle [3]. One of these follicles develops into the dominant follicle, but the regulatory processes guiding this are not well understood. Circulating FSH levels then gradually decrease and, and except for a brief small further surge during ovulation, continue to diminish throughout the remainder of the cycle until the late luteal phase.

Serum LH begins to increase in the late follicular phase of the menstrual cycle, but in distinction to FSH, it continues to slowly increase throughout the follicular phase until it surges for 1–3 days in the middle of the cycle preceding ovulation [3]. LH then gradually decreases to its lowest levels in the late luteal phase.

Both LH and FSH are released in a pulsatile fashion by gonadotropin-secreting cells in the anterior pituitary gland, timed to the pulsatile secretion of hypothalamic GnRH [4]. LH and FSH pulses usually occur over 1–4 hours, depending on the phase of the menstrual cycle [5]. LH is secreted least during the luteal phase, which is attributed to the direct feedback of progesterone produced by the corpus luteum of the ovary on the hypothalamus and pituitary gland [6].

Serum estradiol (E2), estrone (E1), and progesterone are secreted by the ovaries, along with other gonadal sex steroids and nonsteroidal hormones. Circulating E2 levels are lowest during the early follicular phase and begin to increase about 7–8 days before the LH surge. Peak serum E2 levels of 250–350 pg/mL occur on the day before, or on the day of, the LH surge [7]. Serum E2 falls quickly as serum LH peaks, but it increases again about 6–8 days after the LH surge. Serum estrone (E1) levels parallel E2 levels, but at lower levels. About 95% of circulating E2 is produced by the dominant ovarian follicle and corpus luteum, whereas serum E1 is produced by conversion from E2 and from periph-

eral conversion of androstenedione produced by the adrenal glands.

Circulating estrogen levels produced during each menstrual cycle stimulate the skeleton by effects mediated by estrogen receptors on bone cells. Estrogen suppresses bone resorption by osteoclasts and increases bone formation by osteoblasts in women between puberty and menopause, leading to an increase in bone mineral density (BMD) and bone strength. Estrogen stimulates a marked increase in BMD over several years during and after menarche, and BMD generally peaks at different skeletal sites ranging in women from 25 to 35 years. The physiological effects of other hormones produced by the female reproductive system on the skeleton are not as well-defined as for estrogen.

Effects of Estrogen on the Skeleton

Onset of estrogen and other sex steroid hormone secretion during menarche at age 11–13 years stimulates rapid skeletal mineral acquisition, as well as further longitudinal and radial skeletal growth for the next 10 years or so [8]. Women gain about one-third of their peak BMD within the 4 years around the onset of menarche [9]. The early pubertal rapid increase in BMD is followed by further slower increases in BMD and consolidation of skeletal mineral content during the late second and early third decades, until peak BMD is achieved at around age 25–30 years [10–12].

Estrogen plays a major role in regulating the acquisition and loss of bone by the skeleton from menarche through senescence [13]. Onset of estrogen secretion, among other gonadal sex steroids, during puberty is the major factor responsible for skeletal longitudinal and radial growth, as well as significant gain in BMD, until peak BMD is achieved in the third decade, as well as fusing the epiphyses in the late teenage years, leading to cessation of longitudinal growth [14]. Estrogen then helps maintain peak bone density at this peak level until menopause, including during the transient changes in skeletal mineral content associated with pregnancy and lactation [15]. At menopause, decreased estrogen and other gonadal sex steroid production normally leads to relatively rapid bone loss in most women [16]. The most rapid bone loss associated with decreased estrogen levels occurs in the first 8–10 years after menopause, with slower age-related bone loss occurring throughout remaining years of life [17]. Age-related bone loss in women after the early menopausal phase of bone loss is caused by ongoing estrogen and other gonadal sex steroid deficiency, vitamin D deficiency, and secondary hyperparathyroidism [18, 19]. Other factors also contribute to age-related bone loss and osteoporosis, including intrinsic defects in osteoblast function [20], impairment of the growth hormone (GH)/insulin-like growth factor (IGF) axis, age-associated sarcopenia [21], changes associated with senescence including telomere shortening [22–24], and a host of other secondary causes. Further understanding of the relative contributions of estrogen and each of the other factors to development and maintenance of the female skeleton, bone loss, and fracture risk will lead to improved hormonal and other approaches for prevention and treatment of osteoporosis.

Clinical Trials of Hormone Therapy for Osteoporosis

Women's Health Initiative Estrogen Plus Progestin Trial

The Women's Health Initiative (WHI) was an NIH-funded long-term study that evaluated multiple factors governing health in women with aging. One component of this study was a randomized controlled clinical trial that tried to determine the balance of risks and benefits of hormone use in healthy postmenopausal women in the United States [25]. Decades of observational studies had suggested skeletal benefit from hormone therapy, but lingering doubts persisted that hormone therapy might be harmful to at least some postmenopausal women. The study was designed to assess the major health benefits and risks of the most commonly used combined hormone preparation in the United States. at that time. The estrogen plus progestin component of

the WHI was a randomized controlled primary prevention trial of planned duration of 8.5 years in which 16,608 postmenopausal women aged 50–79 years with an intact uterus at baseline were recruited by 40 US clinical centers from 1993 to 1998. Participants received conjugated equine estrogens 0.625 mg and medroxyprogesterone acetate 2.5 mg in the same tablet (n = 8506) or placebo (n = 8102) each day. The primary outcome was coronary heart disease (CHD), including nonfatal myocardial infarction and CHD death, with invasive breast cancer the primary adverse outcome. A global index summarizing the balance of risks and benefits included the two primary outcomes plus stroke, pulmonary embolism (PE), endometrial cancer, colorectal cancer, hip fracture, and death due to other causes.

After a mean follow-up of 5.2 years, the data and safety monitoring board recommended stopping the WHI trial of estrogen plus progestin versus placebo because the test statistic for invasive breast cancer exceeded the stopping boundary for this adverse effect, and the global index statistic supported risks exceeding benefits. The estimated hazard ratios (HRs) (nominal 95% confidence intervals [CIs]) showed increased risk of CHD, 1.29 (1.02–1.63) with 286 cases; breast cancer, 1.26 (1.00–1.59) with 290 cases; stroke, 1.41 (1.07–1.85) with 212 cases; PE, 2.13 (1.39–3.25) with 101 cases; colorectal cancer, 0.63 (0.43–0.92) with 112 cases; endometrial cancer, 0.83 (0.47–1.47) with 47 cases; hip fracture, 0.66 (0.45–0.98) with 106 cases; and death due to other causes, 0.92 (0.74–1.14) with 331 cases (Fig. 12.1). Corresponding HRs (nominal 95% CIs) for the composite outcomes were 1.22 (1.09–1.36) for total cardiovascular disease (arterial and venous disease), 1.03 (0.90–1.17) for total cancer, 0.76 (0.69–0.85) for combined fractures, 0.98 (0.82–1.18) for total mortality, and 1.15 (1.03–1.28) for the global index. Absolute excess risks per 10,000 person years attributable to estrogen plus progestin were seven more CHD events, eight more strokes, eight more PEs, and eight more invasive breast cancers, while absolute risk reductions per 10,000 person years were six fewer colorectal cancers and five fewer hip fractures. The absolute excess risk of events included in the global index was 19/10,000 person years. Even though this study convincingly showed fracture reduction, the weight of evidence suggested that harm from hormone therapy outweighed benefit.

The Women's Health Initiative Estrogen Plus Progestin Trial was further assessed to determine whether the relative risk reduction of estrogen plus progestin on fractures differed according to risk factors for fracture [26]. The main outcome measures were all confirmed osteoporotic fracture events that occurred from enrollment until discontinuation of the trial on July 7, 2002; BMD, measured in a subset of women (n = 1024) at baseline and years 1 and 3; and a global index, developed to summarize the balance of risks and benefits to test whether the risk-benefit profile differed across tertiles of fracture risk. A total of 733 women (8.6%) in the estrogen plus progestin group and 896 women (11.1%) in the placebo group experienced a fracture (hazard ratio [HR], 0.76; 95% confidence interval [CI], 0.69–0.83). The protective effect did not differ in women stratified by age, body mass index, smoking status, history of falls, personal and family history of fracture, total calcium intake, past use of hormone therapy, BMD, or summary fracture risk score. Total hip BMD increased 3.7% after 3 years of treatment with estrogen plus progestin compared with 0.14% in the placebo group ($P < 0.001$). The HR for the global index was similar across tertiles of the fracture risk scale (lowest fracture risk tertile, HR, 1.20; 95% CI, 0.93–1.58; middle tertile, HR, 1.23; 95% CI, 1.04–1.46; highest tertile, HR, 1.03; 95% CI, 0.88–1.24) (P for interaction = 0.54). This study concluded that estrogen plus progestin increased BMD and reduced fracture risk in healthy postmenopausal women. The decreased risk of fracture attributed to estrogen plus progestin appeared to be present in all subgroups of women examined. When considering the effects of hormone therapy on other important disease outcomes in a global model, there was no net benefit, even in women considered to be at high risk of fracture.

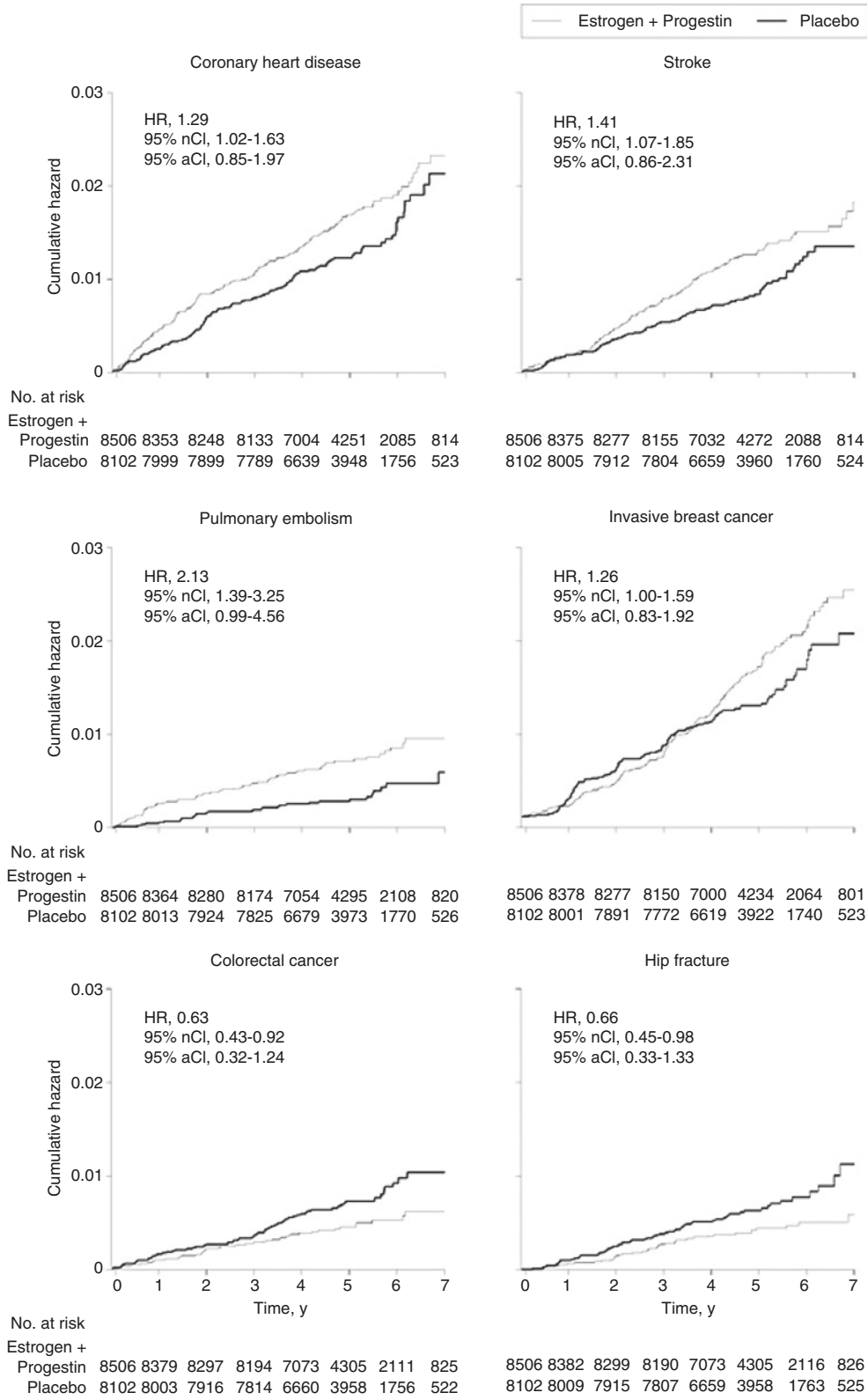


Fig. 12.1 Kaplan–Meier estimates of cumulative hazards for selected clinical outcomes. (Reprinted from Rossouw et al. [25]. With permission from American Medical Association)

Women's Health Initiative Estrogen-Alone Trial

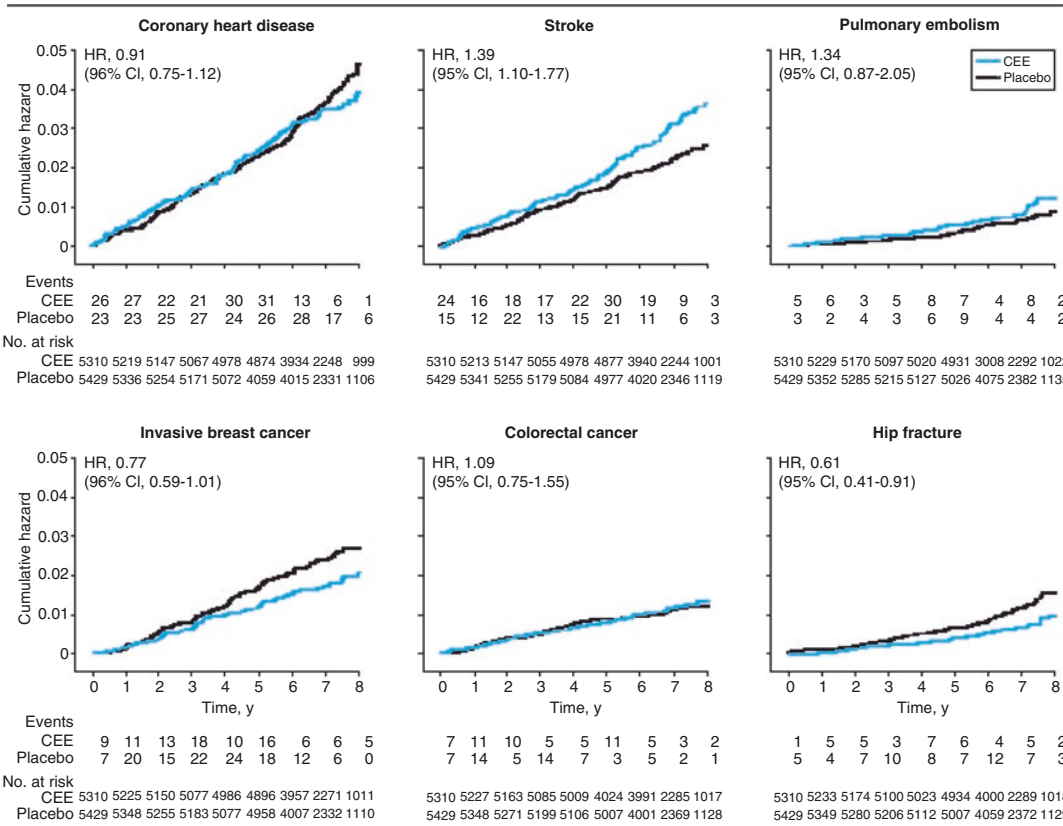
Another component of the WHI was to assess the effects of estrogen therapy alone on major disease incidence rates. A randomized, double-blind, placebo-controlled disease prevention trial with estrogen alone versus placebo was conducted in 40 US clinical centers beginning in 1993 [27]. A total of 10,739 postmenopausal women aged 50–79 years with prior hysterectomy, including 23% of minority race/ethnicity, were enrolled. Women were randomly assigned to receive either 0.625 mg of conjugated equine estrogen (CEE) or placebo each day. The primary outcome was coronary heart disease (CHD) incidence, including nonfatal myocardial infarction or CHD death. Invasive breast cancer incidence was the primary safety outcome. A global index of risks and benefits, including these primary outcomes plus stroke, pulmonary embolism (PE), colorectal cancer, hip fracture, and deaths from other causes, was used to summarize overall effects.

This WHI trial was also stopped early in February 2004. Estimated hazard ratios (HRs) (95% confidence intervals [CIs]) for CEE versus placebo for the major clinical outcomes (average follow-up 6.8 years), were CHD, 0.91 (0.75–1.12) with 376 cases; breast cancer, 0.77 (0.59–1.01) with 218 cases; stroke, 1.39 (1.10–1.77) with 276 cases; PE, 1.34 (0.87–2.06) with 85 cases; colorectal cancer, 1.08 (0.75–1.55) with 119 cases; and hip fracture, 0.61 (0.41–0.91) with 102 cases (Fig. 12.2). Corresponding results for composite outcomes were total cardiovascular disease, 1.12 (1.01–1.24); total cancer, 0.93 (0.81–1.07); total fractures, 0.70 (0.63–0.79); total mortality, 1.04 (0.88–1.22), and the global index, 1.01 (0.91–1.12). For the outcomes significantly affected by CEE, there was an absolute excess risk of 12 additional strokes per 10,000 person-years and an absolute risk reduction of 6 fewer hip fractures per 10,000 person-years. The estimated excess risk for all monitored events in the global index was a nonsignificant 2 events per 10,000 person-years. The study concluded that use of CEE increased the risk of stroke, decreased the risk of hip fracture, and did not affect CHD incidence in postmenopausal women with prior hysterectomy over an average of 6.8 years.

Current Status of Postmenopausal Hormone Therapy

After the release of the findings of the WHI postmenopausal hormone and estrogen-alone clinical trials in 2002 and 2004, many postmenopausal women stopped taking hormone or estrogen-alone therapy because of their perceived increased risk. Follow-up analysis of the WHI trial cohorts in 2013 demonstrated that overall mortality increased beginning only after age 60 years [28]. As a consequence, low-dose and transdermal estrogen became more commonly used to treat vasomotor and genitourinary symptoms in postmenopausal women in their sixth decade. Systemic estrogens currently used include oral medications, transdermal patches, sprays, or gels, and vaginal rings. FDA-approved indications for hormone or estrogen therapy include vasomotor symptoms, prevention of bone loss, premature hypoestrogenism, and genitourinary symptoms. The FDA currently advises use of hormone or estrogen therapy mainly for women in their sixth decade who are unable to tolerate significant hot flashes or other symptoms, and that hormone therapy should be used at the lowest dose possible for as short a time as possible.

Standard-dose hormone and estrogen therapy prevent bone loss in postmenopausal women by decreasing bone resorption and reducing bone remodeling [29–32]. Multiple randomized, controlled trials and observational studies have shown that standard-dose hormone therapy prevents postmenopausal osteoporotic fractures, including hip, vertebral, and nonvertebral fractures [26, 27, 33–36]. Low-dose formulations, including conjugated estrogens at 0.3 mg each day, oral 17β-estradiol less than or equal to 0.5 mg each day, or estradiol patch of 0.025 mg, and ultralow dose estradiol patch 0.014 mg, have not been shown to reduce fracture risk, although no studies have been adequately powered to show this. Discontinuation of treatment with hormone or estrogen leads to a rapid loss of benefit, but



CEE indicates conjugated equine estrogen; HR, hazard ratio; CI, confidence interval. Events shown are occurring during 1-year intervals through year 8 and beyond year 8.

Fig. 12.2 Kaplan–Meier estimates of cumulative hazards for selected clinical outcomes. (Reprinted from Anderson et al. [27]. With permissioin from American Medical Association)

there is not a rebound increase in vertebral or other fractures after discontinuation of treatment [26, 27, 37–41].

The North American Menopause Society (NAMS) 2017 Hormone Therapy Position Statement updated previous position statements, and identified future research needs [42]. An Advisory Panel of clinicians and researchers expert in the field of women's health and menopause reviewed the 2012 Position Statement, evaluated new literature, assessed the evidence, and reached consensus on recommendations, using the level of evidence to identify the strength of recommendations and the quality of the evidence. Hormone therapy (HT) was felt to remain the most effective treatment for vasomotor symptoms (VMS) and the genitourinary syndrome of menopause (GSM) and shown to prevent bone loss and fracture. The risks of HT differed depending on type, dose, duration of use, route of administration, timing of initiation, and whether a progestogen is used. Treatment should be individualized to identify the most appropriate HT type, dose, formulation, route of administration, and duration of use, using the best available evidence to maximize benefits and minimize risks, with periodic reevaluation of the benefits and risks of continuing or discontinuing HT.

For women aged younger than 60 years or who are within 10 years of menopause onset and have no contraindications, the benefit–risk ratio is most favorable for treatment of bothersome VMS and for those at elevated risk for bone loss or fracture. For women who initiate HT more

than 10 or 20 years from menopause onset or are aged 60 years or older, the benefit–risk ratio appears less favorable because of the greater absolute risks of coronary heart disease, stroke, venous thromboembolism, and dementia. Longer durations of therapy should be for documented indications such as persistent VMS or bone loss, with shared decision making and periodic reevaluation. For bothersome GSM symptoms not relieved with over-the-counter therapies and without indications for use of systemic HT, low-dose vaginal estrogen therapy or other therapies were recommended. Multiple other national and international professional organizations and societies, including the US Endocrine Society [43], American Association of Clinical Endocrinologists [44], American College of Physicians [45], and American College of Obstetrics and Gynecology [46] have given their own recommendations regarding HT.

Selective Estrogen Receptor Modulators (SERMs)

Selective estrogen receptor modulators (SERMs) interact with the estrogen receptor to improve bone density and reduce fractures. Actions of SERMs are similar to estrogen in some tissues, but different in other tissues. Part of the variable effects of SERMs compared to estrogen is due to the fact that there are two forms of the estrogen receptor (ER), ERα and ERβ. Estradiol remains the major endogenous ligand for this receptor, but 27-hydroxycholesterol has been identified as another endogenous ligand [47].

ERα and ERβ have different structures, ligand affinities, tissue distributions, transcriptional properties, and biological roles. The presence of two ERs provides greater flexibility for regulation of estrogen and SERM actions in different tissues, including the skeleton.

SERMs directly bind to ERα and/or ERβ in target cells and exert estrogen- or antiestrogen-like actions in affected tissues. These agents exert estrogenic benefits in certain tissues, and minimize estrogenic risks in other tissues. Binding of an SERM to ERα and/or ERβ in the cytoplasm causes a conformational change in the ER, which results in dissociation of associated heat shock chaperone proteins and release of the monomeric receptor from the apo-ER complex. The conformational change results in altered interactions with complexed coactivator or corepressor proteins [48], with subsequent monomeric ER translocation from the cytoplasm to the nucleus, followed by dimerization with a second monomeric ER before binding to specific DNA sequences in the regulatory promoter regions of target genes. Homodimeric binding of the ER to these promoter regions causes initiation or suppression of gene transcription [49].

McDonnell et al. [50] and others showed that a series of SERM ligands formed distinct ER-bound complexes, with each ligand causing slightly different conformational changes. X-ray crystallography quantitatively assessed the conformational changes induced by agonist or antagonist binding to the ER ligand binding domain [51]. Initial structural evidence for the antagonist-bound ER conformation was obtained for the SERM tamoxifen [52], showing that tamoxifen blocked ER binding to nuclear receptor cofactor proteins [51]. Subsequent investigation showed that ER binding by many different SERMs caused development of the classical antagonist-bound ER conformation [51]. SERMs may also produce cell modulation through non-ER pathways, such as through androgen or progesterone receptors, when combined with SERM metabolites that have non-ER binding activities [53].

Each ER ligand has SERM activity intrinsic to the ligand. Tissue-specific actions of SERMS are thought to be due to unique ER conformational changes caused by SERM ligand binding in different tissues, resulting in a variety of specific interactions with other proteins within the cell. However, conformational change alone may not explain all the actions of SERMs on target cells. Work in mice with targeted deletion of the ERα aminoterminal A/B domain suggested that stimulation of ERα by SERMs resulted in minimal activation of the aminoterminal activation domain AF-1 to preserve beneficial vascular effects, but minimize effects on sexual tissues [54].

Human ERα and ERβ greatly differ in their target genes, transcriptional potency, and cofactor-binding capacity, and are differentially expressed in various tissues. In classical estrogen response element (ERE)-mediated transactivation, ERβ has a markedly reduced activation potential compared with ERα, but the mechanism underlying this difference was not initially obvious. Zwart et al. [55] showed that the binding of steroid receptor coactivator-1 (SRC-1) to the AF-1 domain of ERα is essential, but not sufficient, to facilitate synergy between the AF-1 and AF-2 domains, which is required for full agonistic response to 17β-estradiol. Complete synergy is achieved through the distinct hinge domain of ERα, which enables combined action of the AF-1 and AF-2 domains. The AF-1 domain of ERβ lacks the capacity to interact with SRC-1, which prevents hinge-mediated synergy between AF-1 and AF-2, thereby explaining the reduced 17β-estradiol-mediated transactivation of ERβ. Transactivation of ERβ by 17-estradiol requires only the AF-2 domain. A weak agonistic response to tamoxifen occurs for ERα, but not for ERβ, and depends on AF-1 and the hinge-region domain of ERα.

Functions of ERα and ERβ have been investigated in bone, breast, uterine and genitourinary tissues, brain, and other tissues. Because of the widely variable tissue effects of SERM ligands in different tissues, it is difficult to reach conclusions about the complete clinical activity of SERMs without conducting the appropriate clinical trials and monitoring adverse events in different tissues.

Multiple SERMs have been developed for different purposes in the United States, with raloxifene approved for prevention and treatment of postmenopausal osteoporosis and prevention of high risk ER-positive breast cancer in postmenopausal women. Bazedoxifene monotherapy is approved for treatment of postmenopausal osteoporosis in Europe, and bazedoxifene in combination with conjugated estrogens for treatment of menopausal flushes and for prevention of postmenopausal osteoporosis in the United States. Tamoxifen is approved for adjuvant and neoadjuvant treatment of postmenopausal ER-positive breast cancer, prevention of high-risk breast cancer, breast ductal carcinoma in situ to prevent invasive disease, metastatic breast cancer, gynecomastia, and treatment of malignant neoplasms of the endometrium of corpus lutei. Ospemifene is approved for treatment of moderate to severe dyspareunia due to vulvar and vaginal atrophy associated with menopause in the United States. Clomiphene is approved for treatment of female infertility due to ovulatory disorder. Other SERMs remain under development.

The initial SERMs were used as antiestrogens beginning almost 60 years ago [56], with the concept of selective estrogen receptor modulation introduced about 25 years ago [57]. A variety of SERMs with special tissue selectivity have been under clinical investigation for prevention and treatment of a variety of diseases [58]. SERMs may increase the risk of postmenopausal hot flashes, night sweats, leg cramps, deep venous thrombosis, or bone pain in some patients, particularly during the first few months of drug exposure.

Because currently available SERMs do not fully treat symptoms of the menopause, research continues to identify the optimal SERM for postmenopausal women, which would improve hot flashes, reduce vaginal atrophy, and prevent bone loss and fractures, while protecting the uterus, mammary gland, and cardiovascular system. If an ideal SERM cannot be found, as appears increasingly likely, SERMs may be used in postmenopausal women in tissue selective estrogen complexes, in which an SERM is combined with estrogen, in order to obtain the beneficial effects of each component, with improved overall tolerability [59]. SERMs may potentially be used in men to treat osteoporosis, syndromes associated with secondary hypogonadism, or possibly prostate cancer, but none are currently approved.

A number of SERMs have been clinically investigated since clomiphene, the first drug in this class, was introduced many years ago. Many of these have had their clinical investigation discontinued due to various adverse effects or lack of efficacy compared to available SERMs. Published clinical trials over the last decade have focused mostly on raloxifene, bazedoxifene,

bazedoxifene in combination with conjugated estrogens, lasofoxifene, arzoxifene, tamoxifen, and ospemifene.

Raloxifene

Raloxifene is a polyhydroxylated nonsteroidal benzothiophene compound with a benzothiophene core with high affinity for both ERα and ERβ [60], which was originally investigated for breast cancer prevention in the early 1980s. It acts as a partial estrogen agonist in bone, thereby preventing vertebral fractures and loss of bone mineral density when given at the approved oral dose of 60 mg each day [61, 62].

The effect of raloxifene on BMD, serum lipid concentrations, and endometrial thickness was studied in 601 postmenopausal women [61]. Participants were randomly assigned to receive 30, 60, or 150 mg of raloxifene or placebo each day for 24 months. The women receiving each of the three doses of raloxifene had significant increases from baseline values in BMD of the lumbar spine, hip, and total body, whereas those receiving placebo had decreases in BMD. At 24 months, the mean (± SE) difference in the change in BMD between the women receiving 60 mg of raloxifene each day and those receiving placebo was 2.4 ± 0.4% for the lumbar spine, 2.4 ± 0.4% for the total hip, and 2.0 ± 0.4% for the total body ($P < 0.001$ for all comparisons). Serum concentrations of total cholesterol and low-density lipoprotein cholesterol decreased in all the raloxifene groups, whereas serum concentrations of high-density lipoprotein cholesterol and triglycerides did not change. Endometrial thickness was similar in the raloxifene and placebo groups at all times during the study. The proportion of women receiving raloxifene who reported hot flashes or vaginal bleeding was not different from that of the women receiving placebo. The study concluded that daily therapy with raloxifene increases bone mineral density, lowers serum total and low-density lipoprotein cholesterol, and does not stimulate the endometrium.

The Multiple Outcomes of Raloxifene Evaluation (MORE) study was a multicenter, randomized, blinded, placebo-controlled trial randomizing 7705 women aged 31–80 years in 25 countries who had been postmenopausal for at least 2 years and met World Health Organization criteria for osteoporosis [62]. The study continued for up to 36 months for primary efficacy measurements and nonserious adverse events, and up to 40 months for serious adverse events. Participants were randomized to 60 mg or 120 mg each day of raloxifene or to placebo. All women received supplemental calcium and cholecalciferol. Incident vertebral fractures were determined radiographically at baseline and at scheduled 24- and 36-month visits. Nonvertebral fractures were ascertained by interview at 6-month-interim visits. Bone mineral density was determined annually by dual-energy X-ray absorptiometry (DXA). At 36 months, of the evaluable radiographs in 6828 women, 503 (7.4%) had at least one new vertebral fracture, including 10.1% of women receiving placebo, 6.6% of those receiving raloxifene 60 mg each day, and 5.4% of those receiving raloxifene 120 mg each day (Fig. 12.3). Risk of vertebral fracture was reduced in both study groups receiving raloxifene. For the 60 mg each day group: relative risk [RR], 0.7 (95% confidence interval [CI], 0.5–0.8). For the 120 mg each day group: RR, 0.5 (95% CI, 0.4–0.7). Frequency of vertebral fractures was reduced both in women who did and did not have prevalent fracture. Risk of nonvertebral fracture for raloxifene versus placebo did not differ significantly: RR, 0.9 (95% CI, 0.8–1.1 for both raloxifene groups combined). Compared with placebo, raloxifene increased BMD in the femoral neck by 2.1% (60 mg) and 2.4% (120 mg), and in the spine by 2.6% (60 mg) and 2.7% (120 mg) ($P < 0.001$ for all comparisons). Women receiving raloxifene had increased risk of venous thromboembolism versus placebo: RR, 3.1 (95% CI, 1.5–6.2). Raloxifene did not cause vaginal bleeding or breast pain and was associated with a lower incidence of breast cancer. The study concluded that in postmenopausal women with osteoporosis, raloxifene increased BMD in the spine and femoral neck, and reduced risk of vertebral fracture.

Raloxifene was shown to be more effective than tamoxifen, a related SERM, in reducing the risk of ER-positive breast cancers in high-risk

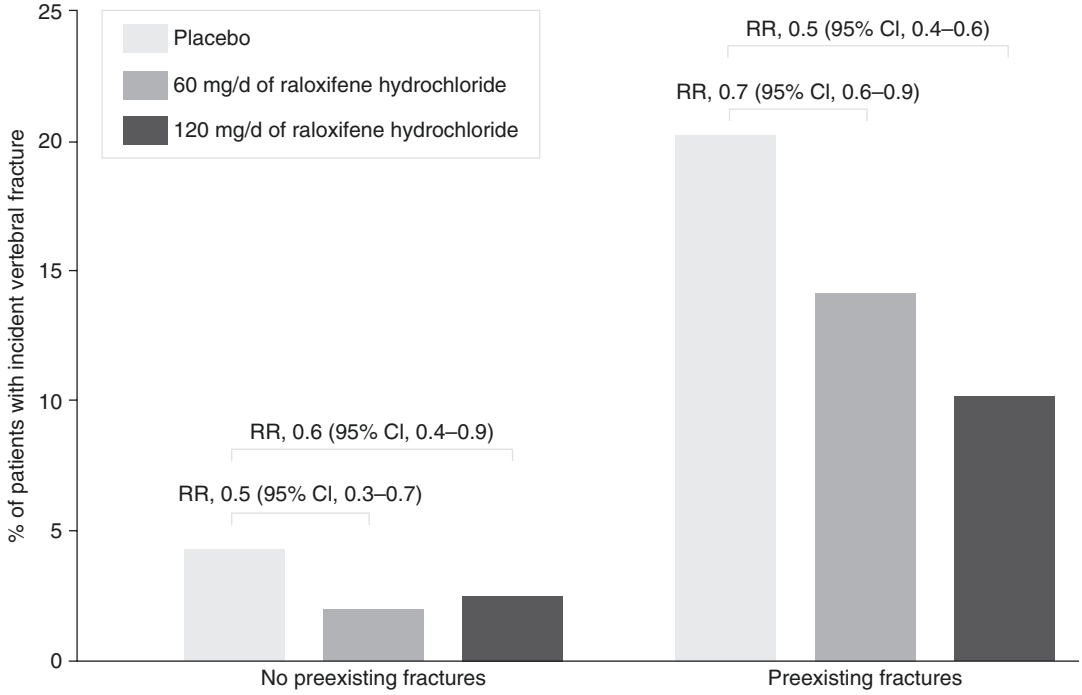


Fig. 12.3 Reduction in new vertebral fractures among 6828 women who completed the study. (Reprinted from Ettinger et al. [62]. With permission from American Medical Association)

postmenopausal women [63]. Neither drug reduced cardiovascular risk in this trial, however. The major clinical trials assessing cardiovascular risk reduction with raloxifene included the Raloxifene Use in the Heart (RUTH) study [64] and Study of Tamoxifen and Raloxifene (STAR) study [65]. The RUTH study evaluated the effects of raloxifene 60 mg each day versus placebo in 10,101 postmenopausal women of mean age 67.5 years with coronary heart disease or multiple coronary heart disease risk factors over a follow-up period of 5.6 years. This study showed that raloxifene reduced the risk of invasive breast cancer, but not noninvasive breast cancer. Raloxifene reduced the risk of clinical vertebral fractures, but not nonvertebral or hip fractures. Unfortunately, raloxifene did not reduce the primary endpoint risk of coronary events or stroke, but was associated with a statistically significant increased risk of stroke mortality and venous thromboembolism.

The STAR study evaluated the effects of raloxifene 60 mg each day versus tamoxifen 20 mg each day in 19,747 postmenopausal women of mean age 58.5 years with high risk of breast cancer over a follow-up period of 5 years [65]. The study showed that raloxifene and tamoxifen caused similar reductions in the risk of invasive breast cancer, with the tamoxifen group having a nonsignificant decrease in noninvasive breast cancer. Neither drug reduced the risk of noninvasive breast cancer in postmenopausal women.

Gushima et al. [66] showed that raloxifene caused translocation of ER-α into nucleoli in breast cancer cell lines, but not other cell types. Mutation analysis showed that helix 12 of ERα was essential for raloxifene-induced nucleolar translocation. This effect, which appeared to be specific to raloxifene, may explain at least part of raloxifene's ability to suppress growth of breast cells.

While raloxifene decreased the incidence of osteoporosis and invasive breast cancer, it also increases the risk of venous thromboembolism and fatal stroke in women with, or at high risk for, coronary heart disease. Grady et al. [67]

assessed treatment effects of raloxifene on overall and cause-specific mortality by performing a pooled analysis of mortality data from the large clinical trials of raloxifene (60 mg each day) versus placebo. This study analyzed data from the Multiple Outcomes of Raloxifene Evaluation/Continuing Outcomes Relevant to Evista studies, with 7705 postmenopausal osteoporotic women followed for 4 years, and a subset of 4011 participants followed for an additional 4 years, with 110 deaths during follow-up. The analysis also included the Raloxifene Use for the Heart trial, with 10,101 postmenopausal women with coronary disease or multiple risk factors for coronary disease followed for 5.6 years, with 1149 deaths during follow-up. Cox proportional hazards regression models compared mortality by treatment assignment in a pooled analysis of the trial data. All-cause mortality was 10% lower among women assigned to raloxifene 60 mg each day versus placebo (relative hazard 0.90; 95% CI, 0.80–1.00; $P = 0.05$). This lower overall mortality was primarily due to lower rates of noncardiovascular deaths, especially lower rates of noncardiovascular, noncancer deaths. The study did not identify mechanisms by which raloxifene reduced the risk of noncardiovascular deaths.

Raloxifene has been shown to affect body composition [68]. In a randomized, double-blind, placebo-controlled trial involving 198 healthy postmenopausal women aged 70 years or older, participants were randomly assigned to receive raloxifene 60 mg or placebo each day for 12 months. At 12 months, fat-free mass increased by a mean of 0.83 ± 2.4 kg in the raloxifene group versus 0.03 ± 1.5 kg in the placebo group ($P = 0.05$), and total body water increased by a mean of 0.6 ± 1.8 L in the raloxifene group versus a decrease of 0.06 ± 1.1 L in the placebo group ($P = 0.02$). Muscle strength and power were not significantly different with raloxifene treatment. The study concluded that raloxifene significantly increased fat-free mass and water content compared to placebo. Because fat-free mass positively correlates with bone mass, this effect of raloxifene might help improve BMD and reduce fractures.

Bazedoxifene

Bazedoxifene is an SERM approved in Europe for the treatment of postmenopausal osteoporosis, and in the United States for prevention and treatment of menopausal flushes and for prevention of postmenopausal osteoporosis. In a 2-year phase III study, bazedoxifene prevented bone loss, reduced bone turnover, and was well tolerated in early postmenopausal women with normal or low BMD [69].

The 3-year, randomized, double-blind, placebo- and active-controlled clinical trial [70] randomized healthy postmenopausal women with osteoporosis (55–85 years of age) to bazedoxifene 20 or 40 mg each day, raloxifene 60 mg each day, or placebo. The primary endpoint was incidence of new vertebral fractures after 36 months, with secondary endpoints including nonvertebral fractures, BMD, and bone turnover markers. Among 6847 subjects in the intent-to-treat population, the incidence of new vertebral fractures was significantly lower ($P < 0.05$) with bazedoxifene 20 mg (2.3%), bazedoxifene 40 mg (2.5%), and raloxifene 60 mg (2.3%), compared to placebo (4.1%), with relative risk reductions of 42%, 37%, and 42%, respectively (Fig. 12.4). The treatment effect was similar among subjects with or without prevalent vertebral fractures ($P = 0.89$ for treatment by baseline fracture status interaction). The incidence of nonvertebral fractures with bazedoxifene or raloxifene was not significantly different from placebo. In a post hoc analysis of a subgroup of women at higher fracture risk (femoral neck T score ≤ -3.0 and/or ≥ 1 moderate or severe vertebral fracture or multiple mild vertebral fractures; $n = 1772$), bazedoxifene 20 mg showed 50% and 44% reduction in nonvertebral fracture risk relative to placebo ($P = 0.02$) and raloxifene 60 mg ($P = 0.05$), respectively. Bazedoxifene significantly improved BMD and reduced bone marker levels ($P < 0.001$ vs. placebo). The incidence of vasodilatation, leg cramps, and venous thromboembolic events was higher with bazedoxifene and raloxifene compared to placebo. Christiansen et al. reported the 3-year safety data for this phase III trial with bazedoxifene separately [71]. In con-

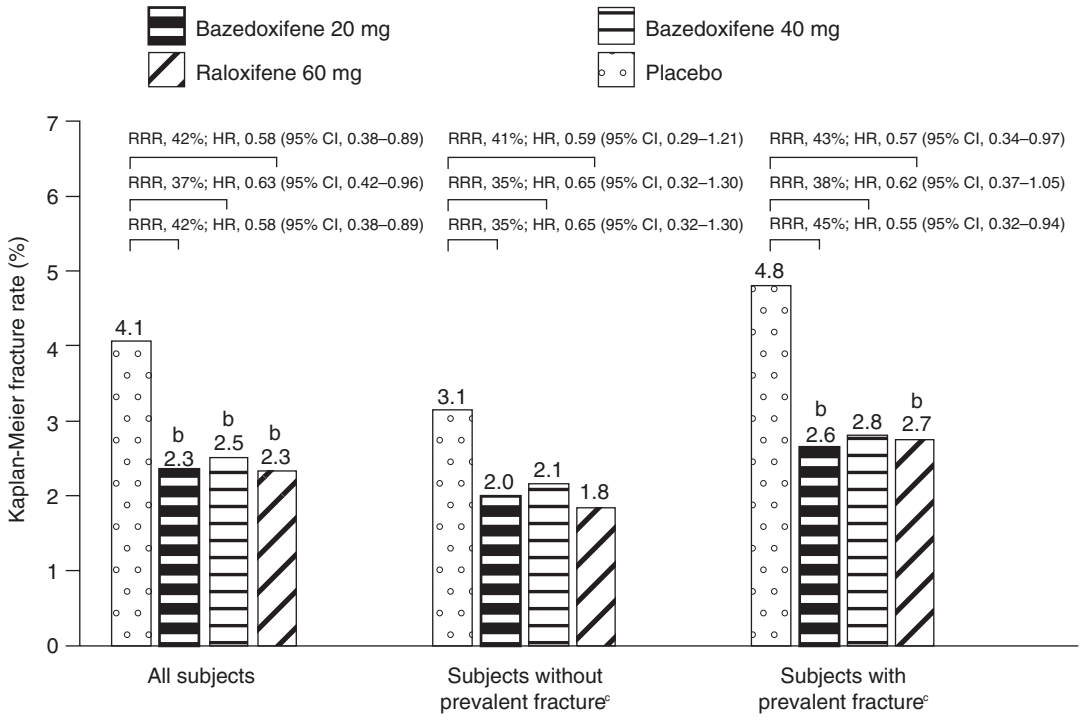


Fig. 12.4 Incidence of new vertebral fractures and corresponding fracture risk reduction by baseline prevalent fracture status. [a]RRR, relative risk reduction; HR, hazard ratio; CI, confidence interval. [a]Intent to treat population; $n = 6847$. [b]$p < 0.05$ versus placebo. [c]$p = 0.89$ for treatment by prevalent fracture status interaction. (Reprinted from Silverman et al. [70]. With permission from John Wiley & Sons, Inc.)

clusion, bazedoxifene significantly reduced the risk of new vertebral fracture in postmenopausal women with osteoporosis, and decreased the risk of nonvertebral fracture in subjects at higher fracture risk.

The 2-year extension of the 3-year study [72, 73], and subsequently the 4-year study extension [74], showed that bazedoxifene sustained efficacy in preventing new vertebral fractures in postmenopausal women with osteoporosis and in preventing nonvertebral fractures in higher-risk women over up to 7 years.

Conjugated Estrogens/Bazedoxifene

Conjugated estrogens/bazedoxifene is the first tissue selective estrogen complex therapy that reduces vasomotor symptoms and prevents postmenopausal bone loss without stimulating the breast and endometrium. Gallagher et al. [75] analyzed changes in BMD and bone markers using pooled data from two phase III trials using this agent. Selective Estrogens, Menopause, and Response to Therapy (SMART)-1 [76] and SMART-5 [77] were randomized, double-blind, placebo- and active-controlled studies conducted in postmenopausal nonhysterectomized women. BMD and turnover marker data were pooled for women given conjugated estrogens (0.45 or 0.625 mg) plus bazedoxifene 20 mg or placebo each day over 12 months. Sensitivity analyses were conducted using baseline Fracture Risk Assessment Tool score, age, years since menopause, body mass index, race, and geographic region. There were 1172 women, mean age 54.9 years, mean 6.21 years since menopause, mean lumbar spine, and total hip *T* scores −1.05 and − 0.58 included. Of these, 58.8% had a Fracture Risk Assessment Tool score less than 5%, indicating low fracture risk. At 12 months, adjusted differences (vs. placebo) in BMD change in the groups taking conjugated estrogens 0.45 or 0.625 mg plus bazedoxifene 20 mg each

day were 2.3% and 2.4% for lumbar spine, 1.4% and 1.5% for total hip, and 1.1% and 1.5% for femoral neck (all $P < 0.001$ vs. placebo). These increases were unrelated to baseline Fracture Risk Assessment Tool score, age, years since menopause, body mass index, or geographic region. Both doses reduced bone turnover markers ($P < 0.001$). The study concluded that conjugated estrogens/bazedoxifene significantly improved BMD and turnover in a large population of younger postmenopausal women at low fracture risk, and was a promising therapy for preventing postmenopausal bone loss.

Tamoxifen

Tamoxifen, a synthetic antiestrogen, increases disease-free and overall survival when used as adjuvant therapy for primary breast cancer. Love et al. [78] evaluated the effects of tamoxifen on BMD of the lumbar spine and radius and on biochemical measures of bone metabolism in 140 postmenopausal women with axillary-node-negative breast cancer, in a 2-year randomized, double-blind, placebo-controlled trial. In the women given tamoxifen, the mean BMD of the lumbar spine increased by 0.61% per year, whereas in those given placebo it decreased by 1.00% per year ($P < 0.001$) (Fig. 12.5). Radial BMD decreased to the same extent in both groups. In a subgroup randomly selected from each group, serum osteocalcin and alkaline phosphatase concentrations decreased significantly in women given tamoxifen ($P < 0.001$ for each variable), whereas serum parathyroid hormone and 1,25-dihydroxyvitamin D concentrations did not change significantly in either group. In postmenopausal women, treatment with tamoxifen is associated with preservation of the BMD of the lumbar spine. These effects continued to be preserved at 5 years of treatment [79]. Whether this favorable effect on BMD is accompanied by a decrease in the risk of fractures has not been determined.

If inherited variants in candidate genes involved in tamoxifen metabolism predict clinical outcomes of treatment of breast cancer with tamoxifen, then it is possible that genes involved in ER signaling or tamoxifen metabolism could also affect tamoxifen effects on bone. In a prospective multicenter clinical trial,

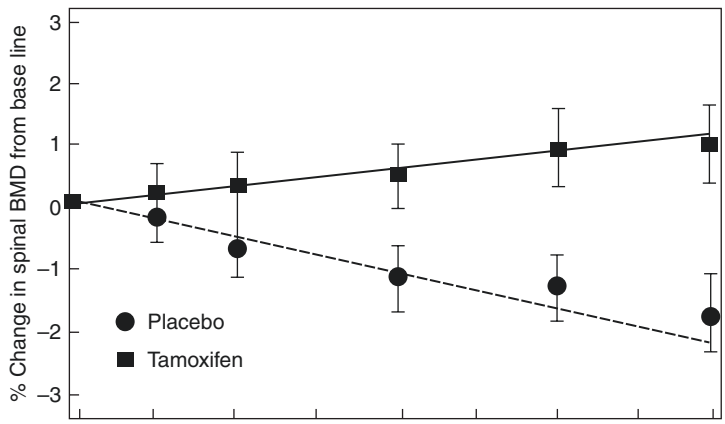


Fig. 12.5 Change in mean (± SE) lumbar-spine bone mineral density (BMD) in women with breast cancer given tamoxifen or placebo for two years. The solid and dashed lines represent the mean regression lines for the tamoxifen and placebo groups, respectively, as determined from the individual regression lines for each woman (only women with ≥3 data points were included in this analysis). (Reprinted from Love et al. [78]. Copyright © 1992 Massachusetts Medical Society. Reprinted with permission from Massachusetts Medical Society)

297 women starting tamoxifen therapy for the first time had their lumbar spine and total hip BMD values assessed by DXA at baseline and after 12 months of tamoxifen therapy [80]. Single-nucleotide polymorphisms (SNPs) in the genes for ERα, ERβ, and cytochrome P450 2D6 were tested for associations with menopausal status, previous chemotherapy, and mean percentage change in BMD over 12 months. The percentage increase in BMD was greater in postmenopausal women and in subjects who had previously been treated with chemotherapy. No significant associations were found between the tested SNPs and either baseline BMD or change in BMD with 1 year of tamoxifen therapy. The study concluded that the evaluated SNPs in these genes did not influence BMD response in tamoxifen-treated subjects.

Ospemifene

Ospemifene is FDA approved for the treatment of moderate-to-severe dyspareunia, a symptom of vulvovaginal atrophy, due to menopause. Preclinical and clinical data suggest that ospemifene may also have an effect on bone health in postmenopausal women. In vitro data suggest that ospemifene may mediate a positive effect on bone through osteoblasts [81]. Ospemifene effectively reduced bone loss and resorption in ovariectomized rats, with activity comparable to estradiol and raloxifene. Clinical data from three phase 1 or 2 clinical trials (two placebo- and one raloxifene-controlled) found ospemifene 60 mg each day to have a positive effect on the biochemical markers for bone turnover in healthy, postmenopausal women with significant improvements relative to placebo and comparable to raloxifene. No bone density or fracture data are available for ospemifene.

Other SERMs

Small clinical trials of several other SERMs, including toremifene, ormeloxifene, pipendoxifene, and fulvestrant are at various stages of development or have been conducted for prevention and treatment of breast cancer and postmenopausal osteoporosis. Each of these SERMs has unique features that endow them with specific characteristics potentially useful for various clinical applications.

Fulvestrant is currently approved for use in postmenopausal women with hormone receptor positive advanced breast cancer that has progressed on treatment with endocrine therapy [82]. Fulvestrant is a pure estrogen antagonist that avoids the risk of detrimental side effects of selective ER modulators such as tamoxifen, which has partial agonist activity. Fulvestrant appears to be well tolerated. Due to its unique mode of action, fulvestrant lacks cross-resistance with existing SERMs.

Conclusion

Estrogens and SERMs have potent skeletal effects that inhibit bone loss and reduce fracture risk. Estrogen at full-strength doses appears to have a stronger effect on BMD, and SERMs are less potent due to their partially antagonistic effects on the ER. The effects of estrogen on skeletal physiology are well known. Individual SERMs have unique tissue-specific activities that require verification in clinical trials, as the clinical profiles of SERMs are moderately variable. The future of SERMs may be rich with possibilities, but successful clinical application has been slowed by their variable tissue-specific effects.

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The Effects of Androgens on Bone Metabolism: Clinical Aspects

13

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Abbreviations

ADT	Androgen deprivation therapy
AIS	Androgen insensitivity syndrome
AR	Androgen receptor
BMD	Bone mineral density
DHEA	Dehydroepiandrosterone
DHT	Dihydrotestosterone
DXA	Dual-energy X-ray absorptiometry
E2	Estradiol
ERα	Estrogen receptor α
FEA	Finite element analysis
IGF	Insulin-like growth factor
IGFBP	Insulin-like growth factor binding protein
IL	Interleukin
LS	Lumbar spine
PCOS	Polycystic ovarian syndrome
QCT	Quantitative computed tomography
RANKL	Receptor activator of nuclear factor kappa B ligand
SARM	Selective androgen receptor modulator
SD	Standard deviations
SERM	Selective estrogen receptor modulator
SHBG	Sex hormone-binding globulin
T	Testosterone
TGF	Transforming growth factor

Key Points

- Androgens appear to determine overall skeletal size but have a more limited impact on bone mineral density and attainment of peak bone mass. Androgens also contribute to bone strength indirectly via their impact on lean muscle mass development.
- Testosterone has modest antiresorptive effects in addition to a minor role in mediating bone formation.
- Hypogonadism in men results in a rapid phase of bone loss, similar to that which occurs in early menopause. This bone loss is associated with an increased risk of fracture.
- The decline in androgen levels, particularly free testosterone levels, that occurs with aging is paralleled by a decline in bone mineral density and an increase in the risk of fracture and development of frailty.
- Testosterone replacement in men with hypogonadism obviates some of these negative skeletal effects. Treatment of men with a normal age-related decline in testosterone levels shows less robust benefits.

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Introduction

Androgens are 19 carbon molecules that circulate in both men and women, albeit to varying degrees, mostly in the form of testosterone (T), dihydrotestosterone (DHT), androstenedione, and dehydroepiandrosterone (DHEA). In men, 95% of T is made in the testes, with the remaining T produced in the adrenal glands from DHEA and androstenedione precursors (Fig. 13.1). About 8% of T is peripherally converted by the enzyme 5-α-reductase to DHT, a derivative more potent than T given its much higher affinity to the androgen receptor (AR) [1]. A smaller proportion of T (~0.2%) is converted to estradiol (E2) under the actions of the microsomal P450 aromatase, the enzyme also involved in the aromatization of circulating DHEA to estrogens. In contrast, most of the T produced by the ovaries in women is aromatized to E2, with only a small proportion of T and DHT originating from the adrenal glands present in the circulation. As with other steroid hormones, most circulating T (~60–65%) is strongly bound to sex hormone-binding globulin (SHBG), with the remaining T bioavailable in either free or albumin-bound forms (Fig. 13.1) [1, 2].

Until recently, the working hypothesis had been that T was the leading gonadal steroid impacting bone metabolism in men, with age-associated declines in men mimicking early menopausal changes in women. This hypothesis was indirectly supported by the observation that male hypogonadism is associated with a period of increased bone turnover, with bone resorption significantly surpassing the degree of bone formation, ultimately leading to bone loss [3, 4].

Arguably the first counter to this hypothesis came with a 1994 report of a male with homozygous loss of estrogen receptor α (ERα) who displayed normal pubertal development and secondary sexual characteristics, but who had unfused epiphyses, continued linear growth into adulthood, osteopenia, and evidence of high bone turnover [5]. Since then, evidence has accumulated that similar to women, estrogen is the main regulator of bone metabolism in men [6]. Further, more recent data has demonstrated the presence of local aromatization of testosterone to estradiol in bone tissue [7–9].

The above issues regarding an important role for estrogen in male skeletal biology notwithstanding, much work has clearly demonstrated that androgens play an important role in bone

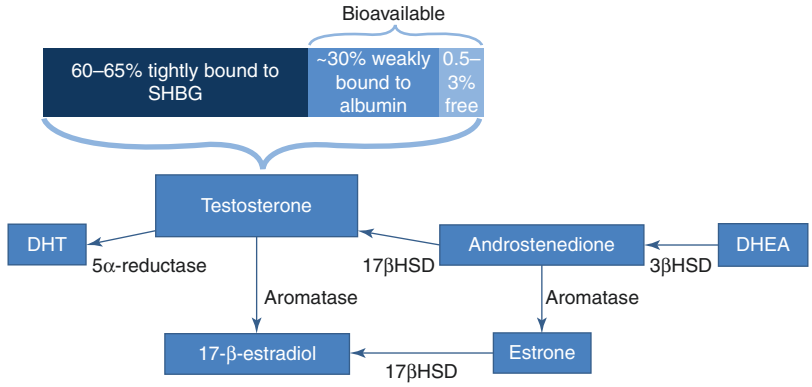

Fig. 13.1 Androgen metabolism and testosterone availability in the circulation. Testosterone is produced in the gonads and adrenal glands from DHEA and androstenedione precursors. Conversion to DHT occurs in peripheral tissues by the enzyme 5-α-reductase; conversion to estradiol occurs dominantly in the gonads under the actions of the microsomal P450 aromatase, the same enzyme involved in aromatization of peripheral circulating androstenedione to estrone. Most circulating testosterone is strongly bound to SHBG, while the remaining is bioavailable in either free or albumin-bound forms. 3βHSD: 3β-hydroxysteroid dehydrogenase; 17βHSD: 17β-hydroxysteroid dehydrogenase; DHEA: dehydroepiandrosterone; DHT: dihydrotestosterone; SHBG: sex hormone-binding globulin

growth and remodeling, a role that is best appreciated clinically in states of androgen deficiency or inefficiency. In addition, advances over the past three decades have enhanced our understanding of androgen actions in bone including the identification of ARs in various bone cells including osteoblasts and osteocytes, and have permitted the molecular and cellular characterization of T action on the skeleton [10–12].

Androgen Effects on Bone Modeling

Skeletal Development During Pubertal Growth

Androgens are responsible for a number of the differential pubertal developments that distinguish boys and girls, including skeletal growth. Specifically, during growth in males, periosteal apposition occurs more rapidly than at the endosteum. In the growing male skeleton, this differential bone accrual results in greater linear growth and cortical thickness, albeit at the expense of increased cortical porosity [13–16]. This is in contrast to the mechanism of skeletal growth in pubertal girls, in which a decrease in endocortical expansion is the main determinant of cortical thickness. Ultimately these differences translate into men having both greater bone width and larger cross-sectional bone area as compared to women, with both factors providing the male skeleton with greater compressive and bending strength and stiffness [17]. In addition, androgens appear to influence both trabecular thickness and trabecular bone volume [18].

This bone modeling continues during early adulthood to confer further increases in bone strength [19, 20]. Other androgenic effects include an increase in lean mass that provides increased mechanical loading to further increase bone strength.

Available data in men with a history of delayed puberty have provided contradictory results with respect to attainment peak bone mass. Thus in one report, 18 adult men with history of constitutionally delayed puberty had significantly lower bone mineral density (BMD) at the lumbar spine (LS) and distal radius as compared to men who underwent normal puberty; furthermore, when followed over time, the men with constitutional delay did not attain the same peak bone mass. In another cohort, however, while areal BMD by dual-energy X-ray absorptiometry (DXA) was similarly lower in men with constitutional delay, volumetric BMD derived from DXA data points showed no significant differences when compared to control subjects [21, 22]. These data further confirm the impact of androgens on bone size is comparatively greater than on bone mineral density.

Lessons Learned from Rare Diseases

Aromatase Deficiency or the Case of a "Pure Androgenic Skeleton"

As noted previously, the P450 aromatase enzyme is responsible for gonadal and peripheral conversion of androgens to estrogens. It is encoded by a single gene, CYP19A1, located on chromosome 15q21.2 [23]. A limited number of cases of aromatase deficiency, in which a mutation in CYP19A1 leads to a nonfunctional enzyme and consequently to estrogen deficiency, have been reported.

Although females with CYP19A1 deficiency are identified at birth due to the presence of ambiguous genitalia and are subsequently treated with estrogen replacement, males are phenotypically normal until early adulthood and thus can provide better attestation to the natural history of estrogen absence on male skeletal growth by allowing for the identification of pure androgenic effects on the growing skeleton. Notably, these skeletal sequelae are virtually identical to those observed by Smith et al. in the case of homozygous loss of ERα [5].

As expected, CYP19A1 deficient males have undetectable circulating estradiol levels with concomitant testosterone and other androgen levels that are normal or elevated. These men present with continued linear growth into adulthood with heights consistently >3 standard deviations (SD) above normal means and with open unfused epiphyses [24, 25]. Ultimately, these abnormalities lead

to gross skeletal deformities such as kyphoscoliosis, pectus carinatum, and genu valgum. Bone mineral content is diminished, with reported BMD Z-scores measured by DXA in one patient of −2.98 at the LS and −3.56 at the hip [26]. In this same patient, there was a significant elevation in serum biochemical markers of bone formation but a much less robust elevation in markers of bone resorption. Similar skeletal findings were noted in an untreated woman with aromatase deficiency with evidence of low bone mineral density [27].

Interestingly, affected individuals do not appear to have an increased rate of fractures, perhaps attesting to the resilience of this "pure androgenic skeleton" with a propensity for deformities rather than fractures. Given the rarity of this condition, however, whether fracture rates will remain normal over the lifespan will require careful monitoring.

Collectively, these observations demonstrate the importance of androgens for skeletal growth but also highlight the need for estrogen for skeletal maturation. In sum, androgens appear to have a greater role in determining bone size with less of a role for attainment of peak bone mass.

A Skeleton Without Androgens: Androgen Insensitivity Syndrome

Another rare condition that offers the opportunity to differentially appreciate the effects of estrogens and androgens on bone metabolism is androgen insensitivity syndrome (AIS), also known as testicular feminization. AIS is caused by mutations in the AR which decrease the receptor's affinity for and response to androgens, thereby leading to complete or partial loss of androgen action [28]. In the case of complete AIS, affected subjects have a 46,XY karyotype but are phenotypically female and undergo breast development during puberty [29, 30]. These individuals possess functional testes and thus have high levels of both T and estrogens present at the onset of puberty. They, however, experience primary amenorrhea in addition to the absence of androgenic sexual characteristics such as hair distribution [31, 32].

In persons affected by AIS, the morphology of diaphysis is similar to the female skeleton with preferential endosteal expansion and a resultant smaller marrow space and lower cortical thickness compared to men [33, 34]. Skeletal height is intermediate between normative male and female patterns [30].

A number of case series and case reports have evaluated bone mineral content in subjects with AIS. In individuals with complete AIS, BMD at both the LS and hip is consistently low as compared to age-matched controls [35–37]. The lower BMD values, however, do not appear to be as dramatic as those seen in subjects with aromatase deficiency. In one report of ten young patients with complete AIS, six of whom had undergone gonadectomy and were receiving estrogen replacement therapy, both areal and volumetric LS BMD values were significantly lower than in controls; BMD was also lower than normative female and normative male cohorts [37]. Although the sample size did not provide enough power to detect differences in fractures, no differences in fracture rates were noted. Similar results have been reported for other cohorts. In one cohort of 18 individuals, subjects compliant with estrogen replacement showed lesser BMD deficits as compared to those with poor estrogen replacement compliance [38]. In individuals with partial AIS, no differences in BMD when compared to control subjects were identified [35, 36, 38].

These observations provide good insight into the role of T in the male skeleton. Lack of androgen action does not delay epiphyseal closure but does yield a shorter stature than normal male counterparts. In addition, loss of androgen action results in lower BMD when compared to either male or female cohorts, irrespective of estrogen replacement, with individuals affected by complete AIS having lower BMD than those with partial androgen insensitivity [38, 39].

Overall, these findings suggest that androgens likely have a direct effect on bone mineral density via their actions on the level of the AR, in addition to their "indirect" effects at the ER following aromatization.

Androgen Effect on Bone Remodeling

Relative Roles of Estrogen and Testosterone on the Male Skeleton

Following the initial report by Smith et al. describing one patient with homozygous loss of the ERα receptor [5], there was considerable debate regarding the potential role for estrogen as the primary regulator of bone metabolism in men.

To address this question directly, an initial study performed in elderly men by Falahati-Nini et al. used a GnRH agonist concomitantly with an aromatase inhibitor to suppress endogenous testosterone and estrogen production, respectively. Subjects were then randomized to receive either T patches alone, estrogen patches alone, both T and estrogen patches or no hormone replacement [40]. As expected, in the group that did not receive hormone replacement, there was a significant increase in biochemical markers of bone resorption, an effect that was completely prevented by the use of both T and estrogen replacement. Interestingly, however, the use of estrogen replacement alone was able to prevent most of the rise in bone resorption, whereas T alone was much less potent. Serum osteocalcin levels were only slightly diminished with either estrogen or testosterone alone, whereas levels of serum amino-terminal propeptide of type I collagen (P1NP) were sustained with estrogen but not T [40].

Subsequently, these earlier findings were confirmed in an elegant study by Finkelstein et al. in which 202 healthy men aged 20–50 years were made similarly hypogonadal using the combination of a GnRH agonist and an aromatase inhibitor. The men were then randomized to receive varying doses of testosterone gel formulation (0, 1.25 g, 2.5 g, 5 g, or 10 grams daily) for a total duration of 16 weeks. As expected, all participants had suppressed estradiol levels and showed an appropriate dose–response relationship to the administered T dose in their respective blood T levels. Importantly, areal BMD assessed by DXA declined by approximately 1–2% in all T dosing groups at all skeletal sites, while trabecular spine BMD assessed by quantitative computed tomography (QCT) declined by approximately 4–5% in each group. Collectively, these results demonstrated that the absence of estradiol in men leads to bone loss and that varying T replacement doses (including supraphysiologic dosing) was unable to limit this bone loss [41, 42]. There was, however, an inverse dose–response relationship between T levels and the percent change in the bone resorption marker C-telopeptide of type 1 collagen (CTX), which seemed to decrease as the T dose was increased. The relative contributions of T on bone remodeling in young adult men were also evaluated in a study by Leder et al. in which subjects were treated with GnRH suppression in addition to a T patch, with or without concomitant aromatase inhibitor therapy, over a 12-week period. When compared to treatment with GnRH suppression alone, the GnRH + T group had a significantly smaller increase in urinary levels of the bone resorption marker deoxypyridinoline. Although a trend toward increases in bone formation markers was suggested in the group treated with GnRH suppression alone, there were no statistical differences noted [43]. Collectively, these observations suggest a contributory antiresorptive role of T, independent of its aromatization to estrogen.

Male Hypogonadism

States of male hypogonadism, whether primary or acquired, and particularly with complete androgen deprivation, are associated with a significant reduction in BMD and consequently with an increased risk of fracture [44]. Such states include primary gonadal failure, secondary hypogonadism due to pituitary insufficiency or hypothalamic malfunction, and hypogonadism due to chemical castration/androgen deprivation therapy (ADT) or surgical castration. The underlying etiology of the hypogonadism, however, bears little weight on the deleterious effects of the hypogonadism on the skeleton [45–50].

Despite the lack of large prospective studies, there is good evidence for increased fracture risk

in hypogonadal men, irrespective of BMD [51, 52]. In general, however, when compared to age-matched controls, men with hypogonadism do have significant reductions in BMD following androgen loss, particularly at sites rich in trabecular bone such as the LS [2, 53]. Bone turnover studies reveal increases in bone resorption with concomitant increases in bone formation, particularly early in the disease process, although the relative increases in bone formation are proportionally much lower than the increases seen in bone resorption [54–56]. Other studies, however, have shown a low bone turnover state [57].

The skeletal impact of the slow decline in testosterone levels that occurs in men during the normal aging process, however, appears to be somewhat different. Bioavailable levels of both T and estradiol decline significantly in aging men and at levels that are disproportionate to the levels of total T and total estradiol, as a result of an increase in SHBG production [58, 59]. Notably, bioavailable T levels decrease to an even greater extent than bioavailable estradiol levels, raising the possibility that declines in both sex steroid levels may contribute to the bone loss that occurs in aging men [39]. Despite this well-documented decline in testosterone levels with aging, the role of testosterone in age-related bone loss in men remains less clear than that of estrogen.

The decline in T levels parallels a decline in both cortical and trabecular BMD, with an overall rate of decline of approximately 1–2% per year (vertebral trabecular bone decline of ≤2% per year; radial cortical bone loss of 0.5–1% per year) [60–62]. Data from bone biopsy studies have generally shown an age-associated decrease in trabecular width and number, decrease in cortical thickness, and an increase in trabecular separation and cortical porosity [63]. The changes in bone remodeling responsible for these observations are yet to be clearly identified as histomorphometric data have provided conflicting results. Whereas some reports have suggested that age-related bone loss in men is associated with high bone turnover marked by significant bone resorption and formation, other studies have reported low rates of bone formation in aging men [63–65]. Epidemiologic evidence of increased fracture risk, however, is more consistent. Some studies revealed a higher incidence of hypogonadism found in a cohort of elderly men with hip or vertebral fragility fractures as compared to the general population [130]. Furthermore, men with hip fractures have increased bone resorption associated with hypogonadism [66].

Studies which have evaluated T and estradiol levels in men and assessed for a correlation with BMD measurements have yielded somewhat inconclusive results. Initially, some studies identified a strong correlation between both total and free T levels and bone loss with age, particularly at primarily trabecular sites [67, 68]. Several more recent studies that have employed more sensitive assays for estradiol in order to better measure the relatively low circulating estradiol levels in men [68, 69], however, have found that bioavailable estradiol levels were better correlated with BMD than either total or bioavailable T levels [58, 59, 70–72]. Notably, this finding was seen even in a cohort of men with hypogonadism and total T levels less than 300 ng/dL, in which serum estradiol levels were better predictors of BMD than serum T levels [70].

Finally, controversy remains regarding the effects of declining T levels on calcium and vitamin D metabolism as well as intestinal calcium absorption. Observational studies suggest that men with hypogonadism have a negative calcium balance, a finding possibly due to decreased 1,25-dihydroxyvitamin D levels and thus lower intestinal calcium absorption. These parameters improved following T replacement [73]. These observations, however, have not been validated in prospective controlled studies.

Castration

Biochemical castration is achieved via the use of GnRH agonists which, alone or in combination with antiandrogens, have been increasingly used to treat prostate cancer in men.

Both surgical and biochemical castration result in a phase of rapid bone loss in men due to the rapid decline in sex steroids, particularly in trabecular bone likely due to its comparatively

larger surface area as relative to cortical bone. In the acute phase, biochemical markers reveal an increase in bone resorption relative to bone formation, a finding which appears to be quite similar to that seen during the early perimenopausal period of rapid bone loss that occurs in women [2, 74]. The use of ADT in men with prostate cancer, for example, can result in 5–10% loss in BMD within the first year, although the rate of decline slows down subsequently [75]. Of importance, however, is that the loss of androgens in men is also accompanied by a decline in both lean and total muscle mass, a factor which contributes to an increased risk for falls and fracture. Histomorphometric data show an increase in osteoclastic resorptive surface as also seen in bone biopsies obtained during early menopause [76].

Paracrine and Autocrine Regulation

Bone remodeling is the result of an intricate coupling between osteoclast-mediated bone resorption and osteoblast-mediated bone formation. This coupling makes use of a host of paracrine modulators between these two cell lineages, the discovery of which has dramatically increased in the past two decades and has begun to allow for a better understanding of the influence of sex steroids on bone remodeling.

In addition to direct activation of the AR, the effects of androgens on bone cells seem to be additionally mediated through their effects on growth factors including transforming growth factor (TGF)-β, insulin-like growth factors (IGFs), and cytokines such as interleukin (IL)-6. Testosterone, not unlike estrogen, primarily inhibits bone resorption. In vitro, testosterone has been shown to weakly stimulate osteoblast proliferation but also to limit osteoblast apoptosis [8, 77–79].

Following activation of AR and ERα, sex steroids increase the production of reactive oxygen species through the action of cytoplasmic kinases. This inhibits the expression of receptor activator of nuclear factor kappa B ligand (RANKL) and production of IL-6, eventually leading to suppression of the differentiation along the osteoclast lineage and therefore ultimately decreasing osteoclast activity. Orchiectomized rats show an increase in RANKL levels and a consequent increase in bone resorption [1, 80]. Estrogens seem to play a major role in this pathway, as evidenced by the fact that RANKL levels are inversely proportional to estradiol levels in men receiving ADT [81]. Expression of IL-6 has been demonstrated in vitro to be inhibited by treatment with DHT or T and in vivo to be stimulated following orchiectomy [82]. TGF-β in bone stimulates osteoblasts and suppresses osteoclast activity. It has been demonstrated that androgens can stimulate TGF-β gene expression [77, 83]. Furthermore, TGF-β levels are significantly reduced in orchiectomized rats, a result which can be prevented by T replacement [84]. Androgens may also exert effects on osteoblast activity by modulation of members of the IGF family of ligands, the IGF receptor family, and the IGF binding proteins (IGFBPs) [82].

Androgen Effects on Bone in Women

As presented above, there is good evidence that androgens play a major role in periosteal apposition and likely also in increasing bone formation and limiting bone resorption. There remains, however, limited evidence of any role for androgens, particularly T, in postmenopausal skeletal remodeling in women [85].

DHEAS is the predominant circulating androgen in women, with levels similar to those in men [86]. Early studies investigating the effects of androgens on the female skeleton focused on hirsute but otherwise healthy premenopausal women with endogenous androgen excess [87]. Affected women were found to have significantly elevated circulating levels of both T and DHEAS (two-fold or higher), but similar estrogen levels as compared to control women of similar age. Single energy QCT evaluation showed significantly higher trabecular BMD in women with androgen excess, with a less pronounced increased in cortical BMD. Notably, however, no correlation between BMD and levels of individual either

T or DHEAS was identified. A subsequent larger study which examined the correlation between androgen levels in circulation and BMD in perimenopausal women found that although free T and estrogen levels showed some correlation, the strongest and most consistent correlation for BMD across all measured sites was SHBG levels [88]. Interestingly, this finding is consistent with later studies performed in men, as detailed below.

There are, however, many potential confounding factors to all such association studies in women. First, the skeletal effects of circulating hyperandrogenism do not solely reflect the results of AR activation. In fact, DHT is the only androgen that exclusively activates the AR, with other androgens able to exert their effects "indirectly" through the ER due to aromatization. This is particularly true in women, in whom the effects of estradiol on the skeleton may completely mask those of endogenous androgens. A second potential confounder relates to the effects of androgens on body composition. In women affected by a hyperandrogenic state such as hirsutism or polycystic ovarian syndrome (PCOS), the increase in BMD observed may, at least partially, be reflective of increased body mass [74]. Notably, obese patients with PCOS have higher bone mineral density when compared to nonobese PCOS patients. This observation raises the possibility that aromatization of androgens in adipose tissue may impact bone remodeling. In women with hyperandrogenism, the use of antiandrogen treatments for hirsutism or acne has yielded conflicting results with respect to bone loss and may depend at least partially on the specific agents used for treatment [89]. Whereas use of spironolactone in combination with a progestin resulted in loss of BMD at the LS, monotherapy with flutamide, another androgen receptor antagonist, did not [90, 91]. In addition, the bone loss seen with use of GnRH agonists was prevented by concomitant use of spironolactone, but not by concomitant use of flutamide [89, 92]. Finally, the purity of assays measuring either free T or free estrogen needs to be taken into account when evaluating these studies. In general, steroid assays are quite intricate due to both the nature and low circulating concentrations of these molecules. While assays continue to improve with respect to their reporting accuracy, studies with earlier available assays may potentially have a significant amount of measurement variability which may impact the reliability of the reported findings [3, 93].

Skeletal Effects of Androgen Replacement

Testosterone Replacement in Men with Hypogonadism

In men with overt hypogonadism of any age, T replacement has been shown to improve BMD, particularly over the first 2 years of treatment [94]. Newer studies have demonstrated improvement in bone microarchitecture as well as bone strength and mechanical properties following T replacement [95–99]. As an example, in 21 adult men with hypogonadotropic hypogonadism, T replacement for 2 years increased both cortical and trabecular BMD up to 13% in those with open epiphyses. In the subset of subjects with fused epiphyses, a 4% increase in cortical BMD but no change in trabecular BMD was noted [100]. Similar results were seen in a similar subsequent study in which 16 men with hypogonadotropic hypogonadism were treated with T replacement therapy [101].

In comparison, evaluation of men with acquired hypogonadism who had an initial 10% BMD loss at the LS following a decline in T levels showed that T replacement resulted in an initial 5% increase in whole body BMD, with the most marked increases seen in sites with the greatest percentage of trabecular bone, where the noted increases were as high as 14% [95]. These results have been subsequently validated in a number of other studies which have also noted that the most significant increases in BMD occur during the first year of treatment [97]. This increase continues during the second year of treatment, but bone mineral density stabilizes thereafter. Studies using QCT assessment show a greater increase in trabecular bone than what is

seen by DXA, although single energy QCT does not account for changes in bone marrow fat with androgen treatment [3, 102].

The basis for this increase in bone mass with T treatment of hypogonadal men is unclear. Whereas some studies have shown that T treatment causes a decrease in bone resorption and possibly bone formation markers, other studies have suggested that treatment with either T or human chorionic gonadotropin causes an initial increase in bone formation markers [95, 96, 103–105]. T has also been shown to increase skeletal calcium uptake in prepubertal boys [94]. Finally, T treatment in hypogonadal men can also result in increases in both lean mass and muscle strength, thereby potentially contributing to mechanical loading, improvements in bone strength properties, and fracture risk reduction [96, 99, 103, 104, 106].

Testosterone Treatment in Elderly Men

In contrast to men with significant hypogonadism due to an underlying disease as discussed above and in whom T replacement shows unequivocal skeletal benefits, studies which have evaluated T treatment either in eugonadal men or in men with normal age-related declines in T levels have shown contradictory results.

In a placebo-controlled trial which included 70 men aged 65 years and older with total T levels less than 350 ng/dL, men who received intramuscular T enanthate 200 mg every 2 weeks for 36 months had a 10% increase in BMD at the LS and an approximately 2% increase in BMD at the total hip, but no change in BMD at the femoral neck. Treated men also had a decrease in bone resorption markers as well as in levels of bone-specific alkaline phosphatase, but osteocalcin levels were unchanged. Of note, however, is that T levels achieved at the end of the trial were supraphysiologic. In addition, the trial was associated with both a higher rate of erythrocytosis and a larger increase in prostate volume when compared to other trials of T replacement [107].

In another placebo-controlled trial of 87 men aged 65 years or older with low bioavailable T levels at baseline study entry, the use of a transdermal T patch at a dose of 5 mg/day for 24 months showed a modest but statistically significant BMD increase of 1% at the femoral neck as compared to a decline in BMD at the femoral neck in the placebo group. No changes in BMD were noted at other sites. Of note, men included in the study had total T levels which were in the low-normal to slightly low range [108]. Finally, in another open-label replacement study which included 60 obese middle-aged men with a mean age of 57 years and total T values of less than 320 ng/dL, the use of the long-acting preparation T undecanoate for 36 months also showed increases in areal BMD at both the LS and femoral neck at a rate of 5% per year [109].

In contrast to the above findings, however, a 36 month placebo-controlled trial of transdermal T therapy in 108 men aged 65 years or older with baseline T levels of less than 475 ng/dL showed no significant changes in LS BMD (4.2% increase vs. 2.5% in the placebo group) [110]. No changes in bone formation or resorption markers were noted for the duration of the study, confirming previous results. Similarly, no change were seen in either bone resorption or formation markers in a short-term (9 weeks) open-label study in which 27 men aged 70 years or older with total T levels of less than 350 ng/dL were treated with either intramuscular or transdermal T [111]. In comparison, when a transdermal T formulation was given for 12 months to a larger cohort, there was a slight 0.3% increase in femoral neck BMD in treated subjects, as compared to a decline of 1.2% in the control group; there was, however, a significant increase in muscle strength in the treatment group [112]. Notably, a more recent placebo-controlled trial by the same group, in which transdermal T gel was used in 131 men aged 65 years or older with T levels less than 350 ng/dL, showed significant increases in BMD at both the femoral neck (1.4%) and LS (3.2%), but a decline at the distal radius (−1.3%). No differences in bone turnover markers were observed over the trial duration [113].

More recently, a series of multicenter placebo-controlled testosterone replacement trials (collectively known as the T-Trials) which included 211 men aged 65 years and older with symptomatic hypogonadism and T levels less than 275 ng/dL, performed a subset analysis that examined bone microarchitecture and finite element analysis (FEA). Transdermal T gel provided for 12 months resulted in an increase in trabecular volumetric BMD at the spine by 7.5% and at the hip by 1.8%. Additionally, bone strength as estimated by FEA was increased by 10.8% as compared to a 2.5% increase in the placebo group. Interestingly, however, areal BMD at the LS was increased by only 1.2%, with no significant differences in BMD observed at other sites [114].

In another study, intramuscular T supplementation provided for 6 months to eugonadal men with osteoporosis diagnosed due to vertebral compression fractures showed antiresorptive effects, as evidenced by a reduction in urine and serum markers of bone resorption as well as an increase on LS BMD by 5% [115].

Taken together, these data show that T does have an antiresorptive effect, and that T supplementation in elderly men with low-normal or low testosterone levels can modestly improve areal BMD by DXA, with possibly more significant improvements in bone microarchitecture and mechanical properties. However, a number of questions remain unanswered. These include: what levels of T should be used as a lower limit for initiation of T replacement; what is the optimal T value (or T value range) that should be targeted; and most importantly, does T replacement provide any fracture benefit and, if so, what are the comparative benefits relative to the potential risks for harm? Together, the available data suggest that T therapy must be both individualized and closely monitored, particularly as it pertains to the potential risks T therapy may impose.

Testosterone Use in Women

Given that the benefits of estrogen as an antiresorptive agent are well established, little effort has been made to evaluate skeletal outcomes associated with the use of T in women. Thus, T therapy for female bone health cannot be recommended, although it is notable that postmenopausal women treated with estrogen plus methyltestosterone showed a greater increase in the bone formation marker osteocalcin as compared to women treated with estrogen alone [116].

DHEA Supplementation

Animal studies using DHEA supplementation have shown significant improvement in a number of age-related variables, including cardiovascular disease. Such findings have led to the promotion of DHEA and DHEA-S as antiaging agents [108].

As an example of the potential skeletal effects of DHEA in a preclinical animal model, orchiectomized rats treated with DHEA showed a reduction in bone resorption markers that was partially reversed with antiandrogen treatment. This suggested a pure androgenic benefit and raised the question as to whether supplementation with DHEA would be of skeletal benefit in both men and women [117].

To assess this directly in humans, a placebo-controlled trial of 280 healthy elderly men and women aged 60–79 years was undertaken. Subjects were evaluated with areal BMD by DXA at baseline and then again following 12 months of DHEA supplementation at a dose of 50 mg/day. In men, DHEA treatment resulted in no significant differences in BMD when compared to placebo. In comparison, women aged <70 years had a significant increase in BMD at the femoral neck, while women aged >70 years had a significant increase in BMD at the distal radius and a nonsignificant BMD increase at the hip. A significant suppression of the bone resorption marker, serum CTX, was also seen in the older women, but no changes were noted in the biochemical markers of bone formation [118].

In an uncontrolled study of 18 elderly men and women aged 64–82 years treated with DHEA 50 mg daily for 6 months, there was a modest increase in LS BMD which was more significant

in men than in women, with no other changes seen at any other site. There was no observed effect on bone turnover markers [119].

Finally, in another placebo-controlled trial, the effects of DHEA supplementation for 24 months on BMD were evaluated in 87 elderly men and 57 elderly women with low DHEA-S levels. Men received DHEA supplementation at a dose of 75 mg/day and had a lower rate of decline in femoral neck BMD compared to placebo; however, no differences were seen at other sites. In comparison, treatment of women with DHEA supplementation at a dose of 50 mg/day resulted in a BMD increase at the radius by 2.5%, but no differences in the rate of BMD decline at either the femoral neck or LS compared to placebo [108]. Whether the skeletal effects of DHEA might reflect the conversion of DHEA to estrone and/or estradiol is unclear. Formal evaluation of DHEA provided in the presence or absence of concomitant aromatase inhibitor therapy would likely be needed to clearly demarcate any effects on the skeleton that reflect the actions of DHEA alone.

Modulators of Androgen Action in Men

Modulators of androgen action include AR antagonists, estrogen receptor antagonists, selective estrogen receptor modulators (SERMs), aromatase inhibitors, and 5α-reductase inhibitors. Their use in men for different indications has been increasing. However, only a limited number of studies have evaluated their effects on bone.

Anastrozole, an aromatase inhibitor, was studied at a dose of 1 mg daily in a placebo-controlled trial which included 69 elderly men aged 60 years or older with low T levels (total T values of less than 350 ng/dL). Relative to placebo, anastrozole treatment for 12 months resulted in significant BMD loss at the LS when assessed by both DXA and QCT. Hip measurements showed a nonsignificant decline and bone turnover markers did not change. Notably, T levels in the treated group increased by 50% and were restored to physiologic levels [120].

Finasteride, a 5α-reductase inhibitor, does not seem to impact either BMD or bone turnover when used alone. However, finasteride did not inhibit the BMD improvement when used in conjunction with T when studied in one of the aforementioned placebo-controlled trials [107, 121, 122].

The SERM raloxifene has been studied for the prevention of bone loss in men treated with GnRH agonists for prostate cancer where it was shown to improved femoral neck BMD and to prevent a decline at BMD at the LS [123]. When used in otherwise healthy elderly men, however, raloxifene had equivocal effects on bone turnover [124].

Finally, selective androgen receptor modulators (SARMs) have been evaluated in both preclinical and a limited number of early phase clinical studies. SARMs are ligands that bind to the AR but induce tissue-selective activation. Efforts in the past two decades have been made to develop nonsteroidal SARMs which have salutary effects on muscle function, physical performance, and possibly bone formation but which spare the prostate, heart, and liver. Such agents would in theory eliminate the dose-limiting adverse events of androgen replacement. A number of these agents have been evaluated in preclinical studies and moved through phase I and phase II trials [125]. In one study, SARM treatment of rats for 16 weeks showed increases in bone mass and strength thought to be due to AR modulation of osteoblast function [126]. Other studies evaluating SARMS have shown prevention of bone loss in either orchiectomized or ovariectomized rats, as well as increases in bone strength [127]. Many of these agents, however, have shown either an increased risk of adverse events or a lack of efficacy when studied in clinical trials [128, 129].

Conclusion

Androgens play an important role in skeletal development, particularly in determining bone size in adults, although their impact on bone maturation and peak bone mass is less clear. Most

evidence, however, is indirect and derived from states of androgen insensitivity or deficiency, either in vitro or in vivo. In addition, androgens contribute to bone remodeling in the developed skeleton either directly via their action at the AR in osteoblasts and/or osteocytes, or indirectly via aromatization to estrogens as well by their effects on maintaining lean muscle mass and function.

Restoring physiologic levels of androgens, particularly T, when deficient in adolescents and young adults is paramount to preventing bone loss and fractures. In aged men, however, treatment of the age-related decline in T levels has shown equivocal benefits on bone metabolism. Although recent data suggest improvements in bone microarchitecture in elderly men following T replacement, fracture data are needed to permit comparison of the potential skeletal benefits against recognized potential thromboembolic and prostate-related adverse events common to this population.

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14 Bisphosphonates: Mechanisms of Action and Role in Osteoporosis Therapy

Arthur C. Santora II and Anupa Sharma

Key Points

- All bisphosphonates inhibit osteoclast-mediated bone resorption, reducing the accelerated rate of bone remodeling found in osteoporotic postmenopausal women and hypogonadal men into the range found in eugonadal younger adults.
- All bisphosphonates approved in the United States, Europe and Japan for the treatment of osteoporosis in postmenopausal women have been shown to decrease the risk of vertebral fractures and most have been shown to decrease the risk of hip and non-vertebral fractures in placebo-controlled studies 3–6 years in duration.
- The pharmacokinetic characteristics of bisphosphonates are similar to each other but differ substantially from most other drugs.
- Because the half-life of bisphosphonates on bone surfaces is approximately 1 month, daily, weekly or monthly dosing will result in the same steady-state level of drug on the surface of bone – and the effects on bone resorption – if the average dose/day is the same.
- Bisphosphonates on bone surfaces may be incorporated into newly forming bone where they are not pharmacologically active until they are released by new bone resorption. Their half-life in bone is approximately 5 years.
- After long-term treatment (3–5 years), bisphosphonates released from the bone matrix are pharmacologically active and will slow, but generally not fully prevent bone loss after treatment is interrupted for a "drug holiday".
- The adverse drug reactions (ADRs) associated with bisphosphonate use are generally consistent with the class, although frequency may vary with route of administration (GI ADRs with oral dosing and acute-phase response-like reactions with intravenous dosing). Prescribers should be familiar with ADRs described in approved Prescribing Information of each drug.

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Introduction

The definition of osteoporosis as "…a disease characterized by low bone mass, microarchitectural deterioration of bone tissue leading to enhanced bone fragility, and a consequent increase in fracture risk" [1] provides a framework for understanding both its pathophysiology and pharmacologic effects of a treatment. "Bone fragility" is synonymous with "decreased bone strength" in this definition. Fractures are the most important consequence of osteoporosis but not part of its definition. Drugs for the treatment of osteoporosis may reduce fracture risk by increasing bone mass, improving bone microarchitecture or both. While bisphosphonates produce small increases in the mass of cancellous bone of vertebrae and at the ends of long bones where most fractures occur in osteoporotic patients, these increases appear too small to account for the reduction in both vertebral and non-vertebral fracture risk observed in clinical trials. The most important effect of bisphosphonates on bone strength is likely due to their effect on the microarchitecture of bone. Bisphosphonates have been shown to prevent the loss of both cancellous and cortical bone in people with normal bone mass who are rapidly losing bone (e.g., women after menopause or hypogonadal men), thereby preventing osteoporosis that would have occurred in the absence of treatment. While it is anticipated that preventing osteoporosis in non-osteoporotic postmenopausal women who are losing bone should reduce future fracture risk, no adequate fracture endpoint clinical trials have been conducted. This chapter will include a review of the chemistry and mechanism of action of bisphosphonates at a molecular, cellular and tissue level that have come from both non-clinical and clinical studies. The clinical pharmacology (absorption, distribution, metabolism and elimination) will be reviewed, with an emphasis on the relevance to long-term treatment and prevention of osteoporosis with bisphosphonates. Finally, the effects of multi-year bisphosphonate treatment on fracture risk, bone mineral density (BMD) and bone turnover, as well as recognized and potential adverse drug reactions will be reviewed. The focus of this chapter is on the treatment of osteoporosis in postmenopausal women and the treatment of osteoporosis in men. The bisphosphonate mechanism of action review supplements the detailed reviews of Combination Anabolic/Antiresorptive Therapy in Osteoporosis (Chap. 18), Osteoporosis in Men (Chap. 20), Glucocorticoid Induced Osteoporosis (Chap. 21) and Safety Considerations for Osteoporosis Therapy (Chap. 24).

Bisphosphonate Chemistry and Physicochemical Properties

Bisphosphonates are not recognized to exist in nature and were initially synthesized in the late 1800s and developed for their ability to chelate calcium and inhibit crystallization of insoluble calcium salts including hydroxyapatite [2]. Etidronate was first synthesized by German chemists in 1865 [3]. Several were proposed as detergent additives and the initial human application was as components of toothpaste to inhibit dental calculus formation [4]. Bisphosphonates, previously referred to as diphosphonates, are analogs of pyrophosphate, but unlike the P-O-P bonds of pyrophosphate, the P-C-P bonds of bisphosphonates (Fig. 14.1) are both chemically stable and not hydrolyzed by any enzymes in humans or other vertebrates. The bisphosphonate backbone provides high affinity for hydroxyapatite surfaces in bone or sites of ectopic calcification. An hydroxyl (OH) group at the R^1 position results in higher affinity for hydroxyapatite than either hydrogen (H) or chlorine (Cl) [5]. The R^2

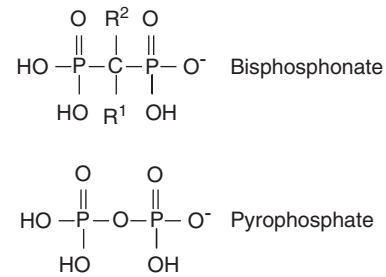


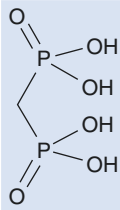
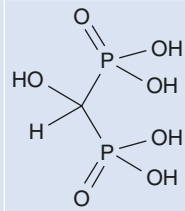
Fig. 14.1 Bisphosphonate and pyrophosphate structure

moiety may also affect affinity for bone hydroxyapatite but is the major determinant of a bisphosphonate's intracellular pharmacodynamic effects on osteoclasts.

More than 10 bisphosphonates have been used in either radionuclide bone imaging or in the treatment and prevention of bone disease in humans. Tables that follow include the generic names of marketed products (that include salt), name(s) of the anionic or acid form that generally appear in medical literature and the structures and chemical names of the acid forms of each bisphosphonate [6].

Bisphosphonates used in ^{99m}Tc bone imaging are presented in Table 14.1. Medronate sodium (methylene bisphosphonate, MDP) as a ^{99m}Tc complex localizes to hydroxyapatite on bone surfaces and identifies sites of osteoblast bone formation. Oxidronate sodium-[(1-hydroxymethylidene) bisphosphonate] complexed with ^{99m}Tc has properties like MDP.

Table 14.1 Bisphosphonates used in ^{99m}Tc bone imaging

Medronate sodium	Oxidronate sodium
	
Methylenebisphosphonic acid	(1-hydroxymethylidene) bisphosphonic acid
Other names: methylenediphosphonate and MDP	

Bisphosphonate drugs are used clinically to either inhibit hydroxyapatite formation in diseases characterized by soft tissue mineralization or inhibit osteoclast bone resorption in diseases in which bone resorption exceeds bone formation. BPs are generally divided into nitrogen-containing (N-BP) and non-nitrogen-containing bisphosphonates (non-N-BP) based on their molecular mechanisms of action as inhibitors of bone resorption.

Table 14.2 presents the structure and names of the most commonly studied non-nitrogen-containing bisphosphonates: etidronate, clodronate and tiludronate. Etidronate inhibits both bone mineralization and osteoclastic bone resorption and was first used as a treatment of myositis ossificans to inhibit calcification. An intermittent dosing regimen is approved for the treatment of osteoporosis in Canada and many European countries, but not approved in the United States. While clodronate has been studied as a treatment of osteoporosis, it is not approved for that use in the United States or Canada. Its most common use is as a treatment of cancer metastatic to bone outside North America. Tiludronate has been studied as a treatment of osteoporosis but a dose that reduced fracture risk in osteoporotic patients was not found.

N-BPs with a non-aromatic nitrogen-containing R^2 moiety are shown in Table 14.3. Alendronate and ibandronate are both marketed as oral formulations for the treatment and prevention of osteoporosis. Ibandronate is also marketed as an intravenous (IV) solution administered every 3 months. Pamidronate was not developed as an oral product due to esophageal toxicity but

Table 14.2 Non-nitrogen-containing bisphosphonates

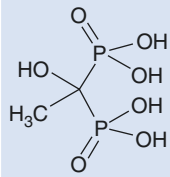
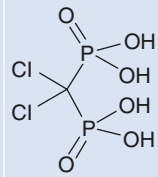
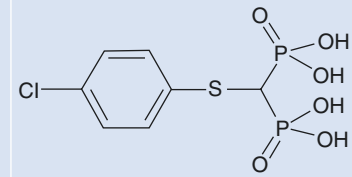
Etidronate disodium	Clodronate disodium	Tiludronate disodium
		
(1-hydroxyethylidene) bisphosphonic acid	(dichloromethylene) bisphosphonic acid	[(4-chlorophenyl)thio] methylene] bisphosphonic acid
Other names: EHDP and HEBP		

Table 14.3 Nitrogen-containing bisphosphonates with a non-aromatic nitrogen R^2 moiety

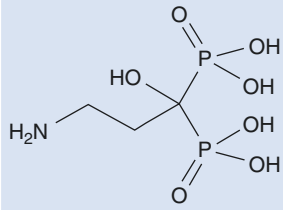
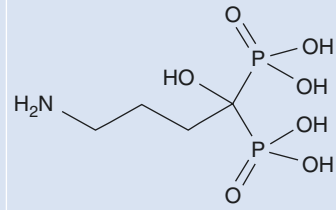
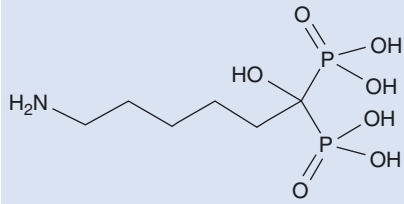
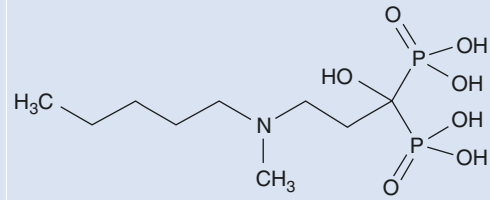
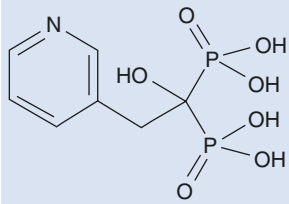
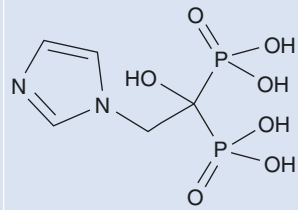
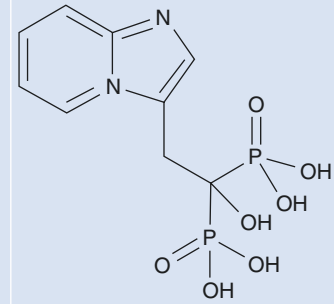
Pamidronate disodium	Alendronate sodium
	
(3-Amino-1-hydroxypropylidene) bisphosphonic acid	(4-Amino-1-hydroxybutylidene) bisphosphonic acid
Neridronate sodium	Ibandronte sodium
	
(6-Amino-1-hydroxyhexylidene) bisphosphonic acid	[1-Hydroxy-3-(methylpentylamino) propylidene]bisphosphonic acid

Table 14.4 Nitrogen-containing bisphosphonates with an aromatic nitrogen R^2 moiety

Risedronate sodium	Zoledronic acid	Minodronic acid
		
[1-Hydroxy-2-(3-pyridinyl) ethylidene] bisphosphonic acid	[1-Hydroxy-2-(1*H*-imidazol-1-yl) ethylidene] bisphosphonic acid	(1-Hydroxy-2-imidazo[1,2-*a*] pyridin-3-ylethylidene) bisphosphonic acid
	Other names: zoledronate	Minodronate

has been studied as an intravenous formulation in osteogenesis imperfecta. Neridronate is marketed in several European countries for osteogenesis imperfecta but has not been adequately studied in osteoporotic adults.

N-BPs with an aromatic nitrogen are presented in Table 14.4. Risedronate and zoledronate (zoledronic acid) (intravenous infusion) are marketed worldwide for the treatment and prevention of osteoporosis. Minodronate is a common osteoporosis drug in Japan but not marketed in either North America or generally in Europe.

The affinity of BPs for human bone particles [7] is generally proportional to their affinity for inorganic hydroxyapatite [8]. In bone particles, Ki's based on competitive binding with [^{14}C] alendronate ([^{14}C]ALN) (Ki's are estimates of the Kd of each bisphosphonate based on the concentration that inhibits 50% of [^{14}C]ALN binding, the Kd of [^{14}C]ALN binding and [^{14}C]ALN con-

centration [7]) were alendronate 61 μM, ibandronate 116 μM, risedronate 85 μM and zoledronate 81 μM. The Ki for clodronate, 806 μM, was substantially higher and consistent with its ten-fold lower potency as an inhibitor of bone resorption in vivo [9]. Tiludronate has an intermediate Ki of 173 μM. A hydroxyl group is present at the R^1 position of bisphosphonates with higher affinity for hydroxyapatite and absent in lower affinity bisphosphonates. The affinity of clodronate for inorganic hydroxyapatite crystals was also lower than that of alendronate, but only four-fold lower. Affinity of bisphosphonates for bone is only one factor that determines the potency of bisphosphonates as inhibitors of bone resorption. Their molecular mechanism of action-based potency is of equal importance.

Bisphosphonate Molecular Mechanisms of Action

While bisphosphonates were initially studied as inhibitors of mineralization, this is an unwanted side effect of a drug intended to prevent bone resorption. Inhibition of mineralization appears to be a physicochemical phenomenon that occurs at the surface of hydroxyapatite crystals at sites of bone formation and ectopic mineralization in some diseases. While several secreted proteins (e.g., tissue-nonspecific alkaline phosphatase [10]) are required for normal bone mineralization, bisphosphonates are not recognized to interfere with their function [11]. Affinity of a bisphosphonate for hydroxyapatite in bone and its concentration at sites of bone formation determine whether inhibition of mineralization occurs in vivo.

Once bisphosphonates were shown to inhibit bone resorption [12, 13], they were screened to identify potential candidates for human diseases characterized by high rates of osteoclastic bone resorption through in vitro assays using isolated osteoclasts and in vivo measures of their ability to inhibit bone resorption [14, 15]. In vivo assays were also used to eliminate bisphosphonates that inhibit mineralization at doses like those that inhibit bone resorption. Etidronate was shown to inhibit mineralization at doses required to inhibit resorption [16] while clodronate could inhibit bone resorption at a dose that did not inhibit mineralization. However, the molecular mechanism of actions of osteoclast inhibition was not established until the late 1990s.

Non-nitrogen-Containing Bisphosphonates

Non-N-BPs etidronate and clodronate were shown to inhibit the growth of amoebae of the slime mold *Dictyostelium discoideum* that was studied as an osteoclast analog because it accumulated bisphosphonates via pinocytosis [17]. Non-N-BPs are incorporated into adenine nucleotides that are non-hydrolysable analogs of ATP. They also inhibit aminoacylation of tRNA. The consequence of these effects is cell death [18]. This mechanism is also observed in mammalian cells including osteoclasts [19, 20]. In contrast, incorporation of N-BPs into analogs of ATP is either absent or minimal. Additional details on the biochemistry of non-N-BP inhibition of osteoclasts may be found in a review by Rogers et al. [21].

Nitrogen-Containing Bisphosphonates

N-BPs were shown to be potent inhibitors of bone resorption in the early 1980s, but their molecular mechanism of action was not elucidated until the late 1990s. N-BPs were initially shown to inhibit several metabolic pathways important for osteoclast differentiation and activity including several protein tyrosine phosphatases [22] but inhibition only occurred at high concentrations and did not correlate with the relative potency of N-BP inhibition of bone resorption.

Studies reported in the late 1990s indicated that N-BPs reversibly inhibit osteoclast activity and at high doses trigger apoptosis by reducing geranylgeranyl diphosphate (GGPP) leading to insufficient geranylgeranylation of regulatory

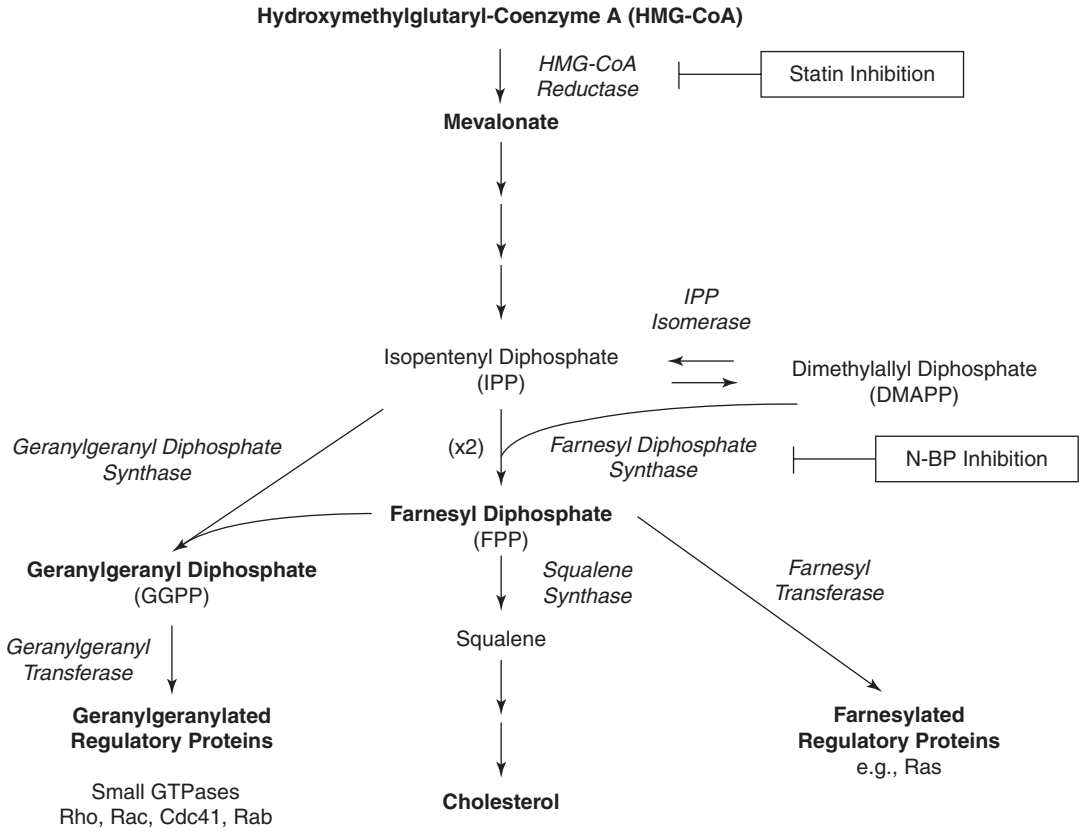


Fig. 14.2 Protein isoprenylation via the mevalonate pathway. Intermediates or side products of the mevalonate pathway for cholesterol biosynthesis include the 15-carbon intermediate farnesyl diphosphate (FPP) and 20-carbon isoprenoid geranylgeranyl diphosphate (GGPP). Several intracellular regulatory proteins are modified by transferring a farnesyl or geranylgeranyl group to a carboxyl terminal cysteine residue with the isoprenoid geranylgeraniol starting with mevalonate. N-BPs specifically block farnesyl diphosphate synthase resulting in depletion of GGPP and FPP. Inadequate geranylgeranylation of small GTPases results in loss of osteoclasts ruffled border and reversible inhibition of bone resorption that can be reversed (in vitro) by restoring GGPP by adding geranylgeraniol. High concentrations of N-BPs result in osteoclast apoptosis that can also be blocked by adding geranylgeraniol. Specific non-BP inhibitors of farnesylation do not block osteoclastic bone resorption and farnesol cannot block N-BP inhibition of osteoclasts

proteins required for normal cytoskeletal function and survival [23, 24]. As illustrated in Fig. 14.2, N-BPs specifically inhibit farnesyl diphosphate synthase, a key enzyme in the mevalonate pathway, required for the biosynthesis of cholesterol and the isoprenoid lipids geranylgeranyl diphosphate (GGPP) and farnesyl diphosphate (FPP) [25–27]. Non-N-BP bisphosphonates do not inhibit farnesyl diphosphate synthase.

The importance of the mevalonate pathway in N-BP molecular mechanism of action of N-BP inhibition of osteoclasts emerged over several years. The cholesterol synthesis pathway was identified as a site of bisphosphonate action when the N-BP incadronate [28] was shown to inhibit squalene synthase. However, alendronate and pamidronate were very weak inhibitors of squalene synthase even though both inhibited total sterol biosynthesis (168 and 420 nM IC_{50}, respectively) [28], suggesting they may inhibit enzymes involved in the synthesis of farnesyl diphosphate from mevalonate. Demonstration that statins (HMG-CoA reductase inhibitors) which inhibit mevalonate synthesis could inhibit monocyte/macrophage cell lines [24] and bone resorption in vitro provided further support to the importance of this metabolic pathway in N-BP action. However, inhibition of cholesterol synthesis in

osteoclasts was not the mechanism of N-BP action, as osteoclasts have an alternate source of cholesterol from low-density lipoproteins (LDL) and supplementation with cholesterol has no effect on the inhibitory action of alendronate on bone resorption [23].

In addition to its role as a precursor of cholesterol, farnesyl diphosphate is converted to geranylgeranyl diphosphate (Fig. 14.2). Geranylgeranyl transferase attaches geranylgeranyl moieties (geranylgeranylation) to a variety of regulatory proteins including Rho and other small GTPases required for osteoclast cytoskeleton function and mTOR [29], Bim [30] and MST1 [31] that are required for osteoclast survival. Farnesyl transferase results in the farnesylation of other cellular proteins.

The ability of N-BPs to inhibit farnesylation and geranylgeranylation was initially shown in a monocyte/macrophage cell line [24]. Inhibition of cell death due to N-BPs in macrophages could be partially suppressed by farnesyl diphosphate or geranylgeranyl diphosphate. Subsequent studies in osteoclasts showed that inhibition by N-BPs could be prevented by the addition of geranylgeraniol, which is converted intracellularly to geranyl diphosphate [23]. The addition of farnesol, which can be converted intracellularly to farnesyl diphosphate, can restore the function of the monocyte/macrophage cell line but it cannot restore the function of osteoclasts treated with N-BPs or prevent osteoclast apoptosis [31].

It is important to keep in mind that N-BPs' inhibition of farnesyl diphosphate synthase, reduction in geranylgeranyl diphosphate and reduction of geranylgeranylation of regulatory proteins is dose-dependent. Lower doses produce reversible inhibition of osteoclast function. Only high doses of N-BPs sufficiently deplete geranylgeranyl diphosphate to produce apoptosis [32].

Bisphosphonate Pharmacology at a Bone Cell and Tissue Level

Knowledge of the pharmacokinetics of bisphosphonates is critical to understanding their effects on bone cells and the function of bone related to its biomechanical properties and reservoir of calcium and to a lesser extent, phosphate. The following sections review the absorption, disposition, metabolism and elimination (ADME) of bisphosphonates. Citation of the source of data presented include publications in the peer-reviewed medical literature when available. In some cases, the only source of data is the product labeling approved by regulatory agencies. Product labeling approved by a regulatory agency is a synopsis of information about a drug product that has generally undergone review of source data regulatory agency scientists that is as rigorous as that of a peer-reviewed publication.

Absorption

Bisphosphonates as a class have low bioavailability following oral administration. Moreover, food and beverages other than water reduce bioavailability observed after an overnight fast up to 90%. Bioavailability reported in product labeling is generally based on dosing following an overnight fast and 2–5 hours before a standardized breakfast. Under these conditions, bioavailability of alendronate averaged 0.64% for doses ranging from 5 to 70 mg, risedronate 30 mg was 0.63% and ibandronate 2.5 mg was 0.6% [33–35]. Solutions of alendronate have the same bioavailability as tablets. While alendronate and risedronate absorption is dose-proportional, the fractional absorption of ibandronate is nonlinear at doses between 50 and 150 mg [36]. The AUC values (adjusted for dose), relative to the 50-mg dose, were 130% for the 100-mg and 191% for the 150-mg dose.

The bioavailability of the non-N-BP etidronate (3–7%) [37] is substantially higher than that of N-BPs. Greater bioavailability was observed with higher doses. Clodronate bioavailability (1–2%) [38] is only slightly greater than that of N-BPs. Higher bioavailability of non-N-BPs may be related to higher doses (mg/kg) of non-N-BP studied.

Absorption in a "real world" setting may differ from standard conditions (fasting, several hours before food) as all bisphosphonates have food interactions and fractional absorption may not be constant among the doses that have been studied. Bioavailability is about 40% lower when

alendronate is taken 1 or ½ hour before a meal. Risedronate bioavailability is reduced by 55% if taken ½ hour before breakfast and 40% when taken 1 hour before breakfast. Ibandronate bioavailability is decreased substantially if taken less than 1 hour before a meal. Risedronate sodium is available as a delayed-release (enteric-coated) tablet intended to be taken with food. The bioavailability of the risedronate sodium 35 mg delayed-release tablet administered after a high-fat breakfast was like risedronate sodium 35 mg immediate-release tablet dosed 4 hours before a meal in one study and was approximately two- to four-fold greater than the immediate-release 35 mg tablet administered 30 minutes prior to a high-fat breakfast [39].

Distribution

A model for the pharmacokinetics of bisphosphonates is shown in Fig. 14.3. As the first N-BP developed for the treatment of osteoporosis, alendronate PK is the most thoroughly studied and is the focus of this presentation. The PK of other N-BPs and Non-N-BPs is similar, although some quantitative differences will be discussed. Elimination of alendronate after intravenous dosing has been studied in postmenopausal women [40] and PK and metabolism using [^{14}C] alendronate studied in women with breast cancer [41]. Data from animal studies are presented as some PK parameters cannot be assessed in humans.

Following IV administration in animals and humans, BPs are cleared from plasma with a half-life of 1–2 hours [42]. Less than 5% is found in non-calcified tissues 1 hour following an IV dose in animals. In contrast, 30% of alendronate found in bone 5 minutes after an infusion in rats, approximately 55% in bone after 1 hour, and approximately 62% at 6, 24 and 72 hours after infusion [43]. Less than 1% is present in non-calcified tissue 6 or more hours after infusion.

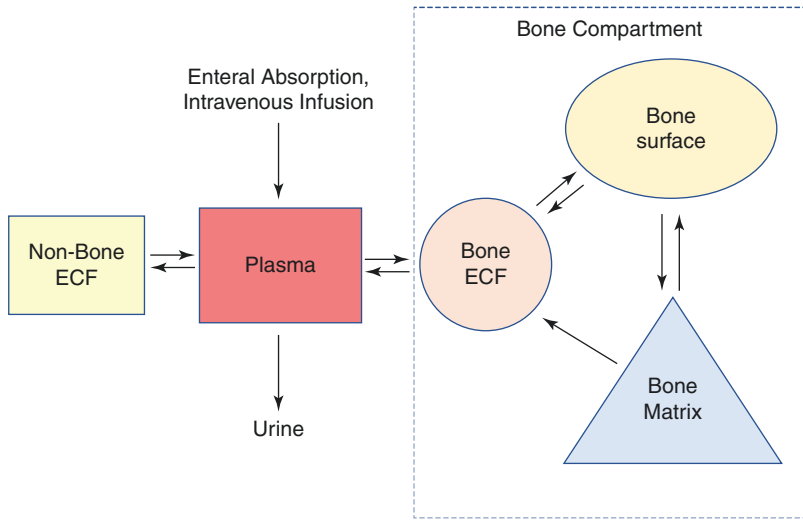


Fig. 14.3 Bisphosphonate pharmacokinetics model. Bisphosphonates (BPs) enter blood plasma after enteral absorption or intravenous administration and rapidly equilibrate with the Non-Bone ECF (extracellular fluid). Intracellular accumulation is negligible. BPs in Plasma flowing to the kidneys is eliminated in urine. Bisphosphonates in Plasma flowing to the Bone Compartment (dotted rectangle) initially enter Bone ECF. They may either bind to hydroxyapatite on Bone Surfaces or exit the Bone Compartment and re-enter systemic Plasma. The affinity of BPs for hydroxyapatite and very high "concentration" of binding sites on Bone Surfaces greatly favors binding to Bone Surfaces. Bone Surface BPs at sites of new bone formation may be incorporated into mineralized Bone Matrix. Other Bone Surface BP re-enter Bone ECF and may either re-bind to a Bone Surface or re-enter systemic Plasma. BPs in mineralized Bone Matrix may be resorbed by osteoclasts and re-enter Bone ECF or Bone Surface, but do not leave the Bone Matrix unless resorbed by osteoclasts

Distribution of bisphosphonates in bone is related to a combination of blood flow and rate of bone formation and resorption. It is illustrated by the localization of radionuclide in ^{99m}Tc-MDP bone imaging. In one report of inadvertent intra-arterial injection, the great majority of uptake was in the forearm distal to the injection site [11]. In studies in mice, 4 hours after dosing, [^{3}H]alendronate is found on bone surfaces preferentially localized under osteoclasts but not within osteoclasts or other bone cells [44]. Seven weeks post-dose, [^{3}H] alendronate is found within recently mineralized bone matrix, with very little remaining on bone surfaces.

Following an oral or intravenous dose, bisphosphonates are rapidly cleared from plasma and ECF and are found only on bone surfaces. Alendronate (and other N-BPs) are relatively tightly bound thus bone lining cells, osteoblasts and osteocytes are exposed to alendronate in Bone ECF. Osteoclasts are the only bone cell exposed to high levels of BPs. The resorption lacuna under an osteoclast is isolated and acidified to promote resorption of the hydroxyapatite. BPs on the bone surface are also mobilized and their concentration in resorption lacunae has been estimated to reach 1 mM [11]. While anionic bisphosphonates do not readily cross the plasma membrane of most cells, they enter osteoclasts through endocytosis of resorbed bone components in resorption lacunae. In animal studies, [^{3}H]alendronate is found with osteoclasts 12–15 hours after it is administered but not found within osteoblasts [44].

Metabolism

There is no apparent metabolism of N-BPs in humans or other animals studied. Non-N-BPs may be incorporated into analogs of ATP that are not hydrolysable and impair a variety of an osteoclast's metabolic processes [21]. As this occurs only in osteoclasts that take up non-N-BPs metabolism does not play an important role in the clearance of bisphosphonates.

Elimination

Renal excretion is the only route of elimination and occurs through both glomerular filtration and tubular secretion. The secretory transport mechanism has not been characterized but appears to be shared by alendronate and etidronate [42]. Bisphosphonates are not metabolized and the fraction excreted via the GI tract much less than 1% [43, 45].

Bisphosphonates differ from most drugs because approximately 50% of an absorbed dose is initially distributed to bone surfaces with a large fraction of that pool retained in mineralized matrix of newly formed bone of the skeleton, then slowly resorbed and eliminated by renal excretion over many years. BPs are not pharmacologically active if they remain within the mineralized bone matrix. However, if released by an osteoclast resorbing mineralized bone matrix containing BPs, the BP released may inhibit the activity of that osteoclast or be redistributed to other bone surfaces and inhibit other osteoclasts. "Recycled" BPs have the potential to add to the effect of continued treatment or provide persistent inhibition of bone resorption for years after the end of dosing.

Figure 14.3 provides a model of bisphosphonate pharmacokinetics that integrates data from clinical and non-clinical studies to describe elimination of bisphosphonates. It also provides a framework for estimating the skeletal accumulation of bisphosphonates over time and effects of prior treatment on bone resorption when treatment is either continued or interrupted. Features of the model follow.

The Bone Compartment (Fig. 14.3) contains three pools of BPs. Approximately 99% of BPs in the body more than 1 day after administration are in the Bone Compartment, and the great majority is on initially on a Bone Surface. Non-calcified tissue does not retain BPs. BPs enter the Bone Compartment by moving from blood Plasma in arteries entering bone to Bone ECF. BP in Bone ECF may bind to hydroxyapatite on the Bone Surface or re-enter venous blood Plasma leaving bone. The high affinity of BPs for hydroxyapatite

on Bone Surfaces and very high Bone Surface area results in retention of almost all BPs entering the Bone Compartment on the Bone Surface. BPs on the Bone Surface may re-enter Bone ECF but – due to the compartmentalization of BPs in bone – more often bind to a different Bone Surface rather than enter venous blood Plasma and exit the Bone Compartment to the systemic Plasma.

BPs on a Bone Surface where new bone matrix is calcifying are "trapped" in the Bone Matrix. BPs in Bone Matrix cannot re-enter either Bone ECF or Bone Surface pools unless an osteoclast resorbs the mineralized Bone Matrix that contains them. There is no physicochemical diffusion of Bone Matrix BPs to other pools. While BPs within Bone Matrix do not degrade over time, they are not pharmacologically active unless they are resorbed by an osteoclast. BPs on a Bone Surface not undergoing new bone formation may diffuse back into the Bone ECF. Thus, the local rate of new bone formation determines where in the skeleton BPs are deposited in new Bone Matrix. More BP accumulates in cancellous Bone Matrix where bone remodeling rates are high, and less BP accumulates on periosteal surfaces or in cortical bone where remodeling rates are relatively low. ^{99m}Tc-MDP bone scans illustrate wherein the skeleton bisphosphonates initially distribute and may subsequently accumulate [11].

Elimination kinetics of bisphosphonates are difficult to measure in humans for two reasons: (1) the concentrations in plasma are generally below the limit of accurate quantitation and (2) subjects must be followed for years to ensure that the terminal phase of elimination has been reached. As bisphosphonates are not metabolized and the only route of elimination is renal, measurement of total BP in 24-hour urine collections may be used to estimate the daily elimination rate. Terminal elimination half-life of alendronate was 200 days in rats and 3 years in dogs [42, 46]. Terminal elimination of BPs in humans has only been adequately studied for alendronate. Excretion of alendronate after IV administration daily for 4 days was tracked by measuring 24-hour excretion for up to 2 years [40]. Terminal elimination half-life was calculated in each patient by log-linear regression of the percentage retained versus time curve between days 240 and 540. Intermediate elimination half-lives were estimated after subtracting the residual retention. Approximately 50% of the alendronate dose was retained for 1 week and 17% of the dose retained at 1 week was excreted over the next 6 months. Over the next 12 months, only 2% of the alendronate retained at month 6 was excreted. The terminal elimination half-life calculated from the mean residual whole-body retention was 10.5 years (95% CI 5 7.9–13.2 years). An alternate regression method for calculating terminal elimination half-life provided a similar estimate (mean 10.9 years, range 5.4–19 years). The half-life for the first three phases were calculated as 0.80 days (days 4–7), 6.6 days (days 9–16) and 35.6 days (days 30–180). Elimination of risedronate has been evaluated in relatively short studies, with only 28 days of follow-up. Excretion described in Pharmacokinetics section of the U.S. Prescribing Information [34] notes that approximately half of the absorbed dose is excreted in urine within 24 hours. However, 85% of an intravenous dose was recovered in urine over 28 days. A "terminal exponential half-life" was reported as 561 hours (23.4 days). A 28-day follow-up is far too short to allow an estimated terminal elimination that includes BP in mineralized bone. Thus, what is reported as "terminal exponential half-life" probably represents the half-life of elimination of risedronate on the surface of bone. The true terminal elimination half-life of risedronate and other N-BPs is unknown but probably like that of alendronate.

Matching the data derived from elimination studies with the model results in several predictions. Over weeks to perhaps a few months after administration, excretion of BP reflects its concentration on a Bone Surface. Measuring excretion shortly after a dose of BP (e.g., weeks to a few months) provides a rough estimate of its half-life on the Bone Surface. In the study of alendronate, the half-life of the penultimate elimination phase was 35.6 days [40] and in the study of risedronate, the half-life measured over 28 days post administration was 23.4 days [34]. In summary,

the half-life of BPs on the Bone Surface is estimated to be 5 weeks for alendronate and 3 weeks for risedronate. In the absence of head-to-head data from an elimination study of any two bisphosphonates, it is not possible to know whether true differences exist.

The rate of accumulation of a bisphosphonate may be estimated if its bioavailability and long-term fractional skeletal retention (i.e., after 4–5 bone surface half-lives) are known. For alendronate with an oral bioavailability of 0.64% and 33% retention 6 months after IV administration [40], the skeletal accumulation would be about 21 μg per day after a 10 mg daily oral dose. Adjusting for the observed terminal elimination half-life, over 5 years of alendronate 10 mg daily (or 70 mg weekly), the total skeletal accumulation would be about 40 mg and over 10 years of 10 mg daily, about 75 mg.

The half-life of a BP in the Bone Matrix is substantially shorter than the terminal elimination half-life from the body, for two reasons. First, only 50% of BP leaving the Bone Compartment is excreted in urine. The other 50% re-enters the Bone Compartment and may be re-deposited in newly mineralizing Bone Matrix. Second, a portion of BP released from Bone Matrix binds to another Bone Surface and may be re-deposited in newly mineralizing Bone Matrix. After dosing is stopped, the concentration of BP on Bone Surfaces that is derived from resorption of alendronate with the Bone Matrix decreases with the same half-life as the terminal elimination half-life. To estimate the amount of BP recycled from bone during or after long-term treatment only the prior dose, duration of prior treatment, fraction of absorbed dose retained in bone and terminal elimination half-life are needed.

During long-term dosing, the BP re-cycled from the Bone Matrix represents an additional source of BP reaching the Bone Surface. The total BP reaching the Bone Surface may be estimated based on the BP in the Bone Matrix from prior administration, its half-life in the bone matrix and the current BP dose. It is estimated that after 10 years of treatment with oral alendronate (10 mg daily), the amount of alendronate released daily from the skeleton would be approximately 25% of that absorbed from the gastrointestinal tract [33]. If dosing were continued after 10 years of treatment, a 10-mg daily dose would have the effect of 12.5 mg, and if interrupted after 10 years of treatment, the post-treatment effect from re-cycled alendronate would be like a 2.5-mg daily oral dose. After 5 years of treatment with alendronate 10 mg daily, the post-treatment effect from re-cycled alendronate would be like a 1.5-mg daily oral dose. Based on the results of a dose-ranging study of osteoporotic women treated with 1, 2.5, and 5 mg daily [47], a 1.5-mg daily dose of alendronate would slow, but not fully prevent bone loss at the spine and proximal femur.

Pharmacokinetics of Bisphosphonates Administered at Weekly, Monthly, and Yearly Intervals

All the N-BPs administered orally (alendronate, risedronate, ibandronate and minodronate) were initially studied for the treatment of osteoporosis using a daily dosing regimen. Weekly dosing was proposed as it is more convenient, likely to be similarly effective as daily dosing and likely to have better gastrointestinal tolerability than daily dosing [48]. In addition, the early elimination half-life from the Bone Surface – the site of pharmacological activity – was approximately 3 (risedronate) to 5 weeks (alendronate) [40]. Thus, the concentration of alendronate on Bone Surfaces should vary only slightly when alendronate was dosed once weekly. Prior to clinical studies in osteoporotic subjects, studies in animals indicated that the effect of alendronate on bone mass was similar when dosed weekly or eight times per month if the same cumulative dose per month was used. When weekly dosing regimens of alendronate, risedronate and minodronate were developed, the cumulative dose was held constant. As fractional bioavailability of alendronate and risedronate of daily and weekly doses do not differ across the 5-mg daily to 70-mg dose range [45, 49], 35-mg weekly should result in the same cumulative absorption as 5-mg daily for 7 days and a 70 mg weekly dose would equal 10 mg daily for 7 days.

Therapeutic equivalence studies with BMD endpoints demonstrated equivalent BMD changes with daily versus weekly regimens of each drug [50–52]. As the 2.5 mg daily dose of ibandronate failed to reduce the risk of non-vertebral fractures, doses higher than 75 mg (2.5 mg/d × 30 d) were studied during the development of a once-monthly regimen. The cumulative absorbed dose of ibandronate 150 mg chosen for once-monthly treatment is estimated to be approximately four-fold higher than the cumulative absorbed dose with 2.5 mg daily for 30 days. First, the 150-mg monthly dose is two-fold greater than 2.5 mg daily for 30 days. Second, the fractional absorption of ibandronate is nonlinear at doses between 50 and 100 mg [36]. The AUC values, relative to the 50-mg dose, were 130% for the 100-mg and 191% for the 150-mg dose. The four-fold greater cumulative absorbed dose achieved with ibandronate 150 mg monthly dose produced greater lumbar spine and proximal femur BMD changes versus 2.5 mg daily after both 1 and 2 years of treatment [53, 54].

Zoledronate (zoledronic acid injection) is the only N-BP dosed by intravenous infusion once a year for the treatment of osteoporosis. Pharmacokinetic profile has only been studied for 28 days post infusion and an apparent elimination half-life during that brief follow-up was > ½ year [55]. While no adequate studies of zoledronate terminal elimination half-life have been published, it is likely to be similar to that of alendronate because its fractional uptake by bone, renal elimination and initial (28-day) pharmacokinetic profile are similar to alendronate.

Pharmacokinetics of Bisphosphonates After Treatment Is Discontinued

During chronic treatment with a bisphosphonate administered daily or weekly for at least 6 months concentrations on bone surfaces reach a steady state as reflected by biochemical markers of bone resorption that are constant during multi-year treatment. After discontinuation of bisphosphonate treatment, the concentration of drug on the surface of bone gradually decreases as BPs on bone surfaces are covered by newly formed bone matrix or dissociate, re-enter the general circulation and are terminally eliminated by the kidney (as illustrated in Fig. 14.3). Bone resorption increases at a rate proportional to its half-life on the surface of bone (estimated to be 5 weeks for alendronate and 3 weeks for risedronate). In a study of postmenopausal women treated for a median of 5.2 years, discontinuation of alendronate resulted in a mean increase of urine NTX/creatinine (N-terminal cross-linked telopeptides of type I collagen/creatinine) of 44.2% in 3 months and 63.6% in 12 months [56]. Serum CTX (C-terminal cross-linked telopeptides of type I collagen) increased 100.7% and 165.8% over the same periods. Zoledronic acid is administered once yearly as a single 5 mg IV resulting in a large increase in bone surface concentrations and a large decease in bone resorption. Serum CTX decreased (94%) as did urine NTX/creatinine (67%) 1 week after administration in a study of postmenopausal women [57]. Suppression of bone resorption waned over 3 months to approximately 80% and 57%, respectively. Six months after administration serum CTX was 74% less than baseline. In a separate study of osteopenic postmenopausal women, the decrease in serum CTX was 86% (1 month), 66% (12 months), and 48% (2 years) after a single dose of zoledronic acid 5 mg IV [58]. The same pattern of prompt reduction of bone resorption followed by gradual increase a stable level that is about 60% below baseline (prior to the initial dose) was observed after each of three annual doses of zoledronic acid [59]. The effect of a single dose of zoledronic acid (1, 2.5, or 5 mg IV) on BMD and biochemical makers of bone remodeling over 5 years was studied in a 3-year placebo-controlled study of postmenopausal women with a 2-year open extension [60]. Serum CTX decreased 74% from baseline in the 5 mg group at 1 year and remained below baseline after 2 (53%), 3 (42%), 4 (29%), and 5 (27%) years.

Inhibition of resorption a year or more after the last dose of a bisphosphonate is likely due to recycling of the drug deposited within the bone matrix, and the magnitude of the persistent effect

a function of the cumulative dose and potency of the bisphosphonate.

Bisphosphonate Mechanism of Action on Bone Strength

Several bisphosphonates have been shown to reduce the risk of fractures in osteoporotic postmenopausal women that are summarized later in this chapter. While this effect must be mediated through an increase in bone strength, it has not been established precisely how they increase bone strength. There are several potential mechanisms.

Bisphosphonates Strengthen Bone by Increasing Bone Mass

Several non-clinical and clinical studies have demonstrated that treatment with bisphosphonates prevents loss of bone due to estrogen deficiency. Bones of animals treated with bisphosphonates are stronger in ex vivo biomechanical testing in both bone loss prevention and osteoporosis treatment models. The currently marketed N-BPs have all been shown to reduce the risk of vertebral compression fractures, and several – alendronate, risedronate and zoledronate – have been shown to reduce the risk of hip and/or non-vertebral fractures in postmenopausal osteoporotic women. However, several lines of evidence indicate that changes in bone mass (as measured by DXA BMD) are only part of the story [61]. Small changes in BMD result in greater than expected reductions in fracture risk [62, 63] and fracture risk at spine and proximal femur is reduced long before maximal changes in BMD occur [64–66].

Bisphosphonates Strengthen Bone by Improving Bone Architecture

The strength of cancellous (trabecular) bone is determined in large part by the connectivity of the "plates" and "struts" of trabecular bone and is one feature of bone microarchitecture [67]. Trabecular connectivity decreases when cancellous vertebral bone is lost in osteoporosis and the trabecular connectivity of cancellous vertebral bone better correlates with compressive strength than simple assessment of bone mass [68]. Bisphosphonates have been demonstrated to preserve trabecular structure in animal studies. While the same preservation should occur in osteoporotic patients treated with bisphosphonates bone samples needed to test this hypothesis can't be obtained in human studies. Thus, preservation of trabecular connectivity remains a theoretical mechanism.

Bisphosphonates Reduce Abnormally High Bone Remodeling

While normal bone remodeling is essential to ensure the availability of calcium and to repair fatigue damage in bone, the high rate of bone remodeling in postmenopausal osteoporotic women (on average, three-times the premenopausal rate [69]) results in both progressive declines in trabecular and cortical bone mass. It also reduces bone strength by impairing microarchitecture through both loss of trabecular connectivity and creating stress risers at sites of bone resorption [70] where the resorption lacuna causes focal thinning of a plate or strut. Moreover, the material property of bone is (transiently) impaired as recently formed bone at sites of remodeling is relatively weak until fully mineralized [71]. While these hypotheses are very reasonable, they are very difficult to test experimentally. Bisphosphonates have been shown to reduce remodeling quickly to normal premenopausal rates and to produce some of their effect on BMD by closing the remodeling transient [72, 73]. The hypothesis that treatment with bisphosphonates increases bone strength by reducing both stress risers and the proportion of newly mineralized bone explains why reductions in fracture risk occur more quickly than changes in BMD. Unfortunately, it will remain an untestable theory until non-invasive in vivo micro-CT can measure bone microarchitecture and local density with at least ten-fold greater resolution.

Treatment of Osteoporosis with Bisphosphonates

Etidronate

Etidronate is a non-nitrogen containing bisphosphonate approved for treatment of postmenopausal osteoporosis in Canada and Europe in the early 1990s, but not in the United States. The phase III study of 429 postmenopausal osteoporotic women with 1–3 prior vertebral compression fractures utilized an intermittent cyclical treatment for 2 years. Each cycle included oral etidronate 400 mg daily for 14 days followed by calcium 500 mg (as carbonate) daily for 74 days, with cycles repeated during chronic treatment. While the vertebral fracture rate was lower in the etidronate group (42.3 vs. 62.9 per 1000 patient-years) after 2 years of treatment [74], the incidence was not different when treatment was extended to 3 years [75]. A meta-analysis of 13 clinical trials of intermittent cyclical etidronate, including 1010 study participants, suggested a reduction in vertebral fractures with a pooled relative risk of 0.6 (95% CI 0.41–0.88) and demonstrated no effect on non-vertebral fractures with a pooled relative risk of 1.00 (95% CI 0.68–1.42). Cyclic intermittent etidronate increased bone density in the lumbar spine and femoral neck after 3 years of treatment by 4.27% (95% CI 2.66–5.88) and 2.19% (95% CI 0.43–3.95), respectively [76]. A subsequent meta-analysis of eight studies that used different selection criteria reported lower risk of vertebral fractures (RR = 0.59; 95% CI 0.36–0.96) [77]. Neither hip nor other non-vertebral fracture risk was reduced. Due to its limited efficacy, use is no longer common.

Alendronate

Alendronate is approved worldwide for the treatment of osteoporosis in postmenopausal women (10 mg daily tablet, 70 mg weekly tablet and 70 mg oral solution or effervescent tablet). It is also approved for the treatment of osteoporosis in men and glucocorticoid-induced osteoporosis (GIOP) although the GIOP dose for men and premenopausal women is 5 mg daily and for postmenopausal women not receiving estrogen is 10 mg daily [78]. Alendronate is also approved for prevention of osteoporosis in postmenopausal women at 5 mg daily and 35 mg weekly doses.

Initial approval was based on pooled data from two identical double-blind, placebo-controlled studies together enrolling 994 postmenopausal women with spine BMD T-score ≤ -2.5 treated with alendronate (5 or 10 mg daily for 3 years or 20 mg for 2 years followed by 5 mg for 1 year) or placebo [79]. Treatment with alendronate was associated with a 48% reduction in the proportion of women with new vertebral fractures (3.2% vs. 6.2% in the placebo group; $p = 0.03$). Risk reduction was consistent in women < or ≥65 years and in those with or without prior fractures. A meta-analysis of 5 phase II or III studies of postmenopausal women treated with alendronate (or placebo) for at least 2 years found a non-vertebral fracture relative risk of 0.71 ($p = 0.048$) in those treated with alendronate [80].

The fracture intervention trial (FIT) was conducted to assess the effect of alendronate on fractures in 6459 postmenopausal women aged 55–81 years old with low femoral neck bone mineral density [64, 81]. FIT included two substudies. The FIT vertebral fracture study included 2027 women with a femoral neck BMD ≤ 0.68 g/cm^2 (Hologic DXA) and at least 1 prior vertebral fracture (confirmed by spine radiographs) who were randomly assigned placebo (1005) or alendronate (1022) and followed for 36 months. The dose of alendronate was initially 5 mg daily and was increased to 10 mg daily at 24 months, with maintenance of the double-blind. Seventy-eight (78) of women in the alendronate group had one or more new morphometric vertebral fractures compared with 145 in the placebo group (RR = 0.53; 95% Cl 0.41–0.68). Whereas, 23 women in the alendronate group and 50 women in the placebo group developed clinical (symptomatic) vertebral fractures (RR 0.45; 95% CI 0.27–0.72). The risk of any clinical fracture, which was the main secondary endpoint, was lower in the alendronate than in the placebo

group (139 [13.6%] vs. 183 [18.2%]; RR = 0.72; 95% CI 0.58–0.90). The relative hazards for hip fracture and wrist fracture for alendronate versus placebo were 0.49 (95% CI 0.23–0.99) and 0.52 (95% CI 0.31–0.87), respectively [81].

The FIT clinical fracture study enrolled 4432 postmenopausal women with femoral neck BMD ≤0.68 g/cm^2 (Hologic DXA) but without a baseline vertebral fracture [82]. The intent of the study was to enroll women with osteoporosis, defined as a baseline femoral neck BMD *T*-score ≤ −2.0 (based on 1992 normative data). However, due to subsequent revisions of normative values for femoral neck BMD [83], 31% of subjects enrolled were found to have femoral neck BMD *T*-scores between −1.6 and −2.0 based on the BMD *T*-score reference range in use when the results of the study were reported [83]. Treatments with alendronate and placebo were the same as the FIT vertebral fracture study, except that treatment was continued at the same dose for a mean of 4.2 years. Clinical fractures, the primary endpoint, occurred in 312 women (14.1%) in the placebo and 272 women (12.3%) in the alendronate group (RH, 0.86; 95% CI 0.73–1.01). A subgroup analysis in subjects with femoral neck BMD *T*-scores < −2.0 (the threshold for diagnosing osteoporosis at the time the study was conducted) found a 22% lower risk of clinical fracture in those treated with alendronate (RH, 0.78; 95% CI 0.65–0.94). An additional subgroup analysis in subjects with a femoral neck BMD *T*-scores < −2.5 found a similar lower risk of clinical fractures in the alendronate group (RH, 0.64, 95% CI 0.50–0.82). In the full group of FIT clinical fracture study subjects, alendronate reduced the overall risk of new radiographic vertebral fractures by 44%: 78 women (3.8%) in the placebo group had ≥1 new fracture versus 43 (2.1%) in the alendronate group (RR 0.56; 95% CI 0.39–0.80; p = 0.001. Unlike non-vertebral fractures, treatment with alendronate was associated with lower risk of vertebral fractures regardless of femoral neck BMD.

A key message from FIT is that alendronate reduces the risk of vertebral morphometric and clinical fractures in postmenopausal women with either severe osteoporosis with a prior vertebral fracture or a femoral neck BMD *T*-score in the osteoporotic range (femoral neck BMD *T*-score below −2.0) but without a prior fracture. Additional clinical trials would be needed to determine whether postmenopausal women who have low normal BMD *T*-scores (osteopenia) at either lumbar spine or total hip DXA regions of interest and no prior vertebral fractures are likely to benefit (lower risk of fracture) from alendronate treatment.

There are two long-term extension studies of alendronate. The 3-year phase III studies were extended to 10 years of treatment with alendronate 5 and 10 mg daily. The group initially treated with alendronate 20 mg daily for 2 years followed by 5 mg daily for 3 years was switched to placebo after a total of 5 years [84]. Treatment with alendronate 10 mg daily for 10 years produced mean increases from baseline in lumbar spine BMD of 13.7% (95% CI 12.0–15.5%), trochanter BMD of 10.3% (95% CI 8.1–12.4%), femoral neck BMD of 5.4% (95% CI 3.5–7.4% percent) and total hip BMD of 6.7% (95% CI 4.4–9.1%). Smaller increases occurred in the 5 mg daily group. The discontinuation of alendronate resulted in a gradual loss of effect, as measured by BMD and biochemical markers of bone remodeling. The FIT long-term extension (FLEX) evaluated randomization of FIT participants initially treated with alendronate to either continuation of alendronate, 5 or 10 mg/d for a total of 10 years, or treatment with placebo after approximately 5 year of treatment with alendronate [85]. A total of 1099 women were eligible and participated in the FLEX trial. The mean age of women was 73 years at FLEX entry and 60% reported a history of clinical fractures since menopause. Changes in spine and proximal femoral BMD in the 5 and 10 mg daily alendronate groups were not statistically significant, and data from the alendronate groups were pooled for comparison with placebo. Continuation of alendronate resulted in maintenance of BMD at proximal femoral BMD sites. Lumbar spine BMD increased by an additional 5.3% in the alendronate groups and by 1.5% in the placebo group. When compared to those continuing alendronate, switching to placebo for 5 years resulted in 2.4%

lower BMD at the total hip (95% CI −2.9% to −1.8%) and 3.7% lower BMD at the lumbar spine (95% CI −4.5% to −3.0%) [85]. Serum CTX, N-propeptide of type I collagen (P1NP) and bone-specific alkaline phosphatase (BSAP) were stable during continued treatment with alendronate (both 5 and 10 mg daily) and increased in patients switched to placebo: CTX by 55.6%, P1NP by 59% and BSAP by 28.1%. Among those who continued treatment with alendronate, there was a significantly lower risk of clinically recognized vertebral fractures (5.3% for placebo and 2.4% for alendronate; RR 0.45; 95% CI 0.24–0.85) but no significant reduction in morphometric vertebral fractures (11.3% for placebo and 9.8% for alendronate; RR 0.86; 95% CI 0.60–1.22).

Risedronate

Risedronate is a nitrogen containing bisphosphonate and is approved for the treatment of postmenopausal osteoporosis (5 mg daily, 35 mg once weekly, 75 mg on 2 consecutive days each month and 150 mg once monthly) and glucocorticoid-induced osteoporosis (5 mg daily) [78]. Risedronate is also approved for the prevention of postmenopausal osteoporosis.

Two, 3-year phase III studies with similar designs – VERT (Vertebral Efficacy with Risedronate Therapy) North American (NA) [86] and VERT-Multinational (MN) [87] evaluated the effect of treatment on vertebral fracture risk. VERT NA enrolled 2458 postmenopausal women <85 years old with either 1 vertebral fracture and Lumbar Spine BMD *T*-score ≤ −2 at baseline or ≥2 vertebral fractures without regard to BMD. Subjects were randomly assigned to receive oral treatment for 3 years with risedronate (2.5 or 5 mg/d) or placebo. Treatment with 5 mg/d of risedronate, compared with placebo, decreased the cumulative incidence of new vertebral fractures (a fracture in a vertebral body normal at baseline) by 41% (95% CI 18–58%) over 3 years. A similar 33% reduction was observed for new or worsening (an additional compression fracture of a vertebral body with a fracture present at baseline) vertebral fractures, the trial's primary fracture endpoint [34]. Over 3 years, the incidence of non-vertebral fractures was reduced by 39% (95% CI 6–61%). VERT MN enrolled 1226 postmenopausal women <85 years old with ≥2 radiographically confirmed vertebral fractures [87]. BMD was not an entry criterion. Treatment and vertebral fracture endpoints were the same as in VERT NA. Over 3 years, the new vertebral fracture risk in the risedronate 5 mg group was reduced by 49% versus placebo (RR 0.51; 95% CI 0.36, 0.73, $p > 0.001$). A similar 46% reduction of the risk of new or worsening vertebral fractures was also observed [34]. Non-vertebral fracture risk was numerically lower (RR = 0.67; 95% CI 0.44, 1.04; $p = 0.063$). A pooled analysis of the two vert studies found a 36% reduction in the relative risk of non-vertebral fractures. The risk of hip fractures was not significantly lower with risedronate group in the pooled analysis.

The Hip Intervention Program (HIP) study of risedronate consisted of 9331 postmenopausal women age 70 years to 89 years [88]. The 3 year study included two subgroups randomly assigned to receive treatment with oral risedronate 2.5 or 5 mg versus placebo: 5445 women aged 70–79 years old who had osteoporosis (indicated by either a femoral neck *T*-score ≤ −4, or femoral neck *T*-score ≤ −3 and at least one non-skeletal risk factor for hip fracture) and 3886 women aged ≥80 years old who had at least one non-skeletal risk factor for hip fracture (BMD not measured) or were osteoporotic (femoral neck *T*-score ≤ −4, or femoral neck *T*-score ≤ −3 with a hip-axis length ≥ 11.1 cm). While the initial analysis plan was to compare the risk of hip fractures in each risedronate group (2.5 and 5-mg) with that in the placebo group, the lower than anticipated hip fracture incidence resulted in a change to a pooled analysis of patients in both the 2.5 and 5-mg groups with the placebo group. The incidence of hip fracture among all the women assigned to risedronate (either 2.5 or 5 mg/d, was 2.8%, as compared to placebo 3.9 (RR 0.7; 95% CI 0.6–0.9). Within the group of women aged 70–79 with osteoporosis, the incidence of hip fracture among those assigned to risedronate was

1.9% versus 3.2% in the placebo arm (RR 0.6; 95% CI 0.4–0.9, $p = 0.02$). In these younger women, the effects of the 2.5-mg and 5.0-mg doses of risedronate were similar; the relative risk of hip fracture for the 2.5-mg dose was 0.5 (95% CI 0.3–0.9) and that for the 5.0-mg dose was 0.7 (95% CI 0.4–1.1). Within the group of women aged ≥80 years, there was no significant effect on risedronate on incidence of hip fracture [88]. The risk of hip fracture in the entire study population was not reported by dose. In an analysis of all the women, the incidence of non-vertebral fractures was 9.4% among those assigned to risedronate, as compared with 11.2% among those assigned to placebo (RR 0.8; 95% CI 0.7–1.0; $p = 0.03$).

A meta-analysis of eight randomized clinical trials demonstrated the pooled relative risk for vertebral fractures in women given 2.5 mg or more of risedronate was 0.64 [CI 95% 0.54–0.77] [89]. In those patients with non-vertebral fractures given risedronate 2.5 mg or more, the pooled RR was 0.73 (95% CI 0.61–0.87). The use of risedronate demonstrated an improved BMD at the lumbar spine, combined forearm and femoral neck and was generally more improved with use of the 5-mg daily dose in comparison to the 2.5-mg dose [90].

Ibandronate

Ibandronate is a nitrogen-containing bisphosphonate approved for the treatment of postmenopausal osteoporosis. The drug was initially approved as a 2.5-mg daily oral dose. However, the drug was not marketed until the approval of additional formulations for treatment of osteoporosis in postmenopausal women: 150 mg oral tablets dosed monthly and 3 mg IV injection dosed every 3 months [78]. Ibandronate differs from the other approved N-BPs as studies of its effect on non-vertebral or hip fractures have not been demonstrated in a clinical trial.

In a phase III trial that enrolled 2946 postmenopausal women with 1–4 vertebral fractures and lumbar spine BMD T score ≤ -2 at one or more L1-L4 vertebrae. Patients received oral ibandronate 2.5 mg daily, intermittent ibandronate 20 mg every other day for 12 doses every 3 months, placebo for 3 years. Over 3 years, the incidence of new vertebral fractures was reduced in patients receiving 2.5 mg daily (4.7%) and intermittent ibandronate 20 mg (4.9%), relative to placebo (9.6%); relative risks were 0.62 (95% CI 43–75; $p < 0.001$) and 0.50 (95% CI 26–65), for daily and intermittent groups, respectively. However, non-vertebral fracture incidences were similar in all groups: 9.1%, 8.9% and 8.2% in the 2.5 mg ibandronate, and intermittent 20 mg ibandronate, and placebo groups, respectively [91]. Explanations for the failure to show non-vertebral fracture risk reduction include an insufficient dose of ibandronate and relatively high mean femoral neck BMD T-score (−2.0), an inadequate number of fractures resulting in limited statistical power or a combination of the three. Whatever the reason, a well-designed non-vertebral or hip fracture endpoint study was never conducted.

Intravenous administration of ibandronate in the Dosing IntraVenous Administration (DIVA) Study, patients received 2 mg injections every 2 months, 3 mg injections every 3 months, or daily oral ibandronate 2.5 mg for a total of 2 years of treatment. The mean lumbar spine BMD increased by 5.1% in the 2 mg arm, 4.8% in the 3 mg arm, and 3.8% in the daily oral arm [92, 93]. No fracture endpoint studies of intravenous ibandronate have been presented.

To evaluate the effectiveness of monthly oral Ibandronate, 1609 postmenopausal women were randomized in the MOBILE (Monthly Oral ibandronate in Ladies) trial to different oral monthly regimens: two 50 mg tablets, one 100 mg tablet, one 150 mg tablet once monthly and compared all other drug groups to patients treated with 2.5 mg daily. All monthly regimens proved to be non-inferior to 2.5 mg daily and the 150 mg monthly dosing was superior to the 2.5 mg daily dosing in regard to increasing lumbar spine bone mineral density [54]. As noted in Bioavailability section of this chapter, greater percentage of an ibandronate 150 mg oral dose is absorbed versus the percentage of a 50 mg dose that is absorbed. Thus, the amount of ibandronate absorbed after a 150 mg monthly dose is about four times more

than the cumulative absorption of ibandronate 2.5 mg daily for 30 days.

Zoledronic Acid (Zoledronate)

Zoledronic acid (zoledronate) is approved for the treatment and prevention (5 mg by intravenous infusion over at least 15 minutes once yearly for treatment and once every 2 years for prevention) of osteoporosis in postmenopausal women. It is also approved to improve bone mass in men with osteoporosis and for the prevention and treatment of osteoporosis in men and women expected to be on glucocorticoid therapy for at least 12 months [78]. Zoledronate is the active moiety although the name zoledronic acid is generally used in the medical literature.

The effect of zoledronic acid on vertebral and hip fractures was shown in a 3-year, double-blind, placebo-controlled study: HORIZON PFT (Health Outcomes and Reduced Incidence with Zoledronic Acid Once Yearly, Pivotal Fracture Trial) [59]. A total of 7765 postmenopausal women, 65–89 years old, femoral neck BMD *T*-score ≤ -2.5, were randomized to receive intravenous (IV) zoledronic acid 5 mg or placebo at baseline, 12 and 24 months. Treatment with zoledronic acid reduced the incidence of vertebral fracture by 70% over 3 years, versus placebo (3.3% in the zoledronic-acid group vs. 10.9% in the placebo group; RR 0.30; 95% CI 0.24–0.38) and reduced the risk of hip fracture by 41% (1.4% in the zoledronic-acid group vs. 2.5% in the placebo group; HR 0.59; 95% CI 0.42–0.83). Non-vertebral fractures, clinical fractures, and clinical vertebral fractures were reduced by 25%, 33%, and 77%, respectively [59].

The HORIZON Recurrent Fracture Trial was a randomized, double-blind, placebo-controlled study of 2127 postmenopausal women (76%) and men (24%) with a recent hip fracture (within 90 days). There was no BMD entry criterion. Patients received yearly zoledronic acid 5 mg IV or placebo infusions. Study duration was event-driven and approximately 4% received four doses, 27% received three doses, 39% received two doses, and 30% received one dose. The incidence of any new clinical fracture was 8.6% in the zoledronic acid group and 13.9% in the placebo group, which is a 35% risk reduction (HR 0.65; 95% CI 0.50–0.84). The incidences of a new clinical vertebral fracture were 1.7% and 3.8% (HR 0.54; 95% CI 0.32–0.92), and the risk of hip fractures was numerically lower (HR 0.7; 95% CI 0.41–1.19; $p = 0.18$). The risk of death was also lower in the zoledronic acid group (HR 0.72; 95% CI 0.56–0.93; $p < 0.01$) [94].

Duration of treatment was examined in cohorts of patients who participated in HORIZON PFT, in two 3-year extension studies. In the first extension, 1233 postmenopausal women who received zoledronic acid for 3 years were randomized to three additional yearly doses of zoledronic acid 5 mg ($n = 616$) or placebo ($n = 617$) [95]. Continued treatment with zoledronic acid was associated with stable BMD while small decreases in proximal femoral BMD. The risk of new morphometric vertebral fractures was reduced by 49% with continued treatment but no effect on non-vertebral fractures was observed. Patients were eligible for the second extension study if they completed the first extension study on treatment and without major protocol violations. The second extension study enrolled 95 patients randomized to treatment with three additional yearly doses of zoledronic acid 5 mg IV and 95 randomly assigned to receive placebo infusions [96]. Continued treatment was associated with less loss of total hip BMD but the between group difference was not statistically significant. The incidence of fractures was low and there were no statistically significant between group differences.

Minodronate

Minodronate is a nitrogen-containing bisphosphonate agent developed and currently approved for use in the treatment of osteoporosis in Japan [97]. It appears to be the most potent N-BP administered orally but has not been compared with zoledronate (intravenous) [98]. The phase III study enrolled 704 postmenopausal women aged 55–80 years old with fragility fractures who were randomized to minodronate 1 mg or pla-

cebo once a day and treated for 2-years [99]. Minodronate reduced the incidence of vertebral fractures by 59% (95% CI 36.6–73.3%). Subjects who completed the 2-year study were invited to participate in an additional 1-year extension in which all subjects were to receive minodronate. Over the third year, new vertebral fracture incidence was similar to that observed in the first 2 years of treatment (no formal statistical comparison). In patients who received minodronate for 3 years, lumbar spine BMD increased 10.4% from baseline. Urine NTX/creatinine decreased 50% versus placebo and approximately 60% from baseline at month 24, and Serum Bone Alkaline Phosphatase decreased 46% from baseline at month 6 and approximately 52% at month 24. Bone turnover markers remained constant thereafter over 3 years [100]. No studies have been conducted in North America or Europe.

Clodronate

Clodronate is a non-nitrogen containing bisphosphonate approved in some countries to reduce the occurrence of bone metastases in post-surgical treatment of breast cancer patients and to treat hypercalcemia of malignancy. Clodronate can be administered orally or intravenously [101]. While not studied in the United States (and not approved), several studies have evaluated its benefit for the treatment of postmenopausal osteoporosis. A 3-year randomized placebo-controlled trial of oral clodronate 800 mg/day included 5596 women ≥75 years old, without regard to BMD or fracture history [102]. Vertebral fractures (at baseline or incident) were not evaluated. While the incidence of hip fractures was not reduced (hazard ratio [HR] 1.02; 95% CI 0.71–1.47), the risk of any clinical fracture was reduced by 20% (HR 0.80; 95% CI 0.68–0.94 [102].

Comparative Efficacy Between Bisphosphonates

Head-to-head fracture endpoint trials of bisphosphonates have not been conducted as it is difficult to demonstrate differences in fracture risk with precision without studying a very large number of osteoporotic patients (20,000 or more). Only BMD changes may be compared in studies of reasonable size (e.g., 1000 subjects). In two 12-month, head-to-head studies of alendronate 70 mg weekly versus risedronate 35 mg once weekly – the doses approved for the treatment of osteoporosis in postmenopausal women – significant differences in bone mineral density were seen as early as 6 months at all BMD sites [103, 104]. The primary endpoint of these trials was a comparison in the change in bone mineral density of the trochanter. Secondary endpoints were differences in bone turn over markers and bone mineral density of total hip, femoral neck, and lumbar spine. Significantly greater increases in hip trochanter BMD were seen with alendronate (3.4%) than risedronate (2.1%) at 12 months (treatment difference, 1.4%; 95% CI 0.8–1.9%) as well as 6 months (treatment difference, 1.3%; $p < 0.001$). Significantly greater gains in BMD were seen with alendronate at all BMD sites measured (12-month difference: total hip, 1.0%; femoral neck, 0.7%; LS, 1.2%) [103]. In the second study, mean increases from baseline in hip trochanter BMD at month 12 were 3.56% (alendronate) and 2.71% (risedronate) (treatment difference 0.83%; 95% CI 0.22–1.45). Treatment differences were maintained during a second year of treatment in both studies [105, 106]. The results were replicated in a second study of identical design. Alendronate also produced greater reductions in bone turnover markers as early as 3 months. Tolerability (all adverse event and gastrointestinal adverse events) was similar for both agents.

Minodronate 1 mg daily and alendronate 5 mg daily were compared in a study of 270 Japanese postmenopausal osteoporotic women, who were randomized to either alendronate 5 mg daily or minodronate 1 mg daily for 12 months. After 1 year of treatment, the lumbar spine BMD increased by 5.86% and 6.29% in the minodronate and alendronate groups, respectively, and the total hip BMD increased by 3.47% and 3.27%, respectively [107].

Comparative Efficacy Between Bisphosphonates and Other Anti-resorptive Drugs

There is not data that support a clinically important effect a bisphosphonate the need for concurrent treatment to treat osteoporosis. Randomized controlled trials of the different bisphosphonates with other anti-resorptive agents, such as raloxifene, denosumab, hormone therapy, and anabolic therapy have been conducted in postmenopausal women evaluating changes in bone mineral density and effects on bone turn over markers.

In the EFFECT (Efficacy of Fosamax versus Evista Comparison Trial), 487 postmenopausal women with *T*-scores <−2 at the lumbar spine or hip were randomized to receive either alendronate 70 mg once weekly and daily placebo identical to Raloxifene or Raloxifene 60 mg daily and weekly placebo identical to alendronate for 12 months. Alendronate demonstrated substantially greater increases in BMD than raloxifene at both lumbar spine and hip sites at 12 months. Lumbar spine BMD increased 4.8% with alendronate versus 2.2% with raloxifene ($p < 0.001$); total hip BMD increased 2.3% with alendronate versus 0.8% with raloxifene ($p < 0.001$). Reductions in bone turnover were significantly larger with alendronate than raloxifene [108].

There was limited analysis of head-to-head trials of different bisphosphonates in comparison to denosumab. One meta-analysis demonstrated that denosumab more effective than both alendronate and risedronate with odds ratio of 1.67, 95% CI 1.06–2.67 and 1.84, 95% CI 1.16–2.92 [109].

A meta-analysis of 1967 patients from eight randomized clinical trials was analyzed and aimed to compare the efficacy of teriparatide versus bisphosphonates in the treatment of osteoporosis. Teriparatide significantly increased the bone mineral density of the lumbar spine, femoral neck, and total hip versus bisphosphonate treatment [110].

Use of Bisphosphonates After Bone Anabolic Drugs

As described in Chap. 18, Combination Anabolic/Antiresorptive Therapy in Osteoporosis Treatment, concurrent treatment with both parathyroid hormone [hPTH(1-84)] and alendronate appears to offer no advantage over either agent given alone [111]. There are no adequate studies of concurrent treatment with any bisphosphonate and ether teriparatide, abaloparatide, or romosozumab. However, treatment with bone anabolic drugs is limited to 1–2 years and the bone anabolic effects are lost if treatment is not followed with an anti-resorptive drug. Clinical trials have demonstrated progressive increases in spine and proximal femoral BMD when hPTH(1–84) is followed by alendronate [112] and fracture risk reduction when abaloparatide [113] and romosozumab [114] are followed by alendronate, as discussed in Chaps. 18 and 19, respectively.

Monitoring Bisphosphonate Therapy in Clinical Practice

The measurements of biochemical markers of bone resorption and formation provide important information on a drug's effects on bone remodeling. Changes in biochemical markers of bone turnover remain key endpoints in phase II dose-ranging clinical trials of osteoporosis drugs. Moreover, the greater decreases in bone turnover markers were associated with greater fracture risk reduction in clinical trials of patients treated with bisphosphonates [115]. However, the utility of biochemical markers to monitor bisphosphonate therapy in individual osteoporotic patients is limited for several reasons. The precision error of the assays used to measure bone resorption (CTX and NTX/creatinine) is good but there is a large diurnal variation (higher in the morning than late afternoon [116] of both serum CTX and urine NTX/creatinine in individuals. Moreover, there are substantial day-to-day variations in bone resorption markers in repeat testing of the same individual. Together, precision error of the methods, diurnal and day to day biological variation make it difficult to accurately measure the magnitude of reduction in serum CTX or urine NTX/creatinine in individual patients. Despite limitations, biochemical markers occasionally measured in clinical practice and failure to observe the anticipated decrease the bone turnovers mark-

ers prompt further investigation to evaluate a cause and consideration of changing treatment [117]. If there is a clinical need (e.g., to assess compliance) to measure the effect of bisphosphonate treatment on bone turnover, serum P1NP (N-terminal pro-peptide of type I collagen) has several practical advantages. The precision error of the method is low, and there is minimal diurnal variation or day-to-day biological variation. The reference range in postmenopausal women is narrower than the other bone turnover markers and the reduction observed with standard treatment doses of oral bisphosphonates is 60–65%. Bisphosphonate effects on bone formation are not direct but linked to their effects on osteoclast-mediated bone resorption, thus they occur about 3 months after the maximal effect on bone resorption. One of most common reasons for small or no apparent decrease in bone turnover is non-compliance. If bisphosphonates are taken with food (rather than fasting in the morning), they will be poorly absorbed. Patients who experience gastrointestinal symptoms may discontinue treatment, yet not report the discontinuation to their physicians. In addition, patients worried about rare potential side effects may decide to forego treatment or discontinue treatment without discussing their fears with the prescribing physician (Chap. 24). Thus, a careful review of actual bisphosphonate use with a patient is the first step in evaluating why bone turnover markers do not decrease during bisphosphonate use.

Selecting Bisphosphonate Therapy

While guidelines provide direction on which patients should receive osteoporosis therapy, they do not provide specific recommendations on what drugs clinicians should prescribe in various situations. Choice of treatment needs to be individualized based on efficacy, cost, safety, and side effect profile [118]. The drugs most commonly used for treatment of osteoporosis are bisphosphonates [3]. All bisphosphonates have been shown to significantly reduce the risk of vertebral, non-vertebral, and hip fractures in random clinical trials of postmenopausal women with osteoporosis [59, 91, 119, 120]. Like all other treatment options, bisphosphonates are associated with both short and long-term safety issues; however, they are often well tolerated, more cost-efficient.

Safety concerns including GI intolerability, osteonecrosis of the jaw, and atypical femur fractures need to be considered by both patients and clinicians before considering bisphosphonate therapy. For patients with gastrointestinal side effects, oral bisphosphonates should be deferred to IV formulations. Due to long-term safety concerns, clinicians should still consider use of bisphosphonates but consider a drug holiday after prolonged duration of use [118]. In younger patients, perhaps use of other options, including hormone therapy or SERM may be appropriate for first-line treatment and then, eventually, these patients may be switched to bisphosphonates. Another indication for bisphosphonate use includes its use after completing 1–2 years of anabolic therapy to maintain bone mineral density gains [118].

Review of Treatment Guidelines

The different medical societies, disease interest groups, and health authorities have different recommendations in terms of their recommendations on the indication, use and duration of treatment in regard to bisphosphonate therapy. In 2014, the National Osteoporosis Foundation (NOF) released the Clinicians Guide to Prevention and Treatment of Osteoporosis. While NOF does not endorse an exact algorithm, the foundation supports the use of bisphosphonates, including alendronate, ibandronate, risedronate, and zoledronate. While they review the indication of each drug, the guide does not comment on duration of treatment and when a drug holiday should be considered [78]. In 2015, ASBMR (American Society for Bone and Mineral Research) released its report on managing osteoporosis in patients on long-term bisphosphonate treatment. The Task Forces suggested approach for long-term therapy and recommendation of drug holiday is based on limited evidence of

vertebral fracture reduction in mostly white postmenopausal women. There is limited evidence on applying this approach to men and patients with glucocorticoid-induced osteoporosis. The Task Force suggests that after 5 years of treatment with oral bisphosphonate or 3 years of treatment of intravenous bisphosphonate, reassessment of risk should be considered. In women at high risk, such as those with high hip *T*-scores, high fracture risk, those with previous osteoporotic fractures or those women who fracture on therapy, they recommend the consideration of treatment for up to 10 years of oral therapy or 6 years of IV therapy. In this subset of patient, the task force comments that while the risk of atypical femur fractures increases with increased duration of use, but not osteonecrosis of the jaw, such rare events are outweighed by vertebral fracture risk reduction. For women not at high risk, a drug holiday of up to 3 years should be considered in those who have completed between 3 and 5 years of treatment [121].

In 2016, AACE released its clinical practice guidelines for the diagnosis and treatment of postmenopausal osteoporosis. They recommend initiation of therapy with one of four agents, which include alendronate, risedronate, zoledronic acid, and denosumab on the basis of their "broad spectrum" of anti-fracture efficacy. The Committee recommends initiating treatment with oral agents in those with lower to moderate risk fracture risk. Those patients with higher risk, those who are forgetful or have trouble coordinating with other agents or those with GI intolerance that may not tolerate or absorb the medication, it is recommended they consider zoledronic acid. They recommend consideration of drug holiday after 5 years of oral therapy in moderate-risk patients, and drug holiday after 6–10 years of stability in high-risk patients. In regard to high-risk patients on the IV formulation, they recommend the consideration of a drug holiday after 3 annual doses in moderate-risk patients and after 6 annual dosages in high-risk patients [122].

In 2017, ACP released its controversial clinical guidelines on the treatment of low bone density or osteoporosis to prevent fractures in men and women. The guideline recommends pharmacologic treatment with alendronate, risedronate, or zoledronic acid to reduce the risk of hip and vertebral fractures in women with known osteoporosis. The use of ibandronate is not included as first-line pharmacologic therapy as its studies have not been shown beneficial in reduction of all fracture types. They also comment that women should be treated for 5 years with the consideration that continuing treatment past 5 years may be beneficial in certain patients; however, they do not comment on who those patients are [89].

Bisphosphonate Adverse Drug Reactions

Use of bisphosphonates for the treatment of osteoporosis is relatively safe and has few common systemic side effects. The full Prescribing Information of each drug should be carefully reviewed for a detailed review of potential adverse drug reactions. The following section reviews several types of potential ADRs that are of most common interest.

Esophagitis and Gastrointestinal ADRs

Oral bisphosphonates are associated with gastrointestinal side effects, including nausea, esophagitis and possibly development of gastric ulcers [123]. The risk of esophageal side effects can be minimized by taking bisphosphonates as stated in Prescribing Information: with a full glass of water, remaining upright (sitting, standing, or walking) after dosing and eating before lying down again. Most important, patients should be made aware of symptoms of esophagitis and interrupt dosing if those symptoms develop. In general, fewer serious gastrointestinal adverse events have been reported with weekly than with daily regimens. Intravenous bisphosphonates are not associated with esophageal or other gastrointestinal side effects.

Acute Phase Response-Like Reactions

While all N-BPs may be associated with an acute phase response-like reaction (APR-like

reaction) when administered at a sufficiently high intravenous dose, [124] they were not observed in clinical studies of oral N-BPs administered daily or weekly. In 30% of patients, use of IV zoledronic acid may be associated with an APR-like event after its first administration. However, many patients do not experience an APR with subsequent infusions. Symptoms begin 12–24 hours after infusion and may include myalgia, arthralgia, low-grade fever, and bone pain, all of which generally resolve after 2–4 days [125]. Adverse events similar to an APR may occur in a small proportion of patients receiving ibandronate 150 mg monthly [35].

Atypical Femur Fractures

The definition of an atypical femoral fracture (AFF) was first proposed by an ASBMR working group in 2010 [126] and with criteria revised in 2013 [127]. An AFF must be in the femoral shaft and have specific radiographic appearance. ASBMR Working Group criteria include the presence of at least 4 of 5 major criteria. Other groups have proposed more rigorous criteria that reflect a lower incidence of AFFs but a greater frequency of bisphosphonate use [128]. In women and men, the age-adjusted relative risk of bisphosphonate use in patients with atypical fracture associated was 55 (95% CI 39–79) and 54 (95% CI 15–192), respectively. In bisphosphonate users, women had a three times higher risk than men (RR = 3.1; 95% CI 1.1–8.4) of developing an atypical fracture [129]. Meta-analyses, which includes 11 studies – 5 case control and 6 cohort studies, have determined an increased risk of femoral shaft and subtrochanteric femoral fractures that have a specific radiographic appearance with use of bisphosphonates [125]. The risk of atypical fractures appears to increase after 4 years of treatment and decline rapidly (by 70% after 1 year) when treatment is discontinued [129]. As AFFs are much less common than hip fractures, the benefit of preventing hip fractures outweighs the risk of AFFs in osteoporotic patients [130]. The pathophysiology resulting in atypical femur shaft fractures remains unclear. Reviews of potential mechanisms are in the ASBMR Working Group publication [127]. The geometry of the proximal femur may also affect the likelihood of developing an atypical femur fracture. The occurrence of proximal femoral varus is described in patients with atypical femoral fractures, and there is weaker evidence that narrow femoral neck and thicker lateral and medical bone cortices of the femoral shaft may predispose patients to developing these types of fractures [131]. Atypical femur fractures were found to be more common with long-term therapy in Asian (China and South East Asia) women when compared to Caucasian women [132]. The risk of atypical femur fractures increases with duration of use, generally after 3–4 years, and increases to further after 6 years [125]. Other identifiable risk factors for the development of atypical femur fractures include a higher body mass index, use of statin, use of oral glucocorticoids and use of proton pump inhibitors [125].

To reduce the risk of AFFs in clinical practice, it is important to establish the diagnosis of osteoporosis based on BMD in the osteoporotic range and to limit the duration of therapy to 3 or 4 years based on placebo-controlled trial data available. Longer term treatment should be prescribed based on individual patient characteristics. As some AFFs present as incomplete fractures, patients should be made aware that new hip or thigh pain may be due to an incomplete AFF and they should be evaluated for an AFF if symptoms develop during long-term N-BP use.

Osteonecrosis of the Jaw

Anti-resorptive related osteonecrosis of the jaw (ONJ) is defined as an area of exposed bone in the maxillofacial region that does not heal within 8 weeks in an individual who has received an anti-resorptive agent. The pathophysiology of ONJ has not been established and many highly speculative hypotheses have been proposed. Potential mechanisms have been reviewed by expert groups [133–135].

This entity was first described with bisphosphonate use in oncology patients treated with very high intravenous doses of bisphosphonates [136,

137]. ASBMR and American Association of Oral and Maxillofacial Surgeons (AAOMS) working groups created position statements on ONJ in 2007 [138] and 2009 [139], respectively. The ASBMR definition of ONJ does not require exposure to bisphosphonates while the AAOMS definition required the use of a bisphosphonate. It is been modified to include the use of denosumab or BPs. The key diagnostic criteria are "exposed bone in the maxillofacial region that has persisted for more than 8 weeks" and "No history of radiation therapy to the jaws." The risk of ONJ is relatively high in cancer patients treated with high doses of either zoledronic acid or denosumab for bone metastases [140]. In one controlled trial of denosumab and zoledronic acid in patients with lung cancer metastatic to bone, the cumulative incidence of osteonecrosis of the jaw was similar between groups (0.7% denosumab vs. 0.8% ZA) [141]. Risk between 1% and 2% per year has been reported in longer studies of zoledronic acid. In contrast, incidence of ONJ in patients with osteoporosis is estimated to be between 0.01% and 0.001% and is based on series of case series, retrospective observation studies and retrospective cohort data [133, 135, 140]. A total of five controlled clinical studies have been evaluated and only two patients (one receiving zoledronic acid and one receiving placebo) developed ONJ. Summarizing the five clinical trials, a total of 5903 patients were treated with zoledronic acid and the incidence of ONJ was less than 1 patient in 14,200 patient treatment years. In the HORIZON study, 7765 postmenopausal women were randomized to zoledronic acid versus placebo, and only two women developed ONJ. One patient was receiving placebo and prednisone whereas the second patient received zoledronic acid and developed a dental abscess [142].

The risk of ONJ in osteoporotic patients is so low that prospective studies of measures that could reduce the risk cannot be conducted. In patients with cancer metastatic to bone, several measures have been shown to reduce the risk of ONJ. First, treatment of any dental conditions that increase the risk of infection (or tooth extraction, e.g., periodontitis or extensive carries) should be aggressively treated, ideally before therapy is initiated. When tooth extractions or other oral surgical procedures during antiresorptive treatment are necessary, the risk of ONJ is lower when measure to prevent infection are followed, including use of antimicrobial mouth rinses, the use of antibiotics before and after oral surgery, and post-surgical follow-up to ensure complete healing has occurred to oral surgery prior to initiation of therapy with antiresorptive therapy [134]. Currently, there is no evidence that interrupting treatment with bisphosphonates in patients requiring oral surgery reduces the risk of ONJ, though this is still often suggested by practicing clinicians as a brief interruption of treatment is unlikely to have negative consequences.

Other ADRs

Other less common adverse events include the association of atrial fibrillation, as suggested in a phase III trial of zoledronic acid versus placebo (1.3% vs. 0.5%) [59] and atrial fibrillation is a labeled potential adverse drug reaction for that drug. However, meta-analysis confirmed no such association with oral alendronate [143]. Musculoskeletal pain (bone, muscle, and/or joint) may develop in patients treated with oral alendronate and other bisphosphonates and may occasionally be severe. While it may occur earlier, onset is generally several months after starting the drug. Pain resolves over 2–3 weeks when treatment is interrupted. While the pain may not recur when treatment is restarted, a subset of patients experience recurrence when rechallenged with the same or another bisphosphonate. Patient should be made aware of this potential ADR and advised to interrupt treatment and contact their health care provider if symptoms develop. Additionally, the development of uveitis, scleritis and orbital inflammation has been established with use of both oral and IV bisphosphonates. In 1054 postmenopausal women, 14 individuals who received IV zoledronic acid 5 mg developed ocular symptoms within 3 days of infusion. The inflammation generally resolved, and no patients had permanent visual impairment [144].

There are additional potential ADRs associated with each bisphosphonate and pharmacovigilance involves continued monitoring of adverse events for each marketed bisphosphonate.

Conclusions

Bisphosphonates are analogs of pyrophosphate that share an affinity for hydroxyapatite. While the P-O-P bond of pyrophosphate may be hydrolyzed, bisphosphonates have a P-C-P structure, and the central carbon may be chemically modified to produce a molecule with a range of pharmaceutical properties. Several nitrogen-containing bisphosphonates (N-BPs) have been shown to both localize to bone surfaces and produce selective inhibition of osteoclastic bone resorption at concentrations that do not inhibit bone mineralization. The molecular mechanism of action of simple bisphosphonates is through incorporation into adenine nucleotides that are non-hydrolysable analogs of ATP. This results in inhibition of a variety of metabolic processes that require ATP and results in osteoclast cell death. N-BPs inhibit farnesyl diphosphate synthase of osteoclasts resulting in decreased osteoclast metabolic activity and decreased bone resorption. High levels of N-BPs may also result in osteoclast apoptosis. At a tissue level, adequate doses of N-BPs result in a decrease in bone resorption that occurs with each bone remodeling cycle and a decrease in the rate of bone remodeling from the accelerated rate found in untreated postmenopausal women with osteoporosis into the range found in premenopausal women. Reduction in the risk of both vertebral fractures and hip fractures has been demonstrated with both oral bisphosphonates administered daily and intravenous bisphosphonates administered one time per year. Daily and either once weekly or once-monthly bisphosphonate regimens result in similar effects on biochemical markers of bone turnover and BMD of spine and proximal femur when the total cumulative dose of drug administered is the same. Except for gastrointestinal adverse reactions that are associated with oral use only and acute-phase response-like reactions associated with intravenous use, the spectrum of adverse drug reactions is similar for oral and IV bisphosphonates. Rare potential adverse reactions have been associated with the use of oral and IV bisphosphonates. Osteonecrosis of the jaw is rare in osteoporosis patients. Atypical femoral fractures appear to be associated with long term use (more than 4 or 5 years) and the risk appears to dissipate quickly when treatment is discontinued. Drug holidays (interruption of therapy after 4 or 5 years of continued use) have been recommended as an empirical method for reducing the risk of adverse events such as ONJ and AFFs. However, it is not known whether a reduction in an adverse risk will occur during a drug holiday. Moreover, it is uncertain how quickly risk of spine, femoral or non-vertebral fractures will increase following the start of a drug holiday. Controlled clinical trial data are needed to provide an evidence-based approach to drug holidays and a very long-term treatment of osteoporosis with bisphosphonates.

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15 Denosumab: Mechanisms and Therapeutic Effects in the Treatment of Osteoporosis

E. Michael Lewiecki

Key Points

- Denosumab is a robust antiresorptive agent that inhibits osteoclast differentiation, activity, and survival
- Denosumab reduces the risk of vertebral fractures, nonvertebral fractures, and hip fractures in postmenopausal women with osteoporosis
- Larger increases in total hip BMD with denosumab are associated with greater reductions in the risk of new or worsening vertebral fractures
- Discontinuation of denosumab should be followed by treatment with another antiresorptive agent

Introduction

Denosumab (Prolia®; Amgen Inc., Thousand Oaks, CA, USA) is a fully human monoclonal IgG2 antibody that binds and inhibits receptor activator of nuclear factor kappa-B ligand (RANKL), the principal regulator of osteoclastic bone resorption. It was initially approved by the US Food and Drug Administration (FDA) in 2010 for the treatment of postmenopausal women with osteoporosis at high risk for fracture, with a dose of 60 mg subcutaneously (SC) every 6 months (Q6M). It was subsequently approved, with the same dose, for treatment to increase bone mass in men with osteoporosis at high risk for fracture, treatment to increase bone mass in men at high risk for fracture receiving androgen deprivation therapy for nonmetastatic prostate cancer, treatment to increase bone mass in women at high risk for fracture receiving adjuvant aromatase inhibitor therapy for breast cancer, and treatment of glucocorticoid-induced osteoporosis in men and women at high risk for fracture [1]. The FDA has defined high risk for fracture as a history of osteoporotic fracture, multiple risk factors for fracture, or failure or intolerance to other available osteoporosis therapy. Another preparation of denosumab (Xgeva®; Amgen Inc., Thousand Oaks, CA, USA) is FDA-approved for prevention of skeletal-related events in patients with multiple myeloma and in patients with bone metastases from solid tumors (120 mg SC every 4 weeks), treatment of adults and skeletally mature adolescents with giant cell tumor of bone that is unresectable or where surgical resection is likely to result in severe morbidity (120 mg SC every 4 weeks with additional 120 mg doses on days 8 and 15 of the first month of therapy), and treatment of hypercalcemia of malignancy refractory to bisphosphonate therapy (120 mg SC every 4 weeks with additional 120 mg doses on

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days 8 and 15 of the first month of therapy) [2]. This is an update on the mechanism of action of denosumab and its clinical applications in the treatment of women and men with osteoporosis.

Mechanism of Action

Denosumab has a high affinity and specificity for RANKL, a homotrimeric protein expressed by osteoblasts, activated T cells, and organs that include lymph nodes, thymus, mammary glands, and lungs [3]. By preventing binding of RANKL to its receptor, RANK, located on the cell surface of mature osteoclasts and their progenitors, denosumab is a potent inhibitor of osteoclast differentiation, activity, and survival (Fig. 15.1). Denosumab mimics the action of osteoprotegerin (OPG), a naturally occurring endogenous product of cells of the osteoblast lineage that is a "decoy receptor" for RANKL. It is the balance of RANKL and OPG that determines the rate of bone resorption, with more RANKL favoring greater bone resorption, and more OPG favoring less bone resorption. Due to the coupling of resorption and formation, when resorption decreases, as with antiresorptive medication, formation decreases as well, and when formation increases, as with osteoanabolic therapy, so does resorption [4]. The skeletal consequences of reducing the rate of bone remodeling with denosumab are an increase in bone mineral density (BMD) and reduction in fracture risk [5].

The pharmacodynamics and pharmacokinetics of denosumab were evaluated in a dose-escalation phase 1 study of 49 healthy postmenopausal women receiving a single dose of SC denosumab (0.01, 0.03, 0.3, or 1.0 mg/kg) or placebo and followed up for 6–9 months [6]. There was a rapid (within 12 hours), dose-dependent, profound (up to 84%), and sustained (up to 6 months) decrease in urinary N-telopeptide (NTX), a marker of bone resorption. Patients in the higher dose groups showed the most prolonged suppression of urinary NTX, with levels returning to baseline after 6–9 months. There was

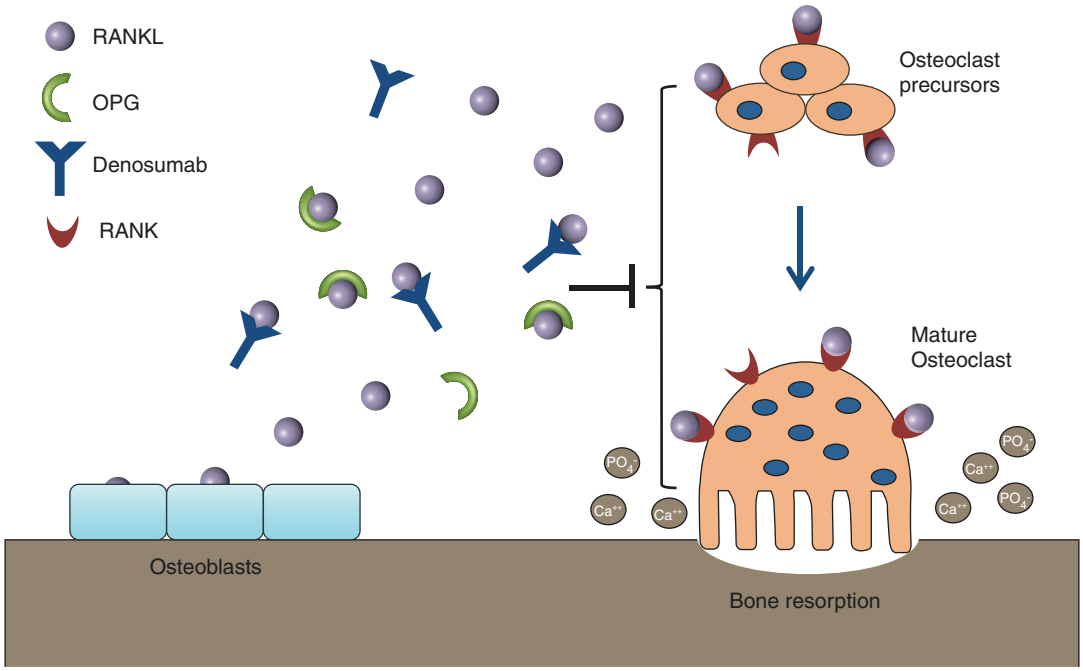

Fig. 15.1 Denosumab mechanism of action. By binding to RANKL, the principal regulator of osteoclastic bone resorption, denosumab reduces osteoclast differentiation, activity, and survival. (Reprinted from Boyce [71].With permission from Springer Nature)

a decrease in levels of serum bone-specific alkaline phosphatase (BSAP), a marker of bone formation, although this fell less rapidly and not to the same magnitude as urinary NTX. Serum intact parathyroid hormone (PTH) levels increased up to threefold after 4 days in the 3.0 mg/kg dose group and returned toward baseline with follow-up. Albumin-adjusted serum calcium levels decreased slightly in denosumab-treated subjects, especially with the higher dose groups, with a maximum decrease of 10% compared with baseline. Serum levels of denosumab were characterized by three phases: a prolonged absorption phase with maximum serum concentration (C_{max}) observed at 5–21 days post-dose with C_{max} increasing as dose increases; a prolonged β-phase, with serum half-life as long as 32 days with the maximum dose; and a rapid terminal phase.

Although the absorption, bioavailability, distribution, and elimination of denosumab are not well defined, it is likely that SC denosumab is absorbed by the lymphatic system, with subsequent drainage into the vascular system [7], distribution that is about the same as the plasma volume, and clearance by the reticuloendothelial system [8]. No significant amount of circulating denosumab is filtered and excreted by the kidneys. With denosumab 60 mg SC Q6M, the dose approved for the treatment of osteoporosis, the median time to maximum concentration (T_{max}) after the first dose is 26 days [9]. The long duration of denosumab activity is probably due to a combination of a long half-life and the potent antiresorptive effect early in the pathway of osteoclast differentiation.

The pattern of BMD response to denosumab, with progressive increases in BMD with up to 10 years of continuous treatment [10], is different than with bisphosphonates, which are associated with increases in BMD for the first 3–4 years of treatment followed by more modest increases or a plateau, as seen in extension studies with 10 years of alendronate [11] and 9 years of annual dosing of zoledronic acid [7]. This has led to speculation that these differences may be due to the pharmacological properties of denosumab that include greater suppression of bone remodeling with a partial release of antiresorptive effect at the end of the 6-month dosing interval [12], greater increase in PTH leading to stimulation of modeling-based bone formation [12], greater access to cortical bone due to its distribution throughout the extracellular space [13], and greater reduction in cortical porosity [14].

Double tetracycline-labeled transiliac bone biopsies in denosumab-treated subjects in phase 3 clinical trials have provided important insights into the mechanism of action of denosumab and an assessment of the quality of bone tissue, including bone microstructure and mineralization [15]. Bone was qualitatively normal after up to 3 years of treatment with denosumab, with biopsies showing normal lamellar bone, normal mineralization with no osteoid accumulation, and no marrow fibrosis. Normal cortical and trabecular microarchitecture was maintained. Histomorphometric indices of bone resorption and formation were markedly reduced, more so than with bisphosphonates. Median eroded surface was reduced by more than 80%, and osteoclasts were absent in more than 50% of biopsy specimens in subjects treated with denosumab. Double labeling was seen in 19% of those treated with denosumab compared with 94% of those treated with placebo. Microcomputed tomography (microCT) showed significantly reduced cortical porosity and increased cortical volumetric BMD after 24 months of denosumab compared with placebo but no significant difference after 36 months. After 10 years of treatment with denosumab, bone biopsies continued to show normal bone histology, low bone remodeling, increased matrix mineralization, and lower mineralization heterogeneity compared with placebo-treated subjects [16].

Landmark Clinical Trials

Phase 2 Study and Extensions

A phase 2 randomized, placebo-controlled, dose-ranging study evaluated the efficacy and safety of denosumab in postmenopausal women with low BMD [12]. Subjects were postmenopausal

women (N = 412), age up to 80 years (mean 63 years), with baseline lumbar spine T-score − 1.8 to −4.0 or total hip or femoral neck T-score − 1.8 to −3.5. They were randomized to receive denosumab 6, 14, or 30 mg SC every 3 months (Q3M) or 14, 60, 100, or 210 mg SC Q6M, open-label oral alendronate 70 mg weekly, or placebo. The primary endpoint was percentage change in lumbar spine BMD at 12 months compared with baseline. Other endpoints included percentage change from baseline in BMD at the total hip, femoral neck, one-third radius, and change in bone turnover markers (BTMs) consisting of urinary NTX, serum C-telopeptide (CTX), and serum BSAP. Long-term effects of treatment were assessed in study extensions, with published reports of data at 2 years [17], 4 years [18], 6 years [19], and 8 years [20].

At 12 months, denosumab was associated with significant lumbar spine BMD increases of 3.0–6.7%, depending on the dose and dosing interval, with smaller significant BMD increases observed at other skeletal sites [12]. In exploratory comparisons, BMD increases at the one-third radius, and total hip appeared to be greater with denosumab 30 mg Q3M and 60 mgQ6M than with open-label alendronate. Decreases of BTMs with denosumab were dose-dependent, rapid, sustained, and reversible. Adverse events (AEs) and serious adverse events (SAEs) were similar in the treatment groups, with the exception of dyspepsia being most common with open-label alendronate. The findings of this study supported further investigation of denosumab for the treatment and prevention of osteoporosis and other diseases associated with bone loss.

Efficacy and safety of 24 months of continuous denosumab treatment were assessed in a prespecified exploratory study [17]. The findings at 24 months supported and extended those of 12 months, with continuing increases in BMD and suppression of BTMs. BMD response at some skeletal sites continued to be greater with denosumab than with open-label alendronate. AEs continued to be generally similar in the placebo, denosumab, and alendronate groups. There were 6 cases (1.9%) of SAEs of infections in the denosumab group (2 cases of diverticulitis, 3 cases of pneumonia, and 1 case of labyrinthitis) compared to none in the placebo group or open-label alendronate group. No neutralizing antibodies to denosumab were observed in the first 24 months of treatment.

For the study extension from 24 months to 48 months, subjects treated with denosumab were reassigned based on the randomization group at enrollment [18]. Patients originally randomized to denosumab 6 and 14 mg SC Q3M and 14, 60, and 100 mg SC Q6M were changed to denosumab 60 mg Q6M. Patients originally randomized to 210 mg SC Q6M were changed to placebo. Patients originally randomized to 30 mg SC Q3M were changed to placebo for 12 months, followed by re-treatment with denosumab 60 mg SC Q6M for 12 months. Subjects receiving open-label alendronate were terminated from the study after 24 months and received no additional drug therapy. The placebo group was maintained for the entire 48 months. Continuous denosumab treatment for 48 months was associated with further increases in BMD at the lumbar spine (9.4–11.8% compared with baseline) and total hip (4.0–6.1% compared with baseline), with continuing suppression of BTMs. Discontinuation of denosumab after 24 months of treatment was associated with BMD decreases of 6.6% at the lumbar spine and 5.3% at the total hip within 12 months of discontinuation. Re-treatment with denosumab12 months after discontinuation increased BMD in a manner similar to initial treatment, with lumbar spine BMD increasing 9.0% and total hip BMD increasing 3.9% compared with original baseline values. BTMs increased to levels higher than baseline after discontinuation of denosumab and decreased with re-treatment. Discontinuation of alendronate at 24 months was followed by a modest decrease in BMD at the lumbar spine by 48 months, with a greater decrease in BMD at the total hip and one-third radius; BTM levels increased, but remained below baseline at 48 months. SAEs were 10.9% (5/46) in the placebo group, 17.8% (56/314) in the denosumab group, and 17.4% (8/46) in the alendronate group. The incidence of malignant neoplasms was balanced among the treatment groups. The overall incidence of infections was

similar in all treatment groups, while infections requiring hospitalization occurred in 3.2% (10/314) of denosumab-treated patients and none of those who received placebo or alendronate. All infections were reported as common community-acquired infections (those identified at 24 months were two cases each of diverticulitis and pneumonia and one case each of atypical pneumonia and labyrinthitis) that responded appropriately to standard antibiotic therapy, with no reports of opportunistic infections.

Of the 262 subjects who completed the 4-year phase 2 "parent study," 200 were enrolled in a single arm extension for an additional 4 years, with all receiving denosumab 60 mg SC Q6M. There were 178 completers at the end of 6 years [19] and 138 at the end of 8 years [20], with 90 subjects receiving 8 years of continuous denosumab. After 8 years of continuous denosumab, BMD increased by 16.5% at the lumbar spine and 6.8% at the total hip, with AEs consistent with previous reports and aging of the study population.

Phase 3 and Extensions

FREEDOM (Fracture REduction Evaluation of Denosumab in Osteoporosis every 6 Months) was the pivotal, 3-year, randomized, placebo-controlled phase 3 clinical trial comparing denosumab 60 mg SC Q6M and placebo, with a primary endpoint of new vertebral fractures at 36 months and secondary endpoints that included nonvertebral and hip fractures [5]. Study subjects were 7868 postmenopausal women (mean age 72.3 years) with osteoporosis (mean baseline lumbar spine T-score = −2.8), 83% of whom completed the 3-year study. Approximately 23% of subjects had at least one prevalent vertebral fracture at the time of entry into the study. All subjects received elemental calcium 1000 mg and vitamin D 400–800 IU daily. It was found that treatment with denosumab significantly reduced the relative risk (RRR) of radiographic vertebral fractures by 68%, with 40% RRR of hip fractures and 20% RRR of nonvertebral fractures compared with placebo. BMD increased by 9.2% at the lumbar spine and 6% at the hip. Increases in total hip BMD explained a considerable proportion of the effect in reducing vertebral fracture risk, with larger increases in total hip BMD associated with greater reduction in the risk of new or worsening vertebral fractures [21], supporting the concept of treat-to-target for osteoporosis [22].There were no significant differences in total AEs, SAEs, or treatment discontinuation between subjects receiving denosumab or placebo. There were no increase in the risk of cancer, infection, cardiovascular disease, or hypocalcemia and no reports of osteonecrosis of the jaw (ONJ) or atypical femur fractures (AFF) in subjects receiving denosumab. There were no subjects with neutralizing antibodies to denosumab. Eczema was reported in 3.0% of subjects in the denosumab group compared with 1.7% in the placebo group ($P < 0.001$). Cellulitis as an SAE was reported in 12 subjects (0.3%) in the denosumab group compared with one subject(<0.1%) in the placebo group ($P = 0.002$), with no significant difference in overall incidence of AEs of cellulitis. There was no evidence that denosumab interfered with fracture healing, even when administered at or near the time of the fracture [23]. The findings of this study led to the FDA approval of denosumab for the treatment of postmenopausal women with osteoporosis at high risk for fracture.

A 7-year FREEDOM extension study was initiated after completion of the 3-year FREEDOM trial to assess the effects of 10 years continuous denosumab in subjects receiving denosumab in FREEDOM and 7 years continuous denosumab in those receiving placebo in FREEDOM. All subjects in the extension received open-label denosumab 60 mg SC Q6M. The primary objective of the extension study was to monitor safety, with secondary endpoints that included changes in BMD and BTMs. The findings of 2 years [24], 5 years [25], and 7 years [10] of the extension study have been published.

Of the 7808 women originally enrolled in FREEDOM, 2626 completed the 7-year FREEDOM extension, with 1343 long-term subjects receiving 10 years of continuous denosumab and 1283 crossover subjects from the placebo group in FREEDOM receiving 7 years of

continuous denosumab [10]. In the long-term group, BMD increased by 21.7% at the lumbar spine, 9.2% at the total hip, 9.0% at the femoral neck, and 2.7% at the one-third radius from the FREEDOM baseline. Sustained reductions in serum levels of CTX and procollagen type 1 N-terminal propeptide (P1NP) were seen. The yearly incidence of new vertebral fractures and nonvertebral fractures remained low during the extension, similar to rates observed in FREEDOM in the denosumab group and lower than that projected to occur in a "virtual twin" placebo cohort. The yearly exposure-adjusted incidence of all AEs was stable. During the extension, there were 5 subtrochanteric or diaphyseal femur fractures reported in the long-term group and 4 in the crossover group, with 2 of these (0.8 per 10,000 participant-years) adjudicated as AFF, 1 in the long-term group and 1 in the crossover group. Also during the extension, there were 13 adjudicated cases of ONJ (5.2 per 10,000 participant-years), 7 in the long-term group and 6 in the crossover group. Two subjects with ONJ were lost to follow-up, with the others having resolution of the ONJ, with resolution of 4 of these cases while continuing denosumab. No subjects developed neutralizing antibodies to denosumab. Transiliac bone biopsies were obtained in 22 subjects with 10 years of continuous denosumab exposure, with 21 of these suitable for histomorphometric analysis. Remodeling activation frequency was low and did not differ from that observed in biopsy specimens of subjects after 2, 3, and 5 years of denosumab treatment.

ADAMO (A multicenter, randomized, double-blind, placebo-controlled study to compare the efficacy and safety of DenosumAb vs. placebo in Males with Osteoporosis) provided data that supported the FDA approval of denosumab for the treatment of men with osteoporosis at high risk for fracture [26, 27]. In this study, 242 men (aged 30–85 years) with low BMD (T-score, based on male reference database, ≤ -2.0 and ≥ -3.5 at the lumbar spine or femoral neck, or previous major osteoporotic fracture and T-score ≤ -1.0 and ≥ -3.5) were randomized to receive denosumab 60 mg SC Q6M or placebo. The primary endpoint was percentage change from baseline of lumbar spine BMD at 12 months. After 12 months, BMD increased by 5.7% at the lumbar spine, 2.4% at the total hip, and 0.6% at the one-third radius in men treated with denosumab compared with baseline (adjusted $P \leq 0.0144$ for differences at all skeletal sites compared with placebo) [26]. Serum CTX was significantly reduced at day 15 for men in the denosumab group compared with placebo ($P < 0.0001$). The BMD and CTX effects were independent of baseline testosterone levels, baseline BMD, age, and estimated fracture risk. The incidence of AEs was similar in the 2 study groups. The study was not powered to detect differences in fracture rates. The increases in BMD and BTM changes were similar to those observed in women in the denosumab group in FREEDOM, suggesting that fracture risk reduction in men is similar to women.

In the second year of the ADAMO study, all participating subjects in both groups received open-label denosumab 60 mg SC Q6M [27]. A total of 228 men were enrolled in the second year of the study with 219 completing the study. The exploratory endpoints for this open-label phase were BMD changes, CTX changes, and safety through month 24. In the long-term group receiving continuous denosumab for 24 months, there was a cumulative BMD increase of 8.0% at the lumbar spine, 3.4% at the total hip, and 0.7% at the one-third radius compared with baseline ($P < 0.01$ for all skeletal sites). There were significant reductions in CTX levels in both groups compared with baseline. AE rates were similar in both groups and no new safety signals were identified.

A phase 3 randomized, double-blind, active-control, double-dummy, non-inferiority study compared the effects of denosumab 60 mg SC Q6M and risedronate 5 mg given orally in 795 patients with glucocorticoid-induced osteoporosis (low BMD or fragility fracture on chronic glucocorticoid therapy with prednisone ≥ 7.5 mg daily or equivalent) [28]. Denosumab was found to be non-inferior and superior to risedronate for effect on lumbar spine BMD at 12 months. The incidence of AEs, SAEs, and fractures was similar between treatment groups.

Safety Concerns of Special Interest

Immune Function

RANKL and RANK are expressed by immune cells that include activated T cells, B cells, and dendritic cells. Gene ablation studies in mice have shown that the complete absence of RANKL during embryogenesis is followed by total absence of lymph nodes [29], suggesting possible adverse immune effects in humans with RANKL inhibition due to denosumab. However, investigation of rodents, cynomolgus monkeys, and humans with inhibition of RANKL has shown no evidence of significant impairment of parameters of immune function [30, 31]. In FREEDOM, numerical imbalances in the incidence of some infections (e.g., cellulitis as an SAE) led to a more thorough analysis of the data to determine whether there was a causal relationship or coincidence in the occurrence of infections in subjects treated with denosumab (Table 15.1) [32]. It was found that SAEs of infections in the gastrointestinal tract, urinary tract, ear, and endocarditis were numerically higher in subjects treated with denosumab compared with placebo, but the number of events was small, and there was no relationship between these events and the timing of dosing or duration of exposure to denosumab. It was concluded that there was no evidence of denosumab causing adverse immune effects resulting in infections. A subsequent analysis of safety observations in FREEDOM followed by 3 years of FREEDOM extension, with some subjects receiving 6 years of continuous denosumab, supported the findings of the previous analysis, with no evidence of increasing trends of imbalances of these low frequency events [33].

Table 15.1 Infections in the FREEDOM trial

	Year 1	Year 2	Year 3
Incidence of serious adverse events of infection by year			
Placebo	42 (1.1%)	49 (1.3%)	47 (1.4%)
Denosumab	55 (1.4%)	58 (1.6%)	54 (1.5%)
Positively identified bacterial infections			
Placebo	10 (0.3%)	12 (0.3%)	10 (0.3%)
Denosumab	12 (0.3%)	15 (0.4%)	19 (0.5%)
Positively identified viral infections			
Placebo	0 (0.0%)	1 (<0.1%)	5 (0.1%)
Denosumab	2 (0.1%)	4 (0.1%)	2 (0.1%)
Positively identified fungal infections			
Placebo	1 (<0.1%)	0 (0.0%)	0 (0.0%)
Denosumab	1 (<0.1%)	0 (0.0%)	1 (<0.1%)

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The incidence of serious adverse events as infections did not increase with longer duration of exposure to denosumab, suggesting there is no causal relationship between treatment with denosumab and risk of infection

Combining Denosumab with Other Biologics

Rheumatoid arthritis is a chronic inflammatory disease that is associated with local and systemic skeletal effects that include focal joint erosions, subchondral joint erosions, periarticular osteoporosis, and systemic osteoporosis [34]. Biologic agents now commonly used to treat rheumatoid arthritis appear to reduce periarticular bone loss, but effects on systemic bone loss are limited [35]. Adverse effects (e.g., risk of serious infections) have been reported with combining biologic agents for rheumatoid arthritis [36, 37], raising concerns of similar consequences when combining denosumab to treat osteoporosis in a patient receiving a biologic agent for rheumatoid arthritis. However, the bulk of evidence to date suggests there is no increase of infection rates in these patients. A 12-month randomized, phase 2, placebo-controlled clinical trial evaluated the effects of denosumab on structural damage, BMD, and bone turnover in 227 patients with rheumatoid arthritis receiving methotrexate [38]. In this study, which included some patients receiving a disease-modifying antirheumatic drug, denosumab inhibited structural skeletal damage in patients for up to 12 months, with no increase in the rates of AEs. In another study conducting a retrospective review of Medicare claims data of 5814 patients with rheumatoid arthritis, it was found that the rate of hospitalization for infections was not increased in patients receiving denosumab plus a biologic agent (most often infliximab or abatacept) compared with those receiving zoledronic acid [39]. These limited data provide reassurance that denosumab may be

safe for use in treating osteoporosis in patients with rheumatoid arthritis receiving a biologic agent, although definitive data are not available.

Atypical Femur Fractures

In FREEDOM extension, there were 2 reported subjects with adjudicated AFF (0.8 per 10,000 participant-years), 1 in the long-term group after 7 years of continuous denosumab and 1 in the crossover group after 3 years of continuous denosumab [10]. There have also been case reports of denosumab-treated patients with AFF [40–44]. Given the rarity of AFF, it is currently not possible to compare the incidence of AFF associated with denosumab vs. patients treated with bisphosphonates or the general population.

Osteonecrosis of the Jaw

In FREEDOM extension, there were 13 adjudicated cases of ONJ, 7 in the long-term group and 6 in the crossover group (5.2 per 10,000 participant-years) [10]. Of the 13 cases, 2 were lost to follow-up and the others resolved, 4 of whom had complete resolution while on denosumab. In a systematic review of 35 randomized clinical trials reporting adverse effects of treatment with denosumab, 7 reported cases of ONJ, all of which were in subjects treated with 120 mg SC every 4 weeks or Q6M, a dose that is higher than that used for osteoporosis [45]. Risk factors for ONJ in that analysis included dental extraction, use of removable dental apparatus, poor oral hygiene, and cancer chemotherapy.

Hypocalcemia

Small asymptomatic decreases in serum calcium have been reported in clinical trials [5, 12]. Symptomatic hypocalcemia has been reported in patients with impaired renal function, especially those with creatinine clearance <30 mL/min and on dialysis [46, 47]. Other patients who are predisposed to hypocalcemia, such as those with hypoparathyroidism, previous thyroid surgery, parathyroid surgery, malabsorption syndromes, or small bowel resection, should have serum calcium closely monitored [1]. Serum calcium should be measured prior to administration of denosumab and pre-existing hypocalcemia, if present, should be evaluated and corrected. Patients should have an adequate intake of calcium and vitamin D.

Studies Comparing Denosumab with Bisphosphonates

The efficacy and safety of denosumab have been compared with alendronate in several studies. DECIDE (Determining Efficacy: Comparison of Initiating Denosumab versus alEndronate) was a 12-month phase 3, double-blind, double-dummy, non-inferiority trial in 1189 postmenopausal women with low BMD (lumbar spine or total hip T-score ≤ -2.0) [48]. Subjects were randomized to receive denosumab 60 mg SC Q6M plus weekly oral placebo or oral alendronate 70 mg weekly plus placebo SC injections Q6M. Changes in BMD, BTMs, and safety measures were assessed. At 12 months, there was a significantly greater BMD increase at the total hip with denosumab compared with alendronate (treatment difference 0.9%, $P < 0.0001$) as well as at other measured skeletal sites, with the treatment difference 1.1% at the lumbar spine and 0.6% at the one-third radius ($P \leq 0.0002$ for all sites). There was greater suppression of BTMs with denosumab and safety profile that was similar for both groups. The study was not powered to compare fracture rates between the 2 groups. STAND (Study of Transitioning from AleNdronate to Denosumab) was a 12-month phase 3, double-blind, active-controlled, double-dummy study in 504 postmenopausal women with low BMD (lumbar spine or total hip T-score −2.0 to −4.0) who had previously been treated with alendronate for at least 6 months (median 36 months) [49]. After a 1-month run-in period during which all subjects received open-label oral alendronate 70 mg once weekly, subjects were randomized to receive denosumab 60 mg SC Q6M once every

6 months plus weekly placebo tablets or to continue oral alendronate 70 mg once weekly plus placebo SC Q6M. Subjects were evaluated for changes in BMD, BTMs, and safety. At 12 months, there was a statistically significant greater increase in BMD at all measured skeletal sites with subjects transitioning to denosumab compared with those continuing alendronate. Total hip BMD increased by 1.90% in the denosumab group compared with 1.05% in the alendronate group ($P < 0.0001$). Median serum CTX levels decreased significantly in the denosumab group compared with the alendronate group ($P < 0.0001$). AEs and SAEs were similar in both groups.

The effects of denosumab have been compared with monthly oral bisphosphonates. The efficacy and safety of denosumab were compared with risedronate in a 12-month randomized open-label study of 870 postmenopausal women aged 55 years and older who were previously suboptimally adherent to treatment with alendronate [50]. Subjects were randomized to receive denosumab 60 mg SC Q6M or oral risedronate 150 once monthly. Changes in BMD, BTMs, and safety were assessed. BMD increases and serum CTX decreases were significantly greater in the denosumab group compared with the risedronate group. AEs and SAEs were similar in both the groups. In another study of similar design, 833 postmenopausal women who had discontinued or were poorly adherent to daily or weekly bisphosphonate therapy were randomized to receive open-label denosumab 60 mg SC Q6M or oral ibandronate 150 mg once monthly [51]. After 12 months, BMD gains and serum CTX decreases were greater in the denosumab group compared with the ibandronate group. AEs were similar in the two groups. The incidence of SAEs was 9.5% in the denosumab group and 5.4% in the ibandronate group ($P = 0.046$), with no clustering of events to explain this difference. In a post-hoc analysis that combined the data from these 2 studies, the incidence of AEs and SAEs in the overall population was similar in the denosumab and monthly oral bisphosphonate groups, except that the proportion of subjects with AEs leading to study discontinuation was lower in the denosumab group compared with the oral bisphosphonate group (0.8% vs. 3.0%, respectively; $P = 0.0013$) [52].

The effect of transitioning from an oral bisphosphonate to denosumab or zoledronic acid was evaluated in a randomized, double-blind study of 643 postmenopausal women [53]. Subjects were randomized to receive denosumab 60 mg SC Q6M for 12 months plus intravenous (IV) placebo or zoledronic acid 5 mg IV plus placebo SC Q6M. BMD increases and CTX decreases were greater in the denosumab than zoledronic acid group. AEs were similar in the two groups. Three patients with adjudicated AFF were reported, two in the denosumab group and one in the zoledronic acid group.

Denosumab and Anabolic Therapy

The DATA (Denosumab And Teriparatide Administration) study provided information comparing the effects of denosumab and teriparatide, alone or combined. In this study, 94 postmenopausal women with osteoporosis were randomized to receive denosumab 60 mg SC Q6M, teriparatide 20 mcg SC daily, or a combination of both [54]. BMD was measured at 0, 3, 6, and 12 months. At 12 months, lumbar spine BMD increased more in the combination group (9.1%) compared with the denosumab alone (5.5%, $P = 0.0005$) or teriparatide alone (6.2%, $P = 0.0139$). A similar pattern was seen for BMD changes at the total hip and femoral neck. In the DATA extension study, the 3 groups continued with the same treatment for an additional 12 months [55]. At 24 months, lumbar spine BMD increased more in the combination group (12.9%) compared with the denosumab alone (4.1%, $P = 0.008$) or teriparatide alone (9.5%, $P = 0.003$) groups, with a similar pattern at the hip. The finding of additive effects on BMD with a combination of denosumab and teriparatide is in contrast to the lack of additive effect in studies combining alendronate with teriparatide [56, 57].

DATA-Switch was a preplanned extension of the DATA study in which women in the combination group for 24 months were switched to

denosumab for an additional 24 months (combination to denosumab group, $n = 23$), those treated with denosumab for 24 months were switched to teriparatide for an additional 24 months (denosumab to teriparatide group, $n = 27$), and those treated with teriparatide for 24 months were switched to denosumab for an additional 24 months (teriparatide to denosumab group, $n = 27$) [58]. The primary outcome measure was change in lumbar spine BMD over 4 years. The observed lumbar spine BMD increase at 4 years was 16.0% in the combination to denosumab group, 14.0% in the denosumab to teriparatide group, and 18.3% in the teriparatide to denosumab group compared with baseline. However, it is notable that there was a transient decrease in BMD at the total hip and femoral neck and progressive bone loss at the one-third radius, when switching from denosumab to teriparatide. This was in contrast to further increases in BMD at all measured skeletal sites when switching from combination or teriparatide to denosumab. Switching from denosumab was associated with a large increase in bone turnover markers, with osteocalcin rising to 275% above baseline and CTX rising to 183% above baseline after 6 months of teriparatide. These findings suggest that switching from denosumab to teriparatide should be undertaken with caution, if at all, and that initial use of anabolic therapy or a combination of an anabolic agent and denosumab may be preferable in high risk patients. Other studies support the concept that treatment sequence is important for optimizing benefits in high risk patients, with an anabolic followed by an antiresorptive agent preferable to an antiresorptive followed by an anabolic agent [59].

Consequences of Denosumab Discontinuation

It was demonstrated in the phase 2 trial of denosumab in postmenopausal women with low BMD that discontinuation of denosumab after 2 years of continuous therapy was followed by a rapid decline in BMD (6.6% at the lumbar spine and 5.3% at the total hip within 12 months of treatment discontinuation) and increase of BTMs to levels above baseline [18]. This raises concern that fracture risk may return to baseline, or perhaps higher than baseline, soon after treatment discontinuation. Published case reports have described multiple vertebral fractures after discontinuation of denosumab [60–63]. The effect of treatment discontinuation on vertebral fractures was evaluated in a post-hoc analysis of data from FREEDOM and FREEDOM extension [64]. This was an analysis of 1475 study participants who discontinued treatment after receiving at least 2 doses of denosumab or placebo and remaining in the study for at least 7 months after the last dose. Vertebral fracture risk increased with denosumab discontinuation to the level observed in untreated subjects, with a majority of those with a vertebral fracture after discontinuing denosumab having multiple vertebral fractures. Of those having a vertebral fracture after denosumab discontinuation, 61% had multiple vertebral fractures, compared with 39% multiple fractures after placebo discontinuation. Fracture rates were low in both groups, with the risk of multiple vertebral fractures 3.4% after stopping denosumab and 2.2% after stopping placebo ($P = 0.049$). The risk of vertebral fractures was greatest in those with a prior vertebral fracture. These data strongly suggest that patients who discontinue denosumab should rapidly switch to another antiresorptive agent and that a "drug holiday" is not appropriate for patients treated with denosumab [65].

A position statement of the European Calcified Tissue Society [66] recommends that patients be evaluated for fracture risk after 5 years of treatment with denosumab. When fracture risk is high, treatment for up to 5 more years, then switching to a bisphosphonate is advised. For patients at low risk of fracture, consider stopping denosumab and switching to a bisphosphonate.

The optimal bisphosphonate regimen after denosumab discontinuation is not known. Alendronate has been shown to maintain BMD in subjects stopping denosumab after 1 year of treatment. In a 24-month, randomized, crossover study comparing denosumab with alendronate in 250 postmenopausal women with low BMD, there were further BMD increases with 1 year of

denosumab after 1 year of alendronate and stability of BMD with 1 year of alendronate after 1 year of denosumab [67]. In a case series of 6 women with postmenopausal osteoporosis treated with 7 years of continuous denosumab in FREEDOM, zoledronic acid 5 mg IV was administered 6 months after the last dose of denosumab [68]. There was a significant decrease in BMD at the lumbar spine and total hip (Fig. 15.2) when BMD was measured 18–23 months after receiving zoledronic acid. It was hypothesized that the disappointing treatment effect of zoledronic acid under these circumstances may have been due to the diminished uptake at bone surfaces due to profound suppression of bone remodeling by denosumab. In another case series, postmenopausal women with osteoporosis completing a clinical trial ending with 2 years of open-label denosumab, preceded by 1 year of romosozumab or placebo, were offered follow-up treatment with IV zoledronic acid or oral risedronate [69]. Women who chose zoledronic acid, given after a median delay of 65 days from the end of the trial, had the best outcomes for maintaining BMD, with 73% retention of the prior BMD increase at the lumbar spine and 87% retention of the prior BMD increase at total hip. Subjects receiving no follow-up treatment had 10–20% retention of the prior BMD increase; those who chose risedronate had 41–64% retention of the prior BMD increase. While some BMD loss may be inevitable when switching from denosumab to a less robust antiresorptive agent, the preferred strategy based on the limited data now available may be to administer zoledronic acid 7–8 months after the last dose of denosumab or alendronate starting 6 months after the last dose. The strategy of switching to another antiresorptive agent after denosumab discontinuation was validated in follow-up of subjects completing the DATA and DATA-Switch studies [70]: those who received antiresorptive therapy maintained BMD while those who did not experienced BMD loss.

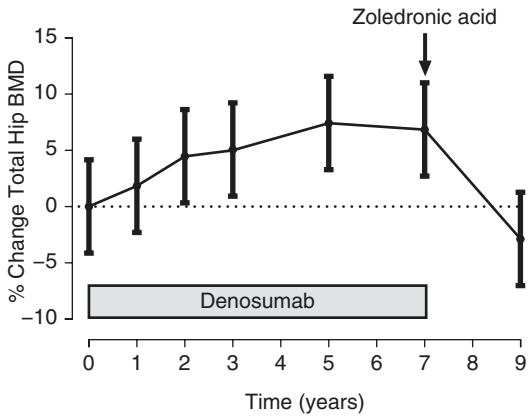

Fig. 15.2 Treatment with zoledronic acid after denosumab. After long-term treatment with denosumab, zoledronic acid administered 6 months after the last dose of denosumab was followed by a decrease in BMD. This may be a consequence of profound reduction in the rate of bone remodeling with denosumab that limits the skeletal uptake of zoledronic acid. It suggests that a delay in zoledronic acid dosing to longer than 6 months after the last dose of denosumab may be more effective. (Reprinted from Reid et al. [69]. With permission from Springer Nature)

Summary

Denosumab is a highly effective agent for the treatment of osteoporosis in postmenopausal women and men, with a favorable safety profile in appropriately selected patients. It also has applications for the management of other conditions characterized by bone loss and skeletal fragility. There are uncertainties regarding the optimal duration of treatment. Unlike bisphosphonates, it is not retained in the skeleton and its therapeutic effects are rapidly reversed with discontinuation. When denosumab therapy is stopped, it should be followed by another antiresorptive agent. Teriparatide after denosumab, a treatment sequence that should likely be avoided, has been associated with progressive or transient bone loss.

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The Parathyroid Hormone Receptor Type 1

16

Thomas J. Gardella

Key Points

- The parathyroid hormone receptor type 1 (PTHR1) is a class B G protein-coupled receptor.
- The PTHR1 binds two distinct peptide ligands, PTH and PTHrP, to regulate two distinct biological processes, calcium homeostasis and tissue development, respectively.
- Novel analogs of PTH or PTHrP can bind to distinct PTHR1 conformations and induce altered functional responses, including prolonged signaling from endosomes.
- A number of diseases of bone and mineral ion physiology are linked to defects in the PTHR1 signaling system.
- The PTHR1 is a key target of interest for pharmaceutical development.

Introduction

The type-1 parathyroid hormone receptor, or PTHR1, mediates the actions of two structurally related, but genetically distinct peptide ligands – parathyroid hormone (PTH) and parathyroid hormone-related protein (PTHrP). Upon binding these ligands, the PTHR1 couples to a heterotrimeric G protein (Gα/β/γ) to thereby activate downstream signaling responses in the target cell. The PTHR1 can couple to multiple G proteins, but the most efficient coupling is to G protein heterotrimers containing the stimulatory alpha subunit, Gαs, which mediates positive activation of the adenylyl cyclase (AC)/cAMP/protein kinase A (PKA) signaling cascade. The biological roles of PTH and PTHrP are markedly distinct. PTH is a secreted hormone that functions to maintain blood calcium and phosphate homeostasis by regulating mineral ion fluxes in the bone and kidney. PTHrP, on the other hand, is a morphogenetic factor that acts in paracrine fashion to regulate cell differentiation programs in developing tissues, most notably in the growth plates where it slows the conversion of proliferating chondrocytes into end-stage hypertrophic during endochondral bone formation [1]. The complex and critical nature of the overall biology controlled by the PTHR1 is reflected by the various diseases that can arise with perturbation in the system, as, for example, with mutations in the genes for the receptor or its ligands, as discussed in a later section of the chapter. It is therefore not surprising that considerable research effort has been directed at understanding the molecular mechanisms by which the PTHR1 functions, in terms of binding its two ligands and mediating

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the signaling responses that give rise to the specific biochemical and/or growth-related changes in the various target cells.

Structural Overview of the PTHR1, a Class B GPCR

The PTHR1 is a member of the G protein-coupled receptor (GPCR) superfamily of integral membrane proteins, and it specifically belongs to the class B subgroup of GPCRs. The primary structure of the PTHR1 and its basic protein domain organization is shown in the snake plot diagram of Fig. 16.1. The class B subgroup of GPCRs is comprised of 15 receptors, each of which binds a moderately sized peptide hormone ligand. These receptors include, in addition to the PTHR1, the receptors for glucagon, glucagon-like peptide-1 (GLP-1), calcitonin, corticotropin-releasing factor (CRF), secretin, and several other peptide hormone ligands. Other than employing the same basic seven-transmembrane domain helical protein architecture used by all GPCRs, class B GPCRs share no direct amino acid sequence homology with the other GPCRs, of which there are a total of some 800 encoded in the human genome and which are grouped, based on amino sequence homology, into four main classes: A, B, C, and F [2, 3]. The class B receptors as a group exhibit ~30% overall amino acid sequence identity and are characterized by a number of highly conserved signature residues that are located at dispersed sites throughout the sequence, but mainly in the large N-terminal extracellular domain (ECD) portion of the receptor, and in the transmembrane domain (TMD) portion that contains the seven-transmembrane helices and interconnecting loops. A hallmark feature of the class B receptors is the absolute conservation of six cysteine residues in the ECD. These cysteines form a disulfide bond network that maintains a specific tertiary fold that is used in common for the ECDs of each of the class B GPCRs. The PTHR1 ECD contains four asparagine residues that are glycosylated during intracellular processing and transport to the plasma membrane. A unique feature of the ECD of the PTHR1 in humans and other mammals is a 44-amino acid segment, Ser61-Gly105, that is inserted between the first and second cysteine residues. An equivalent segment is not present in the PTHR1 of lower vertebrates nor in any other class B GPCR including the so-called PTHR2 subtype, which binds a distinct peptide ligand called TIP-39 and likely functions in the neuroendocrine system rather than in bone or calcium physiology, as will be discussed later. The inserted segment of the mammalian PTHR1 is encoded by a separate gene exon, called E2, and does not contribute to receptor function, as it can be deleted without affecting ligand binding or downstream signaling [4].

Ligand Pharmacology at the PTHR1

The endogenous ligands for the PTHR1, PTH, and PTHrP are straight-chain polypeptides of 84 and 141 amino acids, respectively. Structure-activity relationship studies on PTH began in the 1970s with the determination of the amino acid sequence of the native hormone extracted from bovine parathyroid glands [5, 6] and the subsequent chemical synthesis of a bioactive PTH peptide, the N-terminal PTH (1-34) fragment [7]. Characterization of this synthetic PTH (1-34) peptide and its truncated fragment derivatives in cells or membranes prepared from bone and kidney tissue made it clear that the first 34 amino acids of PTH contain sufficient information for productive and high-affinity interaction with the PTH receptor [7, 8].

PTHrP was discovered in the late 1980s as the hypercalcemia-causing factor secreted by many tumors in late-stage malignancy, which induced effects similar to those observed with hyperparathyroidism or with high-dose administration of PTH [9]. As with PTH, synthetic N-terminal PTHrP peptides, such as PTHrP (1-34) or PTHrP (1-36), which is often used since it is thought to represent an endogenous cleavage fragment of the precursor peptide, mimic the actions of the full-length polypeptide in cell- and membrane-based functional assays [10, 11]. Further studies on truncated fragments defined the N-terminal and C-terminal portions of PTH (1-34) and

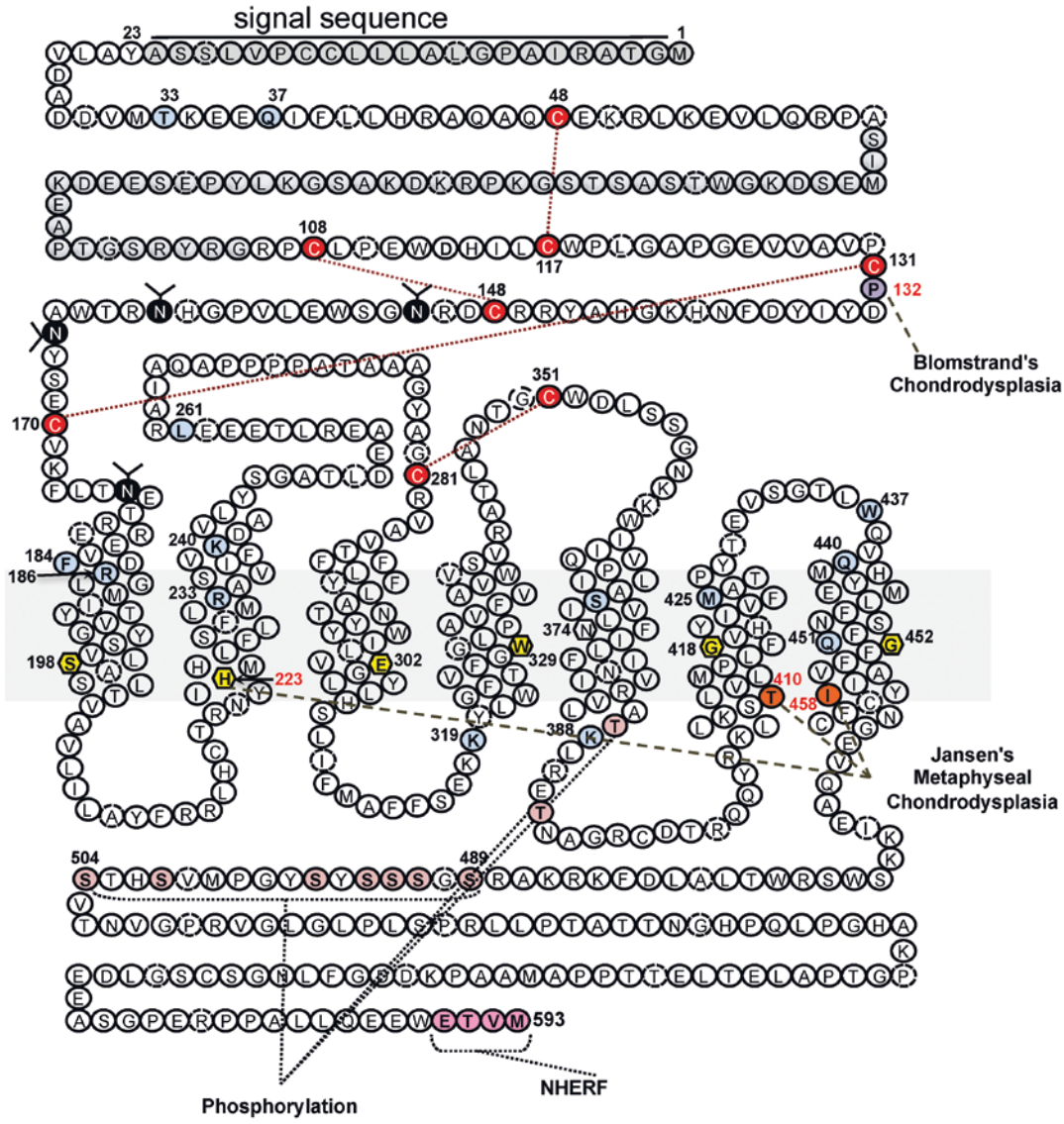


Fig. 16.1 Primary structure of the human PTH-1 receptor. The hPTHR1 amino acid sequence is displayed as a snake plot to illustrate the overall domain organization and location of selected key residues. These include the eight extracellular cysteines that form a disulfide bond network (connecting dotted lines), the four glycosylated extracellular asparagines; Pro132 at which loss-of-function mutations occur in Blomstrand's lethal chondrodysplasia (compound homozygous) and in failed tooth eruption (heterozygous); His223, Thr410, and Ile458 at which activating mutations occur in Jansen's metaphyseal chondrodysplasia; cytoplasmic sites of serine and threonine phosphorylation; the C-terminal residues that mediate PDZ-domain interactions with NHERF proteins; and residues involved in direct ligand interaction (filled shaded circles with position numbers). The residue shown as a filled hexagon in each transmembrane domain helix is the residue in that helix that is most conserved among the class B GPCRS [127]. (Adapted from Gardella et al. [128].With permission from Elsevier)

PTHrP (1-34) as being critically important for induction of receptor activation and receptor binding, respectively (Fig. 16.2a). These findings led to the development of PTH (7-34)- or PTHrP (7-34)-based peptide analogs as competitive antagonists for the PTH receptor [12–15]. Short N-terminal PTH fragments that lack the major C-terminal determinants of receptor binding located in the [15–34] region are generally inert, due to a loss of affinity interactions. However,

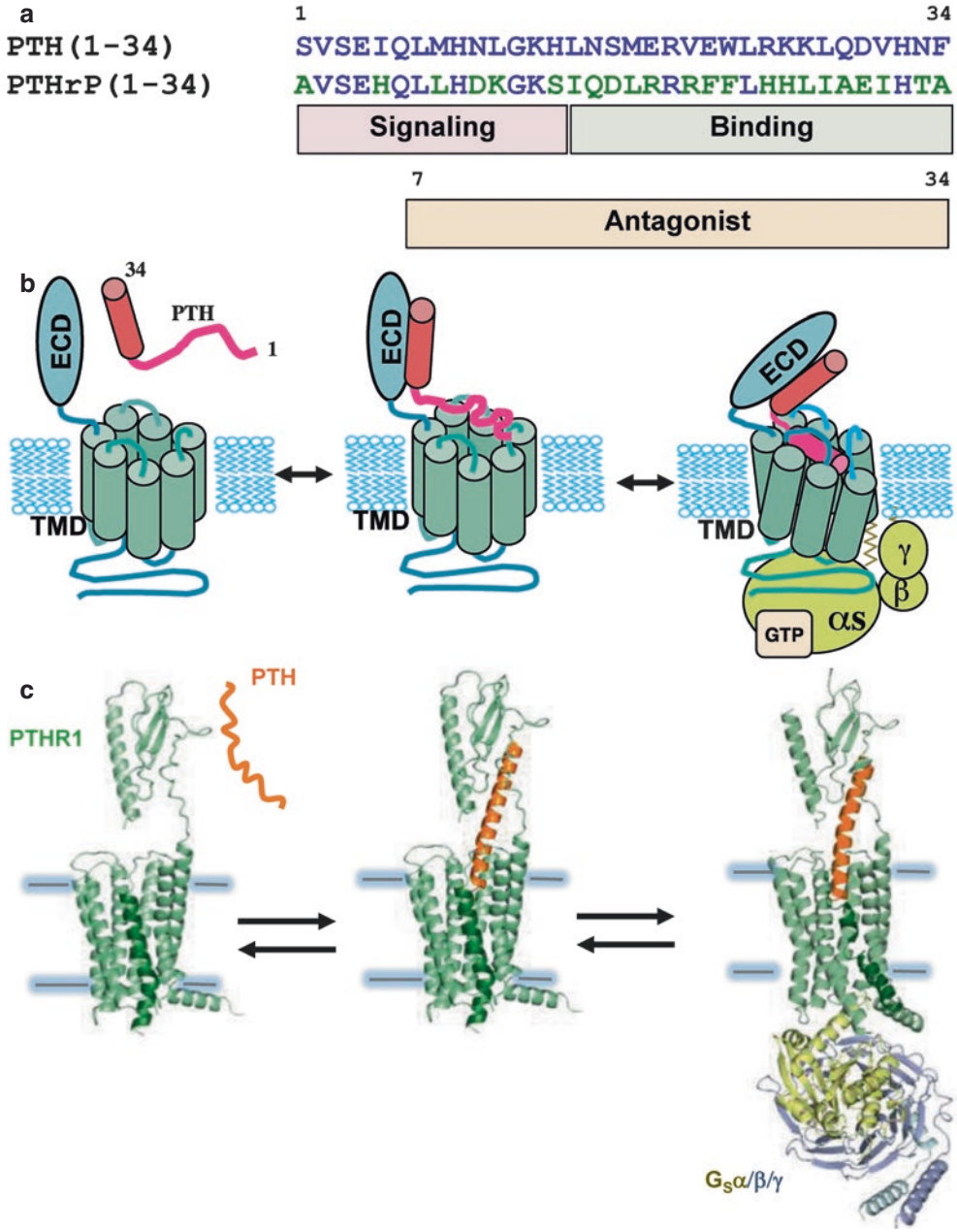


Fig. 16.2 Ligand-binding mechanisms at the PTHR1. (**a**) Sequence of PTH (1-34) and PTHrP (1-34) bioactive peptides. PTH residues are shown in blue and PTHrP residues in green with residues that are identical to those in PTH in blue. The principal N-terminal signaling and C-terminal binding domains and the PTH (7-34) antagonist scaffold are also indicated in the schematic. (**b**) The two-domain model of ligand binding and activation at the PTHR1, as originally developed by cross-linking and mutagenesis data obtained for the PTHR1: the C-terminal portion of PTH (1-34) first docks to the amino-terminal extracellular domain (ECD) of the receptor to provide affinity interactions; then, the amino-terminal portion of the ligand engages the transmembrane domain (TMD) portion of the receptor to induce conformational changes that enable G protein coupling. (**c**) Refinement of the two-domain model based on recent high-resolution X-ray-crystal and cryo-EM structures obtained for the PTHR1. The ligand, PTH (1-34) is shown in orange, and it binds as a nearly linear α-helix and makes extensive contacts with exposed extracellular surfaces in both the ECD and TMD receptor regions. Ligand binding results in an outward movement of the cytoplasmic termini of several of the TM helices, particularly TM6, to thus open a cavity that will accommodate the G protein. (Models of Panel **c** are adapted from Ref. [41] for the G protein-uncoupled states; and from Ref. [42] for the G protein-coupled state)

modified N-terminal peptides based on the PTH (1-11) and PTH (1-14) scaffolds have been developed that function as potent PTH receptor agonists [16–19]. The amino acid modifications in these N-terminal peptides enhance, either directly or indirectly, the productive interaction of the ligand fragment with the receptor.

The basic pharmacological work on ligand binding mechanisms conducted with synthetic bioactive PTH peptides and responsive cells and tissues facilitated the subsequent cloning of the cDNA encoding the PTHR1 in 1991 [20]. Studies on this cDNA revealed the PTHR1 (1) to be a GPCR, (2) to bind both PTH (1-34) and PTHrP (1-34) ligands with near equal affinity, and (3) to represent a distinct GPCR subgroup, now termed the class B GPCRs, as discussed earlier.

Evolutionary Origin and Gene Divergence of the PTHR1

The critical nature of the bone and mineral ion processes regulated by the PTHR1 suggests an early evolutionary origin. This notion is indeed supported by genomic studies conducted in various species. In humans, the gene for the PTHR1 resides on chromosome 3 (locus 3p22-p21.1). The gene spans a ~26 kilobase (kb) DNA segment [21]. The predicted transcript consists of 14 coding exons and 2 noncoding exons. The genes for the other class B GPCRs generally exhibit a similar intron/exon organization, suggesting they evolved from a common ancestral gene [22]. The PTHR1 is present in all vertebrate species and can be traced back via genomic analysis to at least the emergence of the early chordates (~530 million years ago). Homologous coding sequences have thus been found in several invertebrate species, including the amphioxus, *Branchiostoma floridae,* and the tunicate, *Ciona intestinalis* [23]. Sequences exhibiting ~70% amino acid similarity to at least portions of the human PTHR1 have also been identified in the genomes of some insects, including the red flour beetle (*Tribolium castaneum*) and honey bee (*Apis mellifera*) although not detected in the fruit fly (*Drosophila melanogaster*) [24, 25]. The biological function of any such invertebrate PTHR-like sequence is unknown.

Two rounds of whole genome duplication during metazoan evolution are thought to underlie the diversification of the primal PTHR coding sequence into what can now be seen in fish and variably in other vertebrate species, as four PTH receptor subtypes or orthologs – PTHR1, PTHR1b (formerly PTHR3, found only in fish), PTHR2 and PTHR2b [26, 27]. The PTHR1b (PTHR3) subtype has only been characterized in fish, as a corresponding sequence is not detected in higher vertebrates, including humans [28]. The close sequence homology and responsiveness to PTH ligands confirm fish PTHR1b to be a close ortholog of the PTHR1 [27]. The PTHR2 is present in mammals, including humans, in which it shows 51% amino acid identity to the PTHR1, and it is also found in fish, but not in birds [26]. The PTHR2 does not interact efficiently with either PTH or PTHrP, but instead responds to a distinct endogenous peptide ligand, called TIP-39. This ligand is a 39-amino acid peptide that shares some trace homology with the N-terminal (1-34) regions of PTH and PTHrP [29]. While TIP-39 potently activates the PTHR2, it is inactive on the PTHR1, albeit it does bind to the PTHR1 with moderate affinity. The biological roles of the PTHR2 and TIP-39 are not fully delineated but appear to involve actions in the neuroendocrine system [30], including pain and fear responses [31, 32].

Mechanisms of Ligand Binding at the PTHR1

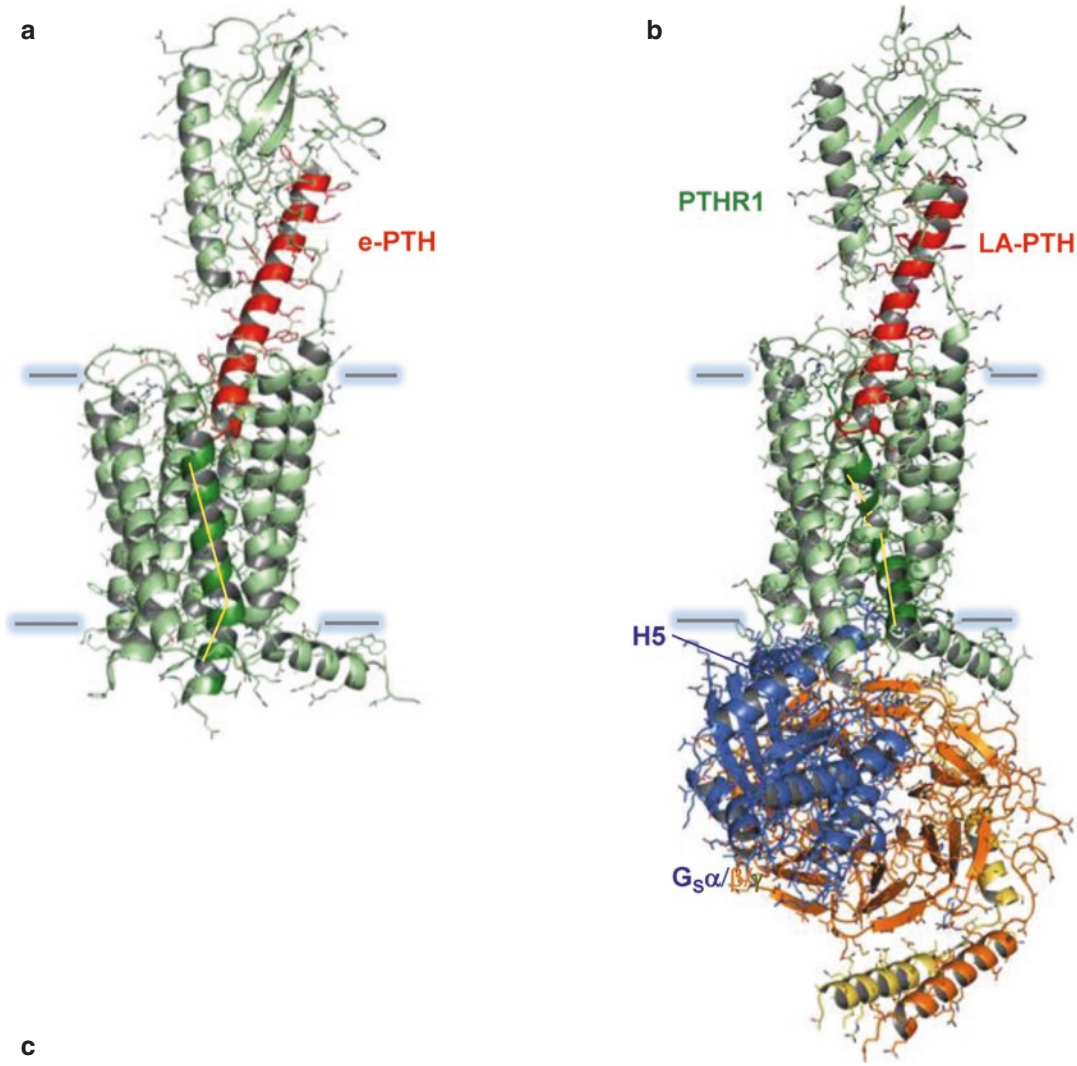
Consistent with the capacity of both PTH (1-34) and PTHrP (1-34) to bind to the PTHR1 with similar affinities and activate cAMP-based signaling responses with similar potencies, the two peptides exhibit considerable amino acid sequence homology in their amino terminal portions, particularly in the N-terminal (1-13) portions where 8 of the first 13 residues are identical (Fig. 16.2a). Early studies aimed at elucidating key sites of binding and activation for the PTHR1 employed a combination of

site-directed mutagenesis and synthetic peptide design approaches to alter targeted residues in the ligand and receptor [4, 33–35]. These functional approaches were complemented by a parallel use of photoaffinity cross-linking methods to directly map sites of proximity between ligand and receptor [36–40]. Together, these studies established that PTH (1-34) binds to the PTHR1 via a two-site mechanism that involves (1) an initial binding interaction between the [15–34] portion of the ligand and the ECD portion of the receptor and (2) a subsequent signaling interaction between the N-terminal (1-14) portion of the ligand and the TMD region of the receptor (Fig. 16.2b). This two-site mode of binding was also found to be used by other class B GPCRs and their cognate peptide ligands. The general model for PTHR1 is now verified and can be further refined by information gained from recent high-resolution crystal and cryo-EM structures that have been determined for the PTHR1 (Fig. 16.2c). Key findings from these structural structures are discussed in detail in the next section.

High-Resolution Molecular Structures of the PTHR1

The first high-resolution three-dimensional structures of the PTHR1 were reported in 2018 and early 2019. Two structures were obtained using two complementary approaches (Fig. 16.3). The structure reported by Ehrenmann et al. was derived using conventional X-ray crystallography methods [41], while the structure of Zhao et al. was derived using cryogenic electron microscopy [42]. The former technique required the introduction of various mutations and protein modifications to stabilize the otherwise flexible receptor protein sufficiently to allow crystal formation. The latter technique does not require or involve formation of crystals and so derives the structure of the receptor in a near-native state, although in general, cryo-EM structures are typically obtained at lower resolution than X-ray crystal structures. Nevertheless, both PTHR1 structures were resolved at a high enough resolution – 2.5 Å for the crystal structure and ~3.0 Å for the cryo-EM structure - to not only reveal the basic overall topology of the protein architecture but also enable a direct mapping of key molecular interactions that mediate peptide ligand binding and receptor activation. Each structure was obtained as a complex with a high-affinity PTH peptide analog, a PTH (1-34) analog called ePTH in the X-ray crystal structure, and a long-acting PTH/PTHrP hybrid analog called LA-PTH [43] in the cryo-EM structure. The cryo-EM structure further includes a coupled heterotrimeric G protein, Gαs/β/γ,and so appears to reflect a true active-state PTHR1 configuration. In contrast, the X-ray crystal structure was obtained with a PTHR1 variant that is defective for signaling due to the thermostabilizing mutations and does not include a coupled G protein, and so it seems more consistent with an intermediate state of agonist activation.

Ligand Binding Mode The overall protein architecture of the PTHR1 and the mechanisms of peptide ligand binding and activation that can be inferred from these structures are largely consistent with the two-site model established by the prior mutational and cross-linking approaches. The general mechanisms of binding and activation are also highly similar to those established for several other class B GPCRs for which high-resolution structures have been obtained, with hormone specificity being maintained by amino acid variations at sites that serve as critical determinants of hormone recognition [44]. The basic mechanism involves binding of the peptide ligand in a linear α-helical configuration. For PTH (1-34), the C-terminal (15-34) portion of the ligand helix fits into a hydrophobic groove in the receptor's ECD [45], while the N-terminal portion of the ligand, residues 1-14, extends down into the core of the TMD bundle. Binding to the ECD is stabilized by hydrophobic interactions formed between Trp23, Leu24, and Leu28, one helical face of the ligand, and complementary nonpolar surfaces lining the ECD binding groove. The side chain of Arg20 in the ligand makes extensive interactions with polar residues located at one end of the ECD. Residues, Arg20, Trp23, Leu24, and Leu28 are highly conserved in PTH and PTHrP ligands, while residues on the



c

PTH(1-34)	SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNF
PTHrP(1-36)	AVSEHQLLHDKGKSIQDLRRRFFLHHLIAEIHTAEI
ePTH	zVbEIQLMHQrAKWLNSnERVEWLRKKLQDVHNY
LA-PTH	AVAEIQLMHQRAKWIQDARRRAFLHKLIAEIHTAEI

z=ACPC, b=Aib, r=homoarginine, n=norleucine,

Fig. 16.3 High-resolution molecular structures of the PTHR1 in complex with PTH (1-34) analogs. (**a**) Structure of the PTHR1 in a non-signaling partially activated state obtained by X-ray crystallography [41]. Structure of the PTHR1 in a G protein-coupled, active state obtained by cryogenic electron microscopy [42]. The PTHR1 in the crystal structure contains thermostabilizing mutations that block signaling. In each complex, the ligand is shown in red, the receptor in green, and the α, β, and γ subunits of the heterotrimeric G protein in blue, orange, and yellow, respectively. The yellow line traces the path of TM helix 6 and highlights the pronounced bending and unwinding of the helix in the active-state structure to open a cytoplasmic cavity that accommodates helix 5 (H5) of Gαs. (**c**) Sequences of the ePTH and LA-PTH analogs contained in the structures of (**a**) and (**b**), respectively, with PTH residues shaded blue, PTHrP residues shaded green, and modified residues purple (ACPC and Aib are amino cyclopentane carboxylic acid and amino-isobutyric acid, respectively). (The structural models were generated from the protein database coordinate files 6FJ3 from Ref. [41] for the G protein-uncoupled states and 6NBF from Ref. [42] for the G protein-coupled state)

opposite ligand helix face, which is mostly polar, are less well conserved and are not critical for binding. Previous X-ray crystal structures of the isolated ECD in complex with PTH (15-34) or PTHrP (12-34) showed slight differences in the binding poses utilized for the two peptide fragments [46], which is consistent with the notion that PTH and PTHrP do not interact identically with their shared receptor [47].

The N-terminal (1-14) region of the ligand forms multiple contacts with various side chain and peptide backbone functional groups that are displayed over the surfaces of the orthosteric ligand-binding pocket formed on the extracellular face of the receptor's TMD bundle. These interactions lead to the induction of the conformational changes involved in receptor activation and G protein coupling. Overall, the total ligand-receptor contact surface is extensive and involves almost every residue in the PTH (1-34) peptide ligand, numerous cognate amino acid residue side chains, and backbone functional groups located in both the ECD and TMD portions of the receptor. These contacts together provide the complex with overall stability and affinity of ligand binding, while at the same time providing a mechanism of activation as well as ligand escape once the activation process is complete.

Mechanism of Receptor Activation In the structures, ligand residues Val2-Glu4 extend the deepest into the core of the TMD helical bundle and contact the floor of the orthosteric cavity (Fig. 16.4). Previous ligand and receptor mutagenesis studies show that the first nine amino acids of PTH contain critical determinants of receptor activation [48]. In both the X-ray crystal and cryo-EM structures, a number of specific contacts occur between conserved residues in the 1–9 region of the ligand and residues in the orthosteric pocket. Some, if not all, of these contacts are likely to play some role in triggering the conformational changes involved in receptor activation. One key set of interactions is a network of hydrogen bonds and Coulombic interactions that is formed by the side chain carboxylate of glutamate-4 in the ligand and polar residues projecting from several of the transmembrane helices (TMs), including Tyr195 in TM1, Arg233 in TM2, and Gln451 in TM7 (Fig. 16.4b, c). Dynamic interactions within this polar network are thus predicted to play key roles in triggering the conformational rearrangements in the hepta helical bundle involved in activation. One critical step in the conformational rearrangement is the pronounced bending and partial unwinding at the midpoint of the helix 6, which occurs around Pro415 and results in the wide outward movement of the cytoplasmic end of TM6 as well as TM7 (Fig. 16.2). These outward movements, in turn, open a cavity on the cytoplasmic face of the TMD bundle that serves as the principal docking site for the G protein and specifically accommodates helix 5 of the alpha subunit. The coupled G protein then undergoes a conformational change that leads to an exchange of GTP guanine nucleotide for the GDP bound within Gα, followed by the release of the activated G protein and activation of downstream effectors, which, for the PTHR1 and Gαs, is adenylyl cyclase (AC). Activation of AC in turn increases the intracellular levels of cAMP, a second-messenger signaling molecule that activates protein kinase A (PKA), which in turn further activates a variety of downstream mediators of the amplified signaling cascade, including other secondary kinases and gene transcription regulators [49].

Regulation and Termination of Signaling

The signaling responses activated by the PTHR1 in any given target cell must be tightly regulated in terms of amplitude and duration in order for the system to achieve normal physiologic adaption. This regulation involves multiple subcellular processes. A key early step is the phosphorylation of a number of hydroxyl-bearing residues in the C-terminal cytoplasmic tail of the receptor. The principal sites of phosphorylation are seven serines that lie in a cluster – Ser489-Ser504 – in the proximal region of the C-terminal tail, although recent mass spectroscopy analyses have revealed Thr387 and Thr392 in intracellular

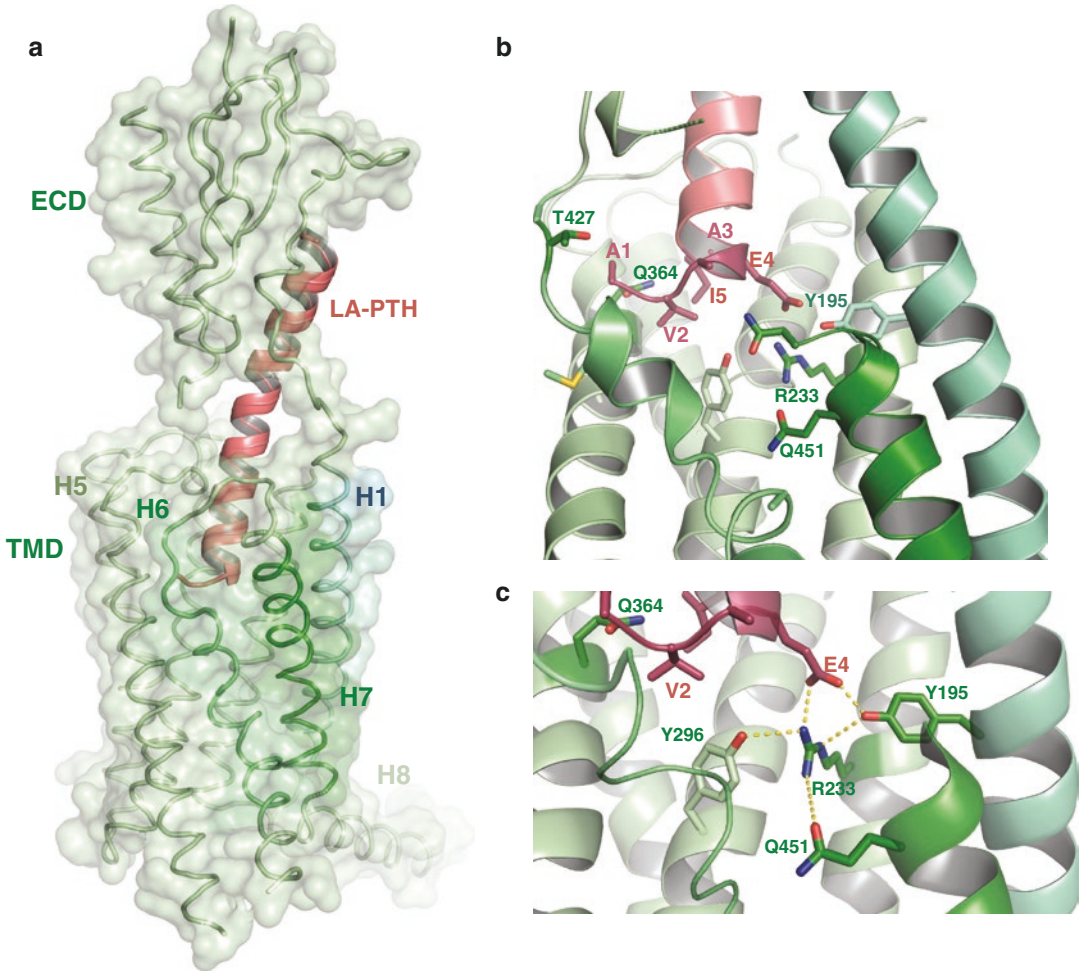


Fig. 16.4 Critical ligand interaction determinants in the TMD region of the PTHR1. (**a**) Cryogenic electron microscopy structure of the PTHR1 in complex with LA-PTH showing the N-terminal (1-14) segment of the ligand entering into the core region of the receptor's TMD bundle [42]. (**b**) Close-up view of the orthosteric ligand-binding pocket within the receptor's TMD bundle showing ligand residues Ala1-Glu4 making multiple contacts to residues dispersed within the cavity. (**c**) Hydrogen-bond network involving the side chain carboxylate atoms of Glu4 and polar groups of Tyr195 in TM1 and Arg233 in TM2, which together with Gln451 in TM7 form a polar network that is predicted to rearrange as a key step in the receptor activation process. (Adapted from Zhao et al. [42]. With permission from The American Association for the Advancement of Science)

loop (ICL3) as additional phosphorylation sites (Fig. 16.1) [50, 51]. G protein receptor kinases (GRK)-2 and GRK-5 mediate serine phosphorylation. The key consequence of phosphorylation is the recruitment of a β-arrestin molecule to the activated receptor, which promotes the internalization of the receptor to endosomal vesicles via a process of clathrin-coated pit (CCP)-mediated endocytosis. Classically, the recruited β-arrestin was thought to compete with and displace the bound G protein, which would further promote signal termination. Recent structural data, however, indicate that at least for some GPCRs, both β-arrestin and a G protein can bind simultaneously [52]. In fact, for the PTHR1, evidence has been presented that β-arrestin promotes Gαs-mediated cAMP signaling and extends the duration of the signaling response, as will be discussed later [53]. In any case, PTHR1 internalization occurs soon (within minutes if not seconds) after

initial agonist binding, with the CCPs pinching off from the plasma membrane as vesicles and the vesicles transiting along the endocytic network. During this movement, the vesicle interior progressively acidifies, which, for the PTHR1-PTH(1-34) complex, destabilizes the interaction to result in the release of the ligand from the receptor and hence signal termination. Vesicle acidification is mediated by the vacuolar ATPase proton pump, the activity of which is stimulated by cAMP/PKA-dependent phosphorylation. This cAMP/PKA-mediated phosphorylation-induced vesicle acidification provides the PTHR1 signaling system with a negative feedback mechanism of regulation [54].

Another step in signal regulation that occurs in endosomes and which is promoted by or at least coincident with vesicle acidification is the engagement of the ligand-receptor complex with retromer, which is an assembly of vesicle transport proteins that act to sort the endosomal cargo, i.e., the receptor, to either the lysosomal pathway for degradation or to the recycling pathway for transport back to the cell surface [55, 56]. A further mechanism by which PTHR signaling can be regulated involves ubiquitination of the receptor on several cytoplasmic lysines, particularly Lys388 and Lys484. Ubiquitination does not appear to be required for internalization, or to have a direct effect on cAMP signaling, but it can modulate signaling through the mitogen-activated protein kinase (MAPK) cascade [51]. Yet another mechanism by which PTHR1 signaling can be regulated, or at least modulated, involves interaction with the Na(+)-H(+) exchanger regulatory factor (NHERF) family of proteins, which mediate protein-protein interactions with the actin cytoskeletal network and associated cytosolic and plasma membrane proteins. For the PTHR1, these interactions occur via PDZ-domain recognition motifs that reside in the C-terminal tail of the receptor (Fig. 16.1). The effects of PTHR1-NHERF interactions are best seen in the studies on the capacity of PTHR1 signaling to promote the retrieval of the sodium-dependent phosphate transporter, NPT2A, from the apical surface of renal proximal tubule cells [57 58], which underlies the potent phosphaturic effects of parathyroid hormone.

Altered Modes of PTHR1 Signaling by Conformational Selectivity

A series of recent studies have shown that structurally distinct peptide ligands of the PTHR1, including unmodified PTH (1-34) and PTHrP (1-36) peptides, as well as analogs designed with various natural and non-natural amino acid substitutions, can bind to the PTHR1 via different mechanisms to thereby induce different types of signaling responses in target cells. One type of variation in signaling response is a shift in the second-messenger signaling pathway activated, as in the relative decrease in signaling via the Gαq/phospholipase C/inositol triphosphate/intracellular calcium/protein kinase C pathway versus the Gαs/AC/cAMP/PKA pathway that is seen with PTH analogs having serine at position 1 replaced by a bulky residue, such as tryptophan [59, 60]. A second type of signaling variation involves differences in the duration of the cAMP response that is activated by a given PTH ligand analog [61]. Such temporal differences in signaling arise from the different affinities with which the analog ligands bind to the G protein-uncoupled PTHR1 conformation, called R^0, which is an intermediate receptor state that can isomerize to the active G protein-coupled receptor conformation, called RG (Fig. 16.5a). Ligands with a range of R^0 affinities have been identified (Fig. 16.5b), and the duration of the cAMP signaling responses induced by these ligands, which is assessed using kinetic FRET-based cAMP sensors or luciferase-based GloSensor reporter assays, correlates strongly with their respective R^0 binding affinities. A high R^0 binding affinity thus results in a prolonged cAMP response.

An example of an altered mode of signaling based on this concept is highlighted by a particularly long-acting PTH analog, called LA-PTH [61, 62]. LA-PTH is a PTH/PTHrP hybrid molecule comprised of the PTH (1-14) domain joined to the PTHrP (15-36) sequence and further modified with nine amino acid substitutions located in both the N-terminal PTH and the C-terminal PTHrP portions of the peptide (Fig. 16.5c). The prolonged signaling properties of this ligand are due to its capacity to bind to the R^0 PTHR1

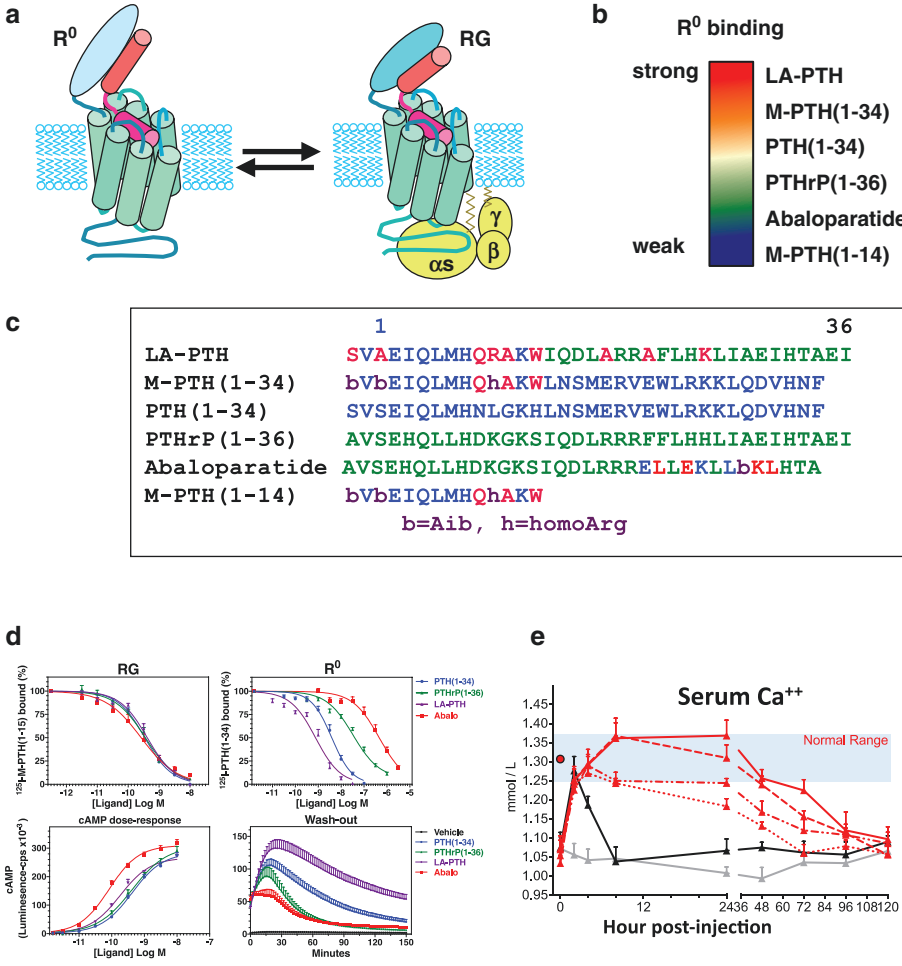


Fig. 16.5 Conformational selectivity and temporal bias of PTHR1 ligands. (**a**) Schematic of the R^0 and RG PTHR1 conformations and their reversible isomerization. (**b**) Relative affinities of various PTH ligand analogs for the R^0 conformation displayed as a heat map. (**c**) Primary structures of the ligands displayed in (**b**). (**d**) PTHR1-binding and cAMP signaling properties of LA-PTH, abaloparatide, PTH (1-34), and PTHrP (1-36) in HEK293 cells or membranes (binding). Binding to the RG (*top-left*) and R^0 (*top-right*) PTHR1 conformations assessed by competition methods using two conformation-selective radioligands – ^{125}I-PTH (1-34) for R^0 and ^{125}I-M-PTH (1-15) for RG. The ligands show similar affinities for RG but disparate affinities for R^0 with LA-PTH binding with highest affinity and abaloparatide binding with weakest affinity. Dose-response analysis of intracellular cAMP levels, measured via the luminescence-based GloSensor cAMP reporter, reveals similar potencies for the ligands (*lower left*); however, time course analysis after washout of the ligand, added previously to the cells for 15 minutes at its EC_{50} concentration, reveals cAMP signaling to be dramatically prolonged for LA-PTH and only transient for abaloparatide (*lower right*). (**e**) Upon single injection at several doses, LA-PTH (red) is markedly more effective than PTH (1-34) (black) at normalizing serum calcium levels in parathyroidectomized mice, a model of hypoparathyroidism. (**d** Adapted from Hattersley et al. [64]. With permission from Oxford University Press; **e** Adapted from Bi et al. [65]. With permission from American Society for Bone and Mineral Research)

conformation, which enables it to remain bound to the receptor even after the G protein has been activated and released from the complex. Classically, G protein release results in a shift of the receptor from the high-affinity RG conformation to the low affinity, uncoupled conformation called R. LA-PTH, and structurally related analogs of this class, can thus remain bound to the PTHR1 through multiple rounds of G protein coupling and release, resulting in prolonged signaling responses [47, 63].

In addition to the long-acting PTH analogs that exhibit high-affinity binding to R^0 as well as RG, other analogs were found that bind with high affinity only to RG. As a result, these RG-selective or RG-biased ligands stimulate relatively transient cAMP responses in cells. Among these analogs are the modified N-terminal PTH fragments, such as M-PTH (1-14), PTHrP (1-36), and a PTHrP (1-34) analog called abaloparatide, which is now in use as a therapy for osteoporosis, as discussed later. Pharmacological assays of receptor binding and cAMP signaling performed on several of these conformation-selective analogs are shown in Fig. 16.5d [64]. These studies illustrate how LA-PTH, PTH(1-34), PTHrP(1-36), and abaloparatide bind with comparably high affinities to the RG conformation of the PTHR1 but display widely divergent affinities for R^0 and that the RG-selective analogs, such as abaloparatide, induce shorter-duration cAMP responses than do the R^0-selective ligands, such as LA-PTH.

Such altered modes of binding and cAMP signaling observed in vitro have been shown to translate into altered effects in vivo. Thus, injection of LA-PTH into animals produces markedly sustained increases in blood calcium levels (Fig. 16.5e) [43, 62, 65]. Overall, these observations suggest a potential strategy, based on PTHR1 conformational selectivity, for optimizing the efficacy of PTH ligands for the treatment of various diseases of the bone and mineral metabolism. Indeed, LA-PTH has shown promise in preclinical tests conducted in rodent models of hypoparathyroidism (Fig. 16.5e), which supports a possible use of this analog as an alternative mode of treatment for this disease.

Further support for the notion that developing new PTH ligand analogs with selective binding to either the R^0 or RG PTHR1 conformation, and hence, with temporal signaling bias, can be a means to improve ligand therapeutic efficacy comes from findings with abaloparatide. This PTHrP (1-34) analog was found in clinical tests to be at least as effective as PTH (1-34) at preventing fractures in women with osteoporosis [66]. Given the efficacy of abaloparatide as an osteoporosis therapy, the findings suggest the general hypothesis that a ligand that binds selectively to the RG PTHR1 conformation with relatively weak binding to R^0, to thus induce potent but transient cAMP signaling responses, would be a more effective therapy for osteoporosis than would a ligand that binds with high affinity to both conformations and mediates prolonged signaling responses. This hypothesis is grounded on the well-established dogma that pulsatile administration of PTH is required to achieve a net anabolic bone response, while continuous treatment promotes a net bone-catabolic response [67, 68]. The corollary to this is that R^0-biased analogs, like LA-PTH, could be a path toward new treatments for hypoparathyroidism. Further concepts and findings relating to the development of PTHR1 ligands as therapeutics are discussed in a later section of this chapter.

Prolonged PTHR1 Signaling from Endosomes

Of considerable interest to these receptor-based mechanistic studies is the role that PTHR1 internalization plays in regulating the duration of the signaling response induced by a given PTH agonist ligand. A series of experiments explored this subject by applying methods of fluorescent microscopy to track PTHR1 signaling complexes in live cells, as well as kinetic FRET methodologies to measure in real-time second-messenger signaling output as well as the assembly of multimolecular signaling protein complexes. These studies together provide strong support for the hypothesis that the PTHR1 can mediate prolonged activation of cAMP signaling via Gαs from within

the endosomal compartment [56, 69, 70]. These breakthrough findings made with the PTHR1 were initially seen as contrary to the dogma that GPCR internalization was a key step in the signal termination process. It now has been shown, however, that a number of other GPCRs, including the β_2 adrenergic receptor, a prototypical class A GPCR, can activate G protein signaling via such a non-canonical, endosomal pathway. Conceptually, such findings open new possibilities for developing novel ligand analogs that are tailored to induce one selective type of signaling response versus another in a target cell of interest and thus potentially improving efficacy and minimizing adverse effects that might otherwise be induced by therapeutic agents targeted to a given GPCR [52, 71].

Diseases Caused by Disruptions in the PTHR1 Signaling System

A number of diseases of the bone and mineral ion metabolism are caused by defects in the PTHR1 signaling cascade. Hypoparathyroidism is a state of chronic hypocalcemia that most commonly arises from damage or loss of parathyroid gland tissue as a negative consequence of neck surgeries, i.e., thyroidectomies, but has also been linked to loss-of-function mutations in the genes encoding PTH and the PTHR1 as well as gain-of-function mutations in either the calcium-sensing receptor or its cognate G protein, Gα11 [72, 73]. In PTH, a heterozygous dominant Cys→Arg mutation at position 18 of the 31-amino acid prepro signal sequence (position −13 relative to Ser1 of the mature peptide) was identified in a patient with hypoparathyroidism; the mutation blocks hormone secretion in the parathyroid glands and is dominant to the wild-type allele [74, 75]. In the receptor, a homozygous mutation of Arg186→His was identified in several members of a family exhibiting hypocalcemia with elevated PTH [76]. The Arg186 residue is located at the extracellular end of the first transmembrane helix and in a segment that contributes importantly to ligand binding (Fig. 16.1) [77, 78]. Notably, the affected patients did not exhibit any abnormality in skeletal development, which suggests that the mutation selectively impairs interaction with PTH and not PTHrP. In support of this interpretation, a number of heterozygous loss-of-function mutations in PTHrP have been linked not to hypocalcemia but to brachydactyly type E, in some cases together with short stature and/or a failure in tooth eruption (FTE) [79–85]. Moreover, a number of heterozygous loss-of-function mutations in the PTHR1, excluding the Arg186→His allele mentioned earlier, have been identified in patients with FTE [86–92]. These phenotypes of brachydactyly and FTE are explained by haploinsufficiency of either the PTHrP ligand or the receptor in the primordia of the developing skeleton and teeth and highlight the critical roles that the PTHrP and the PTHR1 play in regulating cell differentiation processes in these tissues, as further dissected using genetically engineered mice [86, 93–95]. Moreover, homozygous or compound heterozygous loss-of-function PTHR1 mutations in humans result in the neonatal lethal condition of Blomstrand's chondrodysplasia [96, 97]. One point mutation identified in the compound heterozygous state is Pro132→Leu located in the ECD (Fig. 16.1). The same mutation has been found in the heterozygous state in patients with FTE [89].

Jansen's metaphyseal chondrodysplasia (JMC) is a rare disease caused by heterozygous gain-of-function mutations in the PTHR1. The patients exhibit defects in skeletal development and bone metabolism that result in dwarfism, limb deformities, hypomineralization of the bone matrix, and craniofacial abnormalities. The patients also exhibit defects in renal handling of mineral ions, reflected by conditions of hypercalcemia with low serum PTH, hyperphosphaturia, and nephrocalcinosis [98]. Of interest, whereas as the various loss-of-function mutations in the PTHR1 that have been identified in patients with FTE map to dispersed sites throughout the receptor, the mutations that cause JMC have been found only at three sites, His223, Thre410, and Ile458, which are each located at the cytoplasmic base of a TMD helix, TM2, TM6, and TM7, respectively (Fig. 16.6a) [99].Quite interestingly, the recent X-ray crystal and cryo-EM structures

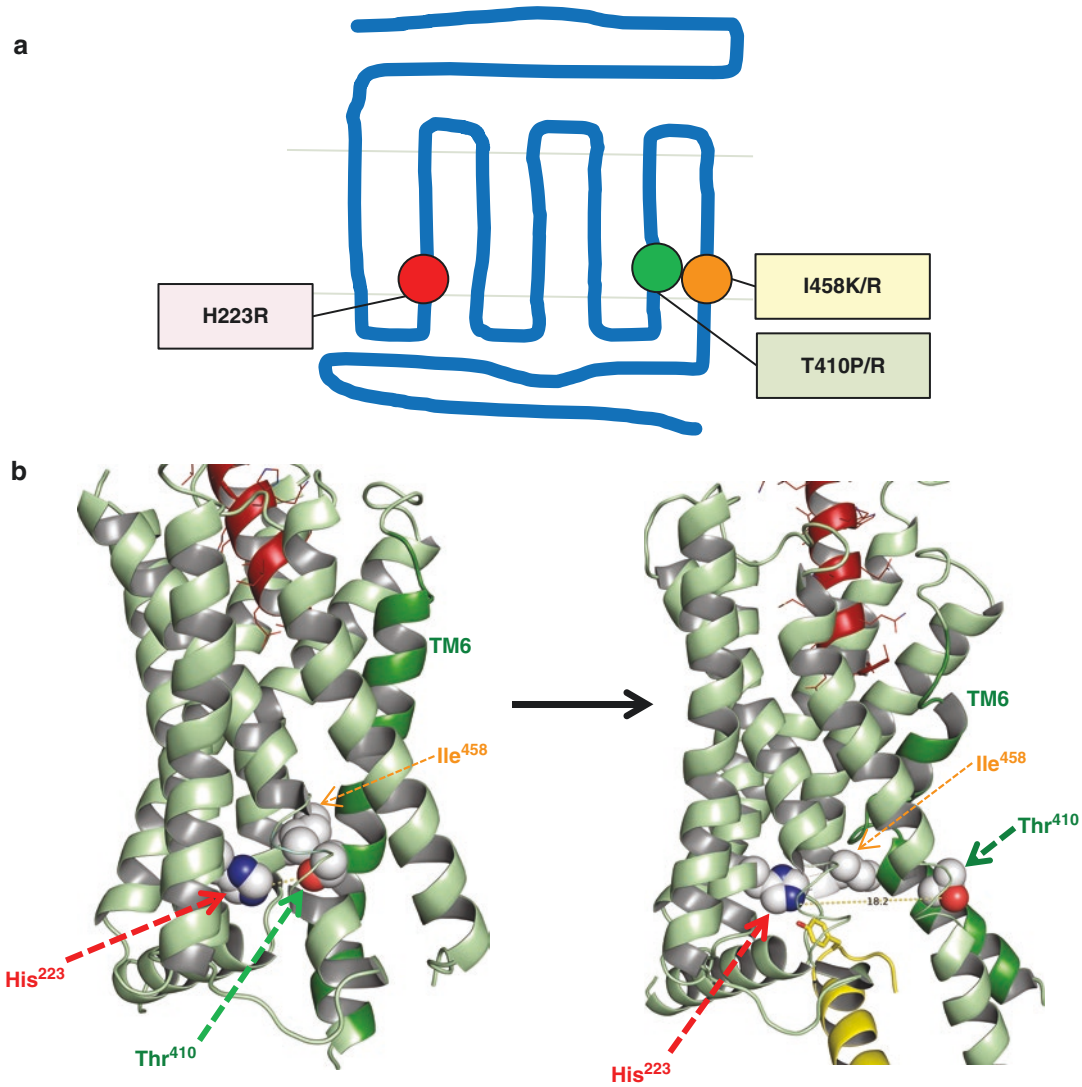


Fig. 16.6 Sites of PTHR1 mutations in Jansen's metaphyseal chondrodysplasia. (**a**) Location of the three residues in the PTHR1 at which mutations cause JMC, displayed in two-dimensional receptor representation to show that each residue is located at the cytosolic base of a transmembrane domain helix (TM): His223 in TM2, Thr410 in TM6, and Ile458 in TM7. (**b**) Display of the three residues mutated in JMC in the three-dimensional structures of the PTHR1 in the inactive [41] and active [42] states. The three residues are localized to a conserved micro-domain that acts as a switch to control the outward movements of the cytoplasmic ends of several of the TM helices, particularly TM6, that occurs during receptor activation. The Jansen mutations, including His223→Arg, Thr410→Pro, and Ile458→Arg, perturb this switch mechanism, causing the receptor to adopt the active-state (open) conformation even in the absence of a bound agonist ligand. In the active-state structure, the C-terminal portion of helix 5 of Gαs is shown in yellow, with the side-chain of Tyr391 in stick format to highlight its proximity to His223R of the receptor. (The structural models of panel B were generated from the protein database coordinate files 6FJ3 from Ref. [41] for the inactive state (left), and 6NBF from Ref. [42] for the active state (right))

of the PTHR1 reveal that these three residues are located close to each other at the base of the TMD helical bundle and are directly involved in or adjacent to a micro-domain switch component of the receptor that controls the movements of the helices and the cytoplasmic face of helical bundle during receptor activation (Fig. 16.6b) [41]. This switch incorporates a hydrogen-bond net-

work that is highly conserved in the class B GPCRs [100] and, which in the PTHR1, directly involves His223 and Thr410, along with Glu302 in TM3 and Tyr459 in TM7. The mutations of JMC can thus be predicted to impact this H-bond network so as to facilitate the outward movements of the helices that lead to receptor activation and G protein coupling in the absence of a bound agonist ligand.

Other Diseases Linked to PTHR1 Mutations

Enchondromatosis (Ollier disease/Maffucci syndrome) is a rare disease characterized by cartilage tumors of the bone and has been associated with four PTHR1 mutations, Gly121→Glu, Ala122→Thr, Arg150→Cys, and Arg255→His, located in the ECD or extracellular loop-1 portions of the receptor. The mechanism by which these mutations result in cartilage tumors in bone is unclear, as studies in vitro provide evidence for both gain-of-function and loss-of-function effects [101, 102]. Eiken syndrome is a very rare skeletal dysplasia associated with a homozygous-recessive, non-sense mutation, Arg485→stop, which truncates the receptor's C-terminal tail [103]. The phenotype is a markedly delayed ossification of the skeleton, opposite to that of Blomstrand's disease. This seems consistent with a gain-of-function effect, albeit a mild one, since no disease is seen in the heterozygous state and the homozygous phenotype is distinct from that of JMC. While the mutation can be predicted to alter interactions with cytoplasmic effectors and scaffolding proteins, and hence, the sub-cellular trafficking and signaling properties of the receptor, this remains to be determined.

The PTHR1 as a Target for Therapeutic Ligands

The PTHR1 has long been of interest as a therapeutic target for diseases of the bone and mineral ion metabolism. The capacity for PTH to stimulate bone formation makes it of high interest as a treatment for osteoporosis. As early as 1929, Fuller Albright made the observation that daily administration of parathyroid gland extracts to rats increased the radiologic bone mineral density and the number of bone trabeculae [104]. This was followed in the 1970s by studies in humans in which a synthetic PTH (1-34) peptide was injected once-daily in patients with osteoporosis and again found to promote substantial increases in trabecular bone mass [105, 106].Three decades later, a large clinical trial was conducted in osteoporotic women, and this positively established that daily injection of PTH (1-34) increased bone mineral density and reduced the risk of new bone fracture [107]. These data led to the US FDA approval of PTH(1-34) as the first bone anabolic therapy for osteoporosis, and this peptide is currently marketed for that indication by Eli Lilly and Company under the trade name Forteo. More recently, in 2015, abaloparatide was developed and approved by the US FDA as an alternative anabolic therapy for osteoporosis, and this PTHrP (1-34) analog is now marketed by Radius Health Inc. under the trade name Tymlos. Abaloparatide was originally developed through a strategy in which analogs of PTHrP (1-34) containing modifications that altered the amphipathic nature of the C-terminal α-helical binding region of the peptide helical were assessed in animals for the capacity to increase bone mineral density without increasing serum calcium, so as to thus minimize risk of adverse hypercalcemia [108, 109]. That abaloparatide may induce less of a bone-catabolic response, relative to the anabolic response, is indeed suggested by clinical studies that included a comparison to PTH (1-34). The studies thus found a lower incidence of hypercalcemia in subjects treated with abaloparatide versus PTH (1-34) ($P = 0.006$) as well as lower serum levels of the collagen breakdown product CTX-1, while beneficial effects on bone structure were comparable [66]. As suggested earlier, a capacity of abaloparatide to induce a relatively favorable outcome in bone formation versus bone resorption parameters could be related to its selectivity in binding to the RG PTHR1 conformation, vs. the R^0 conformation and thereby activating only transient signaling responses in target osteoblastic cells.

As mentioned earlier, LA-PTH is a PTH ligand that binds with a relatively high affinity to the R^0PTHR1 conformation and hence mediates prolonged cAMP response in cells, potentially from endosomes, properties that suggest that LA-PTH could be used as a hormone-replacement therapy in hypoparathyroidism. Indeed, LA-PTH has been shown to be considerably more effective than PTH (1-34) in normalizing blood calcium levels in parathyroidectomized mice [65] as well as in thyroparathyroidectomized rats (Fig. 16.5e) [43]. The analog thus represents a new preclinical candidate therapy for this disease. Natpara is the trade name of the native PTH (1-84) peptide that is currently marketed in the US by Takeda Corporation for the treatment of hypoparathyroidism. This peptide, administered by a once-daily subcutaneous injection, is clearly effective at normalizing serum calcium levels in patients for extended periods. Yet the clinical trial data suggest that improvements in the degree of constancy in maintaining daily serum calcium levels, as well as in the extent that urine calcium excretion is reduced, would be desirable [110]. An alternative approach that has shown promise in clinical trials is the continuous delivery of PTH (1-34) by an insulin infusion pump [72]. Each of these approaches utilizes a PTH peptide that is of unmodified sequence and hence binds to the PTHR1 via a conventional mode of interaction. Whether or not a therapy based on the altered mode of PTHR1 interaction seen with LA-PTH and analogs of that class will provide advantages to either of these native-PTH peptide-based therapies, in terms of the control of serum and urine calcium levels, remains to be seen.

Antagonists and Inverse Agonists for the PTHR1 Consistent with the critical role that residues at the N-terminus of PTH and PTHrP peptides play in receptor activation, N-terminally truncated peptides that lack as many as the first six residues retain adequate binding affinity for the receptor but are markedly deficient for inducing activation. Peptides such as PTH (7-34) and the analog [$Nle^{18,18}$,$dTrp^{12}$,Tyr^{34}]-bovine PTH (7-34) thus function as effective competitive antagonists and inhibit binding of PTH (1-34) to the wild-type PTHR1 in vitro and in vivo [14]. Such analogs could potentially be used to treat diseases of PTH or PTHrP ligand excess, as occurs in primary hyperparathyroidism and in the hypercalcemia of malignancy. However, efficacy of these antagonist ligands in vivo tends to be weak, likely due, at least in part, to a rapid clearance of the small-sized peptide from circulation [111]. Further studies in vitro have revealed that a subset of the PTH and PTHrP antagonist analogs, including [Leu^{11},$dTrp^{12}$,Tyr^{36}]-PTHrP (7-36), behave as inverse agonists on the constitutively active PTHR1 mutants identified in patients with JMC. These inverse agonist ligands dose-dependently depress the basal cAMP signaling activities of the mutant receptors that are otherwise elevated, as compared to the wild-type PTHR1 and assessed in transfected COS-7 and HEK293 cells [112–114]. The mechanism at the receptor level by which these ligands achieve their inverse agonist effect is unclear, although the Gly^{12}→dTrp substitution is known to be required for the effect. The functional properties of these analogs suggest a possible path toward the first therapy option for patients with JMC, for which there is currently no effective treatment available. A promising advance toward this goal has been made with [Leu^{11},$dTrp^{12}$,Tyr^{36}]-PTHrP (7-36), which was tested in transgenic mice that express the PTHR1-H223R allele of JMC via the collagen Ia promoter and hence in osteoblasts and osteocytes. These mutant mice exhibit marked increases in trabecular bone mass, as well as elevations in bone turnover markers [115]. The inverse agonist analog administered by twice-daily injection for 2 weeks starting at day 14 resulted in significant reductions in trabecular bone mass and in the levels of bone turnover markers, as compared to vehicle-injected control mice [116]. Although JMC is a complex disease that involves defects in early skeletal development as well as in the homeostatic control of mineral ion levels, the results at least suggest the possibility that an inverse agonist ligand could be developed as a future treatment option for this disease.

Small-Molecule Ligands Targeted to the PTHR1

The involvement, direct or indirect, of the PTHR1 in a number of bone and mineral ion diseases, including osteoporosis, hyperparathyroidism, hypoparathyroidism, and JMC, places the receptor in a position of considerable interest as a drug-discovery target. A small-molecule compound that mimics the actions of PTH in bone and kidney would be particularly desirable for both osteoporosis and hypoparathyroidism, as current therapies require daily injection of a gut-labile peptide. The PTHR1, however, has been a challenging target for small-molecule drug discovery, as efforts to date have yielded only a handful of reported compound ligands. The key molecules and representatives of the types of compound ligands reported are shown in Fig. 16.7a. Included are both agonists and antagonists. The agonist compound, AH3960, reported by GSK Corporation in 2006, stimulates cAMP formation in cells at doses of about 10 micromolar or higher and is thus at least 1000-fold weaker than PTH (1-34) [117]. Tests in animals were not reported. More recently, the agonist compound PCO371 was reported by Chugai Corporation and was shown to stimulate cAMP formation in cells at concentrations in the low-micromolar range [118] (Fig. 16.7b). PCO371 is thus slightly more potent than AH3960, with which it shares no obvious structural similarity. Moreover, PCO371 is effective in vivo, as it was shown to raise blood calcium levels in TPTX rats (Fig. 16.7b). The calcemic response to PCO371 was prolonged, likely due to an extended pharmacokinetic profile, and because of that, PCO371 is being developed as a candidate therapy for hypoparathyroidism and not for osteoporosis [118].

The first non-peptide antagonist compound identified for the PTHR1 was SW106, discovered by Bristol-Myers Squibb Corporation in 2007. It was identified by screening a compound library for agents that could inhibit the binding of a radiolabeled PTH (1-14) analog [119]. SW106 acts as a competitive inhibitor of the PTHR1. Also in 2007, the James Black Foundation reported a broad set of antagonist compounds distinct from SW106. The most effective of these agents antagonized the cAMP-stimulating actions of PTH (1-34) in HEK293 cells transfected with the human PTHR1 with inhibitory constants in the 10–100 nanomolar range, while studies in vivo were not reported [120].

It is of considerable interest to elucidate the molecular modes of action of such compounds on the receptor. Mutagenesis-based mapping studies have established that SW106, AH-3960, and PCO-371 each bind to the TMD portion of the receptor and not to the ECD region [114, 118].This is shown by the capacity of each of the compounds to be as effective on a PTHR1 construct that lacks the ECD as it is on the intact PTHR1. PCO371 was found to be inactive on the human PTHR2, which is 51% identical to the PTHR1 in amino acid sequence. A mutational search of the divergent residues in the PTHR2 that were involved in this resistance to the drug identified Leu369 in the middle of TM6 [118]. This residue corresponds to proline-415 in the PTHR1. Whether PCO371 directly contacts Pro415 cannot be discerned from the functional data reported. Quite interestingly, however, a proline at this site in TM6 is conserved in most other class B GPCRs and is thought to play a key role in mediating the helix coil transition and helix kinking that occurs in TM helix 6 during receptor activation. Ultimately, crystal structures may be needed to learn the precise mode of action of these small-molecule ligands and to thus reveal whether they bind within the orthosteric pocket used by the peptide ligand or to an allosteric site located outside of the pocket and potentially involving the lipid-facing surfaces of the TMD bundle, as found for small-molecule ligands bound to several other class B GPCRs [44].

Evidence for Receptors That Bind C-Terminal Regions of PTH and PTHrP

The findings with the cloned PTHR1 and synthetic PTH (1-34) and PTHrP (1-34) peptides raised questions as to the biological roles of the

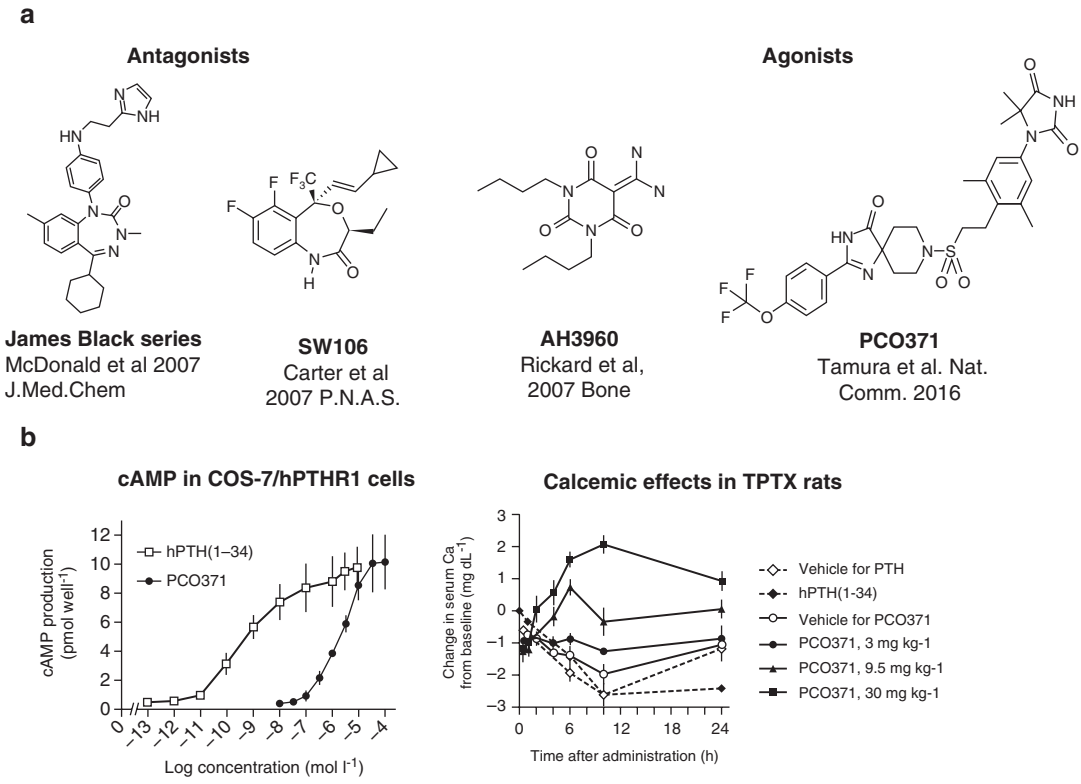


Fig. 16.7 Small-molecule ligands for the PTHR1. (**a**) Structures of small-molecule antagonist and agonist compounds identified for the PTHR1 through high-throughput screening approaches. (**b**) The agonist compound PCO371 stimulates cAMP signaling responses in COS-7 cells expressing the human PTHR1 to the same maximum level as induced by PTH (1-34), albeit with a potency that is about three log-orders weaker than that of PTH (1-34) (*left*). In thyroparathyroidectomized (TPTX) rats, PCO371 induces sustained elevations in serum calcium levels (*right*), presumably due to slow elimination from circulation. Consequently, PCO371 is in development as a candidate treatment for hypoparathyroidism. (**a** Adapted from Ref. 117–120]; **b** Reprinted from Tamura et al. [118]. With permission from Creative Commons License 4.0:https://creativecommons.org/licenses/by/4.0/)

C-terminal portions of the native PTH and PTHrP polypeptides, i.e., the residues located beyond residue 34. At present, these roles, if any, remain poorly understood. It is noteworthy that none of the other peptide ligands that bind to the other class B GPCRs have comparable C-terminal extensions, as each is about 30–40 amino acids in length. Nevertheless, a number of studies conducted on C-terminal fragments of PTH and PTHrP provide evidence for binding activity as well as certain biological responses [121–124] even in vivo [125]. It is quite clear that such effects are not mediated through the PTHR1 since at least some responses, such as the induction of apoptosis by PTH (7-84), can be observed in cells derived from mice genetically ablated for the PTHR1 [126]. The actions are thus thought to involve some other cell surface receptor or binding protein, which remains to be identified. Whether or not any such action provides a means for the native peptides to achieve an additional level of biological regulation, perhaps to complement those actions induced via the PTHR1, also remains to be established.

Conclusions

The PTHR1 is a class B GPCR that plays critical and fundamental roles in biology and hence has been extensively investigated at the basic molecu-

lar level. Defects in the PTHR1 signaling system are associated with a number of diseases of skeletal development and/or mineral ion homeostasis, including Jansen's metaphyseal chondrodysplasia and primary failure of tooth eruption, caused by gain-of-function and loss-of-function mutations in the PTHR1, respectively. New insights into fundamental mechanisms of PTHR1 function can now be gained from the recent X-ray crystal and cryo-EM structures reported for the PTHR1. These structures help to reveal how the receptor engages its peptide ligand, PTH or PTHrP, and then undergoes a process of conformational change to transmit the extracellular hormonal signal to intracellular G proteins and other effectors. The intrinsic conformational plasticity of the PTHR1 has provided a means by which structurally modified PTH and PTHrP peptide analogs that induce unique modes of downstream signaling can be developed. Among such ligands is a novel long-acting PTH analog, called LA-PTH, which holds potential as a new candidate in hormone-replacement therapy for hypoparathyroidism. A shorter-acting PTHrP analog, abaloparatide, has also emerged as an effective bone-anabolic agent and is now in use to treat osteoporosis. Perhaps due to its relatively complex mode of ligand binding and activation, the PTHR1 has been a challenging target for small-molecule discovery efforts. Yet several such compounds have been identified, and the new molecular receptor structures and mechanistic insights bring promise that new paths will be opened that lead to the development of additional ligands that target this receptor and can be used to treat more effectively diseases of the bone and calcium metabolism.

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PTH and PTHrP Analogs: Treatment of Osteoporosis

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Key Points

- Teriparatide and abaloparatide are the two osteoanabolic drugs available for treatment of osteoporosis.
- The PTH/PTHrP analogs represent a therapeutic approach to osteoporosis that significantly improves microarchitectural, geometric, and other properties of bone with the potential to reconstruct the skeleton in a disease characterized by abnormal skeletal microstructure.
- Since PTH/PTHrP analogs and antiresorptives operate by completely different mechanisms, the rationale for combination therapy is attractive to maximize the therapeutic benefits.

Introduction

Until 2002, antiresorptive agents defined our pharmacological approach to osteoporosis.With the introduction of teriparatide, PTH (1-34), and more recently abaloparatide as treatments for osteoporosis, we now have available a class of drugs that reduce fracture risk by completely different mechanisms. By stimulating bone formation to a greater extent and earlier than bone resorption, teriparatide and abaloparatide improve not only bone mineral density (BMD) but also other properties of bone. These other properties include skeletal microarchitecture and bone size. These features confer upon PTH analogs that have been developed for osteoporosis the potential to reconstruct the skeleton [1]. Since PTH and antiresorptives operate by completely different mechanisms, the rationale for combination therapy is attractive. Further work has provided new insights into how antiresorptive agents and PTH can be used in sequence or in combination for maximal therapeutic benefits.

Parathyroid Hormone as an Anabolic Agent

In primary hyperparathyroidism, a disorder of chronic, continuous secretion of excess PTH, catabolic effects are seen commonly at cortical sites such as the distal one-third radius.

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Nevertheless, even in primary hyperparathyroidism, a clue to the anabolic actions of PTH can be appreciated by its salutary effects at the lumbar spine, a site that is endowed preferentially with cancellous bone [2]. The typical pattern of bone density in primary hyperparathyroidism is relatively well-conserved lumbar spine density with more evident reduction of bone mineral density at the distal one-third radius. This is particularly noteworthy in postmenopausal women with primary hyperparathyroidism who are not receiving estrogens. In these estrogen-deficient individuals, one would expect early and preferential reduction of lumbar spine bone density since sex steroid deficiency is classically associated with rapid cancellous bone loss. Greater insight into the anabolic potential of PTH came with the recognition that this property could be distinguished from its catabolic proclivities when PTH is used in low doses and intermittently [3]. Subsequent animal and then human studies confirmed the point that PTH is a potent anabolic agent when it is used intermittently and in low doses. PTH is currently available in many countries as the recombinant human PTH (1-34) fragment known as teriparatide. Abaloparatide, a PTHrP (1-34) analog, is approved in the USA as a treatment for postmenopausal osteoporosis. It contains the first 22 residues of the PTHrP molecule, but then differs by the strategic placement of different residues to amplify an interaction with a specific conformation of the PTH receptor [4]. The PTH receptor type 1 (PTHR1) mediates the skeletal effects of both teriparatide and abaloparatide. The molecules showed a different osteoanabolic profile, although they act on the same receptor. The work of Hattersley et al. [5] demonstrated that abaloparatide has greater affinity for the RG conformation of the PTH1 receptor than the R0. The RG conformation of the PTH1 receptor is associated with a much more transient interaction with its cognate ligands (e.g., PTH and PTHrP) than the R0 conformation in which the interaction is longer lived. Theoretically, the affinity of a PTH or PTHrP ligand for the RG conformation would help to define its actions as less catabolic. The distinction between abaloparatide and teriparatide is not so much in the relative affinities of these two ligands for the RG conformation but rather in the markedly reduced affinity of abaloparatide for R0 [6, 7]. This leads to a substantially greater relative affinity of abaloparatide for the RG than the R0 conformation of the PTH/PTHrP receptor (Fig. 17.1).

Teriparatide leads to a rapid increase in bone formation markers followed thereafter by increases in bone resorption markers. The discordant kinetics of PTH actions on these reflectors of bone formation and bone resorption, respectively, are compatible with the idea that PTH initially stimulates processes associated with bone formation. At some time later, generally increased bone turnover is stimulated. This sequence of events with bone formation preceding bone resorption has led to the concept of the "anabolic window," a period of time when the actions of PTH are maximally anabolic [8] (Fig. 17.2). It is noteworthy that this concept is itself not a permanent one because eventually, bone turnover returns, after several years, to the baseline level. Although the concept of the anabolic window is supported by many observations, in many different clinical trials, the mechanistic basis is not clear. The time course of the change in bone turnover markers, coupled with histomorphometric observations, suggest that several mechanisms are likely to account for the anabolic action of these PTH peptides. The initial action in which bone formation is seen rather exclusively argues for a direct modeling effect of PTH, namely, to stimulate bone formation on quiescent surfaces. Subsequently, remodeling-based processes seem to be predominant, but during this remodeling period, bone formation is still stimulated to a greater extent than bone resorption. Thus, in this context, bone remodeling is not a negative experience because bone formation exceeds bone resorption [9]. Finally, bone turnover trends toward baseline values with the anabolic window becoming more and more narrow until such time that the anabolic effect of PTH is no longer seen.

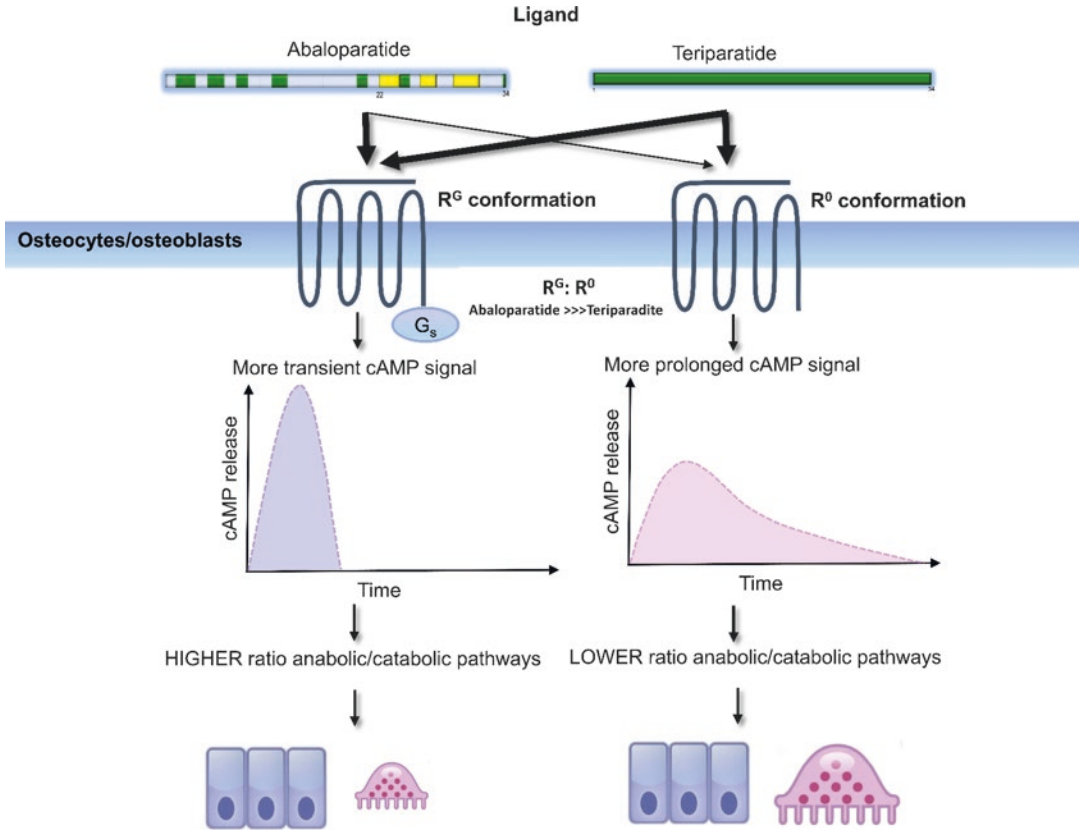

Fig. 17.1 Binding selectivity for the different conformational states (RG and R0) of the PTH/PTHrP receptor. (Adapted from Tay et al. [4]. With permission from John Wiley & Sons)

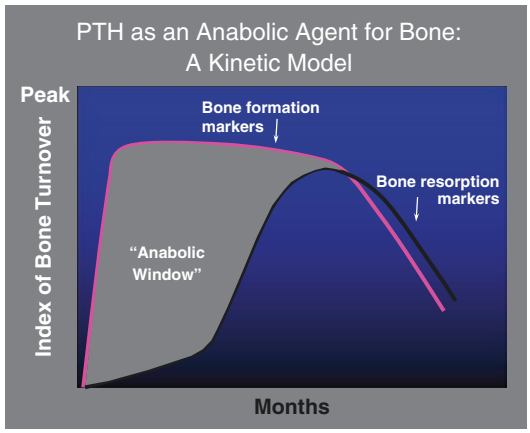

Fig. 17.2 Teriparatide anabolic window. Based on the difference in kinetics of changes between bone formation and bone resorption markers, an "anabolic window" is formed during which the actions of the parathyroid hormone are believed to be maximally anabolic

This concept of the anabolic window can be applied to both teriparatide and abaloparatide. They each seem to follow the same chronology of change in bone markers. Relative to teriparatide, however, abaloparatide does not stimulate bone resorption and bone formation to the same extent. Translating this difference in the context of the anabolic window, it would appear to be wider for abaloparatide than teriparatide [10] (Fig. 17.3).

Many technological approaches have demonstrated the beneficial effects of teriparatide on the properties of the skeleton, such as bone mineral density, microarchitecture, collagen maturity, bone geometry, and overall bone strength [11–17]. At a cortical skeletal site, such as the distal one-third radius, PTH typically does not increase bone density. In fact, there is often a small decline in BMD at that cortical site in association with an

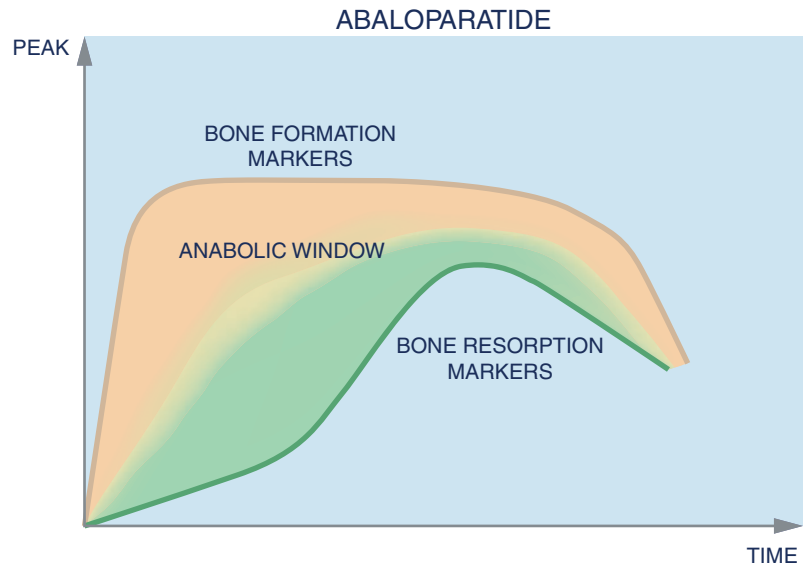

Fig. 17.3 Abaloparatide anabolic window. Based on the difference in kinetics of changes between bone formation and bone resorption markers, an "anabolic window" is formed during which the actions of the parathyroid hormone are believed to be maximally anabolic

increase in cortical porosity. However, the decline in BMD can be misinterpreted because it is well-known that teriparatide reduces non-vertebral fracture risk. This apparent paradox, namely, fracture reduction at site(s) at which bone mineral density declines, can be accounted for by several observations. First, the increase in porosity is seen only in the inner one-third of the cortical compartment, where the biomechanical effects or cortical porosity is minimal. In addition, microarchitecture improves [13–16]. These microarchitectural changes strengthen the cortical bone despite the small reduction in bone density [13, 14]. By finite element modeling, which takes all these changes into account, Keaveny et al. [11] have shown that biomechanical properties of the vertebrae are strengthened by teriparatide and that the strength to density ratio is improved. Despite an increase in radius cortical porosity, the whole-bone stiffness and failure load estimated by finite element analysis are maintained or increased [18–20].While these observations have been made repetitively and extensively for teriparatide, one would anticipate that similar studies with abaloparatide, when and if conducted, would not be qualitatively different. PTH and PTHrP peptides clearly improve bone strength through several different mechanisms that improve bone quality.

Indications for Treatment

Teriparatide is indicated in postmenopausal women and men with osteoporosis who are at high risk for fracture or who have failed or been intolerant to previous osteoporosis therapy. It is also indicated in glucocorticoid-induced osteoporosis. Abaloparatide is indicated, at this time, only in postmenopausal women at high risk for fracture. To help select patients for osteoanabolic therapy, useful guidelines have been published [21]. Patients who have already sustained an osteoporotic fracture are among the highest-risk groups because the likelihood of sustaining another fracture is very high [22]. In many countries, in fact, a previous osteoporotic fracture is a requirement for treatment with teriparatide. However, the *T*-score itself, even without an osteoporotic fracture, can confer high risk, especially if the *T*-score is very low (i.e., <−3.0). Age of the patient is also important because it confers greater risk for any given *T*-score. A 75-year-old woman with a *T*-score of −2.5 is at greater risk for a fracture than a 55-year-old woman with the same *T*-score. While these indications are straightforward, it is not always clear when teriparatide or abaloparatide should be used since the major clinical trials with the two major bisphosphonates, alendronate and risedro-

nate, also were shown to be effective in patients whose osteoporosis was just as severe as those for whom teriparatide is indicated. This discussion has to take into account facts that favor a bisphosphonate (lower cost, oral and intravenous routes of administration) versus those that would favor teriparatide or abaloparatide (actual incremental gains in bone tissue per se). Other potential candidates for anabolic therapy are patients in whom one might consider a bisphosphonate but who cannot tolerate the drug. In addition, patients who fracture while on antiresorptive therapy could be considered to be at even higher risk and thus be candidates for teriparatide or abaloparatide. Both drugs are approved for 2 years of therapy. Lifetime exposure to osteoanabolic therapy for osteoporosis is limited to 2 years. Someone, for example, who has received 2 years of teriparatide therapy is not recommended for abaloparatide [23].The reason for the strong recommendation limiting exposure of both osteoanabolic agents to no more than 2 years may be due to the fact that both drugs have a "black box" warning. The "black box" warning calls attention to the fact that in rats exposed to either of these agents, at 3–60 times the human dose for 2 years (equivalent to 75 years of human life), osteosarcoma develops [24, 25]. We can speculate on the relevance of this animal model to potential human toxicity, because it is noteworthy that the non-human primate, the cynomolgus monkey, does not develop osteosarcoma under similar conditions [26]. Moreover, after 16 years of experience throughout the world in well over two million patients, the incidence of osteosarcoma is well below what one might expect coincidentally. Finally, the profound differences in skeletal metabolism between the rat and primate with the rat growing forever with modeling as the primary skeletal dynamic versus the adult human in which growth has ceased and remodeling is the primary skeletal dynamic raise further questions as to the relevance of the rat model to the human, with regard to osteosarcoma.

Teriparatide as Monotherapy in Postmenopausal Osteoporosis

The randomized, double-blind, pivotal clinical trial of Neer et al. [27] showed that women with advanced osteoporosis and multiple fragility fractures had a lower incidence of vertebral and non-vertebral fractures when treated subcutaneously with either 20 or 40 μg of daily teriparatide versus placebo. Over a follow-up period of 21 months, BMD increased by an average of 10–14%. Total hip BMD also improved, but more slowly and to a smaller extent (approximately 3%) in comparison to the lumbar spine. At 20 μg of teriparatide, BMD did not change at the distal radius. The most important findings of the teriparatide trial by Neer et al. were significant reductions in new vertebral and non-vertebral fractures (Fig. 17.4). At the end of the treatment period, patients were enrolled in a follow-up study, and vertebral radiographs were repeated 18 months later [28]. It appeared that the history of prior teriparatide exposure was associated with continuous fracture protection compared to the pla-

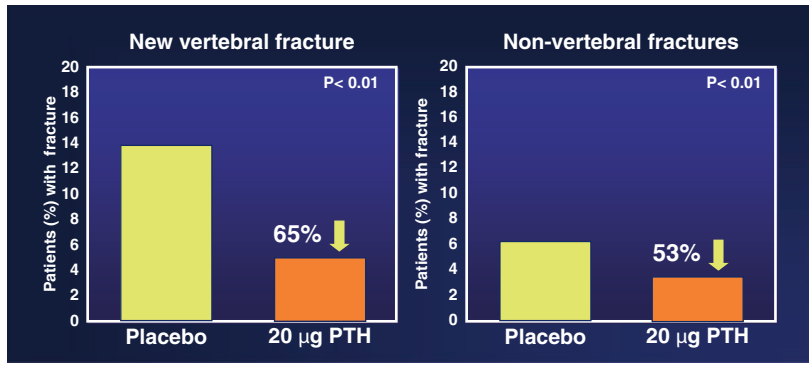

Fig. 17.4 Fracture incidence reduced with teriparatide. Fracture incidence after treatment with teriparatide. As shown for the registered 20 μg dose, teriparatide reduces the incidence of vertebral and non-vertebral fractures significantly. (Based on data from Ref. [27])

cebo group, but other osteoporosis drugs were permitted during the follow-up period [29]. Post hoc analysis of this study showed that the reduction in fracture incidence was not related to the number, severity, or sites of previous fractures [30]. Efficacy was also independent of age and initial BMD [31] and was not affected by reduced renal function [32].

Other studies have confirmed the efficacy of teriparatide at 20 mcg daily to reduce vertebral and non-vertebral fracture [29, 33–35]. Moreover, in a comparator trial with alendronate, Miller et al. [36] showed that teriparatide is associated with a significant reduction in back pain. Chen et al. [37] related the change in BMD with teriparatide to the reduction in fracture risk, similar to analyses relating change in bone mineral density to reduction in fracture risk for antiresorptive agents [38–40]. The 20 mcg dose was chosen over the 40 mcg dose because there was a somewhat higher incidence of side effects (e.g., hypercalcemia) at the higher dose without any difference in efficacy. Finally, a feature of teriparatide that continues to distinguish this drug from all antiresorptive agents is its ability to improve skeletal microstructure (Fig. 17.5).

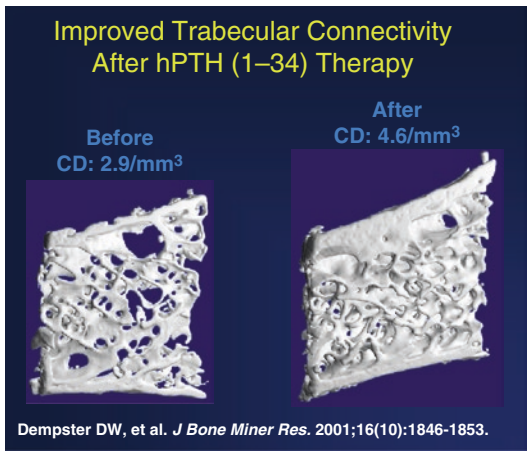

Fig. 17.5 Microarchitectural changes with teriparatide. After therapy with teriparatide, there are marked changes in trabecular and cortical architecture as shown in this study by Dempster et al. (Adapted from Dempster et al. [79]. With permission from John Wiley & Sons)

Teriparatide in Men with Osteoporosis

In 1986, Slovik et al. demonstrated that in men with idiopathic osteoporosis, daily subcutaneous injection of teriparatide combined with daily 1,25-dihydroxyvitamin D markedly increased spinal BMD during 1 year of treatment [41]. In the first randomized, double-blinded controlled trial of teriparatide in men, Kurland et al. studied 23 men with 400 U/day of teriparatide (equivalent to 25 μg/day) or placebo for 18 months [42]. The men who received teriparatide demonstrated an impressive 13.5% increase in lumbar spine bone density. Hip BMD increased significantly but more slowly and to a smaller extent in comparison to the lumbar spine. Cortical bone density at the distal radius did not change as compared to placebo. Bone turnover markers rose quickly and substantially in the men treated with teriparatide, with bone formation markers rising and peaking earlier than bone resorption markers. In a larger trial of 437 men that was the counterpart of the pivotal trial of Neer et al. in postmenopausal women, Orwoll et al. [43] followed a protocol that was essentially identical to the study of Neer et al. BMD increased significantly in the 20 μg treatment group by 5.9% at the lumbar spine and by 1.5% at the femoral neck. These increases were independent of gonadal status. Although fractures could not be assessed during the short 11-month trial, they were assessed in a follow-up observational period of 30 months. Two hundred and seventy-nine men from the original cohort had lateral thoracic and lumbar spine X-rays, 18 months after treatment was stopped. In the combined teriparatide treatment groups (20 and 40 μg), the risk of vertebral fracture was reduced by 51% ($p = 0.07$). Significant reductions were seen in the combined group as compared to the placebo group when only moderate or severe fractures were considered [6.8% versus 1.1%; $p < 0.02$] [44]. As was the case in the observational follow-up period in postmenopausal women, a substantial number of male study subjects in all groups (25–30%) reported use of antiresorptive therapy during the follow-up period. Men treated with placebo utilized anti-

resorptive therapy to a greater extent than those who were treated with either dose of teriparatide (36% versus 25%). Even though the data for men are sparse compared to women, it seems nevertheless clear that teriparatide is as effective in men as in postmenopausal women [45].

Teriparatide in Glucocorticoid-Induced Osteoporosis

Glucocorticoid treatment reduces bone formation directly by impairing osteoblast differentiation and indirectly by reducing intestinal absorption of calcium and renal tubular calcium reabsorption [46]. In this context, teriparatide is an attractive option for the treatment of glucocorticoid-induced osteoporosis (GIO).

In 1998, Lane et al. for the first time evaluated hPTH (1–34) in GIO. Teriparatide added to hormone replacement therapy in postmenopausal women on corticosteroids was associated with significantly increased BMD at lumbar spine more than with hormone therapy alone [47].

Saag et al. showed more definitively the efficacy of teriparatide in GIO by a direct head-to-head comparison with alendronate. Teriparatide, 20 mcg/day, significantly improved BMD at lumbar spine, total hip, and femoral neck and reduced the risk of vertebral fractures compared to alendronate 10 mg/daily [48, 49]. The study population was predominantly female (81%), but the efficacy in men was also confirmed by Glüer et al. in an 18-month trial comparing teriparatide 20 mcg/daily to risedronate 35 mg/week. Teriparatide significantly improved lumbar spine BMD, microstructure, and bone strength measured by high-resolution QCT [50].

Sequential and Combination Therapy with Teriparatide and an Antiresorptive Agent

Previous Use of an Antiresorptive

As many as 50% of patients who are considered candidates for teriparatide have previously been treated with bisphosphonates or other antiresorptives. Cosman et al. [51] treated postmenopausal women, previously treated with estrogen for at least 1 year, with teriparatide. Increases in vertebral BMD proceeded promptly and linearly during the entire 3-year study. Ettinger et al. [52] studied the influence of previous exposure of raloxifene or alendronate. Fifty-nine postmenopausal women with *T*-scores ≤ -2.0 had been treated for an average of 28 months either with raloxifene or alendronate. In most respects, subjects were well matched in terms of age, BMI, and *T*-scores. Similar to the study of Lindsay et al. for estrogen, raloxifene did not impede the effects of teriparatide to increase BMD rapidly and linearly. In contrast, alendronate was associated with a 6-month delay before BMD in the lumbar spine began to increase. After 18 months, lumbar spine BMD increased by 10.2% in the prior raloxifene-treated group compared to only 4.1% in the prior alendronate-treated subjects ($p < 0.05$). The alendronate-treated group showed an initial decline in hip BMD at 6 months, but at 18 months, the mean total hip BMD was not different from baseline. During teriparatide treatment, bone markers in prior alendronate patients increased later and peaked at about one-third lower levels as compared to prior raloxifene-treated patients. These results imply that the potency of the antiresorptive to control bone turnover can determine the early response to teriparatide. Therefore, the type of prior antiresorptive therapy could influence the rate at which teriparatide increases bone mineral density [53]. Cosman et al. [54] have helped to refine this point in a study of teriparatide in postmenopausal women who also had previously received alendronate for the same period of time. In contrast to the study of Ettinger et al., their subjects responded to teriparatide with rapid increases in BMD. To account for these differences, it is noteworthy that the baseline bone turnover markers prior to the initiation of teriparatide therapy were markedly different in the two studies. In the study by Ettinger et al., bone turnover markers were almost completely suppressed. In comparison, in the study by Cosman et al., bone turnover markers were not suppressed to the same extent. Therefore, it is distinctly possible that it is not so much the specific antiresorptive used prior to teriparatide that dictates the subsequent densitometric response to teriparatide, but rather the extent to which bone turnover is reduced. To sup-

port this idea, the response to teriparatide has been shown to be a function of the level of baseline bone turnover in subjects not previously treated with any therapy for osteoporosis: the higher the level of turnover, the more robust the densitometric response to teriparatide [42]. Other studies make the point that teriparatide improves BMD in postmenopausal women regardless of previous long-term exposure to antiresorptive therapies, but prior exposure can attenuate modestly and in the short-term the densitometric response to teriparatide [55]. Duration of previous antiresorptive therapy and latency time between stopping previous bisphosphonate therapy and starting teriparatide does not impair the BMD response at any skeletal site [56].

Different results were found in subjects previously treated with denosumab. Postmenopausal osteoporotic women treated with denosumab for 24 months and then switched to teriparatide for an additional 24 months experienced progressive or transient bone loss [57]. In particular, lumbar spine BMD decreased in the first 6 months followed by increases, resulting in a 14.0% increase at 48 months. Total hip BMD transiently declined and then between 36 and 42 months started to increase, resulting in a 2.8% increase at 48 months. At the distal radius, there was a progressive decrease of BMD, resulting eventually in only a −1.8% at 48 months. As noted earlier, declines in distal radial bone density with teriparatide are commonly seen in subjects who have not previously been treated with any agent. However, in these subjects, there is a reduction in the estimated bone strength [58]. In view of these findings, the use of teriparatide in subjects previously treated with denosumab should be avoided, whereas the use of a combination of denosumab and teriparatide might be an option, as reported below.

Concurrent Use of Anabolic and Antiresorptive Therapy

Since the advent of osteoanabolic therapy in 2002, many investigators have been intrigued by the possibility that combination therapy with an antiresorptive and teriparatide could be more beneficial than monotherapy with either agent. The rationale for this expectation is that the mechanisms of action are very much different from each other and, theoretically, could be additive. Simply put, if bone resorption is inhibited (antiresorptive) while bone formation is stimulated (anabolic), the "therapeutic window" could be substantially wider than with either agent alone. Despite the intuitive appeal of this reasoning, important data to the contrary have been provided by Black et al. [59] and by Finkelstein et al. [60]. These two groups independently completed trials using a form of PTH alone, alendronate alone, or a combination of a PTH form and alendronate. Black et al. studied postmenopausal women with 100 μg of PTH (1-84). The study of Finkelstein et al. involved men treated with 40 μg of teriparatide. Both studies utilized DXA and QCT to measure areal or volumetric BMD, respectively. With either measurement, monotherapy with PTH exceeded densitometric gains with combination therapy or alendronate alone at the lumbar spine. Measurement of trabecular bone by QCT, in fact, showed that combination therapy was associated with substantially smaller increases in BMD than monotherapy with PTH. Bone turnover markers followed the expected course for anabolic (increases) or antiresorptive (decreases) therapy alone. However, for combination therapy, bone markers followed the course of alendronate, not PTH therapy, with reductions in bone formation and bone resorption markers.This suggests that the impaired response to combination therapy, in comparison to PTH alone, might be due to the dominating effects of the antiresorptive agent to suppress bone dynamics when both drugs are used together. Finkelstein et al. showed similar results in women, namely spine, femoral neck, and total hip BMD increased more in women treated with teriparatide alone compared to the combination therapy of alendronate and teriparatide [61].

Since we do not have data referent to other aspects of bone quality, such as actual bone strength, it may be premature to reach the conclusion that combination therapy is necessarily not as good as or even inferior to monotherapy. For

example, if an antiresorptive were not as powerfully suppressive as is alendronate on bone turnover, would the attenuation still be appreciated? Deal et al. argue, to this point, that under certain circumstances, combination therapy can appear to be beneficial to monotherapy [62]. In a 6-month clinical trial, Deal et al. showed that combination therapy with teriparatide and raloxifene may have more beneficial effects on hip bone density than monotherapy with teriparatide in postmenopausal osteoporosis. Bone formation markers increased similarly in both groups. Bone resorption markers, however, were reduced in the combination group. BMD increased to a similar extent in the lumbar spine and femoral neck in both groups, but the increase in total hip BMD was significantly greater in subjects treated with both teriparatide and raloxifene. The effect of raloxifene, a less potent antiresorptive than alendronate, appears to allow teriparatide to stimulate bone formation, unimpeded, but does impair the ability of teriparatide to stimulate bone resorption. These actions may, thus, expand the anabolic window over that which is seen with teriparatide alone.

Combination therapy may be beneficial in specific circumstances, as suggested by Cosman et al. [63] in a 1-year study comparing combination therapy of zoledronic acid 5 mg (one intravenous infusion) plus daily teriparatide 20 mcg versus either agent alone. Levels for both resorption and formation markers increased more in the teriparatide alone group than in the combination therapy. Combination therapy was most advantageous with regard to the increase in BMD when both spine and hip sites are considered, but it was not superior to monotherapy in either the specific instance of the lumbar spine or the hip.

Returning to the idea that different mechanisms of action might be advantageous for combination therapy, denosumab and teriparatide would be particularly attractive in concept. Since denosumab inhibits RANK-L, a key intermediate in a catabolic pathway for parathyroid hormone, this combination could amplify the anabolic actions of PTH while exploiting the antiresorptive actions of denosumab. In a series of studies by Leder et al., it was shown that teriparatide and denosumab together increase BMD at the spine, hip, and femoral neck to a greater extent in postmenopausal women with osteoporosis than either agent alone [64, 65]. In addition to the densitometric advantage, combination therapy with denosumab and teriparatide was associated with greater improvements in skeletal microstructure. By finite element analysis, bone strength was also improved at radius and tibia sites with combination therapy [19].

Abaloparatide as Monotherapy in Osteoporosis

Abaloparatide was tested in a phase 2 trial of 222 postmenopausal women with osteoporosis, randomized to 20, 40, or 80 μg of abaloparatide, teriparatide, or placebo for 24 weeks. Compared with placebo, abaloparatide increased BMD of the lumbar spine, femoral neck, and total hip in a dose-dependent manner [66]. The pivotal trial (ACTIVE) was a randomized, placebo-controlled, and open-label active-controlled trial in postmenopausal women with osteoporosis. Abaloparatide 80 μg was compared to placebo (blinded) or teriparatide 20 μg (open label) for 18 months [10]. Abaloparatide significantly reduced the risk of new vertebral and nonvertebral fractures compared with placebo.The reduction in fracture risk due to abaloparatide was not related to the baseline fracture risk [67]. ACTIVE was designed to be followed after 18 months of abaloparatide with 24 months of alendronate in both the treatment and placebo arms of the blinded study. The primary 6-month endpoint demonstrated continued efficacy of abaloparatide/alendronate to be superior to placebo/alendronate [68]. The exploratory 24-month endpoint gave similar results [69].

Safety

Discussed earlier is the reason why teriparatide and abaloparatide carry with them black box warnings. There is no clinical evidence, however, that this cautionary note related to osteosarcoma

in rats has relevance to human subjects. The clinical trials have observed vasoactive complaints, namely, dizziness, headache, and palpitations in a small number of subjects receiving teriparatide or abaloparatide [10, 27]. There is also a small incidence of mild hypercalcemia that has been observed with both agents. Referencing the observations of hypercalcemia, concerns about nephrolithiasis, nephrocalcinosis, and hypercalciuria are included as cautionary notes.

Consequences of Discontinuing Osteoanabolic Therapy

Since osteoanabolic therapy is approved for only 2 years, there are obvious concerns regarding the consequences of discontinuing therapy. Some a priori concerns relate to the fact that new bone matrix is not fully mineralized following PTH therapy [70]. Therefore, the newly formed bone matrix could be at risk for rapid metabolism if a period of consolidation with an antiresorptive is not used. Although the treatment with PTH (1-84) analog is not the focus of this chapter, with a stronger experimental design, the PaTH study has provided prospective data in a rigorously controlled, blinded fashion to address this issue [71]. Postmenopausal women who had received PTH (1-84) for 12 months were randomly assigned to an additional 12 months of therapy with 10 mg of alendronate daily or placebo. In subjects who received alendronate, there was a further 4.9% gain in lumbar spine BMD, while those who received placebo experienced a substantial decline. By QCT analysis, the net increase over 24 months in lumbar spine BMD among those treated with alendronate after PTH (1–84) was 30%. In those who received placebo after PTH (1-84), the net change in bone density was only 13%. There were similar dramatic differences in hip BMD when those who followed PTH with alendronate were compared to those who were treated with placebo (13% versus 5%). The results of this study establish the importance of following PTH therapy with an antiresorptive agent. Several trials have confirmed a marked decrease in BMD after discontinuation of teriparatide therapy [72–75], while also demonstrating that an antiresorptive may be beneficial by helping to optimize densitometric gains [28, 73–75]. .Moreover, denosumab, after 24 months of teriparatide, results in further substantial increases in lumbar spine and total hip BMD [57]. As noted earlier, the ACTIVExtend trial also documents the same beneficial effects of antiresorptive therapy following abaloparatide [68].

Future Perspectives

In the future, PTH may be modified for easier and more targeted delivery. Less frequent administration of PTH, such as once weekly, might also be an effective treatment option. In a randomized, double-blind, placebo-controlled trial, 578 Japanese patients with vertebral fracture were randomly assigned to receive once-weekly subcutaneous injections of teriparatide (56.5 μg) or placebo, for 72 weeks. Once-weekly injections of teriparatide reduced the risk of new vertebral fracture compared to the placebo group ($p < 0.01$). Adverse events were more frequent in the teriparatide group, but they were generally mild [76]. Also, non-vertebral fracture efficacy was not demonstrated under these conditions. Cosman et al. [54] have reported on the use of cyclical 3-month courses of teriparatide against a backdrop of continued alendronate use. In comparison to regular, uninterrupted teriparatide use, the cyclic administration of teriparatide was associated with similar densitometric gains. Of further interest was the observation that with sequential 3-month cycles of teriparatide, bone formation markers that fell quickly when teriparatide was stopped were stimulated to the same degree with each cycle. On the other hand, bone resorption markers showed smaller increases with successive cycles. This observation gives credence to the idea that the anabolic window is actually expanded when teriparatide is used in this context [77]. Cosman et al. [78] have shown that during long-term alendronate therapy, a rechallenge with PTH after 12 months off PTH increases bone formation, bone resorption, and BMD to a similar extent as during the first course

of PTH administration. These data suggest that a future paradigm might be a second course of PTH given 12 months after a first course of therapy in patients who remain at high fracture risk.

Conclusions

Although antiresorptives remain the mainstay of osteoporosis treatment, the advent of osteoanabolic skeletal agents first with teriparatide and now with abaloparatide is changing our approach to therapy. Teriparatide and the PTHrP analog abaloparatide are promising treatment options in many clinical situations. With regard to these two agents, there is now available, for the first time, a therapeutic approach to osteoporosis that significantly improves microarchitectural, geometric, and other properties of bone. These changes in bone quality induced by osteoanabolic therapy are attractive considering the goal of therapy for osteoporosis, namely, to improve the basic underlying microarchitectural abnormalities that give rise to skeletal fragility.

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18

Combination and Sequential Osteoanabolic/Antiresorptive Therapy in Osteoporosis Treatment

Benjamin Z. Leder

Key Points

- The combination of teriparatide or other PTH-analogs and oral or intravenous bisphosphonates have not shown significant benefits compared to monotherapy.
- Conversely, the combination of teriparatide and denosumab increases bone density, improves skeletal microarchitecture, and augments estimated bone strength more than either of the drug alone.
- The mechanism underlying the efficacy of the denosumab/teriparatide combinations appears to be related to the capacity of denosumab to fully inhibit teriparatide-induced bone resorption while not interfering with teriparatide-induced modeling-based bone formation.
- The use of antiresorptive agents after osteoanabolic therapy is associated with continued anti-fracture efficacy, further increases in bone mass, and improvements in skeletal microarchitecture.
- Increases in bone mineral density are blunted when osteoanabolic therapy is used after bisphosphonate therapy.
- Patients who directly transition from denosumab-to-teriparatide experience rapid and significant high-turnover bone loss, and thus this particular sequential approach should be avoided.

Introduction

In contrast to many chronic conditions, such as hypertension or type 2 diabetes, that often require more than one drug to achieve clinical goals, the standard of osteoporosis care has historically involved the use of a single drug at a single dose. And despite the fact that several new antiresorptive and osteoanabolic agents have been introduced over the past decade, it remains the case that no single agent can cure osteoporosis. Thus, the need for more effective therapeutic regimens remains pressing, especially for those at the highest risk of fragility fracture.

An additional challenge to managing patients with established osteoporosis is an increasing reluctance to treat patients with antiresorptive medications for more than 3–5 years due to the concern over uncommon but serious side effects such as atypical femur fracture and osteonecrosis

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of the jaw, as well as the long-standing regulatory 2-year limit on parathyroid hormone receptor targeted osteoanabolic therapies [1–3]. Thus, it is expected that over a lifetime, the use of more than one medication will be required for many patients with established disease. In this setting, investigators have focused on evaluating the efficacy of combining antiresorptive and osteoanabolic drugs, an approach that was hypothesized to benefit from contrasting the drugs' differing mechanisms of action, as well using these medications in specific sequences. This chapter evaluates the available evidence concerning the differential effects of the various sequential and combination osteoporosis treatment strategies and details some of the important pharmacological and clinical distinctions between the various approaches.

Mechanisms of Current Osteoporosis Medications

Osteoanabolic Drugs

Currently, there are two available osteoporosis medications that can be classified as "anabolic" based on their capacity to increase osteoblastic bone formation. They are *teriparatide*, a parathyroid hormone (PTH) analog comprising the first 34 amino acids of the endogenous hormone and *abaloparatide*, a 34-amino-acid peptide that shares significant homology to parathyroid hormone-related protein (PTHrP). Both of these drugs target the same receptor. The anabolic potential of both teriparatide and ababloparatide appears to be dependent on intermittent administration as sustained receptor activation favors bone resorption over formation [4, 5].

One of the key unresolved questions concerning the mechanisms of these agents is what portion of their osteoanabolic effects are mediated through the initial stimulation of bone resorption: via the release of preformed growth factors from the bone matrix or via communication from osteoclasts to osteoblasts [6] versus direct stimulatory effects on osteoblasts, osteocytes, and bone lining cells [6, 7]. This question has direct relevance to the efficacy of combination therapy regimens, in that if resorption-dependent mechanisms were dominant, one would expect that combination strategies that more fully block bone resorption would be ineffective whereas if direct stimulatory effects on osteoblasts, osteocytes, or lining cells were dominant, combination strategies that more fully block bone resorption would be more effective than those that do so only partially. It has also been recently suggested that the subtle distinction in the pharmacological effects of PTH analogs may be based on their relative binding affinities to different PTH/PTHrP receptor conformations. Specifically, preclinical studies have suggested that PTH/PTHrP analogs distinguish between the two distinct receptor conformations (R^o and RG) and that more efficient binding to R^o leads to sustained signaling whereas more efficient binding to RG results in more transient signaling [8, 9]. It is thus conceivable that different signaling outputs triggered by the differential binding affinities of abaloparatide and teriparatide to the RG conformation, for example, may account for some of the observed differences in bone resorption rates and the incidence of hypercalcemia between these two agents [10]. Irrespective of mechanisms, however, it is well established that net skeletal effects of the currently approved PTH and PTHrP analogs are to increase trabecular bone mass and improve trabecular microarchitecture while concomitantly increasing cortical bone porosity [11–14]. It is also established that the subsequent clinical consequences of these skeletal changes are, in turn, an increase in bone strength and a clinically significant reduction in the risk of vertebral and nonvertebral fragility fractures in osteoporotic patients [15–22].

Antiresorptive Drugs

Antiresorptive medications act by inhibiting osteoclastic resorption of previously formed bone. The most commonly used antiresorptive medications are nitrogen-containing bisphosphonates that act by binding to hydroxyapatite and inhibiting the enzyme farnesyl diphosphate synthase in the cholesterol biosynthetic pathway,

suppressing protein geranylgeranylation, and hence osteoclastic bone resorption [23]. The various oral and intravenous bisphosphonates bind to hydroxyapatite with distinct affinities, and while they persist in bone for prolonged periods, there are differences in the endurance of their pharmacological effects (zoledronic acid > alendronate > ibandronate > risedronate) and these differences may account for their different pharmacological properties when combined with anabolic agents (as discussed in detail below) [24]. Denosumab is a monoclonal antibody that inhibits the binding of receptor activator of NFκB (RANK)-ligand to its osteoclast-derived receptor, RANK, thus inhibiting osteoclast formation, activation, and survival [25, 26]. Denosumab is the most rapidly acting and potent antiresorptive drug currently available but, unlike bisphosphonates, its effects are immediately reversible and rates of bone resorption and formation "rebound" to levels above the patient's original baseline levels when it is discontinued [27–32]. Estrogens and selective estrogen-receptor modulators exert their skeletal effects through binding to the estrogen receptor (ER)-α, playing a key role in both osteoblast and osteoclast biology. In the pharmacologic setting, however, these agents act primarily as antiresorptive drugs by suppressing stromal cell, osteoblast, and lining cell production of RANKL, increasing osteoblastic production of osteoprotegerin, directly suppressing the production of pro-resorptive cytokines, and promoting osteoclast apoptosis [33, 34]. Like denosumab, the antiresorptive effects of estrogens and selective estrogen-receptor modulators are immediately reversible, though a rebound phenomenon is not observed [35–37].

Combination Antiresorptive and Osteoanabolic Treatment

While the combination of multiple antiresorptive drugs has been studied in previous decades, these trials generally did not show a clinical benefit, and the introduction of more potent antiresorptives such as zoledronic acid and denosumab further dampened enthusiasm for this approach, leading to a focus on combining drugs of different mechanistic classes [38–46].

Combination of Estrogen or Selective Estrogen-Receptor Modulators and PTH Analogs

Some of the early studies assessing the efficacy of PTH analogs was performed in patients receiving ongoing estrogen administration but the lack of monotherapy comparator groups makes it is difficult to assess the relative benefits of these combinations versus the PTH analog alone [47–49]. In a 6-month double-blind, placebo-controlled trial study of 137 postmenopausal women randomized to receive teriparatide 20 μg daily either alone or combined with raloxifene 60 mg daily, combination therapy was shown to increase total hip BMD more than teriparatide monotherapy, though in this case the lack of a raloxifene monotherapy control group also limits clinical conclusions [50]. Thus, at present there is no conclusive evidence that combining PTH analogs with estrogens or selective estrogen-receptor modulators offers a clinical advantage.

Combination of Bisphosphonates and PTH Analogs

Most of the studies investigating combination osteoanabolic/antiresorptive treatment regimens have involved either teriparatide or PTH-(1-84) combined with the nitrogen-containing bisphosphonates. The first combination studies were performed utilizing the oral bisphosphonate, alendronate, and include the Parathyroid Hormone and Alendronate (PATH) study wherein 238 postmenopausal women with osteoporosis were randomized to receive PTH-(1-84) 100 μg daily, alendronate 10 mg daily, or both for 12 months [51]. As shown in the Table 18.1, 12-month increases in DXA-derived spine areal BMD were similar in all three treatment groups. However, at the total hip, combination therapy increased BMD more than the PTH-(1-84) alone but similarly to alendronate (of note, hip BMD

gains with PTH analogs in the first year of therapy are known to be absent or modest with subsequent larger gains if therapy is continued for 2 years) [54]. Additionally, PTH-(1-84) increased spine trabecular volumetric BMD (assessed by quantitative computed tomography or QCT) approximately twofold more than the combination of both medications. An assessment of biochemical markers of bone turnover in this study demonstrated that combination treatment suppressed bone resorption (serum c-telopeptide or CTX) less than alendronate monotherapy and that bone formation (type I collagen propeptide or P1NP) increased only transiently.

Two similar studies of combined teriparatide and alendronate were performed in postmenopausal osteoporotic women and osteoporotic men. In these studies, subjects were randomized to receive either alendronate 10 mg daily, teriparatide 40 μg daily, or both medications for 30 months, though teriparatide was not started until month 6 [55, 56]. In both of these populations, DXA-derived lumbar spine and femoral neck areal BMD increased more than two-fold more in those treated with teriparatide alone than those treated with alendronate alone or both medications. In another clinical trial utilizing PTH-(1-84), postmenopausal osteoporotic women were randomized to either 6 months of combined PTH-(1-84) and ibandronate 150 mg monthly followed by 18 months of ibandronate alone or two sequential courses of 3 months of PTH followed by 9 months of ibandronate alone and areal BMD of the spine and hip increased similarly in both groups [57].

The combination of teriparatide and the intravenous bisphosphonate, zoledronic acid, was assessed in a 12-month randomized controlled trial of 412 postmenopausal women who received 12 months of teriparatide 20 μg daily, a single infusion of zoledronic acid 5 mg or both [52]. As indicated in Table 18.1, while spine BMD increased more in the combination group at early time points, increases at 12 months were similar in the combination therapy and teriparatide groups. The two drugs together also demonstrated larger initial increases in hip BMD, though by 12 months the increases were similar in the combination therapy and zoledronic acid monotherapy groups. In a pattern that differs from the studies involving alendronate, in the groups receiving both zoledronic acid and teriparatide, while bone resorption (CTX) was suppressed initially, this suppression was not sustained after the first several months and serum CTX levels eventually increased to levels above the original baseline by the end of the 12-month treatment period.

Table 18.1 12-month changes in bone mineral density in three randomized combination therapies or randomized controlled trials

Study	Sample size	Regimen	Lumbar spine (%)	Total hip (%)
Black et al. [51]	119	PTH 1–84	6.3	0.3
	60	Alendronate	4.6	~3
	59	Both	6.1	1.9[b]
Cosman et al. [52]	138	Teriparatide	7.0	1.1[a]
	137	Zoledronic acid	4.4[a]	2.2
	137	Both	7.5	2.3
Tsai et al. [53]	31	Teriparatide	6.2	0.7
	33	Denosumab	5.5	2.5
	30	Both	9.1[a]	4.9[a]

[a]Differs significantly from the other two groups
[b]Differs significantly from PTH-(1-84)

Taken together, it appears that combining PTH analogs with bisphosphonates do not provide significant additive skeletal effects in postmenopausal osteoporotic women. The reasons for this lack of efficacy are currently unclear. Potential hypotheses to explain the apparent counteractive effects of bisphosphonates and PTH analogs are suggested by the observed changes in markers of bone resorption and formation in these studies. Bone turnover marker data suggest that bisphosphonates may blunt the effects of PTH analogs because of the key role that bone resorption plays mediating the anabolic effects of PTH analogs or the inability of bisphosphonates to fully inhibit PTH analog-induced bone resorption (or some combination of both mechanisms).

Combination of Denosumab and Teriparatide

Unlike bisphosphonate-containing combinations, the combination of teriparatide and the RANKL

inhibitor, denosumab increase BMD at the spine and hip at both 1 and 2 years more than either of the drug alone. In the Denosumab and Teriparatide Administration (DATA) trial, 94 osteoporotic postmenopausal women were randomized to receive teriparatide 20 mcg daily, denosumab 60 mg every 6 months, or both for 2 years. Combination treatment increased spine, total hip, and femoral neck BMD more than either group after both 12 and 24 months [53, 58] (Table 18.1, Fig. 18.1). Specifically, the combination of both agents increased spine, total hip, and femoral neck BMD by 12.9%, 6.3%, and 6.8%, respectively, increases that currently cannot be achieved with 2-year courses of any approved single drug [20, 60–64]. Areal BMD at the distal radius increased by slightly more than 2% in both the denosumab and combinations groups, and these increases differed significantly from the decrease of 1.7% observed in the teriparatide group.

In this same study, the effects of these interventions on bone microarchitecture and estimated were assessed by high-resolution peripheral QCT (HR-pQCT) [19]. Total volumetric bone mineral density (vBMD) at the radius and tibia, trabecular vBMD at the radius, and cortical vBMD at the tibia all increased more in women who received both drugs compared to either denosumab or teriparatide alone. Cortical thickness also increased more in the combination group than the other two groups, while cortical porosity increased in a fairly linear fashion over the entire 24-month treatment period in the teriparatide group but was stable in both other groups. Using the engineering technique of finite element analysis to estimate strength at both the radius and the tibia, the advantage of combination therapy is also apparent.

The pattern of changes in biochemical markers of bone resorption and formation induced by combined teriparatide/denosumab suggest a unique mechanism underlying the regimen's distinctive efficacy. As shown in the figure, bone resorption, as assessed by serum CTX, was identically suppressed in women treated with combination therapy and denosumab monotherapy during the initial 24 months of the trial, whereas the changes in bone formation markers differed, in that serum osteocalcin remained stable in the combination therapy group for the initial 3 months of treatment and then declined only modestly thereafter (though not to the level observed in the denosumab monotherapy group) [58]. This divergent pattern in bone resorption and formation marker changes in the combination group suggests that when given together, denosumab fully inhibits teriparatide-induced bone resorption but does not interfere with teriparatide-induced "modelling-based" bone formation (i.e., bone formation that does not require antecedent bone resorption).

In summary, while bisphosphonate-containing combinations with PTH analogs do not lead to additive effects, the combination of denosumab and teriparatide appears to be a promising approach. These rapid and large gains in BMD of the hip and spine suggest that this approach may be particularly useful in patients with severe osteoporosis in whom no single therapy can adequately reduce fracture risk. Larger studies assessing this regimen's capacity to reduce fracture incidence are now needed.

Sequential Approaches to Osteoporosis Therapy

Antiresorptive Agents After Osteoanabolic Agents

When teriparatide and abaloparatide are initiated, bone remodeling is rapidly stimulated but remodeling rates revert to pretreatment levels after 12–24 months of treatment [65]. Despite this pattern of bone cell activity, however, BMD continues to increase over the entire 2-year treatment period, likely due to continued modeling-based bone formation [66]. When PTH analogs are discontinued, BMD begins to revert to pretreatment levels almost immediately though studies have suggested that the antifracture efficacy is maintained for up to 18 months after the drug has been stopped [67, 68]. That said, it is likely that most of the beneficial effects of PTH analogs do eventually dissipate if they are not followed by a potent antiresorptive drug.

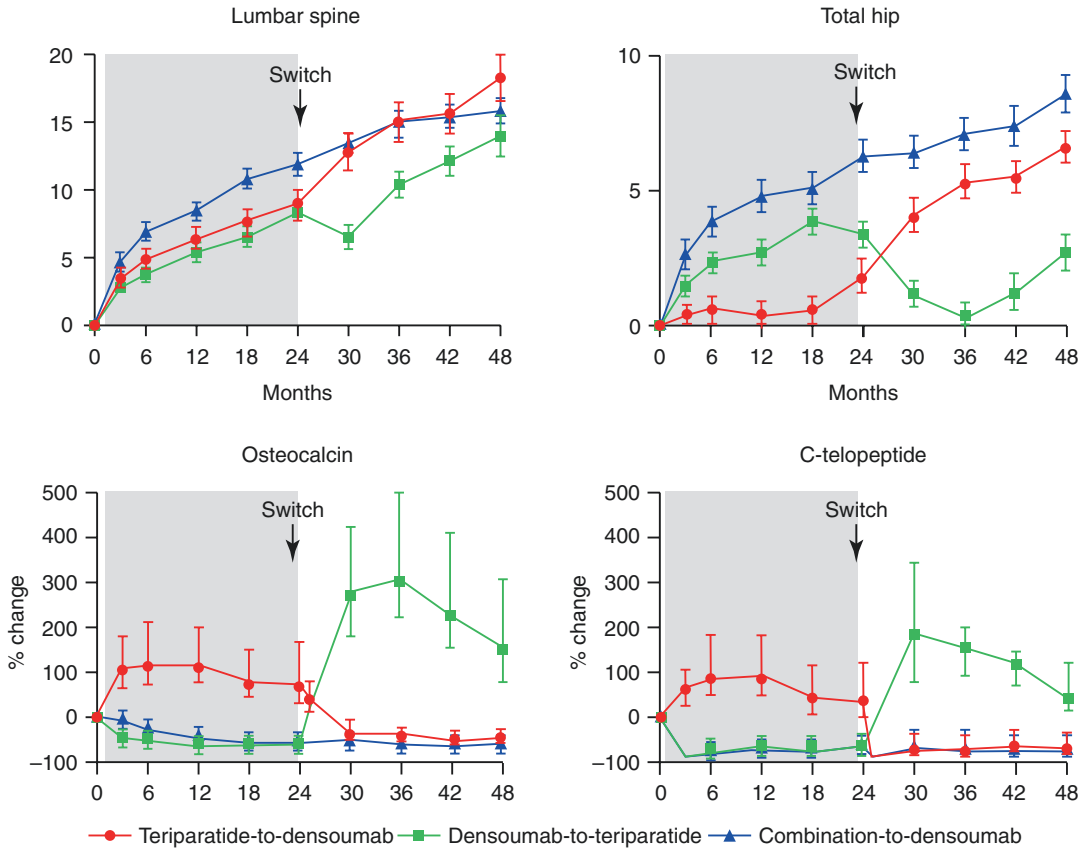

Fig. 18.1 Mean percent change (±SEM) in BMD at the spine and total hip and median percent change in osteocalcin and P1NP (±IQR) over the 48 months of the DATA and DATA-Switch studies. (Based on data from Refs. [53, 58, 59])

The capacity of oral alendronate to prevent post-teriparatide bone loss was studied in several clinical trials and is clearly effective not only consolidating the gains achieved with anabolic therapy but also in further increasing hip and spine BMD [69–71]. Similarly, patients who have been treated with 18-months of abaloparatide experience additional areal BMD gains and maintain a fracture-reduction benefit when switched to alendronate [61]. The selective estrogen receptor modulator, raloxifene, also prevents post-teriparatide bone loss but appears to be less effective than alendronate at further increasing BMD, particularly at the spine [72].

The ability of denosumab to further increase BMD when used after teriparatide was assessed using the *DATA-Switch* study (see Fig. 18.1). In DATA-Switch, postmenopausal women who received 2 years of teriparatide followed by 2 years of denosumab experienced large additional increases in both spine and hip BMD. Specifically, spine BMD increased by an additional 9.4% during the 2 years of denosumab (18.3% total 4 year increase) and total hip BMD increased by an additional 4.8% (6.6% total 4 year increase) [59]. Notably, these post-teriparatide denosumab-induced BMD increases appear to be significantly greater than what can be achieved with bisphosphonates therapy after teriparatide or when denosumab is administered to treat naïve patients [59, 73]. Denosumab was also able to further increase BMD in patients who previously received 2 years of combined teriparatide/denosumab therapy [59].

The skeletal effects of potent antiresorptive therapy, when used after the currently investigational mixed osteoanabolic/antiresorptive agent, romozosumab, have also been studied.

Romozosumab is a monoclonal antibody that inhibits osteocyte-derived sclerostin and has potent but transient osteoanabolic effects and weaker, but sustained, antiresorptive properties [74]. In these studies, denosumab was shown to further decrease bone resorption, augment gains in spine and hip areal BMD, and maintain antifracture efficacy when used after romozosumab [75, 76].

Osteoanabolic Agents After Antiresorptive Agents

Many patients who are treated with osteoanabolic drugs have already been exposed to antiresorptive agents, usually bisphosphonates, often for extended periods. Despite this common pattern of medication sequence, several clinical trials have clearly demonstrated that switching from a bisphosphonate to a PTH analog may result in transient cortical BMD loss and diminished BMD gains at sites of predominantly trabecular bone, such as the lumbar spine [56, 77–81]. For example, in a clinical trial of 59 postmenopausal osteoporotic women who previously received either alendronate or raloxifene for 18–36 months followed by 18-months of teriparatide, spine areal BMD increased more in those who had previously received raloxifene than those who had received alendronate and total hip areal BMD actually decreased by almost 2% in the initial 6 months of teriparatide therapy in those previously treated with alendronate [79]. In a separate study, 24 months of teriparatide was administered to postmenopausal women who were either treatment naïve or had prior bisphosphonate use and areal spine BMD increased more in the treatment-naïve group than those with prior bisphosphonate exposure [82]. This general pattern was also observed in several additional studies involving bisphosphonates [78–81], and blunting was suggested when romosozumab was given after bisphosphonate exposure as well [83].

The transition from denosumab to teriparatide appears to result in a uniquely maladaptive stimulation of high-turnover bone loss. In the DATA-Switch study previously described (Fig. 18.1), women who transitioned from denosumab to teriparatide experienced 6 months of declining areal BMD at the spine, 12 months of declining areal BMD at the hip and femoral neck, and progressive bone loss during all 24 months of treatment at the distal radius [59]. Moreover, at the distal tibia and distal radius, the transition from denosumab to teriparatide resulted in progressive decreases in total volumetric cortical volumetric BMD, progressive increases in cortical porosity, and reduced bone strength as assessed by finite element analysis [84]. Notably, this observed bone loss and deterioration of skeletal microarchitecture was associated with a dramatic stimulation of bone remodeling as serum markers of both bone formation and bone resorption increased by two- to threefold in the 6–12 months after the drug transition and remained elevated even 24 months after the drug transition [59]. This accelerated rate of bone remodeling, accompanied by bone loss, is concerning given that the more modest stimulation of bone turnover that occurs when denosumab is discontinued without a transition to teriparatide has been shown to be accompanied by a rapid and complete loss of denosumab's antifracture efficacy and an increase in the risk of multiple vertebral compression fractures, especially in those with very low BMD and prevalent fractures [85, 86].

Adding One Class of Osteoporosis Medication to Another

Several studies have assessed an overlapping medication approach to osteoporosis therapy. For example, in a clinical trial of 198 postmenopausal osteoporotic women, who initially received 18+ months of either alendronate or raloxifene, it was reported that in the prior-raloxifene group, switching to or adding teriparatide resulted in similar BMD increases, whereas in the prior-alendronate group, adding teriparatide increased spine BMD more than switching to teriparatide [80]. In a separate trial of 125 postmenopausal osteoporotic women who had received 9 months of teriparatide, adding raloxifene for 9 months resulted in larger spine BMD gains and adding alendronate resulted in larger hip BMD gains when compared

to continuing teriparatide alone [87]. Furthermore, in a 12-month extension to this study in which teriparatide was discontinued in all three groups, the greatest BMD increases occurred in the group that received 9 months of teriparatide followed by 9 months of combined teriparatide/alendronate followed by 12 months of alendronate monotherapy [88]. Finally, 126 osteoporotic women who had been taking alendronate for at least 1 year were randomized to 15 months of either continued alendronate plus teriparatide 20 μg daily, continued alendronate plus teriparatide for three 3-month cycles alternating with 3 months of alendronate alone or continuing alendronate alone. Areal BMD of the spine increased similarly in both groups who received teriparatide but did not increase further in the alendronate alone group, while hip BMD increased slightly and similarly in all three treatment groups [89].

Summary

In the past two decades, there has been a large expansion in osteoporosis treatment options, particularly in terms of the various antiresorptive drugs but also among osteoanabolic agents. This expansion has led to increased interest in developing combination and sequential drug approaches with the potential of providing greater benefit than monotherapy. The clinical trials completed to date have demonstrated that when given sequentially, it is preferable to first initiate osteoanabolic therapy and then transition to an antiresorptive agent as this approach leads to the greatest gains in bone mass and, in some circumstances, have been shown to reduce fracture risk in osteoporotic patients. These trials have also demonstrated that while prior bisphosphonate exposure may diminish the efficacy of subsequent PTH analog therapy, it is the specific transition from denosumab to teriparatide that should be unequivocally avoided due to rapid bone induced by accelerated skeletal metabolism—a physiologic state that may have significant negative clinical consequences. Studies assessing combined osteoanabolic/antiresorptive therapy have suggested that while the combination of bisphosphonates and PTH analogs does not result in clinical benefit, the combination of denosumab and teriparatide appears to be uniquely able to increase BMD and improve skeletal microarchitecture as compared to monotherapy. What is now required are larger studies that can adequately assess the comparative effectiveness of the various combination and sequential therapy approaches on clinically important endpoints in patients with established or severe osteoporosis.

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19 Sclerostin Inhibition in the Treatment of Osteoporosis

Roland Baron, Francesca Gori, and Benjamin Z. Leder

Key Points

- High/low bone density in rare human diseases has been linked to mutations in components of WNT signaling.
- In particular, mutation in the receptors LRP 5 or 6 decreases their affinity for an endogenous WNT inhibitor, sclerostin, thereby activating locally WNT signaling, inducing a high bone mass phenotype.
- Other mutations affect the expression (Sclerosteosis) or transcriptional regulation and expression of sclerostin (van Buchem disease).
- Mutations in the sclerostin co-receptor LRP4 decreases sclerostin efficacy and also lead to high bone mass.
- Antibody inhibition or deletion of sclerostin increases markedly bone formation along trabecular and cortical bone surfaces and decreases bone resorption.
- Clinical trials show a rapid but self-regulated increase in bone formation and bone mass with a prolonged decrease in resorption and decreased fracture rate.

The treatment of osteoporosis consists of attempts to increase cortical and trabecular bone mass and improve their microstructure in order to prevent both vertebral and nonvertebral fragility fractures. As discussed in other chapters of this book, several therapeutic options are available to physicians to achieve this goal. On the one hand, and by far the most commonly used approach, it is possible to increase bone mass by inhibiting bone resorption with antiresorptives such as bisphosphonates or antibodies to RANK ligand. On the other, and a preferred choice in severe cases of osteoporosis, one can first stimulate bone formation with osteoanabolics, usually before switching to antiresorptives in order to stabilize and further enhance the gain achieved by these anabolic drugs. Because the bone remodeling mechanism implies activation of bone resorption as a necessary step to initiate bone formation in the

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remodeling units, antiresorptive drugs decrease remodeling activity, and thereby secondarily decrease bone formation, a somewhat undesirable secondary effect of treatment [1]. Our field has therefore long been seeking agents that would directly or indirectly increase bone formation, i.e., osteoanabolic therapies. Consequently, bone anabolics are defined not by their simple ability to increase bone mass (antiresorptives do that as well) but by their ability to increase bone formation, as measured by biochemical markers such as procollagen type 1 amino-terminal propeptide (P1NP) and/or bone-specific alkaline phosphatase (BSAP), and/or histomorphometric parameters (mineral apposition rate (MAR) and bone formation rate (BFR)) on bone biopsies. Furthermore, it has recently been recognized that, even in the adult skeleton bone formation can be enhanced by two, not mutually exclusive, mechanisms. Bone formation can be increased within the bone remodeling unit, increasing the amount of bone formed during the formation phase of the remodeling sequence (remodeling-based bone formation). This mode of bone formation requires a reasonable rate of remodeling to be efficient at the organ level. But bone formation can also increase through direct stimulation of inactive cells lining the so-called "quiescent" bone surfaces (lining cells) in a process called modeling-based bone formation. This latter type of bone forming activity is particularly important for cortical bone, because in the adult, skeleton modeling takes place for the most part along the endosteal and periosteal surfaces of the cortex, even under steady-state conditions, whereas it is far less frequent along trabecular surfaces. To be defined as osteoanabolics, and independent of their effects on bone resorption, these treatment modalities should also result in an increase in bone density, even if, as parathyroid hormone (PTH), they also increase bone resorption [2].

As of today, two bone anabolic pathways have been targeted in the effort to develop osteoanabolic drugs: PTH/PTHrP signaling through their common PTH1R receptor (see Chaps. 16 and 17) and canonical WNT (cWNT) signaling. PTH/PTHrP analog's anabolic properties are in part dependent on increased remodeling-based bone formation, although these agents also increase modeling-based formation [3]. Therefore, bone resorption is also increased, partially limiting PTH's therapeutic window [2, 4] (as discussed in Chaps. 16 and 17). In contrast, as discussed in detail below, bone anabolism seen following activation of the cWNT pathway after treatment with antibodies to sclerostin is partially independent of bone remodeling, as it actually decreases bone resorption and increases both remodeling-based and modeling-based bone formation. This chapter focuses on cWNT signaling in bone as a target for anabolic treatment, and in particular on sclerostin and its inhibition.

WNT Signaling and Sclerostin: Mechanisms of Action

The dominant role of WNT signaling on skeletal homeostasis was discovered through the identification of causal mutations in rare human skeletal diseases. The identification of loss- and gain-of-function mutations in the low-density lipoprotein receptor-related protein 5 (Lrp5), a co-receptor for WNT signaling, which were associated with low bone mass in osteoporosis pseudoglioma syndrome (OPPG) and high bone mass (HBM), respectively [5–7] established the first direct link between WNT signaling and bone mass regulation. More directly relevant to this chapter, mutations causing HBM in two other rare human diseases, sclerosteosis and van Buchem syndrome disease, were identified in a secreted protein, sclerostin, encoded by the *SOST* gene located on human chromosome 17q12-q21 or in the enhancer regulating the transcription of *SOST*, respectively [8–11]. Sclerosteosis is caused by null mutations in *SOST*, whereas van Buchem patients lack an enhancer element required for *SOST* expression. Patients with sclerosteosis or van Buchem disease have bone overgrowth with increased bone mass and bone strength. Establishing a link with WNT signaling, sclerostin is an endogenous inhibitor of the WNT signaling pathway that interacts not only with LRP5/6 but also with another member of this family of receptors, LRP4, which serves as a co-receptor to stabilize sclerostin on the plasma membrane [12–15] (Fig. 19.1). Strikingly, *LRP4*

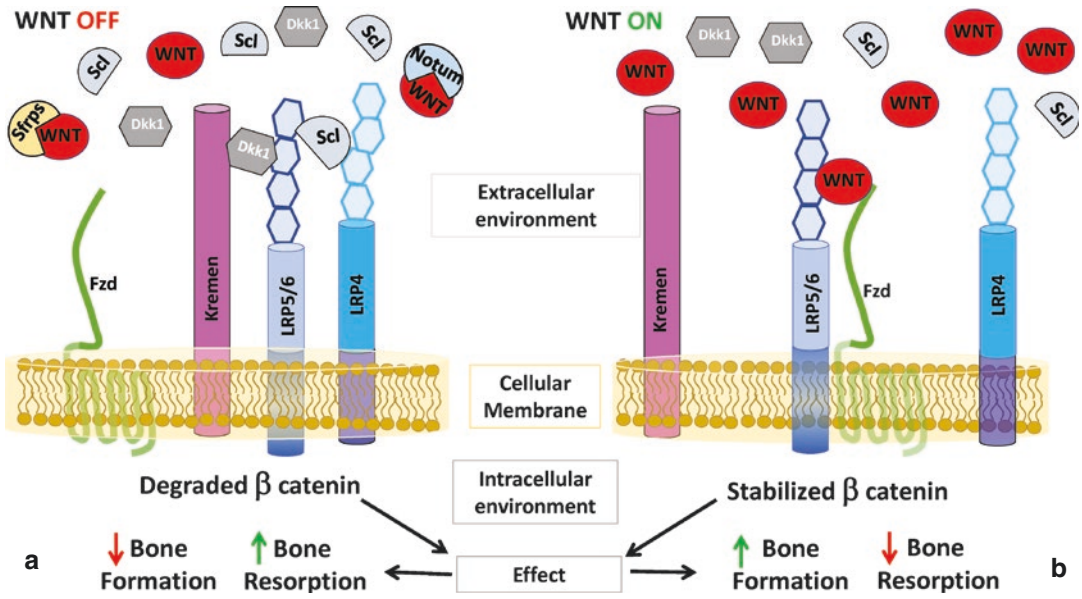

Fig. 19.1 Receptors and endogenous inhibitors of WNT ligands. (**a**) When there is more sclerostin and/or Dkk1 than WNTS, the stoichiometry favors the inhibitors; Dkk1 and sclerostin bind to the beta-propellers of LRP5 and/or 6, preventing the binding of WNT and recruiting Kremen or LRP4, respectively, to the complex; this leads to the internalization and removal of LRP5/6 from the cell surface, such that WNT signaling is not activated, leading to the degradation of β-catenin**,** low bone formation, and high bone resorption. (**b**) When there is more WNTs than sclerostin and/or Dkk1, the stoichiometry favors WNTs, preventing the binding of the inhibitors and allowing binding of WNTs to LRP5/6 and Frizzled (Fzd) receptors complexes; WNT signaling is activated, stabilizing β-catenin to increase bone formation and decrease bone resorption

null mutations were later also found in humans and these patients also exhibited osteosclerotic phenotypes [12–14], establishing even more firmly the link between sclerostin, WNT signaling, and skeletal homeostasis.

Mechanistically, it turned out that the single point mutation initially identified in *LRP5* (G171 V) in HBM families did not, as initially thought, make the receptor constitutively active, but rather decreased its affinity for sclerostin [16] and Dickkopf 1 (Dkk1), another endogenous WNT inhibitor [7, 17]. The importance of the WNT pathway as a major regulator of bone mass in humans and as a potential therapeutic target was further established by several large genome-wide association studies (GWAS) that identified polymorphisms within several members of the Wnt signaling pathway strongly associated with BMD variations, including AXIN1 (a downstream effector), DKK1, LRP5, LRP4, R-spondin 3 (a co-activator), SFRP4 (another soluble inhibitor), and WNT16, among others [18–20].

As a consequence of these findings, intense research was initiated to understand the mechanisms involved and identify potential therapeutic targets in the WNT signaling pathway. While these findings were very encouraging for the prospect of finding novel targets to increase bone formation in patients with low bone mass and bone fragility, several potential hurdles were quickly identified. Among those, the complexity of the WNT pathway(s) and its ubiquitous nature, not to mention the recognized association between WNT activation and certain type of cancers [21], created significant challenges.

The WNT Signaling Pathway

WNT Extracellular Environment

In mammals, 19 secreted WNT proteins have been identified [22–24]. These ligands can bind to a large number of combinations of several

receptors and co-receptors (11 distinct Frizzled (Fzd), LRP4,5,6, Ror2, Ryk). In addition, several families of secreted antagonists (sclerostin, WIFs, WISE, sFRPs, Notum, Dkks) and co-activators (Norrin, R-Spondins) have also been identified and bind to the same set of receptors or ligands, in addition to some others (LGRs for R spondins). Wnt signaling can also be regulated by postranslational modifications of WNT ligands. Indeed, WNTs are acetylated, and this acetylation is critical for their function. Porcupine acetylates and Notum deacetylates Wnts, respectively. So, while from the endoplasmic reticulm porcupine add palmitoleate and shorter *cis* unsaturated fatty acids onto Wnts, required for their activity, in the extracellular matrix, this fatty acid can be removed by Notum leading to inactivation of the Wnt ligands [24, 25]. The balance between the activity of these two enzymes therefore regulates Wnt activity. This complex set of proteins constitutes the extracellular WNT signaling environment, and when a specific ligand binds to a specific set of membrane receptors, it activates intracellular signaling to induce specific changes in target gene expression.

WNT Intracellular Signaling Cascades

WNT activation can regulate two distinct intracellular WNT signaling pathways, depending upon which WNT protein is bound to the cell surface and which receptors are activated. Classically, two pathways are mentioned: the canonical or β-catenin-dependent pathway (in which β-catenin is the main downstream effector) and the noncanonical or β-catenin-independent pathway, which activates several intracellular cascades other than β-catenin cascade. In the canonical WNT pathway, binding of the appropriate WNT proteins to the Fzd-LRP5/6 co-receptor complex recruits and activates the intracellular signaling protein Dishevelled (Dvl) to the cytoplasmic tail of Fzd. Dvl in turn recruits the axin-GSK-3β complex, leading to LRP5/6 cytoplasmic tail phosphorylation and inhibition of the β-catenin destruction complex (axin-GSK3-Ck1-APC), a signal that leads to cytosolic β-catenin stabilization. Stabilized β-catenin accumulates in the cytoplasm and then translocates to the nucleus, where it interacts with Tcf/Lef transcription factors. This molecular complex then binds to specific transcriptional elements to activate transcription of WNT downstream target genes involved in osteoblast commitment and maturation [22]. Activation of canonical WNT signaling also regulates osteoclastogenesis: this occurs mainly through the repression of osteoprotegerin (OPG), decreasing the RANKL/OPG ratio and reducing osteoclast differentiation and activity [26–28]. A direct, i.e., not mediated by changes in OPG production by osteoblasts and osteocytes, effect of cWNT signaling on osteoclasts and osteoclastogenesis has also been proposed as mice lacking β-catenin in osteoclast precursors develop osteopetrosis because of reduced osteoclast number and activity [29]. Thus, cWNT signaling can at the same time activate bone formation and decrease bone resorption, a truly ideal and unique mechanism to increase bone mass.

But WNT ligands can also activate β-catenin-independent signaling cascades, the so-called noncanonical WNT signaling pathway. To activate the noncanonical pathway, WNT proteins, such as WNT 5a, will bind to Frizzled receptors forming either Frizzled homo- or heterodimers among the 11 known receptors or use Ror2 or Ryk as Frizzled co-receptors. Noncanonical WNT signaling can then follow different signaling cascades, the WNT/planar cell polarity (PCP), the WNT/Ca^{2+}, and the WNT-JNK signaling pathways [22]. Although the individual WNT ligands can be predominantly canonical and/or noncanonical, these pathways are not entirely independent from each other, are often activated concurrently, overlapping and influencing each other. So far, most of the literature suggests that activation of noncanonical WNT signaling is linked to increases in osteoclast differentiation and function rather than osteoblasts and bone formation, albeit activation of this pathway in cortical bone appears to also decrease bone formation along the periosteum and the endosteum [30]. Furthermore, with the exception of the WNT5a ligand, no link to BMD has been reported in

GWAS studies and no human mutations associated with skeletal dysplasias or high bone mass reported.

Because the HBM, OPPG, or sclerosteosis mutations affect LRP4, LRP5, LRP6, or sclerostin itself, which are co-receptors and inhibitor, respectively, of canonical WNT signaling, this chapter will be focused only on this pathway. For detailed description and discussion of the role of noncanonical signaling cascades, see Baron and Kneissel [21] and Gori and Baron [22, 31].

Sclerostin: A Target to Activate WNT Signaling Selectively in Bone

Sclerostin binds LRP5 and LRP6r, competing with WNTs for interaction with these receptors and thereby inhibiting cWNT signaling in target cells [10, 16, 32, 33] (Fig. 19.1). Sclerostin also uses LRP4 as a co-receptor [13, 15]. When bound to LRP5/6 and LRP4, sclerostin prevents interaction of LRP5/6 with their cognate Frizzled co-receptors, effectively blocking canonical WNT signaling. In addition, the formation of this complex induces the internalization of the sclerostin-LRP4-LRP5/6 ligand-receptor complex, effectively removing LRP5/6 from the cell surface and thereby further blocking canonical WNT signaling. Most importantly for therapeutic intervention, sclerostin is almost exclusively secreted by osteocytes (Fig. 19.2) [34, 35], acting in part in an autocrine manner (osteocytes express WNT receptors and ligands) but also reaching the bone surface through the osteocyte canalicular network to regulate osteoblast and osteoclast differentiation and function in a paracrine manner.

Given the dominant influence of WNT signaling in bone, it is not so surprising that many of the hormones and cytokines that regulate bone remodeling and skeletal homeostasis do so indirectly, via the regulation of sclerostin expression. For instance, sclerostin is decreased by 1,25(OH)2 vitamin D3 (ref) and PTH (see below) and increased by glucocorticoids and estrogen deprivation [36–39]. Also important is the fact that sclerostin is regulated by mechanical forces, with loading decreasing and unloading or immobilization increasing its expression in osteocytes [40, 41]. Given than osteocytes are essential for bone mechanosensing and express high levels of sclerostin, this represents a finely tuned mechanism by which osteocytes can modulate bone formation in response to changes in mechanical stimulation, likely to regulate locally Wnt signaling and its anabolic/antiresorptive effects [40].

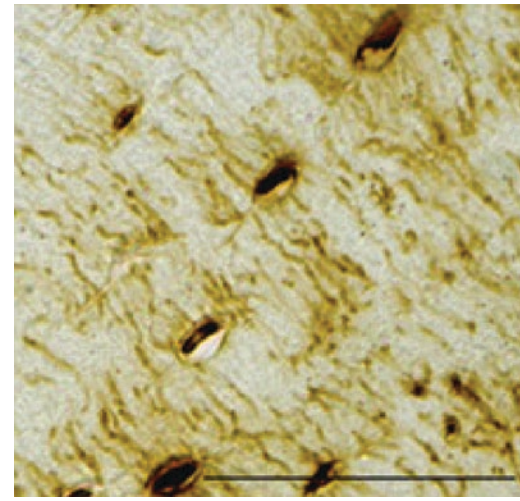

Fig. 19.2 Expression of sclerostin in osteocytes and their canaliculi in a model of unloading in mice. Immunostaining of human osteocytes in cortical bone shows the presence of sclerostin in the cell body and dendrites within the canalicular system. Bar = 50 μm. (Reprinted from Moustafa et al. [73]. With permission from Creative Commons License 2.0: https://creativecommons.org/licenses/by-nc/2.0/)

In addition to secreting sclerostin, osteocytes are an important source of RANKL [42, 43], sclerostin favors osteoclastogenesis by increasing the RANKL/OPG ratio. Consequently, blocking or deleting sclerostin increases bone formation dramatically but also decreases osteoclast surface and activity [44, 45].

In principle, therapeutic intervention could target any of the several steps of the β-catenin-dependent WNT signaling cascade. However, two significant challenges would make this intervention very risky. First, WNT signaling and the β-catenin signaling cascade are ubiquitous, making any intervention prone to unwanted side effects. Second, uncontrolled activation of WNT signaling has been associated with oncogenic

transformation, in particular in the colon [29–31] and also in osteosarcoma [32]. Sclerostin, as a secreted and bone-restricted endogenous inhibitor, exerts a constant negative influence on this pathway to keep it tamed. In this context, sclerostin appears as the ideal therapeutic target for promoting WNT signaling because it could keep WNT activation as restricted to the bone microenvironment as possible.

Thus, as of today, the best opportunity to activate WNT signaling specifically in bone comes from the fact that sclerostin expression is predominantly restricted to cells of the osteoblast lineage, and in particular osteocytes [34, 35, 46]. This exceptional situation opened the possibility that blocking this secreted WNT inhibitor might selectively increase WNT signaling only in bone, offering the theoretical opportunity to safely increase bone mass while leaving other tissues un- or only mildly affected. Moreover, as sclerostin is induced in unloaded bone areas and repressed in loaded areas [40], antagonizing sclerostin may essentially mimic loading, targeting the increase in bone formation to areas that most need it. The observation that sclerosteosis or van Buchem hemizygote carriers, in which the levels of expression of sclerostin are effectively decreased by about 50% have high bone mass but no measurable undesirable adverse effects [47], supported the hypothesis that targeting sclerostin could be both efficient and safe in humans. Thus, reducing sclerostin activity, not entirely as in the sclerosteosis null mutants but partially as in the hemizygotes, should increase bone mass with limited or no adverse effects.

Effects of Sclerostin Overexpression, Deletion, or Antibody Blockade in Animal Models

Mouse models genetically engineered to mimic the mutations seen in sclerosteosis and van Buchem's patients reproduce the HBM phenotypes seen in these patients, while transgenic expression of sclerostin in mice leads to osteopenia [9, 48]. In contrast, targeted deletion of sclerostin in bone increased bone formation in trabecular and cortical bone [48]. In female rats, antibody-based sclerostin inhibition increased bone mass and strength and was able to reverse ovariectomy-induced bone loss [44]. Treatment with antibodies to sclerostin also increased bone formation, bone mass, and strength in aged male rats [49]. In a model of hind limb-immobilization in rats, inhibition of sclerostin resulted in a marked increase in cortical and trabecular bone mass, despite the unloading of the limbs, with high bone formation and lower bone resorption, supporting the concept that sclerostin inhibition mimics to a certain degree bone loading [45]. The ability of sclerostin antibodies to increase bone formation at all skeletal sites was confirmed in other preclinical models, such as type 1 and 2 diabetes or high dose corticosteroid treatment [50]. Consistent with these rodent data, injection of humanized sclerostin-neutralizing antibodies once a month for 2 months in gonad-intact or in ovariectomized nonhuman primates (NHPs) had a marked dose-dependent effect on bone formation, bone mass, and bone strength [51].

Mechanistically (Fig. 19.3), the most striking and important effect of sclerostin antibodies in all these preclinical models, besides the massive increases in trabecular and cortical bone volumes, was the consistent induction of extensive modeling-based bone formation. In NHPs, these effects are unprecedented, with modeling surfaces increasing from 0.6% to 33.7% in trabecular bone and from 6.9% to 76.8% along the endocortical surface [51–53]. This de novo bone formation occurs along otherwise quiescent surfaces covered with bone lining cells, independent of remodeling [44, 49, 51]. Recent studies have clearly documented these cellular effects of sclerostin antibodies in rodents and have detailed the mechanisms involved [54–56]. Consistent with the observed increase in modeling-based bone formation, the increase in bone formation seen after sclerostin antibodies is due more to an increase in the size and activity of lining cells and osteoblasts than to the proliferation of osteoprogenitors, whereas PTH seems to affect mostly this latter mechanism [56–58]. In addition to this

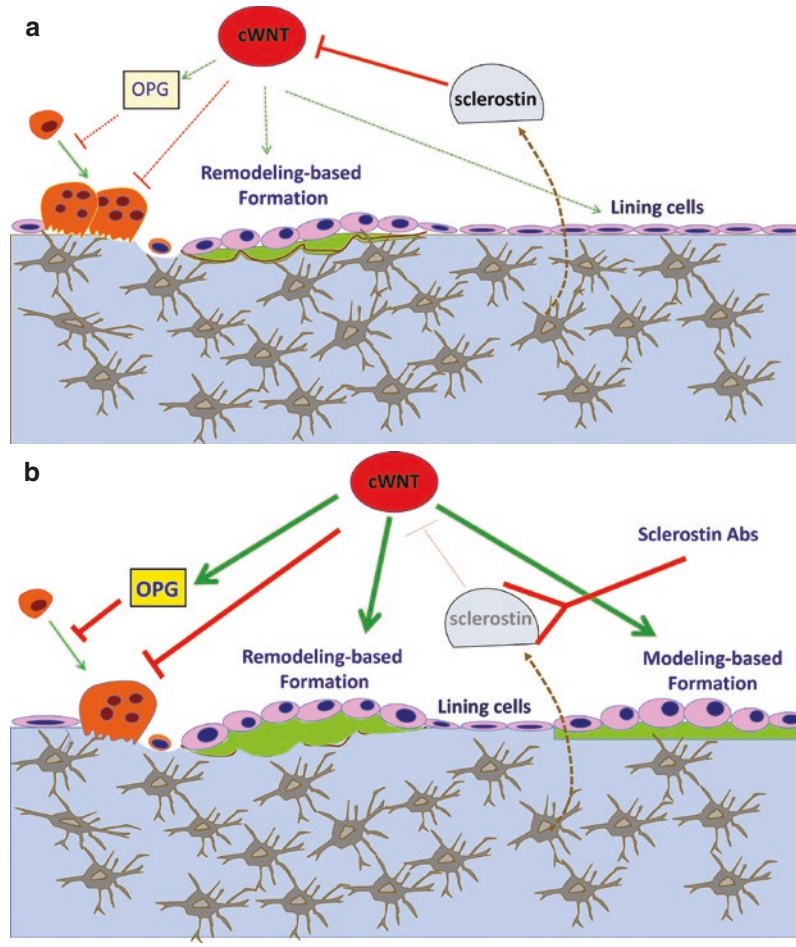

Fig. 19.3 Mode of action of sclerostin (**a**) and effects of sclerostin antibodies (**b**). (**a**) Under steady-state conditions, osteocyte-derived sclerostin blocks the activity of WNTs, and suppresses remodeling- and modeling-based bone formation, while allowing osteoclast differentiation and activity; (**b**) Delivery of antibodies to sclerostin relieves the inhibition exerted by sclerostin on WNT signaling, enhancing bone formation at remodeling sites, and activating bone lining cells to form new bone along previously quiescent surfaces through modeling activity; activation of WNT signaling also increases OPG production by osteoblasts and lining cells, preventing osteoclast differentiation and directly impairing osteoclast function

increase in modeling-based bone formation, inhibition of sclerostin also increases remodeling-based bone formation, but this appears to contribute to a much lesser degree to the overall increase in bone mass [52].

Thus, the blocking of sclerostin stimulates bone formation directly, through bone modeling, i.e., at least in part independent of bone remodeling, and therefore without prior activation of bone resorption. Moreover, inhibition of sclerostin also decreases bone resorption. This should, in theory, lead, like other antiresorptives, to an overall decrease in activation rate and thereby decrease remodeling-based bone formation and bone turnover. The fact that remodeling-based bone formation is also increased by antibodies to sclerostin therefore suggests that, like with inhibitors of cathepsin K [59], these agents uncouple partially bone resorption and bone formation in the remodeling units.

Altogether, the genetic and preclinical studies point to a potent and unique mechanism, both anabolic and antiresorptive, by which sclerostin blockade increases bone mass and strength in both trabecular and cortical bone (Fig. 19.3).

Effects of Monoclonal Antibodies to Sclerostin in Humans

Based on these human genetics and preclinical studies, two monoclonal antibodies against sclerostin have been generated and tested in clinical trials: romosozumab and blozosumab. Because the development of blozosumab has been halted due to manufacturing issues, this section will largely focus on romosozumab.

Early Studies

Padhi et al. reported in 2011 the first human, phase 1 randomized, double-blind, placebo-controlled clinical trial of romosozumab, a humanized monoclonal sclerostin antibody, in healthy men and postmenopausal women [60]. A single subcutaneous dose of Romosozumab quickly increased the bone formation marker P1NP with a peak at about 1 month, returning to baseline after one additional month. Markers of bone resorption were also rapidly decreased by 40–50%, returning to baseline after 50 days (Fig. 19.4a). Although this antiresorptive effect was expected, it remained a surprising finding in humans, particularly in its amplitude. Likewise, the gain in BMD at the lumbar spine and total hip 2 months after this single injection was comparable or even greater than with daily injections of teriparatide [60]. Very similar data were obtained later in the phase 1 clinical trials with blosozumab, another monoclonal antibody against sclerostin [61]. In both instances, no undesirable effects were observed.

The results of subsequent clinical trials confirmed the efficacy of monthly injections of sclerostin monoclonal antibodies over 12 months but revealed an unexpectedly limited duration of the anabolic effect (as assessed by increases in serum markers of bone formation), though the increases in BMD were sustained for up to 2 years [62]. Indeed, the increase in bone formation markers, although robust, peaked at 1–3 months after initiating therapy but declined thereafter despite continued monthly dosing, reaching baseline at 6 months and even decreasing below baseline later. In contrast, the effects on bone resorption markers, although more modest, were durable (Fig. 19.4b). Since both antibodies showed very comparable limitations in two entirely independent trials [63], it must reflect a common biological response to treatment, though the underlying mechanism remains undefined. Despite these limitations, both antibodies showed effects on BMD after 1 year of treatment that were very significant at all sites and stronger that those of teriparatide. Specifically, in the romosozumab trial, 419 postmenopausal women with a T-score of <−2.0 were randomized to receive one of several doses of romosozumab, alendronate, teriparatide, or placebo. In those women who received the romosozumab dose of 210 mg SC monthly (the dose that was subsequently tested in phase 3 trials), spine and total hip BMD increased by 11.3% and 4.1%, respectively, both of which were significantly greater than women receiving either alendronate or teriparatide.

Phase 3 Trials

FRAME Study (Placebo Controlled)

The FRAME study was a phase 3 study assessing the effects of 1 year or romosozumab 210 mg administered by subcutaneous injection every month followed by 1 year of the RANKL-inhibitor denosumab compared to 1 year of placebo followed by a year of denosumab [64–67]. Postmenopausal women with BMD T score between −2.5 and − 3.5 at the total hip or femoral neck were enrolled. By the 12-month timepoint, the women receiving romosozumab had experienced 73% fewer morphometric vertebral fractures compared to placebo, whereas there was no difference in the rate of nonvertebral fractures. At 24 months, the rates of vertebral fractures remained 75% lower in the romosozumab group than in the placebo group whereas the nonvertebral fracture rate, while lower in the romosozumab arm, was not significantly so ($p = 0.06$). The pattern of BMD changes in FRAME were similar to those observed in earlier studies with the total 2 year BMD gains in women treated with romosozumab followed by denosumab approaching 18% at the lumbar spine. The incidence of adverse events and serious adverse events in the FRAME study were balanced between groups. Two patients in the romosozumab experienced osteonecrosis of the jaw and one patient experienced an atypical femoral fracture 3.5 months after starting romosozumab.

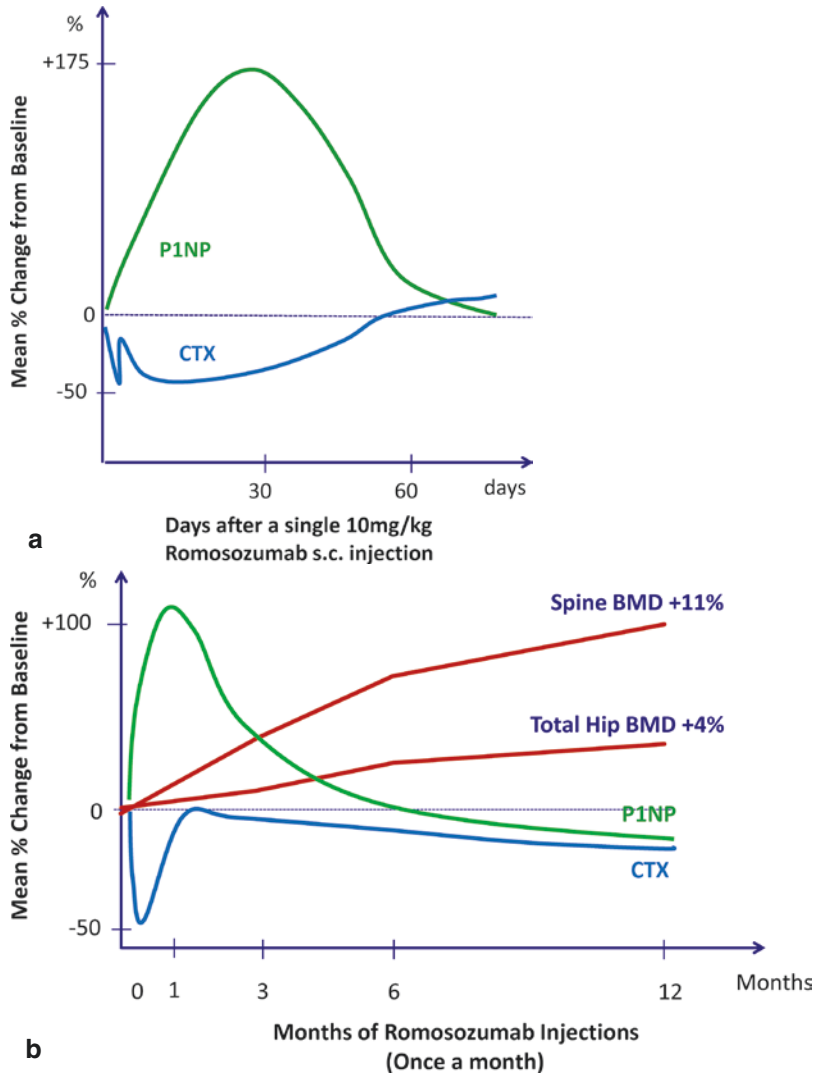

Fig. 19.4 (**a**) Phase 1 effects of one single injection of romosozumab (210 mg) on bone biochemical markers in healthy men and postmenopausal women. P1NP (green line) increases rapidly to reach a peak at 175% of baseline after about 30 days and then returns to baseline by 60 days; CTX (blue line) decreases sharply after the injection, briefly returns close to baseline and then decreases to about 50% of baseline after 20–30 days, returning to baseline after 50–60 days. (**b**) Phase 2 effects of 12 monthly injections of romosozumab (210 mg) on bone biochemical markers and BMD in postmenopausal women. After repeated monthly injections of the antibodies, the bone formation marker P1NP (green line) increases rapidly and reaches a peak at 100% above baseline after 1 month, similar to the effects of a single injection, but then declines progressively, despite continued treatment, first sharply between 1 and 3 months and slowly thereafter, reaching baseline values at 6 months; it then drifts below baseline at 12 months; at the same time, the bone resorption marker CTX (blue line) decreased sharply to reach a 40% nadir after 1 month, bouncing back close to baseline at 3 months but decreasing progressively thereafter to 20–40% below baseline at 6 and 12 months. During this 12 months period, BMD increased at both the spine and the hip (straight blue lines) by 11% and 4%, respectively, at a faster pace during the first 6 months than between 6 and 12 months. Changes observed in the two Phase 3 trials, FRAME (Cosman et al. [64],) and ARCH (Saag et al. [68],) in biochemical markers and BMD followed similar patterns. (**a** Based on data from Ref. [60]. **b** Based on data from Ref. [62])

The lack of a significant reduction in nonvertebral fractures despite the very large BMD increases were difficult to explain, though the authors noted that in a preplanned subgroup analysis, among Latin American study sites the risk of nonvertebral fracture was lower than in other areas and there was no effect of romosozumab on nonvertebral fracture incidence. When these sites in Latin America were excluded, a significant effect was observed on nonvertebral fractures, a finding that should not be over-interpreted.

ARCH Study (Active Comparator)

The ARCH study was a phase 3 randomized controlled trial in which 4093 postmenopausal women with either (1) a total or femoral neck T score < −2.5 and either one or more moderate or severe vertebral fractures or (2) two or more mild vertebral fractures or a T score < −2.0 and either two or more moderate or severe vertebral fractures or recent hip fracture were randomized to receive 1 year of monthly subcutaneous romosozumab (210 mg) or weekly oral alendronate (70 mg) [68]. After the first year, all subjects received open-label alendronate for an additional 3 years. After 24 months, the women randomized to the romosozumab-to-alendronate arm had a 48% lower risk of new vertebral fractures (Fig. 19.5) and a 19% lower risk of nonvertebral fracture compared to the alendronate-alendronate arm, both of which were statistically significant. The benefit of romosozumab on fracture risk appeared to be sustained but not expanded during the 3-year follow-up, when all subjects were receiving alendronate. This study was notable because it is among the only osteoporosis head-to-head trials to show a fracture benefit of one drug versus another and remains the only study to show a nonvertebral fracture benefit of one drug over another. Unlike in the FRAME study, however, in ARCH there was an increased incidence of adjudicated serious cardiovascular adverse events (2.5% versus 1.9% at 12 months). Because vascular safety signals with romosozumab were not noted in FRAME, this was a surprising finding and one that persisted even when all subjects were taking alendronate. Moreover, as discussed below, there was also a numeric imbalance in the much smaller BRIDGE Study of male

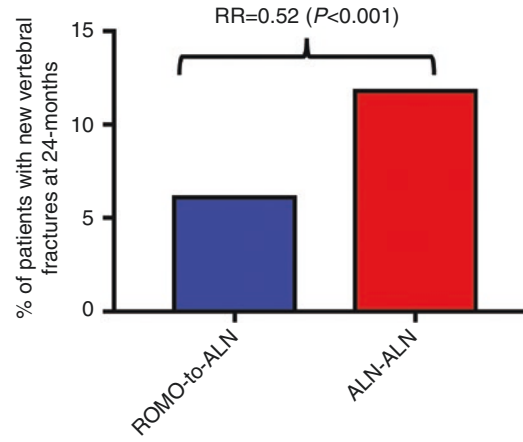

Fig. 19.5 Head-to-head fracture reduction, romosozumab versus alendronate. Incidence of new vertebral fractures in postmenopausal osteoporotic women treated with 1 year of romosozumab followed by 1 year of alendronate versus postmenopausal osteoporotic women treated with 2 years of alendronate in the ARCH study. (Based on data from Ref. [68])

osteoporosis. Potential mechanisms that may underlie a romosozumab-induced increase risk in cardiovascular events have been hypothesized given recent basic studies demonstrating that both canonical and noncanonical Wnt pathways play a role in vascular pathophysiology, but have not been clearly defined [69].

BRIDGE Study (Male Osteoporosis)

The BRIDGE study [70] was a placebo-controlled trial of 245 men aged 55–90 with a T score at the spine, femoral neck, or total hip <−2.5 or > −1.5 with a history of fragility nonvertebral or vertebral fracture randomized to receive either romosozumab or placebo (2:1 randomization) for 12 months. In these men, the increases in BMD were similar to those observed in postmenopausal women with significant increases at the spine and hip of 12.1% and 2.5%, respectively. As noted above, the percentage of subjects experiencing adjudicated serious cardiovascular events was 4.9% in the romosozumab group and 2.5% in the placebo group.

Discontinuing Romosozumab

As discussed in prior chapters, the effects of discontinuing osteoporosis medications differ

depending on their mechanism of action. Bone mineral density gains are generally sustained when bisphosphonates are discontinued whereas gains in bone density are reversible when PTH analogs, hormonal agents, or denosumab are discontinued (albeit to differing degrees). The effects of discontinuing romosozumab were investigated in an extension to the initial 12-month bone mineral density study of 419 postmenopausal women discussed above [71]. Specifically, the women originally randomized to receive one of several doses of romosozumab for 12 months were then extended for an additional 12 months on their assigned therapy after which they were re-randomized to either denosumab or placebo. Focusing on the group that received 210 mg monthly, BMD at the spine and hip continued to increase (albeit quite modestly) during the second year of therapy but after re-randomization women receiving placebo experienced acute bone loss. 12 months after re-randomization, spine BMD had decreased nearly 10% and hip BMD decreased by approximately 5% in the placebo group with BMD at the hip essentially reverting to the pretreatment baseline. Bone formation (as assessed by P1NP), which was below the pretreatment baseline after 24 months of romozosumab, gradually returned to pretreatment levels during the placebo phase. Conversely, bone resorption (as assessed by CTX), which was below the pretreatment baseline after 24 months of romozosumab, increased more rapidly and to a level above the pretreatment baseline. CTX levels remained nearly 50% above pretreatment baseline even at month 36 or 12 months after romosozumab discontinuation. These findings underscore the importance of consistently following romosozumab with an antiresorptive agents, as was done in the large phase 3 trials discussed above.

Sequential Therapy

As discussed in Chap. 18 and in the above section, the utility and necessity of transitioning to an antiresorptive agent after completing anabolic therapy is well-established. Conversely, it has been reported that the effects of using PTH analogs after bisphosphonate exposure results in a significant blunting of the BMD increases, especially at the hip (see Chap. 17). The effect of romosozumab when used after bisphosphonate therapy was investigated in 436 postmenopausal osteoporotic women who had taken an oral bisphosphonate for 3 or more years before enrollment and alendronate the year before enrollment who were then randomized to receive either romosozumab 210 mg monthly or teriparatide for 12 months [72]. In this study, those randomized to romosozumab achieved greater gains in BMD at the spine then those receiving teriparatide (9.8% versus 5.4%). At the hip, women randomized to romosozumab achieved gains of 2.9% versus no gain with teriparatide. Additionally, hip strength (estimated by finite element analysis of QCT images) increased only in the romosozumab group (2·5% versus −0.7% in the teriparatide group). Bone formation (P1NP), which was suppressed at baseline due to the bisphosphonate exposure, increased transiently whereas bone resorption (CTX) decreased transiently. The romosozumab-induced changes of bone turnover in this cohort are difficult to compare to those reported in treated naïve women due to their much lower baseline values, but it should be noted that the BMD gains appear to be still slightly lower than those reported in treated naïve women, and hence some degree of blunting cannot be excluded.

In conclusion, the discovery that Sost mutations in the gene that encodes for sclerostin, Sost, in humans lead to two rare high bone mass conditions, established that sclerostin is a critical regulator of bone mass. Numerous studies in genetically modified animal models have since then demonstrated that sclerostin, mainly secreted by osteocytes, is an antagonist of the canonical WNT signaling pathway, and thereby negatively regulates bone formation and favors bone resorption. Compelling evidence indicates that sclerostin suppresses cell commitment to the osteoblast lineage, prevents bone lining cell activation, osteoblast differentiation and activity, and inhibits late osteoblast differentiation into osteocytes. Consequently, inhibition of sclerostin via specific antibodies markedly enhances bone formation, in particular modeling-based bone for-

mation stemming from the activation of quiescent bone lining cells, as well as remodeling-based formation. In addition, sclerostin antibodies suppress osteoclast differentiation and bone resorption. The combination of these two effects results in an unprecedented increase in trabecular and cortical bone density and bone strength.

In humans, antibodies to sclerostin, and romosozumab in particular, are able to acutely, though transiently, stimulate bone formation while modestly decreasing bone resorption. In this way, romosozumab is first single-agent regimen that has successfully separated effects on bone formation and resorption leading to large and rapid increases in bone mineral density and decreases in fracture incidence. While potential safety issues have emerged related to cardiovascular event, as of the writing of this chapter, romosozumab has been approved for the treatment of severe postmenopausal osteoporosis in Japan and in the United States (approval in Europe is still pending). These approvals are accompanied by specific warnings regarding the potential for increased cardiovascular risk and potential restriction on the appropriate patient populations to be treated.

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Osteoporosis in Men

20

Robert A. Adler

Key Points

- Osteoporosis in men is common with one out of four to six men over the age of 50 experiencing an osteoporotic fracture in their lifetime.
- In men who experience a hip fracture, there is a very high mortality rate (even higher than the rate in postmenopausal women) and survivors often lose independence.
- History, physical exam, modest lab testing, and DXA are indicated in all men who experience an osteoporotic fracture.
- Osteoporosis medications are similarly effective in men as they are in women.

Introduction

Early osteoporosis guidelines did not even mention men, but gradually there has been greater recognition that osteoporotic fractures occur in men as well as women. As the population ages, men are living long enough to fracture; indeed, the incidence of fracture rises markedly after age 80. Despite greater appreciation of osteoporotic fracture risk in men, men are less likely to have attention paid to underlying osteoporosis after a hip fracture or during oral glucocorticoid therapy. In addition, for reasons not yet clear, men do worse after a hip fracture, with higher death rate and lower incidence of postfracture independence. While much of our knowledge about osteoporosis in men relies upon studies in women, there is more evidence than ever to prove that medications that lower fracture risk in women will also do so in men. Consequently, a plan for identifying men at risk, evaluating, and treating men can be constructed. Even with limited evaluation and treatment tools, it should be possible to lower fracture risk and the sequelae of fracture.

Definition and Epidemiology of Osteoporotic Fracture

Osteoporosis can be defined as the occurrence of a low trauma fracture, meaning a fracture after a fall from a standing position. The bone breaks because it is too weak to withstand the force of the fall. With increasing age, more low trauma fractures occur in both sexes, although risk accelerates about 10 years later in men than in women. Surprisingly, based on data from the websites of

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the American Heart Association, the American Cancer Society, and the National Osteoporosis Foundation, the annual incidence of osteoporotic fracture in men is greater than the annual incidence of stroke, myocardial infarction, or prostate cancer. Yet, osteoporosis is generally considered to be a disorder affecting postmenopausal women. Osteoporosis is defined operationally as compromised bone strength due to decreased bone quantity and/or quality. It is much easier to determine quantity, and as in women, osteoporosis is defined by the World Health Organization as a bone mineral density (BMD) by dual energy X-ray absorptiometry (DXA) 2.5 standard deviations below the normal young mean at peak bone mass, a T score ≤ -2.5. The International Society for Clinical Densitometry (ISCD) and other expert groups concluded that for all people, a normal white female database should be used for calculation of the T score [1]. While this decreases the proportion of men who fit this diagnostic criterion for osteoporosis [2], there is evidence that they will have a better response to osteoporosis treatment [3]. A further definition of osteoporosis is derived from fracture risk calculation scores. Those men who have a risk beyond a certain threshold can be described as having osteoporosis. Specifically, the National Bone Health Alliance (NBHA) proposed that the FRAX 10-year fracture risk calculation, based on femoral neck BMD and several validated risk factors (age, previous fracture, parental history of fracture, current smoking, drinking more than three units per day, use of glucocorticoid drugs, and rheumatoid arthritis), be used to define osteoporosis. If a $\geq 3\%$ 10-year risk of hip fracture or $\geq 20\%$ 10-year risk of major osteoporotic fracture (spine, hip, humerus, or wrist) is predicted by the FRAX, then the patient is considered to have osteoporosis [4, 5]. Wright et al. [6] reviewed NHANES data and found that 16% of men over age 50 and 46% of men over age 80 meet these criteria. It is interesting that at age 50, a man has a 13–25% chance of suffering an osteoporotic fracture during his lifetime, [7] compatible with the findings of Wright et al. Other countries may have other thresholds for the diagnosis of osteoporosis, and it is not proven that treating men with osteoporosis by the NBHA definition will lead to fewer fractures. Regardless of the definition used, it is obvious that osteoporosis in men is more common than most patients and clinicians have thought.

As stated above, the increase in fracture risk in men occurs about 10 years later in life than in women [8]. One reason for this is that, in general, men have bigger bones than women. Thus, there is more bone to lose with aging. Second, there is evidence that the changes with aging in bone are somewhat different. In trabecular bone, aging in women leads to fewer trabeculae and the spaces between trabeculae increase, whereas in men there is simply thinning of trabeculae [9]. In cortical bone, men have more periosteal bone formed with aging than do women [10]. Men do not undergo menopause with rapid loss of sex steroids. Instead, there is a gradual diminution of testosterone and estradiol with aging in men. While these differences delay the incidence of fracture, it is interesting that once a hip fracture occurs, men have about twice the mortality rate of women [11]. In addition, those men who survive hip fracture are not likely to regain independence. The seriousness of hip fracture in men is greatly underappreciated. A consequence of this lack of appreciation is that men are less likely to have evaluation and treatment of underlying osteoporosis in situations where there is consensus that osteoporosis and fracture risk should be assessed, such as after a fragility fracture [12] or in those individuals taking oral glucocorticoids [13]. Some years ago, predictions [14] were made on the incidence and costs of osteoporotic fracture in American women and men in 2005 and 2025. At the earlier time, almost 30% of fragility fractures were found to occur in men, and it was predicted that there would be a significant increase in the number of new fractures in men as the population aged, as of 2025. The predictions may have been conservative because of an interesting and troubling recent finding [15]. In the Medicare population, mostly American adults over age 65, the incidence of hip fracture had been declining dramatically from about 2002 to 2012. From 2012 to 2015, the hip fracture incidence remained the same, according to the study by Lewiecki et al. [15]. Potential reasons for this included actual decreases in the diagnosis of

osteoporosis, the number of bone density tests done per year [15], and number of prescriptions for osteoporosis medications [16]. Should this trend continue, as men live long enough to fracture, the incidence, morbidity, mortality, and costs of osteoporotic fractures are likely to rise. Populations in Europe and the rest of the world are also aging. For example, in the European Union, the overall annual incidence of osteoporotic fracture is predicted to rise from 2.5 million in 2010 to 4.5 million in 2025 [17].

Osteoporotic Fractures in Middle – Aged Men

While most fragility fractures occur in men after age 80, middle-aged men may present with vertebral fractures (either clinical or on X-ray) or low BMD. While there are no studies of large populations presenting this way, either of two common disorders is likely to be present, although there are some less likely causes as well. Hypogonadism leads to low bone density and lower muscle mass, and it can present as a fracture in middle age. The diagnosis can usually be made by history, physical examination, and early morning fasting levels of serum testosterone. The site of the defect (at the testes or at the pituitary or above) can be deduced from the levels of the gonadotropins, LH, and FSH. If they are elevated in the face of a low serum testosterone, the diagnosis is primary hypogonadism (a testicular defect). If LH and FSH are not elevated in the face of low serum testosterone, the diagnosis is secondary hypogonadism. The patient may be relatively asymptomatic unless there is a clinical fracture or the patient has had low enough testosterone for long, enough to have decreased libido. It has long been established that the BMD of middle aged and younger men with hypogonadism will respond to testosterone replacement [18]. The impact on fracture risk is unknown, but most experts will follow BMD and other risk factors in such patients to determine if additional therapy is needed.

Another relatively common cause of osteoporosis in middle-aged men is hypercalciuria [19]. Some of the patients may have a history of kidney stones. It is thought that long-standing negative calcium balance predisposes to osteoporosis and may present in middle age with vertebral fractures. Some secondary causes of osteoporosis (see below) may be relatively asymptomatic, such as celiac disease. These secondary causes can be discovered by history, physical examination, and targeted laboratory testing. In addition, there have been a few specific syndromes that have presented as vertebral fractures or osteoporosis in younger men. These include idiopathic osteoporosis characterized by low serum IGF-1 levels despite normal growth hormone secretion [20]. In Belgium, male family members have been found to have low bone mass associated with decreased bioavailable estradiol levels [21].

Screening for Fracture Risk in Older Men

One method to reduce fractures is primary prevention, and the best predictor of fracture in general is DXA. Several organizations and guidelines suggest or recommend DXA screening men at a certain age. For example, the Endocrine Society Male Osteoporosis Guideline [22] suggested DXA testing at age 70; men younger than 70 could be tested if important risk factors or secondary causes of osteoporosis were present. On the other hand, the United States Preventive Services Task Force [23] concluded that there was insufficient evidence to recommend for or against osteoporosis screening by DXA in men. Even in women, the effectiveness of osteoporosis screening has not been supported by many studies demonstrating that such screening leads to fewer fractures. More recently, an important trial from the United Kingdom [24] provided convincing evidence that a screening program in women had important benefits. In the SCOOP study, women were randomized to normal care or a two-step screening procedure. The first step consisted of calculating FRAX using body mass index (BMI). FRAX, described below, predicts the 10-year risk of major osteoporotic fracture (MOF: spine, hip, wrist, or humerus) and hip fracture based on BMD or BMI plus validated

risk factors such as age, prior history of fracture, parental history of fracture, etc. Those women found to be at low risk by FRAX with BMI received no further evaluation. Those whose risk was higher were then invited to have a DXA, and with it either osteoporosis was diagnosed or FRAX was recalculated with BMD. Those women who met treatment thresholds were treated for osteoporosis and over 5 years had fewer fractures than the women who had usual care. Thus, screening worked [24]. There are no large randomized studies in men, and given the lower fracture incidence in men, the size of a randomized, controlled trial similar to SCOOP would have to be much larger and more expensive. In a recent observational study [25] using the very large administrative database from the United States Department of Veterans Affairs, Colon-Emeric et al. demonstrated that overall DXA screening as currently done does not lead to fewer fractures. One important reason for this was the very low initiation of, and persistence with, osteoporosis medication in those men who met criteria for treatment, based on the screening. On the other hand, when prespecified higher risk groups were screened by DXA, fewer fractures occurred over the study period. The men at augmented fracture risk included those aged 80 or older, those on androgen deprivation therapy (ADT) for prostate cancer or on chronic oral glucocorticoid therapy, and those with high fracture risk predicted by FRAX using BMI. Men aged 65 or older were likely to benefit from DXA screening if they had one of several important risk factors (in addition to those already listed [26]): rheumatoid arthritis, use of enzyme-inducing antiepileptic drugs, alcohol abuse, smoking, hyperthyroidism, hyperparathyroidism, chronic liver disease, chronic lung disease, stroke, Parkinson's disease, BMI < 25 kg/m^2, or history of gastrectomy (prior Bilroth or bariatric). The conclusion from this study was that targeted testing with DXA could identify men at the highest fracture risk, leading to fewer fractures [25].

From these studies, a strategy for DXA testing in men can be developed. FRAX can be calculated from history and measurement of height and weight. Other history can be entered into the FRAX website (www.shef.ac.uk/FRAX/) and the 10-year risk of MOF and hip fracture can be determined. For glucocorticoid-induced osteoporosis [27], the American College of Rheumatology defines low risk of fracture as <10% MOF and <1% hip fracture, moderate risk as 10–19% MOF and 1–3% hip fracture, and high risk as 20% or more MOF and 3% or more hip fracture over 10 years. Using these criteria and BMI as a surrogate for BMD, FRAX can be calculated to choose moderate or high risk men for DXA testing. In addition, all men 80 and older, or those on chronic prednisone or ADT, and those over 65 with one of the other important diagnoses could be tested by DXA. The proposed screening test algorithm is shown in Fig. 20.1. There are some caveats to this algorithm. One is that calculations

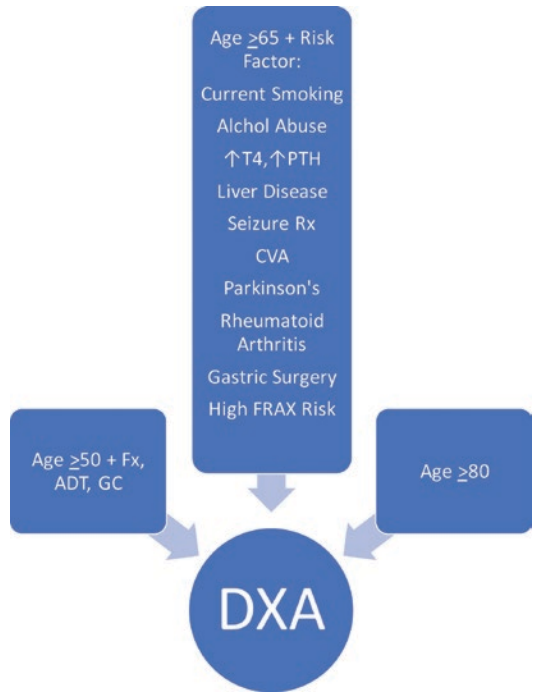

Fig. 20.1 Men who should be tested by DXA. *Abbreviations*: Fx osteoporotic fracture, ADT androgen deprivation therapy, GC oral glucocorticoids >5 mg for >3 months. Risk factors are derived from [26]: current smoking, alcohol abuse, hyperthyroidism, hyperparathyroidism, chronic liver disease, enzyme-inducing antiepileptic drugs, stroke, Parkinson's disease, rheumatoid arthritis, gastric surgery, high risk by FRAX using BMI. (Scheme based on data from Colon-Emeric et al. [25])

simpler than FRAX may be helpful in choosing men for DXA testing. The Osteoporosis Self-Assessment Tool (OST) is based on weight and age [28]. In men, it can predict bone density fairly well, although not in all studies [29, 30]. For determining which men should have a DXA, OST may be used instead of FRAX, because in some studies it has been shown to be as good as, if not better than, FRAX with BMI [31]. A second caveat relates to the treatment threshold for fracture risk based on FRAX. In the United States, patients are treated if the MOF risk is 20% or higher or the hip fracture risk is 3% or higher, based on a cost-effectiveness study [5]. With generic bisphosphonates now very cheap, the cost-effectiveness argument seems less convincing, and in many countries, the threshold for treatment is different from that of the United States. On a practical level, can the clinician convince a man to take medication for a long time if his 10-year hip fracture risk is 3% and his 10-year probability of not having a hip fracture is 97%? On the other hand, if the FRAX or OST calculation leads to a DXA that shows osteoporosis, then the male patient is probably (though not proven) more likely to be willing to begin osteoporosis treatment.

Other Evaluation: History, Physical Examination, Laboratory Testing

If a man has had a fragility fracture and/or has a DXA showing osteoporosis and/or is found to be at high fracture risk by FRAX or other risk calculator, then history and physical examination are important to plan management. It has been said that secondary causes of osteoporosis are more common in men than in women, but there is some overlap between secondary causes and risk factors for fracture [32]. Table 20.1 lists some of the more common secondary causes of osteoporosis in men [33]. Table 20.2 is derived from a study [34] of hip fracture in the MrOS cohort. This multisite observational study of about 6000 older American men has provided a great deal of information about osteoporotic fracture. Among the many lessons from the study, risk factors (as listed in Table 20.2) magnify fracture risk after DXA is measured. For example, if men have a femoral neck T scores < −2.5 and only one of the risk factors listed in Table 20.2, the annual incidence of hip fractures is about 11/1000 patient-years. However, if the men have four or more risk factors with the same T score, the incidence of hip fractures is about 51/1000 patient-years, an almost fivefold difference [34]. Thus, these secondary causes and risk factors may be revealed by the history and physical examination: these are the problems the clinician should be looking for while assessing the patient.

In our Metabolic Bone Disease Clinic, we also watch the patient walk into the examination

Table 20.1 Important secondary causes of osteoporosis in men

Oral glucocorticoid therapy
Androgen deprivation therapy for prostate cancer
Chronic obstructive pulmonary disease
Primary testicular failure
Secondary hypogonadism
Alcohol abuse
Multiple myeloma
Gastrectomy/bariatric surgery
Rheumatoid arthritis
Organ transplantation
Celiac disease, other malabsorption
Hyperthyroidism
Hyperparathyroidism
Inflammatory bowel disease
Mastocytosis
Mobility disorders (e.g., stroke, multiple sclerosis, Parkinson's disease, and spinal cord injury)

Table 20.2 Risk factors that magnify hip fracture risk predicted by DXA

Age > 75 years
Less protein in diet
Any fracture after age 50
Divorce
Tricyclic antidepressants
Hypoglycemic agents
Height loss
Hyperthyroidism
Parkinson's disease
Inability to do chair stands
Decreased cognitive function
Current smoking

Based on work of Cauley et al. [34]

room. We ask the patient to stand up from the chair without using his hands. We enquire about his dental health. Are invasive dental procedures planned? We ask about gastro-esophageal reflux (GERD) and ability to swallow pills and food. We ask about dietary/supplemental calcium and vitamin D as well as general nutrition including protein intake. We ask about exercise, home safety, vision, and falls. Another fracture risk calculator, the Garvan Nomogram [35], adds the number of falls in the past year to the calculation of 5-year and 10-year fracture risk. While some of these questions are appropriate for any general medical visit, it is obvious that others are more specific to osteoporosis. We review and reconcile medications. We ask about over-the-counter medications, supplements, herbals, and illicit drugs. We gently inquire about smoking and drinking. On examination, we are interested in kyphosis, height loss, balance, dental hygiene, tenderness upon spine percussion, and evidence of thyroid hyperfunction or gonadal hypofunction, among other things.

No blood or urine test can be used to define osteoporosis. Some experts measure baseline bone turnover marker levels (particularly those of bone resorption, such as CTX) as a baseline for assessing patients' responses to antiresorptive (antiturnover) therapy. Men who do not suppress CTX after starting antiresorptive treatment may not be adherent or responsive to therapy. This might lead to a different treatment option. Laboratory tests should be done to help make some of the diagnoses listed in Table 20.1 and are shown in Table 20.3. However, there are standard laboratory tests that should be done in all men with elevated risk for osteoporotic fracture. Serum chemistries should include calcium, phosphate, and a measure of renal function. In addition, alkaline phosphatase has been recommended as a marker of bone turnover and bone response to treatment. High quality studies to prove the value of each of these laboratory tests are lacking, but there are good reasons to check them. Serum calcium and phosphate are important for detecting hyperparathyroidism and possibly malabsorption. Renal function must be known before prescribing certain medications. In the U.S. Veterans population, laboratory testing has proven valuable in identifying secondary causes of osteoporosis [32], whereas in the healthier MrOS cohort, routine testing has not yielded similar results [36]. Alkaline phosphatase elevations may be seen in patients with recent fracture and more interestingly, low serum alkaline phosphatase may be a clue to a disorder that, while still unusual, has been reported in patients who appear to have osteoporosis. This is hypophosphatasia, a genetic disorder of low serum alkaline phosphatase leading to variable clinical manifestations, including osteomalacia and low bone density. Hypophosphatasia is very diverse clinically, and its presence may complicate the management of osteoporosis [37].

Table 20.3 Laboratory tests for men with osteoporosis/increased fracture risk

Standard tests for all
Serum calcium
Serum phosphate
Renal function measurement
Serum alkaline phosphatase
Serum 25-hydroxyvitamin D
Complete blood count
24-hour urine calcium
Tests for specific patients, depending on history, physical examination, standard tests
Serum testosterone (possibly free and bioavailable testosterone)
LH, FSH, prolactin
Parathyroid hormone (PTH)
Thyroid function tests (TSH, free T4)
Celiac panel
Serum/urine protein electrophoresis
Tryptase

As in women, vitamin D status should be checked in men with osteoporosis, partly to rule out osteomalacia and also to assure full response to osteoporosis treatment. Although not all studies demonstrate this, some osteoporosis treatments appear to work better if the serum 25-hydroxyvitamin D level is at least 30 ng/ml [38]. A full discussion of vitamin D is beyond this chapter, but suffice it to say that most experts believe that for osteoporosis patients, a level of 30 ng/ml is the appropriate vitamin D goal [39]. An argument can be made to measure a complete blood count, now an automated inexpensive test,

in patients with osteoporosis because multiple myeloma can cause fractures that resemble fragility fractures, and three-fourths of patients with multiple myeloma are anemic. Finally, when the 25-hydroxyvitamin D level is close to 30 ng/ml, a 24-hour urine calcium can be helpful to diagnose hypercalciuria and hypocalciuria, the latter being a consequence of malabsorption.

In addition to DXA, images of the spine may reveal compression deformities. A man who has lost more than 2 inches (5 cm) in height may have no recollection of an acute painful back event but may have wedging in the thoracic or lumbar spine. Vertebral fracture assessment is available on some DXA machines [40]. This technique is efficient because it can be done at the same time as the DXA, provides a good image up to T5, and has a much lower radiation dose than conventional X-rays of the spine. However, there is considerable technician time needed for assessment and reimbursement by insurance is unlikely. Indeed, reimbursement for regular DXA is problematic in the United States. Only men who have fractured, are on glucocorticoids, have hyperparathyroidism, have lost significant height, or are treated with osteoporosis drugs are likely to have reimbursement of DXA testing. This is likely a further manifestation of the incorrect notion that osteoporosis is just a disorder of postmenopausal women.

The Role of Testosterone in Osteoporosis

In parallel to the gradual decline of BMD with aging, there is a gradual decline in serum total testosterone levels [41]. In addition, because sex-hormone-binding globulin rises with aging, free testosterone declines even more dramatically as men age [42]. Some years ago, Khosla and colleagues [43] reported that in aging men, BMD was more strongly associated with levels of bioavailable estradiol than with any measure of testosterone per se. It is important to note that in men, estradiol is produced by aromatization of testosterone, but circulating testosterone is found in so much greater amounts than estradiol that changes in estradiol are not very dependent on the amount of testosterone present. Nonetheless, it is likely that testosterone, by its impact on muscle, plays a role in fracture risk. Binkley, Krueger, and Buehring [44] have postulated that osteoporosis is part of a syndrome of muscle and bone loss that occurs with aging. While there is no consensus on the definition of sarcopenia [45], there is no doubt that the frail, older man with decreased muscle mass and strength is at risk for falls and consequent fracture. We know that patients with decreased mobility with loss of muscle, such as those with hemiplegia from a stroke [46] or with more generalized muscle loss from spinal cord injury [47], are at high risk for osteoporotic fracture. There is some evidence that androgens have direct effect on bone cells [48], and Leder et al. [49] have shown that changes in testosterone have an impact on bone turnover markers.

If by direct or indirect means testosterone deficiency leads to fracture risk, it is possible that testosterone replacement in men with hypogonadism would mitigate the increased fracture risk. It is very plausible but hard to prove that exercise, by improving muscle strength, leads to fewer fractures [50]. Nonetheless, as listed below in the section on nonpharmacologic therapy, weight bearing exercise is advocated for all patients with osteoporosis. Testosterone, by improving muscle mass and strength, would likely also decrease fracture risk, but no firm evidence exists. On the other hand, there is increasing evidence that testosterone replacement leads to increased bone mass. Early and recent studies [51, 52] have shown that testosterone replacement in older men raises BMD by DXA. In studies using testosterone gel replacement, Snyder et al. [52] demonstrated that, compared to men receiving placebo gel, testosterone modestly increased bone density by DXA and volumetric bone density by quantitative computed tomography (qCT). Importantly, testosterone gel increased bone strength determined by finite element analysis (FEA) of qCT images. These findings do not tell us how testosterone had its effects. It could be a direct effect on muscle and/or bone or an effect via the estradiol increase caused by testosterone replacement. Nonetheless, the evi-

dence is stronger now to consider testosterone treatment for some clearly hypogonadal men. As described above, some middle-aged men presenting with vertebral fractures and/or low spine bone density may be hypogonadal, and testosterone replacement is usually indicated. Doing so has been known for years [18] to increase BMD. Although there are no fracture data, many experts would consider treating younger men with hypogonadism and osteoporosis only with testosterone replacement, saving osteoporosis-specific drugs for those who are older, at very high fracture risk, or cannot take testosterone. The long-term safety of testosterone has not been established. In older patients, development of a drug that has anabolic effects on bone and muscle like testosterone but without some of the other androgenic effects (such as stimulation of the prostate) has been a goal not yet attained. As will be shown below, the great majority of men with increased osteoporotic fracture risk, regardless of testosterone status, will be treated with osteoporosis-specific medications.

Summary of Osteoporosis Evaluation in Men

For the man who has fractured or found to have low bone mass by DXA testing, possible vertebral imaging, history, physical examination, and routine/targeted laboratory testing may lead to either a specific cause of osteoporosis or no specific cause. The findings will, of course, help the clinician choose an appropriate course of therapy, including observation if fracture risk is considered to be very low. For the man not at high risk, each clinic visit may reveal a new risk factor, such as commencement of glucocorticoid or androgen deprivation therapy, that would lead to further re-evaluation. For the man remaining at low risk, BMD changes slowly, so long intervals between DXA tests are reasonable. In the United Kingdom, some patients have periodic FRAX risk calculations using BMI. When the risk rises, a DXA is performed and the FRAX is recalculated. This is a reasonable longitudinal follow up in other parts of the world, particularly if it is augmented by general history and physical examination plus longitudinal measurements of height. Newer techniques such as qCT are useful for research but cannot be used clinically because the radiation dose precludes serial measurements in most cases. Particularly for the man who has lost height (>2 inches), an image of the lateral spine, either X-ray or vertebral fracture assessment, should be performed. Osteoporotic fractures occur in men with normal BMD. Indeed because use of the female normative database for DXA means more men who have fractured will have relatively good bone density [2], the clinician must remember that fragility fracture almost always means osteoporosis, regardless of the BMD.

Treatment of Osteoporosis in Men: Nonpharmacologic

Some experts have suggested that the general approach to treatment in the United States—quickly writing a prescription for an osteoporosis drug—is the reason patients do not trust clinicians. Thus, one approach to regaining trust in osteoporosis management is to make pharmacologic treatment a part of a more comprehensive approach. For this reason, nonpharmacologic management will be described first. After all, the purpose of treatment is fracture risk reduction, not just increases in BMD. If one could turn back the clock, then adequate calcium, vitamin D, and exercise throughout life would be a good way to prevent fractures. The higher the maximum skeletal mass, the more there is to lose over time. Interestingly, delayed puberty in male adolescents leads to increased fracture risk later in life [53].

For the adult with increased fracture risk, there has been controversy as to whether calcium and vitamin D in diet and/or supplements actually decrease fracture risk. Part of the problem is that it is exceedingly difficult to design and execute randomized, controlled trials to determine if dietary components affect fracture risk. While there are systematic reviews that support calcium and vitamin D in patients with osteoporosis [54], a recent study [55] of normal populations failed to show that calcium and vitamin D had any

impact on overall fracture risk. Nonetheless, bone is the major source of calcium to maintain the serum calcium, and vitamin D must be adequate to absorb calcium in the gut. Almost all studies of osteoporosis medications have included calcium and vitamin D for both placebo and active drug arms. There is some evidence [38], for example, that patients respond to bisphosphonates better if serum vitamin D levels are 30 ng/ml or greater. In older men [34], lack of protein in the diet appears to be associated with increased hip fracture risk. This finding is consistent with the hypothesis that fracture risk is a component of the sarcopenia noted in many elderly individuals. Consequently, adequate dietary protein, in addition to calcium and vitamin D, is suggested for older men at augmented fracture risk.

Reducing fall risk is very important in older men. Maintenance of muscle strength, improving balance, good vision, and home safety are some of the ways falls can be reduced. Examples of specific techniques include tai chi [56], balance training [57], cataract removal [58], and night lights. From my experience, men avoid walking aids. Convincing a man to use a cane for balance or a walker may be difficult but worthwhile if it decreases fall risk. Reviewing the patient's list of medications is important as well. Any medication that can affect cognition, wakefulness status, or balance should be avoided if possible. Benzodiazepines, hypnotic antihistamines, and powerful antihypertensives potentially raise fall and fracture risk. Men with seizures fall, so good control of seizures is important. Attention must be paid to vitamin D status because some antiepileptic drugs, such as phenytoin and carbamazepine, increase the catabolism of vitamin D [59].

Pharmacologic Treatment of Osteoporosis in Men: Bisphosphonates

Presenting osteoporosis treatment as part of a comprehensive approach to fracture risk reduction takes more time but provides context for the use of medications. In the United States, the first modern bisphosphonate, alendronate, was approved in 1995. Alendronate, risedronate, and zoledronic acid are now widely used in men as approved treatment for osteoporosis. The early studies in men were too small to definitively show fracture risk reduction, but the bisphosphonates raised BMD and affected bone turnover markers similarly in men compared to women [60–62]. A more recent study was designed to use morphometric vertebral fracture risk reduction as the primary outcome. In this 2-year study [63], Boonen and colleagues reported that zoledronic acid administration led to a statistically significant fracture risk reduction with data similar to what has been reported in women. Thus, while there is no large body of randomized, controlled trials showing decreased fracture risk in male patients taking bisphosphonates, there is consistency of the changes in fracture surrogates (DXA, bone turnover markers) with the Boonen study and studies in women. Generic alendronate is inexpensive and generally well tolerated. Thus, it has become the first choice of treatment for most men. The dose is the same in women and men, 70 milligrams (mg) by mouth weekly. Alternatives include oral risedronate 35 mg weekly or 150 mg monthly or intravenous zoledronic acid infusion, with a standard dose of 5 mg yearly. For the patient unable to take oral bisphosphonates because of esophageal dysmotility, Barret's esophagus, GERD (esophageal reflux not under control), or having a large pill burden, the intravenous route is appropriate.

The length of osteoporosis treatment has not been adequately studied in men. Indeed, the only long-term studies involved postmenopausal women taking alendronate [64] or zoledronic acid [65]. A task force [66] empaneled by the American Society for Bone and Mineral Research made recommendations for long-term treatment in postmenopausal women based on these studies. However, they suggested that the approach could be used for men with osteoporosis as well. The approach suggests that after 5 years of oral bisphosphonate treatment or 3 years of intravenous bisphosphonate, the patients should be reassessed. If they remain at high fracture risk or had an osteoporotic fracture before or during treatment, they should be continued on treatment and

reassessed again in 2 years. This is a reasonable approach, and the paucity of data, even in women, prevents stronger recommendations in men. In a recent article [67], this author advocated increasing the intervals between zoledronic acid infusions such that everyone taking bisphosphonates for osteoporosis would be treated for a minimum of 5 years. Assessment by history, physical examination, and DXA should be done at 5 years and every 2–3 years thereafter. The longest treatment study of osteoporosis was 10 years. For the patient still at risk after 10 years of treatment, there is no evidence. One example would be the man on long-term glucocorticoid treatment for an inflammatory condition. The American College of Rheumatology Guideline on Glucocorticoid-Induced Osteoporosis recommends continued treatment for the patient on higher doses of prednisone for as long as it is taken [27].

One minor side effect of oral bisphosphonates is esophageal irritation, which can often be avoided by the patient taking a full glass of water with the alendronate tablet and staying upright or seated for the next half hour. Despite this, some patients will have nonspecific abdominal complaints that are at least temporally related to the bisphosphonates. Such patients are candidates for intravenous zoledronic acid. Two other potential side effects may be very weakly associated with bisphosphonates but have been widely cited, particularly in the lay press: atrial fibrillation and esophageal cancer [68, 69].

There are two serious side effects that have been directly linked to bisphosphonates and probably related to duration of therapy: osteonecrosis of the jaw and atypical femoral fracture (AFF). The reader is directed to chapters covering these side effects as well as pertinent review articles [70–72]. As part of the evaluation prior to bisphosphonate therapy, the patient should be asked about dental status and any invasive procedures that can be done before starting therapy should be done. It is a good idea to look in the patient's mouth for evidence of osteonecrosis of the jaw before and periodically after treatment is started. At this point, there are no definitive methods to predict AFF, but patients of Asian ethnic background, patients with bowed femora, and patients with varus femoral neck to shaft angle may be at higher risk [73, 74]. While on therapy, some patients developing AFF may have a prodrome of groin or thigh pain, which should be investigated with appropriate images [75]. Recently, it has been suggested that patients with hypophosphatasia may be at higher risk for AFF, so a suppressed alkaline phosphatase while on treatment could be a clue [37, 76]. Newer DXA instruments can provide a low radiation dose single energy image of the entire femur. Whether doing this imaging at the time of follow-up DXA will catch early AFF is unknown.

Pharmacologic Treatment of Osteoporosis in Men: Denosumab

Denosumab is a humanized monoclonal antibody directed against RANK Ligand, and it has been shown to increase BMD for up to 10 years in postmenopausal women [77]. In men, the short-term increase in BMD is similar [78]. In men on androgen deprivation therapy for prostate cancer, denosumab has been shown to increase bone density and decrease the incidence of vertebral fractures [79]. Interestingly, denosumab is the only osteoporosis treatment that has been shown to increase BMD in the forearm. While there are no head-to-head fracture trials of denosumab versus bisphosphonates, a study in women on alendronate demonstrated that switching to denosumab increased bone density more than remaining on alendronate [80]. On the other hand, one newly recognized aspect of denosumab therapy, noted in women, should affect consideration of this treatment. When bisphosphonates are discontinued, the terminal half-life of this class of drugs is long, and bone density drops very slowly thereafter. Denosumab is not deposited in bone as are bisphosphonates. With discontinuation of treatment, there is a rapid loss of bone and most troubling are reports of multiple vertebral fractures in women who have recently discontinued denosumab [81, 82]. Thus, men treated with this antibody need to have it administered on time every 6 months. No definitive protocol for discontinua-

tion has been established. Some experts believe that bisphosphonates should be started before the last injection of denosumab. For the man with osteoporosis and limited life expectancy, continuing denosumab indefinitely is reasonable. For the younger man, the rebound fractures upon discontinuation should be discussed prior to choosing this therapy.

Pharmacologic Treatment of Osteoporosis in Men: Anabolic Medications

From a randomized controlled trial [83], there is evidence that teriparatide increases BMD in men, and observational data [84] suggest that fracture risk is reduced. Teriparatide (parathyroid hormone fragment of 34 amino acids), as described elsewhere in this book, improves microarchitecture and builds bone. It is administered as a daily subcutaneous injection using a pen device. In the United States, it is much more expensive than other treatments. While it is less expensive in Europe, it is still orders of magnitude more than oral bisphosphonates. Nonetheless, there is increasing enthusiasm for using anabolic treatment for the first 2 years in patients at the highest risk for osteoporotic fracture. Because bone is lost once teriparatide treatment is discontinued, an antiresorptive agent, usually a bisphosphonate should be started immediately [85]. In an important study in postmenopausal women [86], anabolic treatment for 2 years followed by denosumab treatment for 2 years had a much more robust effect on bone density than denosumab treatment for 2 years followed by 2 years of teriparatide. A conclusion from this study is that high risk patients should not have to "fail" cheaper antiresorptive therapy before starting anabolic treatment. Interestingly, in contrast to the variable results of teriparatide combined with bisphosphonate treatments, teriparatide plus denosumab appeared to have a synergistic effect on BMD when given together over 2 years. Teriparatide continues to have a black box warning because of osteosarcoma seen in a certain strain of rodents who received high dose teriparatide from birth. So far, no osteosarcoma safety signal has been noted in humans on teriparatide [87], but treatment is still limited to 2 years. On the other hand, parathyroid hormone replacement with parathyroid hormone (1-84) in patients with hypoparathyroidism is not limited to 2 years. In any event, teriparatide should be considered for the man with very high fracture risk. It should not be given to men with higher risk of osteosarcoma, such as men with Paget's disease or who have received radiation to bone. Our clinic will also not give it to men with prostate cancer because prostate cancer bony metastases can be osteoblastic, and teriparatide stimulates osteoblasts. In the United States, abaloparatide, a derivative of parathyroid hormone-related protein, has been approved for postmenopausal osteoporosis [88]. A study has been started to determine if it works similarly in men.

Choice of Therapy in Men

There are no head-to-head fracture outcome studies of osteoporosis drugs in men. In glucocorticoid-induced osteoporosis, teriparatide treatment resulted in fewer morphometric and clinical vertebral fractures than alendronate in a 3-year study [89] that included men and women. In a recent study in postmenopausal women, teriparatide treatment resulted in fewer fractures compared to risedronate [90]. In practice, the huge difference in cost often decides the therapeutic choice, and generic oral alendronate is the usual first-line treatment. However, for the man at very high risk for fracture, teriparatide should at least be considered. Whether anabolic treatment first followed by antiresorptive treatment will lead to fewer long-term side effects is unknown.

Conclusions

With increased life expectancy, men require more attention to their osteoporotic fracture risk. After hip fracture, mortality is high, and for those who survive, independence is often lost. Thus, finding men at high fracture risk is important and can be

accomplished with targeted screening. Evaluation is generally straightforward with history, physical examination, a small set of basic laboratory tests, and DXA for the majority of patients. Treatments used in women appear to work equally well in men. Long-term management remains a challenge partly because there are no long-term studies in men. Nonetheless, there is enough evidence to recommend that the man at high risk for osteoporotic fracture be treated and followed regularly.

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Glucocorticoid-Induced Osteoporosis

21

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Key Points

- Glucocorticoid-induced bone loss is the most common secondary cause of osteoporosis.
- Glucocorticoids reduce bone strength more than bone mass
- Prevention of glucocorticoid-induced bone loss should be initiated within a few weeks of glucocorticoid initiation to prevent the loss of bone strength from glucocorticoids
- Treatments to prevent and treat glucocorticoid-induced bone loss are effective and include calcium and vitamin supplementation and either antiresorptive or anabolic agents.

Introduction

Harvey Cushing first described the association between excess endogenous glucocorticoids and fractures in 1932 [1]. By 1954, a few years after the introduction of prednisone to treat rheumatoid arthritis, the deleterious skeletal effects of exogenous glucocorticoids were reported [2]. Glucocorticoid-induced bone loss is now the most common form of secondary osteoporosis, and fractures are glucocorticoids' most common adverse effect [3]. They are among the most common iatrogenic complications of clinical practice as glucocorticoids are used by 0.5–2.5% of adults [4]. Concomitant factors including the underlying disease for which patients are treated, age, baseline bone mineral density (BMD), the hormonal status of the patient, and individual differences in sensitivity to glucocorticoid also play a role in determining whether or not a patient will develop osteoporosis and incident fractures.

Pathogenesis of Glucocorticoid-Induced Bone Loss

Glucocorticoids have both direct and indirect effects on bone and affect both bone formation and resorption. Among the indirect effects, glucocorticoids cause a decrease in intestinal calcium absorption and an increase in the urinary excretion of calcium. Although secondary hyperparathyroidism

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had been thought to play a role in GIOP, elevated parathyroid hormone levels are not consistently found, and histomorphometric analysis of bone biopsies from patients with GIOP reveal decreased bone remodeling rather than the increased remodeling seen with secondary hyperparathyroidism [5]. Glucocorticoids inhibit gonadotopin secretion leading to hypogonadism. Enhanced bone resorption ensues, at least in part, due to enhanced secretion of cytokines such as interleukin-6, tumor necrosis factor alpha, and macrophage-colony stimulating factor (M-CSF) [6].

Bone resorption is coupled with bone formation. A critical system involved in this coupling is the RANK-L (receptor activator of nuclear factor-κB ligand)-RANK-OPG (osteoprotegerin) system. RANK-L is secreted by osteoblasts, then binds to and activates its receptor, RANK, on the surface of osteoclast precursors and induces osteoclastogenesis [7]. OPG is a natural inhibitor of RANK-L, preventing RANK-L from binding to its osteoclast receptor. Glucocorticoids increase the expression of RANK-L and M-CSF [8] and decrease OPG expression in osteoblasts and stromal cells. The consequence of these changes is an initial increase in the number of osteoclasts capable of resorbing bone. Eventually, glucocorticoids deplete the population of osteoblasts as described below, which subsequently leads to decreased RANK-L and M-CSF expression by osteoblasts with a consequent decrease in osteoblast number [9].

However, the most significant mechanism of glucocorticoid-induced bone loss is decreased bone formation. Glucocorticoid exposure leads to a decrease in the activity and lifespan of osteoblasts, both by decreasing osteoblast formation and increasing osteoblast apoptosis [10]. Pluripotent bone marrow stromal cells have the ability to differentiate into a number of cells of the mesenchymal lineage, including either osteoblasts or adipocytes. Glucocorticoids shift the differentiation of pluripotent stromal cells away from osteoblasts toward the adipocyte lineage through regulation of nuclear factors of the CAAT enhancer-binding protein family and by induction of peroxisome proliferator-activated receptor γ2 [11]. Glucocorticoids also suppress canonical Wnt/β-catenin signaling, a key regulator of osteoblastogenesis [12]. The bone morphogenetic protein (BMP) pathway involved in stimulating osteoblast differentiation and bone formation is also suppressed by glucocorticoids [13].

In addition to their effects on osteoblastogenesis, glucocorticoids have effects on bone matrix including the inhibition of type I collagen synthesis and increased collagenase production [14] and on the production of skeletal growth factors through regulation of the transcription of the insulin growth factor I (IGF I) gene and its binding proteins [15].

Osteocytes are thought to participate in the detection and healing of bone microdamage. Accelerated apoptosis of osteocytes could lead to bone microdamage and diminished bone quality and strength independent of BMD [16]. Increased osteocyte apoptosis and necrosis has been documented in patients with GIOP [10].

Another key component in the pathogenesis of glucocorticoid induced osteoporosis, derived from a preclinical animal model, is decreased bone vascularity and blood flow. Reduced blood flow observed in GIOP is mediated by upregulation of endothelin-1, while decreased bone vascularity is mediated by suppression of vascular endothelial growth factor (VEGF) nitric oxide and Hif-1α and an increase in thrombospondin-1 [17–19]. These vascular changes contribute to the decreased bone strength, even preceding changes seen with BMD measurements [20]. This decrease in blood flow was prevented by concurrent administration with PTH or LLP2A-Ale compared to placebo or glucocorticoids alone in a mouse glucocorticoid bone loss model [21].

Glucocorticoid Effects on Bone Mineral Density

Glucocorticoids affect both trabecular and cortical bone mass, however, bone loss is usually most marked in trabecular bone, due to its high surface area and high metabolic activity. Initiation of glucocorticoids results in a rapid decline in bone mineral density (BMD). Subjects treated with

prednisone at 10 mg a day had trabecular BMD decline of 8.2% within 20 weeks, however upon discontinuing the glucocorticoid, there was a 5.2% increase in BMD detected by quantitative computed tomography (QCT), a sensitive measure of trabecular bone density [22]. Also, subjects initiating glucocorticoid therapy showed a −1% change in lumbar spine BMD measured by DEXA at 48 weeks, and −2.92% at 24 months [23, 24]. A number of studies have shown that bone loss is similar in men and women (both postmenopausal and premenopausal women). Fractures are more likely to occur in those with the lowest baseline bone mass, thus most studies demonstrate highest fracture rates in postmenopausal women. Inhaled glucocorticoids can reduce bone mass in subjects that take the medications continuously; however, careful studies have not been performed.

Glucocorticoid Effects on Fracture Risk

Glucocorticoid use increases the risk of both vertebral and nonvertebral fractures. In a study using an administrative claims database in the United States, Steinbuch compared fracture risk in glucocorticoid users to age and sex-matched controls [25]. The adjusted relative risk (RR) among users of glucocorticoids compared to controls was 2.92 for vertebral, 1.68 for nonvertebral, 1.87 for hip, and 1.75 for any fracture. The combined effect of higher dose, longer duration, and continuous pattern of glucocorticoid use further increased RR estimates to seven-fold for hip and 17-fold for vertebral fractures.

Kanis et al. [26] studied the relationship between use of glucocorticoids and fracture risk in a meta-analysis of data from seven cohort studies of 42,000 men and women. Both current and past use of glucocorticoids was an important predictor of fracture risk that was independent of prior fracture and BMD. No significant difference in risk was seen between men and women. For osteoporotic fracture, the range of relative risk was 2.63–1.71 and for hip fracture 4.42–2.48.

The largest study examining the relationship between oral glucocorticoid use and fractures was the United Kingdom General Practice Database (GPRD) study reported by van Staa et al. [27]. This study compared the relative rates of fracture in 244,235 patients receiving oral glucocorticoids to age and sex-matched controls. An average daily dose of prednisolone of 5 mg/day significantly increased the risk of spine and hip fractures. The most important risk factor for fracture in GIOP is the daily, rather than the cumulative, dose of oral glucocorticoids. The risk of nonvertebral fracture increases exponentially for daily doses over 20 mg prednisolone/day [28]. Fracture risk rises within 3 months of starting glucocorticoids and falls after discontinuation within 1 year of stopping therapy. However, this increased risk does not fall back to baseline [27], so that even prior users of glucocorticoids have an increased fracture risk irrespective of BMD [26].

Several epidemiological studies have reported increased risk of lower BMD and fracture in patients using inhaled glucocorticoids [29, 30]. Whether this is due to the glucocorticoids themselves or the underlying disease is controversial. A large cohort study performed by van Staa et al. [30] using the (GPRD) suggests that users of other respiratory medications other than inhaled glucocorticoids also have an increased risk of fracture suggesting that the excess risk appears to be related to the underlying respiratory disease rather than to inhaled glucocorticoid. In a nested case-control study using the Quebec healthcare database, there was no increase in fracture rates among patients receiving three or more respiratory medications, one of which was inhaled glucocorticoids. However, there was a slight increase in risk if the dose was ≥1000 μg of inhaled glucocorticoids for >4 years [31]. A similar study found conflicting results when comparing fracture risk in Tawainese COPD and asthma patients in the National Health Insurance program. The investigators evaluated subjects that were prescribed no inhaled glucocorticoids, low dose (100–250 μg/day), medium dose (250–500 μg/day), or high dose (500 > μg/day). Fracture risk was significantly increased in the medium and

high dose of inhaled glucocorticoids groups compared to the no inhaled glucocorticoids groups [32].

Other concomitant factors that may be associated with bone loss and fractures may include the underlying disease state for which glucocorticoids are given, individual differences in sensitivity to glucocorticoids, and the age and hormonal status of the patient. Although men and women are both susceptible to GIOP, the highest fracture rates are seen in postmenopausal women.

Trabecular Bone Score

Trabecular bone score (TBS) is a recent analysis technique that can be obtained from DEXA scans that estimates 3D components of bone microarchitecture including trabecular number, trabecular separation, and connectivity density. TBS may be a useful tool in the assessment of fracture risk with GIOP. Cross-sectional and retrospective studies of patients receiving oral glucocorticoid therapy, and patients with glucocorticoid releasing adrenal tumors, showed an association between TBS and daily glucocorticoid dose and TBS and 40 month fracture risk [33, 34]. In addition, analysis of women with recent fracture or glucocorticoid use reported lower TBS compared to control subjects, and low TBS was correlated with recent fractures or glucocorticoid use [35]. In addition, Colson reported that compared to control subjects, TBS was lower and BMD of the lumbar spine was not different, in subjects treated with glucocorticoids suggesting that TBS may be a more sensitive measurement of bone health and fracture risk [36]. Additional studies are needed to confirm this observation.

BMD Threshold for Fractures in Glucocorticoid-Induced Osteoporosis

The World Health Organization (WHO) criteria for the densitometric diagnosis of osteoporosis (T-score < −2.5) were developed in 1994 based on the relationship of the prevalence of fractures in postmenopausal Caucasian females to the prevalence of T-scores below a certain level in the same population [37]. The same type of large epidemiological study does not exist for glucocorticoid-treated patients.

To answer the question of whether or not fracture rates occur at a higher bone density or T-score in patients on glucocorticoids, than in postmenopausal women van Staa et al. [38] analyzed the relationship between BMD and vertebral fracture in postmenopausal women taking glucocorticoids. He compared the incidence of fracture in the placebo groups from the risedronate prevention [39] and treatment [40] of osteoporosis trials to the 1-year fracture risk of postmenopausal women not taking glucocorticoids in three other trials. In the BMD threshold analysis, even though the women taking glucocorticoids were younger (64.7 versus 74.1 years old), they had higher mean lumbar T-score (−1.8 vs. –2.6), femoral neck T-score (−1.9 vs. –2.6), and less prevalent fractures (42.9 vs. 58.3%) than the nonglucocorticoid users, the risk of incident fractures was higher in the GC users than the non-GC users[adjusted RR 5.7 (CI 2.57–12.54)]. Thus, fracture incidence was markedly higher in the glucocorticoid users at any given level of BMD.

Diagnosis of GIOP

The fracture risk assessment tool (FRAX) created by WHO in 2008 estimates the 10-year probability of osteoporotic fractures based on the presence of various clinical risk factors, often including femoral neck BMD. Guidelines have been published to help physicians accurately apply FRAX to patients with glucocorticoid therapy. It is an accurate tool for assessing risk when the glucocorticoid dose is between 2.5 and 7.5 mg per day, but may overestimate risk for lower doses and underestimate risk for higher doses. A few shortcomings of FRAX include inability to predict risk with intermittent glucocorticoid therapy and inhaled glucocorticoid use. Glucocorticoid therapy information that is not included in FRAX are past glucocorticoid use, short-term glucocorticoid use, and adrenal failure glucocorticoid replacement therapy [41].

Additional studies showed that FRAX closely predicted fracture risk for glucocorticoid patients regardless of BMD inclusion or gender, but older age decreased FRAX reliability. This observation likely stems from the increased risk of death in patients prescribed glucocorticoids. Overall, FRAX is most accurate for middle doses on glucocorticoids, and less so for high or low dose [42].

Treatment Recommendations

Nonpharmacologic Interventions

The use of systemic glucocorticoids should be minimized whenever possible. Nonpharmacologic interventions such as smoking, alcohol cessation and fall risk assessment should be offered to all patients. Exercise to improve lower extremity strength and balance is particularly important in glucocorticoid-treated patients where myopathy and an increased risk of falls are common. Calcium and vitamin D should be considered necessary but not sufficient for patients receiving chronic glucocorticoids, as they do not reduce fracture risk equal to the degree compared with bisphosphonates. Recommended calcium doses are at least 800–1200 mg/day. Vitamin D should be administered in doses from 600 to 800 IU daily.

A 2-year trial of 65 rheumatoid arthritis patients treated chronically with low-dose prednisone (approximately 5 mg/day) randomized to 1000 mg of calcium carbonate and 500 IU of ergocalciferol versus placebo demonstrated that those given the daily supplements gained 0.7% and 0.9% annually in lumbar spine and greater trochanter bone mineral density (BMD) compared to losses of −2.0 and −0.9% at these sites in the placebo group [43]. A meta-analysis on the effectiveness of treatments for GIOP concluded that calcium plus vitamin D was more effective than no treatment or calcium alone at the lumbar spine [44]. A recent meta-analysis of active vitamin D_3 analogues in GIOP found that they preserve bone density more effectively than no treatment, plain vitamin D_3, and/or calcium [45]. Bisphosphonates, however, were found to be more effective in preserving bone and decreasing the risk of vertebral fractures than active vitamin D_3 analogues.

Bisphosphonates

Bisphosphonates are currently the preferred treatment for GIOP. Most trials have examined their efficacy on BMD as the primary endpoint; however, post hoc analyses consistently support an effect on fracture reduction (mainly in the group at highest risk, postmenopausal women). In the United States, risedronate (5 mg/day) is approved by the Food and Drug Administration (FDA) for the prevention and/or treatment of GIOP and alendronate (5 mg/day for males and premenopausal females and 10 mg/day for postmenopausal females not receiving estrogen therapy) is approved for treatment. Although commonly utilized in clinical practice, neither weekly alendronate nor risedronate have been approved for GIOP. The package labeling for risedronate does state that 35 mg once weekly "may be considered" for prevention of GIOP.

In studies of patients receiving glucocorticoids, oral bisphosphonates that include daily alendronate, daily risedronate, cyclic etidronate, have demonstrated significant increases in BMD at both the hip and spine and reductions in vertebral fracture risk (all compared to calcium and vitamin D alone). In the alendronate GIOP trial reported by Saag, there were too few patients with new vertebral fractures after 1 year, so the fracture reduction was not significant [24]. After 2 years, a significant reduction of 89% in vertebral fractures was demonstrated with alendronate compared to placebo [23]. A pooling of the risedronate prevention and treatment studies also demonstrated a significant 70% reduction in vertebral fractures after 1 year compared to calcium and vitamin D alone [46]. Cyclic etidronate was demonstrated to reduce the risk of new vertebral fractures by 85% over 1 year compared to placebo [47].

A meta-regression analysis [48] comparing the efficacy of therapies used for the treatment of

glucocorticoid-induced osteoporosis determined that bisphosphonates were the most effective class of drugs to preserve vertebral BMD, with an effect size of 1.03 (95% CI, 0.85–1.17) compared to vitamin D (0.46, CI 95%, 0.27–0.62), or calcitonin (0.51, CI 95%, 0.33–0.67) therapy. When combined with vitamin D, the effect size of bisphosphonates further increased to 1.31 (1.07–1.50).

In patients for whom a contraindication to oral bisphosphonates exists, the parenteral bisphosphonates zoledronic acid and pamidronate could be considered. Compared to risedronate, a single infusion of 5 mg zoledronic acid increased BMD and reduced markers of bone resorption for up to 12 months [49]. In a primary prevention study of 32 patients initiating prednisone ≥10 mg/day, patients were randomized to receive either an intravenous infusions of 90 mg pamidronate at baseline or an infusion of 90 mg at baseline followed by 30 mg pamidronate given every 3 months for 1 year or placebo infusions (all groups received calcium carbonate 800 mg/day). BMD changes at the lumbar spine were + 1.7, +2.3, and −4.6% and at the total hip were + 1.0, +2.6, and −2.2% for the three respective groups. The differences between the placebo and both pamidronate groups were statistically significant [50].

Intravenous ibandronate has been studied for glucocorticoid-induced osteoporosis. A total of 115 patients receiving long-term glucocorticoids (average daily dose 10 mg prednisone) were randomized to receive either 500 mg of calcium and 1 μg of alfacalcidiol daily or calcium and infusions of 2 mg intravenous ibandronate every 3 months [51]. At 3 years, BMD was increased 13.3% at the lumbar spine and 5.2% at the femoral neck in the ibandronate group compared to alfacalcidiol group (2.6 and 1.9%, respectively). Although not specifically powered to detect a reduction in fracture risk, the incidence of new vertebral fractures in the alfacalcidiol group (22.8%) was statistically greater than in the ibandronate group (8.6%) a 62% relative risk reduction (p = 0.043). Intravenous ibandronate is not currently approved nor marketed.

Parathyroid Hormone (PTH)

Parathyroid hormone (hPTH 1–34), when administered by daily subcutaneous injection, results in a rapid increase in bone mass, especially in skeletal areas high in trabecular bone [52]. A 12-month trial of females with low bone mass (T-score ≤ −2.5) who were on long-term estrogen and glucocorticoids (mean 8.5 mg of prednisone daily for an average of 13 years) was performed to compare the additive effects of subcutaneous daily human parathyroid hormone (1–34) with placebo [53]. Both groups received 1500 mg calcium and 800 IU vitamin D per day. BMD at the lumbar spine was significantly greater in the combination of PTH and estrogen group (11% increase by dual-energy X-ray absorptiometry and 33% by quantitative CT scan) compared to the estrogen-alone group at 12 months). Increases in femoral neck BMD were significant in the combination group at 24 months. The effect on BMD was sustained for 1 year following the discontinuation of PTH and the continuation of estrogen [54].

In an 18-month long, randomized controlled trial involving 214 women receiving 20 μg of teriparatide and 214 women receiving 10 mg alendronate all of whom received at least 3 months of glucocorticoid therapy before enrolling in the study, women receiving teriparatide showed significantly higher BMD measured at lumbar spine and total hip at 6, 12, and 18 month follow-ups. These women also had lower rates of vertebral fractures, but no difference in overall fractures rates [55].

At the 36 month follow-up on the same cohort, BMD remained significantly increased in the teriparatide group compared to the alendronate group measured at lumbar spine, total hip, and femoral neck; bone markers remained significantly increased compared to baseline in the teriparatide group; and vertebral fractures were significantly lower in the teriparatide group with no change in overall fracture rate [56]. Subjects with very low lumbar spine BMDs, less than −3.0 or with prevalent vertebral fractures may benefit from initial treatment with teriparatide.

However, there are a few contraindications to teriparatide use. These include children or young adults with open epiphyses, patients with Paget's disease, patients with a history of radiation to the skeleton or history of sarcoma. An additional limitation is that treatment with teriparatide is limited to 2 years.

Other Therapies

Other pharmacologic options for the prevention of bone loss include nasal spray calcitonin and estrogen hormone therapy or selective estrogen receptor modulators (SERMs) in women and testosterone in men. Studies of calcitonin in GIOP are limited with conflicting data on its ability to prevent bone loss [57] and no studies demonstrating fracture risk reduction. No studies currently exist examining the role of SERMS in GIOP. A few small studies of estrogen hormone therapy in GIOP have been performed. In one trial of postmenopausal women receiving prednisone for rheumatoid arthritis, those randomized to estrogen hormone therapy had a significant (3.4%) increase in their lumbar spine BMD compared with controls. There was no significant change in femoral neck BMD in either group [58]. Similar small studies of testosterone replacement in GIOP have been performed [59], demonstrating increases in BMD of 5% at 1 year in hypogonadal asthmatic men treated with testosterone compared to controls. Increases in lean body mass (reflecting muscle mass) were also demonstrated in the testosterone treated men.

Due to the increased risks of breast cancer and cardiovascular disease associated with estrogen hormone therapy [60], recommendations for its use as a primary treatment for osteoporosis cannot be made. Similar arguments could be made for testosterone replacement where the long-term adverse effects are unknown [61]. These therapies are most likely to be appropriate in the patient on glucocorticoids where deficiencies of these hormones lead to vasomotor symptoms, loss of libido, etc., and where specific replacement could enhance the patient's quality of life for reasons other than osteoporosis.

Most recently, denosumab, a monoclonal antibody against RANKL, was shown be a viable treatment option for both the prevention and treatment of glucocorticoid-induced bone loss. A multicenter, randomized, double-blind study comparing the effect of denosumab and risedronate on lumbar BMD for patients continuing or initiating glucocorticoid treatment reported denosumab was noninferior and superior to risedronate. The percent change in lumbar BMD was significantly greater for patients treated with 60 mg of subcutaneous denosumab every 6 months for 24 months compared with 5 mg of oral risedronate daily. In addition, markers for bone turnover, CTX and P1NP, were significantly lower at 6 and 12 months after treatment in the group receiving denosumab compared to the risedronate treated group. However, there was no difference in osteoporotic fracture rate between the treatment groups [62]. These results were supported by another randomized, double-blind study evaluating the effects of denosumab on BMD in RA patients [63].

Guidelines for the Prevention and Treatment of GIOP

Based in part on the knowledge that the most rapid loss of bone density is observed upon initiating glucocorticoid therapy, preventive actions are recommended by the numerous professional organizations in which the membership treat the osteoporosis induced by glucocorticoids. Due to the high variability in risk based on age and baseline BMD, and the limited data for younger patients, the American College of Rheumatology recommendations for prevention and treatment vary in accordance with these factors. The one recommendation that is equal across all groups receiving oral prednisone or another glucocorticoid equivalent of ≥2.5 mg/day for ≥3 months is calcium and vitamin D supplementation and lifestyle modification. This is the only preventative treatment for patients of all age groups with low fracture risk. However, for all other groups a combination of supplementation and lifestyle modification with additional pharmacotherapy is

recommended. Individuals in any age group with moderate-to-high risk of fracture should be treated with oral bisphosphonates (Table 21.1). Variations to this regimen include intravenous bisphosphonates for individuals with low compliance, teriparatide in those with contraindications to bisphosphonates, or denosumab if both teriparatide and all forms of bisphosphonates are contraindicated. An additional option is raloxifene, but this is limited to postmenopausal women.

BMD testing should be obtained as soon as possible for patients ≥40 years old receiving glucocorticoid therapy. BMD should be reassessed every 2–3 years if they are treated for GIOP. For patients less than 40 years old, BMD testing is only needed if the patient is at high risk for fractures. BMD does not need to be reassessed for this age group, unless patients are at moderate-to-high risk of fracture risk. However, for all patients, a fracture risk assessment should be done when initiating treatment and every 12 months during treatment [64].

The UK National Osteoporosis Guidelines Group, the International Osteoporosis Foundation, the European Calcified Tissue Society, and the Scottish Intercollegiate Guidelines Network's published frameworks are very similar to the ACR recommendations with minor exceptions. These groups include zolendronic acid as second-line therapy and reserve teriparatide for patients with high risk of vertebral fractures or specific recommendations. Data are sparse for premenopausal women and men under the age of 50, so all guidelines are flexible, with pharmacotherapy recommended when patients have previous fractures or are receiving high doses of glucocorticoids.

None of these guidelines specifically address patients using inhaled glucocorticoids, however, based on the data that patients with chronic lung disease receiving either glucocorticoid or non-glucocorticoid inhalers are at increased risk of fracture [29, 30], measurement of BMD in these patients would seem appropriate with treatment determined by their overall risk factor profile and dose of inhaled glucocorticoids.

Although markers of bone formation and resorption predict fracture risk in chronic glucocorticoid users [65], their clinical utility in GIOP remains investigational.

Table 21.2 provides the author's recommended approach to patients on or beginning glucocorticoid therapy.

Table 21.1 Fracture risk categories

	Adults ≥40 years of age	Adults <40 years of age
High fracture risk	History of osteoporotic fracture T score ≤ −2.5 in postmenopausal women and men GC-adjusted FRAX 10 year risk of major osteoporotic fracture of ≥ 20% and risk of hip fracture ≥ 3%	History of osteoporotic fracture
Moderate fracture risk	GC-adjusted FRAX 10 year risk of major osteoporotic fracture of 10–19% and risk of hip fracture > 1% and < 3%	Z score < −3 or bone loss of ≥ 10% over 1 year with continuing GC treatment of ≥ 7.5 mg/day for ≥ 6 months
Low fracture risk	GC-adjusted FRAX 10 year risk of major osteoporotic fracture of < 10% and risk of hip fracture ≤ 1%	No risk factors

Based on data from Ref. [64]

Table 21.2 Recommendations for the prevention and treatment of GIOP

Minimize dose of systemic glucocorticoids whenever possible
Nonpharmacologic interventions, such as smoking and alcohol cessation, minimization of alcohol intake, fall avoidance strategies, and balance/lower extremity strengthening exercises should be recommended
A daily intake of calcium (1200–1500 mg/day) and serum vitamin D (600–800 IU/day) should be given
Initiate bisphosphonate therapy [risedronate (5 mg/day) or alendronate (5 mg/day in men and premenopausal women or 10 mg/day in postmenopausal women not on estrogen therapy)] in moderate to high risk patients
Consider teriparatide, intravenous bisphosphonates, denosumab, zoledronic acid, or raloxifene if patients have contraindications to, do not tolerate or fail oral bisphosphonate therapy
Monitor clinical fracture risk every 12 months with initial evaluation within 6 months of GC therapy, including FRAX if ≥40 years old Monitor BMD every 2–3 years if ≥40 years old or younger with risk factors

Conclusions

Considerable advances have been made over the past 10 years in the recognition and treatment of GIOP. Antiresorptive therapies for the prevention and treatment of GIOP have advanced such that patients can decide between daily, weekly, monthly, twice a year, or each year dosing. In addition, the anabolic agent teriparatide can both increase bone mass and prevent fractures in GIOP subjects. However, despite the knowledge of the fracture risk associated with glucocorticoids, the availability of effective prophylaxis, and treatment and published guidelines, measurement of bone density and institution of medications to prevent bone loss are suboptimal, including among specialty physicians [66, 67]. Continued education, dissemination of guidelines, and other innovative approaches will be necessary to make a more substantial impact on this disorder.

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Transplantation Osteoporosis

22

Yi Liu and Emily Margaret Stein

Key Points

- Osteoporosis is prevalent among organ transplant candidates. Patients awaiting transplant should be assessed, and treatment initiated if they have osteoporosis.
- Rapid bone loss and fractures commonly occur in the first year after transplant, though rates of bones loss have declined in recent years.
- Bisphosphonates are the most well-studied and consistently effective agents for prevention of bone loss in organ transplant recipients.
- Primary prevention therapy should be initiated immediately after transplantation.
- Long-term transplant recipients should be screened and treated for osteoporosis.

Introduction

The introduction of cyclosporine to transplantation immunology in the early 1980s resulted in marked improvement in short-term graft and patient survival and ushered in a new era for patients with end-stage renal, hepatic, cardiac, pulmonary, and hematopoietic disease. The addition of cyclosporine, and later tacrolimus, to post-transplantation immunosuppression regimens permitted the use of lower doses of glucocorticoids (GCs). Therefore, it was initially expected that glucocorticoid-induced osteoporosis would be less of a problem in the cyclosporine era. During the past two decades, however, it has become clear that, despite reduced GC exposure, organ transplant recipients continue to experience rapid bone loss and fragility fractures [1–4]. Moreover, transplantation-related bone loss and fractures may become increasingly prevalent as more patients are undergoing transplantation each year and survival continues to improve [5]. The epidemiology, natural history, and pathogenesis of bone loss and fracture after various types of organ transplantation will be reviewed. Recommendations for prevention of the acute phase of bone loss after organ transplantation, and treatment of established osteoporosis in organ transplantation candidates and recipients will be summarized.

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Skeletal Effects of Immunosuppressive Drugs

The Bone-Remodeling System

Transplantation osteoporosis, as with most adult metabolic bone diseases, is the result of alterations in the bone remodeling system, an orderly progression of events by which bone cells remove old bone tissue and replace it with new. Thus, it is helpful to review the orderly sequence of events that constitutes normal bone remodeling in order to understand the pathogenesis of transplantation osteoporosis. The two main processes by which remodeling occurs are known as resorption [6] and formation [7]. Conceptually, these processes are somewhat akin to the repair of cracks and potholes that develop in surfaces of highways. Remodeling occurs on the surfaces of both cancellous and cortical bone. The first step is activation of macrophage precursors to form osteoclasts, giant multinucleated cells that excavate or resorb a cavity on the bone surface. Osteoclasts express receptors for receptor activator of NFκB ligand (RANKL) produced by osteoblasts, calcitonin, prostaglandins, calcium, and vitronectin (integrin $\alpha_1\beta_3$). In general, approximately 0.05 mm [3] of bone tissue is resorbed by each osteoclast, leaving small resorption pits on the bone surface called Howship's lacunae. This process takes approximately 2–3 weeks. After a brief rest period known as the reversal phase, local mesenchymal bone marrow stem cells differentiate into osteoblasts that are attracted to the empty resorption pits. There they accumulate as clusters of plump cuboidal cells along the bone surface. Osteoblasts have two major functions. They produce the proteins, both collagenous and non-collagenous, that constitute the matrix of the newly formed bone. Osteoblasts are also responsible for mineralization of the matrix or osteoid, a process that takes place approximately 10 days after the osteoid was synthesized. Osteoblasts express receptors for parathyroid hormone, estrogens, vitamin D_3, cell adhesion molecules (integrins) and several cytokines. The complete remodeling cycle at each remodeling site requires approximately 3–6 months. This process serves to replace old, micro-damaged bone with new and ultimately mechanically stronger bone. RANKL, RANK, and osteoprotogerin (OPG) are three members of the tumor necrosis factor (TNF) ligand and receptor-signaling family that are final effectors of bone resorption [8, 9]. RANKL is expressed in osteoblasts and bone marrow stromal cells. When sufficient concentrations of macrophage colony stimulating factor (mCSF) are present, binding of RANKL to RANK, which is expressed on surfaces of osteoclast lineage cells, through cell-to-cell contact, results in rapid differentiation of osteoclast precursors in bone marrow to mature osteoclasts, increased osteoclast activity, and reduced apoptosis of mature osteoclasts. RANKL is neutralized by binding to OPG, another member of the TNF-receptor superfamily secreted by cells of the osteoblast lineage. Competitive binding of RANKL to either RANK or OPG regulates bone remodeling by increasing (RANK) or decreasing (OPG) osteoclastogenesis. Immunosuppressants exert their effects on bone remodeling by interacting with the RANK/RANKL/OPG system [10].

In normal adults, bone remodeling results in no net change in bone mass. Bone loss develops in any situation in which bone remodeling becomes "uncoupled," such that the rate of resorption exceeds the rate of formation. This most often occurs when the rate of resorption is so elevated that it is beyond the capacity of the osteoblasts to restore the original amount of bone volume. However, bone loss may also develop in the setting of depressed bone formation, such that even normal amounts of resorbed bone cannot be replaced. It is very likely that transplantation-related bone loss results from both a primary decrease in the rate of bone formation and a primary increase in the rate of resorption [1, 11].

Glucocorticoids

Glucocorticoids (GCs), an integral component of most transplant immunosuppression regimens, are notorious for causing osteoporosis. Prednisone or methylprednisolone may be prescribed in high doses (50–100 mg of prednisone

Table 22.1 Glucocorticoid actions that contribute to bone loss

Inhibit bone formation – most important effects that result in a 30% reduction in amount of bone replaced in each remodeling cycle
Reduce osteoblast numbers
Decrease osteoblast replication and differentiation
Shorten osteoblast lifespan
Increase apoptosis of osteoblasts and osteocytes
Inhibit osteoblast function
Reduce synthesis of type I collagen
Decrease synthesis of bone matrix proteins and osteocalcin
Decrease synthesis of IGF-I and inhibit IGF-II receptor expression in osteoblasts – local anabolic regulators that increase Type 1 collagen synthesis
Stimulate bone resorption – effects on resorption are minor and limited to first 6–12 months
Directly increase osteoclast activity
Decrease osteoblast expression of osteoprotegerin (OPG)
Increase osteoblast expression of RANKL
Increase osteoclast maturation and decrease apoptosis
Indirectly increase resorption
Decrease production of gonadal hormones
Decrease intestinal calcium absorption
Increase urinary calcium excretion
Increase secretion of parathyroid hormone

or its equivalent daily) immediately after transplantation and during episodes of severe rejection, with gradual reduction over weeks to months. Total exposure varies with the organ transplanted, the number and management of rejection episodes, and the practice of individual transplantation programs.

GCs cause bone loss and fractures by mechanisms summarized in Table 22.1 and several recent reviews [12–14] and as detailed in Chap. 21. The main effect is an immediate and profound inhibition of bone formation by decreasing osteoblast recruitment and differentiation, synthesis of type I collagen, and induction of apoptosis of osteoblasts and osteocytes both in vitro and in vivo [15].

GCs also increase bone resorption, through both direct effects on osteoclasts and indirect effects. GCs *indirectly* increase resorption by impairing calcium transport across cell membranes, causing reduced intestinal calcium absorption, increased urinary calcium losses and negative calcium balance. Secondary hyperparathyroidism may result, although it is unlikely that it plays a major role in the pathogenesis of associated bone loss when GCs are administered in the absence of calcineurin inhibitors [16]. GCs also cause hypogonadotrophic hypogonadism and reduced secretion of adrenal androgens and estrogens, which may also be associated with increases in bone resorption.

Patients taking GCs generally sustain significant bone loss [13, 14]. Bone loss occurs in all races, at all ages, and in both genders. However, postmenopausal women are at greater risk for fracture than men or premenopausal women, because glucocorticoid-related bone loss is superimposed upon that already sustained because of aging and estrogen deficiency. In general, bone loss is most rapid during the first 12 months and is directly related to dose and duration of therapy. Areas of the skeleton rich in cancellous bone (ribs, vertebrae, and distal ends of long bones) and the cortical rim of the vertebral body are most severely affected and also fracture most frequently.

In recent years, there has been a trend toward more rapid lowering of glucocorticoid doses after transplantation or rejection episodes, and an increase in the use of alternative drugs to treat rejection [17–20]. In more recently transplanted patients who have received lower doses of steroids, significant bone loss persists although it may be less rapid than previously documented [21–24]. Moreover, it should be noted that even rather small doses of GCs are associated with increased fracture risk. A large retrospective general practice database study found that doses of prednisolone as low as 2.5 mg daily were associated with a significant 55% increase in the relative risk of spine fractures; doses between 2.5 and 7.5 mg daily were associated with a 2.6-fold increase in the risk of spine fracture and a 77% increase in the risk of hip fracture [25]. Thus, even in those programs that have embraced the use of lower doses of GCs, there is likely still sufficient exposure in the initial year to cause significant bone loss.

Cyclosporines

Cyclosporine (CsA) is a small fungal cyclic peptide. Its activity depends upon the formation of a heterodimer consisting of cyclosporine and its cycloplasmic receptor, cyclophilin. This cyclosporine–cyclophilin heterodimer then binds to calcineurin [26]. CsA, and similarly tacrolimus, inhibits the phosphatase activity of calcineurin through interaction with distinct domains on the calcineurin subunit [27]. Calcineurin may regulate both osteoblast [28] and osteoclast differentiation [29]. Although the gene for calcineurin, which is integral to the immunosuppressive action of CsA, has been identified in osteoclasts and extracted whole rat bone, it does not appear to be altered by CsA administration [30].

Animal studies suggest that CsA has effects on bone and mineral metabolism that may contribute to bone loss after organ transplantation (Table 22.2) [11, 31]. When administered to rodents in doses higher than those currently used to prevent allograft rejection, CsA causes rapid and severe cancellous bone loss [32, 33], characterized histologically by a marked increase in bone resorption. In contrast to the effects of GCs, bone formation is increased in CsA-treated animals, although insufficiently to compensate for the increase in resorption. The stimulatory effects of CsA on osteoclast formation are likely mediated via T lymphocytes [34–36]. CsA also increases gene expression of osteocalcin and of bone-resorbing cytokines, such as IL-1 and IL-6 [37]. Parathyroid hormone (PTH) may facilitate CsA-induced bone loss [38]. Drugs that inhibit bone resorption, including estrogen, raloxifene, calcitonin, and alendronate, prevent or attenuate CsA-induced bone loss in the rat [39–42]. Similarly, 1,25 dihydroxyvitamin D and prostaglandin E2 also prevent bone loss in CsA-treated rats [43, 44]. In contrast, testosterone does not ameliorate CsA-induced bone loss [45].

Table 22.2 Skeletal effects of cyclosporine (and tacrolimus)[a]

Increase expression of bone resorbing cytokines
Increase expression of osteocalcin
Increase bone resorption
Increase bone formation
Cause rapid, severe cancellous bone loss
Effects mediated by T lymphocytes
PTH may have permissive effect
Bone loss prevented by antiresorptive agents, 1,25 dihydroxyvitamin D

[a]These effects are based primarily on animal studies

Studies examining the effects of CsA on the human skeleton have yielded conflicting results. Several have shown that kidney transplant patients receiving cyclosporine in a steroid-free regimen did not lose bone [46–48]. In contrast, a small study of kidney transplant recipients detected no difference in bone loss between those who received CsA monotherapy and those who received azathioprine and prednisolone [49], and a prospective study found that cumulative CsA dose was associated with bone loss in the 2 years following transplant, independent of the effect of steroids [50].

Tacrolimus (FK506)

FK506 is a macrolide that binds to an immunophilin FK binding protein and blocks T-cell activation in a manner similar to CsA. FK506 has been shown to cause bone loss in the rat model comparable to that observed with CsA [51], and accompanied by similar biochemical and histomorphometric alterations (Table 22.2). In humans, rapid bone loss has been documented after both cardiac [52] and liver transplantation [53], when FK506 is used for immunosuppression. However, other studies suggest that FK506 may cause less bone loss than CsA in humans [54, 55], likely because lower doses of GCs are required for immunosuppression. It remains unclear whether FK506 confers any benefit over cyclosporine with regard to fracture incidence.

Sirolimus (Rapamycin)

Rapamycin is a macrocyclic lactone. Although it is structurally similar to FK506 and binds to the same binding protein, the mechanism by which rapamycin induces immunosuppression is distinct from both FK506 and CsA. When combined with low-dose CsA, rapamycin was bone sparing

in rat studies [56]. In a recent open label study, markers of bone turnover (N-telopeptide and osteocalcin) were lower in kidney transplant recipients who received sirolimus rather than CsA; unfortunately, BMD was not measured, so it remains unclear whether this translated into lower rates of bone loss [57]. However, combining immunosuppressive agents in lower doses may provide hope for achieving adequate immunosuppression while protecting the skeleton.

Azathioprine, Mycophenolate Mofetil, and Other Drugs

Short-term administration of azathioprine is associated with decreases in serum osteocalcin but does not cause bone loss in the rat model [58]. No adverse effects of azathioprine administration alone on bone mass have been reported in human subjects. In the past, azathioprine was frequently used in combination with prednisone and CsA or FK506 to prevent organ rejection. However, it has largely been supplanted by mycophenolate mofetil, which does not have deleterious effects on bone in the rat [59]. The skeletal effects of other immunosuppressant agents are unclear.

Effect of Transplantation on Bone and Mineral Metabolism

Bone Loss Before Transplantation

In many cases, individuals with chronic diseases severe enough to warrant organ transplantation have already sustained considerable bone loss [60–62] (Table 22.3). The majority of candidates for organ transplantation have one or more accepted risk factors for osteoporosis, including debilitation, loss of mobility and physical inactivity, poor nutrition and cachexia. They are commonly exposed to drugs known to cause bone loss, such as GCs, heparin, loop diuretics, excessive doses of thyroid hormone, and anticonvulsants. Postmenopausal women are estrogen deficient, as are many chronically ill premenopausal women. Similarly, men with chronic illness often have hypogonadotrophic hypogonadism [63]. When the disease is present during childhood or adolescence, as is the case with cystic fibrosis or congenital heart disease, peak bone mass, which is attained during adolescence, may be low. Therefore, in caring for organ transplant candidates, it is essential to consider the possibility that bone mass may be reduced before transplantation. Consideration of particular issues related to transplantation of specific organs follows.

Table 22.3 Osteoporosis, fractures, and bone loss in candidates for solid organ transplantation

Type of transplant	Prevalence before transplantation	
	Osteoporosis[a] (%)	Fractures
Kidney[b]	8–49	Vertebral: 3–21% Peripheral: 35%
Heart	4–10	Vertebral: 18–50%
Liver	8–43	Vertebral: 20–25%
Lung	30–35	Vertebral: 14–49%

Based on data from Ref. [2]

[a]Accepted definitions included BMD (by dual X-ray absorptiometry) of the spine and/or hip with Z score ≤ -2 or T score ≤ -2.5

[b]Definition of osteoporosis also included BMD of predominantly cortical sites such as the femoral shaft or proximal radius that are adversely affected by excessive PTH secretion

Kidney and Kidney-Pancreas Transplantation

Skeletal Status Before Transplantation

In patients with severe chronic kidney disease (CKD) or end-stage kidney disease (ESKD), disturbances in calcium and phosphate metabolism, decreased calcitriol synthesis, increased synthesis and secretion of PTH, metabolic acidosis, and defective bone mineralization, result in the complex form of bone disease known as renal osteodystrophy [64], now termed mineral and bone disorders of chronic kidney disease (CKD-MBD). Some form of CKD-MBD is almost universal in patients who undergo kidney transplantation. A given individual may have high bone turnover, due to hyperparathyroidism with or without osteitis fibrosa, low turnover or adynamic bone disease,

osteomalacia, or "mixed" renal osteodystrophy, a combination of one or more of the aforementioned lesions. Type I diabetes, hypogonadism secondary to uremia, and diseases such as systemic lupus erythematosus, common in patients with CKD and ESKD, also adversely affect the skeleton. Several drugs used routinely in the management of patients with renal disease, such as loop diuretics and calcium-containing phosphate binders, can also affect bone and mineral metabolism. In addition, some kidney transplant candidates may have had previous exposure to GCs or CsA as therapy for immune complex nephritis or other diseases and thus may already have sustained significant bone loss prior to transplantation.

Measurement of BMD by dual energy X-ray absorptiometry (DXA) is of limited utility in patients with ESKD as it does not distinguish among the various types of renal osteodystrophy and more importantly, does not discriminate between patients with and without fractures [65]. That being said, several cross-sectional studies have documented that osteoporosis and low bone mass are present in a significant proportion of patients on chronic dialysis (Table 22.3) [66–68].

Not surprisingly, the risk of fracture in patients with ESKD is greatly elevated. Risk of all fractures has been estimated at 4.4–14 times greater than that of the general population [69, 70]. Vertebral fractures were present in 21% of Japanese hemodialysis patients [71]. In one study, 34% of 68 hemodialysis patients had a history of previous fracture [72]. In another prospective study, the incidence of fractures was 0.1 fractures per dialysis year in patients with osteitis fibrosa and 0.2 fractures per dialysis year in patients with adynamic bone disease [73]. Recently, we have appreciated that hip fractures are also twofold more common in patients with moderate-to-severe CKD, who do not yet require dialysis [74] than in those with normal kidney function.

Risk factors for low bone mineral density and fractures include female gender, Caucasian race, hyperparathyroidism, adynamic bone disease, secondary amenorrhea, type I diabetes, older age, duration of dialysis, peripheral vascular disease, prior kidney transplant [75], and diabetic nephropathy [61].

Prevalence of Osteoporosis in Kidney Transplant Recipients

Low BMD measurements have been reported in several cross-sectional studies of patients who have undergone kidney transplantation [2, 3, 76–80] (Table 22.4), although again the prognostic significance of low BMD is unclear in such

Table 22.4 Osteoporosis, fractures, and bone loss after solid organ and bone marrow transplantation

Type of transplant	Prevalence after transplantation		Bone loss: first post-transplant year	Fracture incidence
	Osteoporosis[a] (%)	Fractures		
Kidney[b]	11–56	Vertebral: 3–29% Peripheral: 11–22%	Spine: 4–9% Hip: 8%	Vertebral: 3–10% Peripheral: 10–50%
Heart	25–50	Vertebral: 22–35%	Spine: 2.5–8% Hip: 6–11%	10–36%
Liver	30–46	Vertebral: 29–47%	Spine: 0–24% Hip: 2–4%	Vertebral: 24–65%
Lung	57–73	42%	Spine: 1–5% Hip: 2–5%	18–37%
Bone marrow	4–15	5%	Spine: 2–9% Hip: 6–11%	1–16%

Based on data from Ref. [2]

[a]Accepted definitions included BMD (by dual X-ray absorptiometry) of the spine and/or hip with Z score ≤ -2 or T score ≤ -2.5

[b]Definition of osteoporosis also included BMD of predominantly cortical sites such as the femoral shaft or proximal radius that are adversely affected by excessive PTH secretion

patients. For example, lumbar spine (LS) BMD was below the fracture threshold in 23% of 65 renal transplant recipients studied an average of 4 years after transplantation [81]; female gender, postmenopausal status, and cumulative prednisone dose were independent predictors of low BMD. Similarly, LS BMD was more than two standard deviations below age- and sex-matched controls (Z score ≤ -2.0) in 41% of patients studied 6–195 months after renal transplantation [82], and was directly related to increasing time since transplantation and PTH concentrations. LS and femoral neck (FN) bone density were more than two standard deviations below age- and sex-matched controls in 29% and 11% of 70 kidney transplant recipients studied an average of 8 years after transplantation [83], and was particularly prevalent in women. In a study of male renal transplant recipients, only 17% had normal BMD, 30% had osteoporosis at the hip or LS, 41% including the one-third radius; bone resorption markers were elevated in 48% [84]. Other studies have shown similar results [47, 85, 86].

Mineral Metabolism and Bone Turnover After Kidney Transplantation

The changes in biochemical indices of mineral metabolism and bone turnover after renal transplantation are fairly consistent [87, 88]. PTH levels, usually elevated before transplantation, frequently remain high for some time after transplantation and may never completely normalize [89]. Hypercalcemia and hypophosphatemia, related to persistent parathyroid hyperplasia and elevated PTH levels, occur commonly during the first few months. Persistent elevations in fibroblast growth factor-23 (FGF-23) after transplant have been hypothesized to be related to post-transplant hypophosphatemia [90]. In most patients, these biochemical abnormalities are mild and resolve within the first year. In long-term transplant recipients, persistent elevations in PTH may be associated with reduced hip BMD [89]. Calcitriol production by the transplanted kidney may be inadequate to suppress PTH secretion by hyperplastic parathyroid tissue [91], and treatment with calcitriol may prevent hyperparathyroidism after renal transplantation [92]. Vitamin D deficiency is common and severe in patients after kidney transplantation [93, 94]. In one study [94], the mean serum level of 25-hydroxyvitamin D (25OHD) was 10 ng/ml (25 nmol/L) and one-third of patients had undetectable levels; transplant recipients had significantly lower levels than age-matched controls [94].

Bone Loss After Kidney Transplantation

Prospective longitudinal studies have documented high rates of bone loss after kidney transplantation (Table 22.4), particularly during the first 6–18 months after grafting. Julian et al. were the first to report that LS BMD decreased by 6.8% at 6 months and by 8.8% at 18 months after transplantation [87]. At 18 months, BMD was below the "fracture threshold" in 10 of 17 patients. Several prospective studies have confirmed this pattern of bone loss [21, 95–102], in which the rate of bone loss is greatest during the first 6 months after transplantation and at sites where cancellous bone predominates, such as the LS. The rate of LS bone loss varies between 3 and 10%. There may be a gender difference in the site at which bone is lost [75, 95, 97]; men have been shown to lose more bone at the proximal femur than women in the first few months after transplantation.

The pathogenesis of bone loss after renal transplantation is complex. The majority of studies have found that glucocorticoid dose correlates directly with bone loss. Men and premenopausal women may be at lower, and postmenopausal women at higher risk. There is also some evidence in the literature to support a role for cyclosporine in the pathogenesis of the high turnover state often apparent in renal transplant recipients by 1 year after renal transplantation [103].

In recent years, many centers have stopped using glucocorticoids for immunosuppression in kidney transplant patients after hospital

discharge. These steroid-free regimens may be associated with less bone loss. In one study of patients who did not receive GCs after discharge, spine BMD remained stable and there was a transient 1–2% decrease in BMD at the hip. However, progressive declines occurred at the forearm [104]. Further, high-resolution peripheral CT scans of these patients demonstrated cortical bone loss and a decrease in whole bone stiffness, a surrogate for strength. These findings suggest that even in the absence of glucocorticoids there are ongoing detrimental skeletal effects after renal transplant [104].

Bone Histology After Kidney Transplantation

Before transplantation, classic hyperparathyroid high-turnover lesions are most commonly seen on bone biopsy. However, by 6 months after transplantation, glucocorticoid effects predominate, with osteoblast dysfunction and decreased mineral apposition [87, 105]. In long-term kidney transplant recipients, many of whom had mild renal insufficiency, bone biopsy results were more heterogeneous and included osteoporosis, osteomalacia, and osteitis fibrosa. An increase in osteoblastic activity and mineralization defects were common [106].

Fracture After Kidney Transplantation

Fractures are very common after renal transplantation (Table 22.4), and affect appendicular sites (feet, ankles, long bones, hips) more commonly than axial sites (spine, ribs) [84]. One study determined that nonvertebral fractures are fivefold more common in males aged 25–64, and 18-fold and 34-fold more common in females aged 25–44 and 45–64, respectively, who have had a renal transplant than they are in the normal population [107]. Prevalent vertebral or appendicular fractures were identified in 24% of long-term kidney transplant subjects [78]. Vertebral fractures have been reported in 3–10% of nondiabetic patients after renal transplantation [47, 83]. A cohort study involving 101,039 subjects found that patients who underwent kidney transplant had a 34% greater risk of hip fracture than those who remained on dialysis [61].

Fractures are particularly common in patients who receive kidney or kidney-pancreas transplants for diabetic nephropathy [108–111]. In a retrospective study of 35 kidney-pancreas recipients, approximately half had sustained from one to three symptomatic, nonvertebral fractures by the end of the third post-transplant year [108]. In a nested case-control study, pre-transplant diabetes was associated with a significant increase in fracture after transplantation [112]. This relationship persisted after controlling for several potential confounders, including glucocorticoid use. Although subjects were predominantly kidney transplant recipients, this study also included heart, liver, lung, and heart and lung transplant recipients. Nikkel. et al. performed an analysis of data from the US Renal Data System investigating whether kidney transplant recipients placed on steroid-sparing immunosuppression had lower rates of fracture [113]. They found that fracture rates were 50% lower among patients who did not receive glucocorticoids after hospital discharge.

Cardiac Transplantation

Skeletal Status Before Transplantation

Risk factors common in patients with end-stage cardiac failure that may predispose to bone loss before transplantation include exposure to tobacco, alcohol, and loop diuretics; physical inactivity; hypogonadism; and anorexia that may contribute to dietary calcium deficiency. Hepatic congestion and prerenal azotemia may also affect mineral metabolism, causing mild secondary hyperparathyroidism. Although on average bone density of patients awaiting cardiac transplantation may not differ significantly from normal, it has been observed that approximately 4–10% fulfill World Health Organization criteria for osteoporosis (Table 22.3) [23, 60, 114–117].

Prevalence of Osteoporosis in Heart Transplant Recipients

Osteoporosis and fractures constitute a major cause of morbidity after cardiac transplantation. In cross-sectional studies, the prevalence rate of vertebral fractures in cardiac transplant recipients (Table 22.4) ranges between 18 and 50% and moderate-to-severe bone loss is present in a substantial proportion of subjects at both LS and the femoral neck [107, 114–116, 118–128]. In a cross-sectional study of long-term cardiac transplant recipients, osteopenia or osteoporosis (*T* score less than −1.0) were found in 66% at the femoral neck, and 26% at the LS [129]. Perhaps related to a failure to achieve peak bone mass, adults who receive cardiac transplants as adolescents have significantly lower BMD at LS, FN and one-third radius than age-matched controls [130].

Mineral Metabolism and Bone Turnover After Heart Transplantation

We reported that severe vitamin D deficiency was extremely common among heart and liver transplant recipients at the time of transplantation; 91% of patients had vitamin D insufficiency (25-OHD 20- < 32 ng/ml), 55% had deficiency (25-OHD 10- < 20 ng/ml), and 16% had severe deficiency (25-OHD 10 ng/ml) [131]. Biochemical changes after cardiac transplantation include sustained increases in serum creatinine [132–134] and decreases in 1,25 dihydroxyvitamin D concentrations [133]. Serum testosterone concentrations decrease in men, and may recover by the sixth post-transplant month [132–135]. In one study, testosterone levels were lowest in the first month following transplant, and reflected suppression of the hypothalamic pituitary gonadal axis by prednisone as well as peri-operative stressors [135]. Low total testosterone was also common at 1 and 2 years after transplantation. At these later time points, low testosterone may result from primary gonadal failure [135]. Serum osteocalcin falls precipitously and there is a sharp increase in markers of bone resorption (hydroxyproline and pyridinium crosslink excretion) during the first 3 months with return to baseline levels by the sixth month [132–134]. This biochemical pattern coincides with the period of most rapid bone loss and highest fracture incidence and suggests that the early post-transplant period is associated with uncoupling of formation from resorption, and restitution of coupling when glucocorticoid doses are lowered. There is also evidence for a high bone turnover state later in the post-transplant course perhaps due to cyclosporine, characterized by elevations in both serum osteocalcin and urinary excretion of resorption markers [116, 119, 120, 126, 127, 132, 134, 136]. The increased bone turnover may be due in part to secondary hyperparathyroidism related to renal impairment [120]. Thus, biochemical changes later in the post-transplant course may be mediated, at least in part, by cyclosporine A-induced renal insufficiency, although other etiologies cannot be excluded.

Bone Loss After Heart Transplantation

The pattern of bone loss after cardiac transplantation is similar to that observed after renal transplant. Prospective longitudinal studies have documented rates of bone loss ranging from 2.5% to 11%, predominantly during the first 3–12 months after transplantation (Table 22.4) [52, 133, 134, 136–141]. Although GCs affect the predominantly cancellous bone of the vertebrae to a greater extent than other sites, there is as much or more bone loss at the hip, a site with more cortical bone than the vertebral bodies [23, 133]. Moreover, while bone loss at the LS slows or stops after the first 6 months, femoral neck bone loss continues during the second half of the first year after transplantation [23, 133]. There are very few longitudinal data available on the pattern of bone loss after the first year. However, data suggest that the rate of bone loss slows or stops in the majority of patients, with some recovery at the LS noted during the third year of observation [23, 133]. Bone loss also slows at the hip after the first year; however, in contrast to the spine, there has been no significant recovery by the fourth post-transplant year. The results of a recent study suggest that there may be less bone loss than suggested in literature from the 1980s and early 1990s [23].

Fracture After Heart Transplantation

Fragility fractures are most common during the phase of rapid bone loss that characterizes the first post-transplant year (Table 22.3). In a prospective observational longitudinal study, 36% of patients (54% of the women and 29% of the men) suffered one or more fractures of the vertebrae, ribs, and hip in the first year despite daily supplementation with calcium (1000 mg) and vitamin D (400 IU) [142]. The mean time to first fracture was 4 months, with most patients sustaining their initial fracture during the first 6 months. Lower pre-transplant BMD and female gender were associated with a trend toward increased fracture risk. In men, however, it was the rate of bone loss after transplantation rather than the pre-transplant bone density that was associated with fracture risk. Many of the patients that fractured had normal pre-transplant BMD and thus it was not possible to predict who would fracture on the basis of pre-transplant BMD or any other demographic or biochemical parameter [142]. Two European studies of cardiac transplant recipients reported similar fracture incidence with approximately 30% to 33% sustaining vertebral fractures during the first 3 years [143]. The risk of a vertebral fracture was higher in those patients who had LS *T* scores below −1.0 (hazard ratio 3.1) [143].

In a more recent interventional study, the incidence of vertebral fractures during the first post-transplant year in patients who received only calcium and vitamin D was only 14% [23]. Similarly, in a prospective study of untreated patients only 12% had fractures [144], suggesting fracture rates may be lower than in the past. However, clinical experience suggests that fractures remain a very common and sometimes devastating complication of heart transplantation. A complete bone evaluation including BMD measurements before or immediately after transplantation, as well as aggressive intervention to prevent bone loss and fractures should be considered in all patients regardless of age, sex, or pre-transplant bone density.

Liver Transplantation

Skeletal Status Before Transplantation

Patients with liver failure have multiple risk factors that may predispose to low bone mineral density before transplantation and fracture after transplantation [145–147]. Many patients with end-stage liver disease who are listed for liver transplantation have prevalent osteoporosis (Table 22.3), as evidenced by low bone mineral density (BMD) and fragility fractures [148, 149]. Osteoporosis and abnormal mineral metabolism have been described in association with alcoholic liver disease, hemochromatosis, steroid-treated autoimmune chronic active hepatitis, post-necrotic cirrhosis, and particularly in chronic cholestatic liver diseases such as biliary cirrhosis [150–153]. A study of 58 patients with cirrhotic end-stage liver disease referred for liver transplantation [149], reported that 43% had osteoporosis (defined as *Z* score > 2 S.D. below age-matched controls or presence of vertebral fractures). Serum 25-OHD, $1,25(OH)_2D$, intact PTH, and osteocalcin (a marker of bone formation) were lower and urinary hydroxyproline excretion (a marker of bone resorption) was higher in cirrhotic patients than controls. Male patients had lower serum testosterone levels than controls. A study of 56 liver transplant recipients revealed that 23% had osteoporosis that antedated transplantation [154]. In a recent study of 360 liver transplant candidates, 38% had osteoporosis and 39% had osteopenia [155].

Histomorphometric studies have found that bone formation is decreased in patients with primary biliary cirrhosis, and reflected by low serum osteocalcin levels [156–158]. Another study found biochemical evidence of both decreased bone formation and increased bone resorption in patients with chronic liver disease [148]. However, while serum osteocalcin appears to be a valid marker of bone formation in cholestatic liver disease, the utility of collagen-related markers of bone turnover has recently been called into question. In fibrotic liver diseases, the synthesis of type I collagen is markedly increased. Guanabens et al. have found that collagen-related bone turnover markers appear influenced by liver,

rather than bone, collagen metabolism and do not reflect skeletal turnover in patients with liver disease [156]. Serum osteocalcin and tartrate-resistant acid phosphatase (TRAP) may be more valid markers of bone remodeling activity in this clinical situation.

Mineral Metabolism and Bone Turnover After Liver Transplantation

Studies of calciotropic hormone levels and bone turnover markers after liver transplantation are limited. Compston et al. reported a significant rise in serum-intact PTH during the first 3 months after liver transplantation, although levels did not exceed the upper limit of the normal range [159]. Significant increases in PTH during the first 3–6 months after transplant have been observed by other authors as well [160, 161]. In contrast, intact PTH levels have been reported to be within the normal range in liver transplant recipients in other studies [162–164]. Our study that investigated 25-OHD at the time of transplantation found that liver transplant recipients had significantly lower vitamin D levels than heart transplant recipients. This finding likely relates to disease-specific factors such as malabsorption, and impaired hepatic 25-hydroxylation of vitamin D [131]. Moreover, reduced hepatic production of vitamin D binding protein may lead to an apparent decrease in total (bound and free) serum 25-OHD, but free levels may be normal.

With respect to bone turnover, markers of bone formation (osteocalcin and carboxyterminal peptide of type I collagen) and resorption are higher in liver transplant recipients than in normal controls in most [163–166], though not all, studies [167]. OPG and RANK-L levels are significantly elevated in the first 2 weeks following liver transplant [168]. The balance of the data thus suggests that low bone turnover observed in many patients with liver failure converts to a high turnover state that persists indefinitely after liver transplantation.

As is the case with renal and cardiac transplantation, the independent role of GCs and calcineurin inhibitors in the pathogenesis of bone disease in liver transplant patients is difficult to assess since single drug therapy is uncommon. The mechanism of bone loss after liver transplantation has been studied by transiliac crest bone biopsy after tetracycline labeling in 21 patients, evaluated before and 3 months after transplantation. Before transplantation, a low turnover state was observed, with decreased wall width and erosion depth. Postoperative biopsies showed high turnover with increased formation rates and activation frequency, and a trend toward increased indices of resorption [169], which may have been related to the concomitant increase in PTH concentrations [159] or alternatively to calcineurin inhibitors.

Bone Loss and Fracture After Liver Transplantation

Osteoporosis is also common after liver transplantation, as detailed in several recent reviews [62, 170]. The natural history of bone loss following liver and cardiac transplantation appears similar [143]. Rates of bone loss and fracture vary considerably after liver transplantation (Table 22.3), but were often extremely high, particularly in studies published before 1995 [143, 154, 162, 163, 171–176], in which LS BMD fell by 2–24%, primarily in the initial few months after liver transplantation. Bone loss appears to stop after 3–6 months with gradual improvement by the second and third post-transplant years. Eastell et al. reported that bone mass recovers and bone histology normalizes with increasing survival time after transplantation [171], and other investigators have shown that there is improvement in BMD in long-term liver transplant recipients [177]. This, however, has not been a uniform finding and other studies have found continued losses rather than recovery [162, 178].

More recent studies have found smaller amounts of bone loss. Keogh et al. reported that femoral neck BMD fell by 8% and LS BMD by 2% after liver transplantation [179]. Ninkovic et al. found only a 2.3% loss at the femoral neck, with preservation of LS BMD 1 year after liver transplant [22]. Floreani et al. found increases in BMD at 1 year [160]. Smallwood et al. reported

in a cross-sectional study that lower bone mass following liver transplant was associated with older age, female gender, cholestatic liver disease, and higher prednisone dose [180]. A recent retrospective study found that women receiving cumulative glucocorticoid doses greater than 3500 mg had lower FN BMD at one and 2 years following liver transplant than other patients [181]. Guichelaar et al. followed 360 patients after liver transplant. Higher rates of LS bone loss occurred in patients with primary sclerosing cholangitis, current smokers, younger age, higher baseline BMD, shorter duration of liver disease, and ongoing cholestasis [155].

Fracture incidence is also highest in the first year and ranges from 24% to 65%, the latter in a group of women with primary biliary cirrhosis. The vertebrae and ribs are the most common fracture sites. Again, fracture rates appear to be considerably lower in more recent studies [22]. Whether type of liver disease at baseline predicts fractures is controversial. Some authors report more bone loss and fractures in patients with primary sclerosing cholangitis [155] and alcoholic cirrhosis [182]. Glucocorticoid exposure and markers of bone turnover do not reliably predict bone loss or fracture risk. Older age and pre-transplant BMD at the FN and LS were predictive of post-transplant fractures in recent prospective studies [22, 161]. Vertebral fractures prior to transplant have been shown to predict post-transplant vertebral fractures [143, 183]. In re-transplanted patients, those with primary biliary cirrhosis and those with previous fragility fractures are at increased risk. These patients may always be at risk for fractures as survival rates and duration increase. In a recent study of patients who survived more than 15 years after liver transplantation, 49% had osteoporosis and 30% had sustained vertebral fractures [184].

Lung Transplantation

Skeletal Status Before Lung Transplantation

Hypoxemia, tobacco use, and prior glucocorticoid therapy are frequent attributes of candidates for lung transplantation and may contribute to the pre-transplant bone loss (Table 22.3) particularly common in these patients [185, 186]. Cystic fibrosis (CF), a common reason for lung transplantation, is itself associated with osteoporosis and fractures due to pancreatic insufficiency, vitamin D deficiency and calcium malabsorption, and hypogonadism [187–189]. A greatly increased rate of all fractures and severe kyphosis has been reported in adults with cystic fibrosis [187]. Vitamin D deficiency is extremely common in CF patients, despite supplementation; bone density was significantly lower in D-deficient patients [188]. Two cross-sectional studies have found that low bone mass and osteoporosis are present in 45–75% of candidates for lung transplantation [185, 186]. In both, glucocorticoid exposure was inversely related to BMD. Vertebral fracture prevalence was 29% in patients with emphysema and 25% in patients with CF [185, 186]. Low bone mass is also common in patients with primary pulmonary hypertension prior to lung transplantation; in a retrospective study, 61% had osteopenia at the FN and 72% at the LS. BMD at the FN correlated with functional measures, walking distance, and pulmonary vascular resistance [190]. A cross-sectional study of patients with diffuse parenchymal lung disease presenting for lung transplantation found that 13% had osteoporosis, and 57% osteopenia. Low BMD was associated with lower body mass index, and Hispanic ethnicity [191].

Mineral Metabolism and Bone Turnover After Lung Transplantation

Bone turnover markers are elevated following lung transplant. Increased osteoclastic and decreased osteoblastic activity have been observed in post-transplant bone biopsies of CF patients [192].

Bone Loss and Fracture After Lung Transplantation

Few studies have prospectively evaluated patients after lung transplantation (Table 22.3). A study of 12 patients demonstrated an average 4% decrease in LS BMD during the first 6 months despite calcium

and 400 IU of vitamin D [193]. Two men sustained multiple vertebral fractures. Another study documented decreases of approximately 5% in both LS and femoral neck BMD during the first 6–12 months after lung transplantation and fractures developed in 18% of 28 patients [194]. In a retrospective analysis of 33 lung transplant recipients who had survived at least 1 year after grafting, BMD was markedly decreased and 42% had vertebral fractures [195]. In a 10-year follow-up study of lung transplant recipients, of the 28 (29%) of patients who survived, 11% had prevalent osteoporotic fractures [196]. As many as 37% of lung transplant recipients suffer fragility fractures and significant bone loss during the first post-transplant year despite antiresorptive therapy [197].

Risk factors for fracture and bone loss include female gender, low pre-transplant LS BMD, pre-transplant glucocorticoid therapy, and higher bone turnover after transplantation. Some studies have found that bone loss correlates with GC dose [194], but others have not found this relationship [197].

Bone Marrow Transplantation (BMT)

BMT is performed with increasing frequency and for expanding indications. In preparation for transplantation, patients receive myeloablative therapy (alkylating agents and/or total body irradiation) and commonly develop profound and frequently permanent hypogonadism, which could certainly cause bone loss.

Mineral Metabolism and Bone Turnover After Bone Marrow Transplantation

Bone turnover markers are consistent with the pattern of decreased formation and increased resorption [198] observed in other forms of transplantation during the first 3 months, a pattern consistent with uncoupling of formation from resorption. After 3 months, there was recovery of bone formation markers and generally elevated turnover during the latter half of the year [198]. Similar elevations of bone turnover markers have also been observed by other investigators after BMT [199–201] [202, 203]. Rates of FN bone loss are lower after autologous BMT, about 4%. LS BMD returns to baseline, while FN bone loss persists for 2 years [204].

Cellular or cytokine-mediated abnormalities in bone marrow function after BMT may affect bone turnover and BMD [205]. Osteoblastic differentiation is reduced by damage from high-dose chemotherapy, total body irradiation and treatment with GCs and/or CsA. Colony forming units-fibroblasts (CFU-f) are reduced for up to 12 years following BMT [206, 207]. Long-term survivors have been shown to have persistent abnormalities in bone turnover and vitamin D [208].

Avascular necrosis (AVN) is common, occurring in 10–20% of allo-BMT survivors, at a median of 12 months following transplant [207, 209]. The most important risk factor for the development of avascular necrosis is GC treatment of chronic GVHD. AVN may be related to decreased numbers of bone marrow CFU-f in vitro, but does not appear to be related to BMD [210].

Bone Loss and Fracture After Bone Marrow Transplantation

After transplantation, patients may receive GCs, methotrexate, or cyclosporine A, alone or in combination. The pathogenesis of osteoporosis after allogenic BMT is complex, related to many factors including the effects of treatment and effects on the stromal cell compartment of the bone marrow [77, 202, 206]. Low BMD was first reported after BMT by Kelly et al. [211]. Since then, several cross-sectional studies have confirmed low total body BMD [212, 213] or bone mineral content (BMC) [214] (by DXA) and LS volumetric BMD (by computed tomography) [200] in bone marrow transplant recipients (Table 22.4). However, in one study, only those who were less than 18 years old at the time of transplantation were affected, perhaps because of a failure to achieve optimal bone mass and smaller bone size [212]. Two studies have documented that bone mass is low in hypogonadal women after bone marrrow transplantation [215, 216] and that hormone replacement therapy is associated with significant increases in BMD [215, 216].

With respect to natural history of bone loss after BMT, a study of 9 adults undergoing 6 months of high-dose glucocorticoid and CsA therapy for graft-versus-host disease (GVHD) observed significant LS bone loss [217]. Ebeling et al. found that low BMD antedates BMT, particularly in subjects with prior glucocorticoid exposure and that post-transplant bone loss is particularly severe in patients who undergo allogeneic BMT, probably because of their increased propensity for GVHD [209]. Another study followed a group of patients who had undergone allogeneic BMT for 6 months (n = 44) and 12 months (n = 36) after grafting. Although some received calcium and vitamin D and some received calcitonin, there was no discernable difference in rates of bone loss; therefore, the groups were combined. BMD decreased by approximately 6% at the LS and 7% at the FN [198]. In other studies, LS BMD decreased by 2.2–3.0% and FN BMD by 6.2–11.6% during the first 12–14 months [203, 218]. There appears to be little bone loss after the first year [200]. The significant bone loss that occurs in the femoral neck does not appear to be regained [219]. In a recent retrospective study, risk of fracture incidence was up to 9 times higher in bone marrow transplant recipients compared with an age- and sex-matched reference population [220].

Evaluation and Management of Candidates for Transplantation

Evaluation

There are now abundant data documenting the high prevalence of bone disease in candidates for all types of transplantation. Therefore, the possibility of significant bone disease should be considered before transplantation so that potentially treatable abnormalities of bone and mineral metabolism may be addressed and the skeletal condition of the patient optimized before transplantation (Table 22.5). Risk factors for osteoporosis should be assessed. These include a family history of osteoporosis, history of adult low-trauma fractures, medical conditions (thyrotoxicosis, renal disease, rheumatological, and intestinal disorders), unhealthy lifestyle choices (physical inactivity, dietary calcium and vitamin D deficiency, excessive caffeine and alcohol intake, tobacco use) and exposure to certain drugs (diphenylhydantoin, lithium, loop diuretics, glucocorticoids, prolonged, and large doses of heparin, thyroid hormone). In men, it is important to exclude hypogonadism. A physical examination should focus upon findings that suggest hypogonadism, thyrotoxicosis, and Cushing's syndrome. Risk factors for falling (poor impaired vision, hearing, balance and muscle strength, psychotropic drugs) should also be assessed.

Table 22.5 Skeletal evaluation of the candidate for organ transplantation

In all candidates:
Assess risk factors for osteoporosis, including menstrual history, history of low-trauma fractures.
Measure bone densitometry (BMD) of spine and hip by DXA
Obtain thoracic and lumbar spine radiographs
If BMD testing reveals osteoporosis or if there are prevalent vertebral or nonvertebral fractures:
Serum electrolytes, BUN creatinine, calcium, parathyroid hormone, 25-hydyroxyvitamin D, thyroid function tests (see text)
In men, serum total and/or testosterone, with follow-up FSH, and LH if testosterone is low
Urine for calcium and creatinine

BMD of the spine and hip is the most important test to obtain before transplantation. Radiographs of the thoracic and lumbar spine are also important since risk of future fracture is greater in patients with prevalent vertebral fractures. A battery of biochemical tests is unnecessary if the BMD is normal and supplementation with calcium and vitamin D is planned. However, if the pre-transplant BMD is low, a thorough biochemical evaluation can alert the physician to the etiology of low bone mass and guide appropriate therapy, targeted to the cause. In such instances, the biochemical evaluation should include a chemistry panel (serum electrolytes, creatinine, calcium, phosphorus, alkaline phosphatase), thyroid function tests, intact PTH, and serum 25-OHD. In men, total and free testosterone should be obtained. Markers of bone formation (serum osteocalcin, bone specific alkaline phosphatase and procollagen type 1

amino-terminal propeptide (P1NP), and resorption (C- or N-telopeptide excretion) can also be measured to assess bone turnover status, although this is optional.

Although pre-transplant BMD does not reliably predict fracture in individual patients, low pre-transplant BMD probably increases fracture risk. Individuals awaiting transplantation who meet World Health Organization criteria for diagnosis of osteoporosis (*T* Score < −2.5), osteopenia or low bone mass (*T* score between −1.0 and −2.5) should be evaluated and treated similarly to others with, or at risk, for osteoporosis (Table 22.5).

While on the waiting list for transplantation, rehabilitation therapy should be prescribed as tolerated to maximize conditioning and physical fitness. All transplant candidates should receive the Recommended Daily Allowance of vitamin D (600–800 IU), or as necessary to maintain the serum 25-OHD level above 30 ng/ml (80 nmol/mL) and a daily calcium intake of 1000–1200 mg (depending on menopausal status). Patients should be encouraged to obtain as much of their calcium from diet as possible. Calcium citrate is preferred as a supplement. Many of these patients take proton pump inhibitors before or after transplantation, which can reduce intestinal calcium absorption. Hypogonadal men should also be offered testosterone replacement. Generally accepted guidelines for gonadal hormone replacement should apply to these patients.

Patients who are found to have osteoporosis before transplantation should begin antiresorptive therapy with a bisphosphonate. The pre-transplant waiting period is often long enough (1–2 years) for significant improvement in BMD before transplantation. Patients with CKD-MBD should be managed in accordance with accepted clinical guidelines [221]. A discussion of this topic is beyond the scope of this chapter.

After transplantation, monitoring serum and urine indices of mineral metabolism is less crucial, although it may be useful to detect developing conditions that may contribute to bone loss (vitamin D deficiency or renal insufficiency with secondary hyperparathyroidism. Serum (and urinary) calcium must be monitored frequently if pharmacologic doses of vitamin D or its active 1-hydroxylated metabolites are used, in order to detect hypercalciuria or hypercalcemia. Measurement of BMD should be performed annually for the first 2 years, particularly if the patient remains on GCs. Frequency of follow-up BMD measurement should be based upon whether the patient continues to require GCs and if they are using anti-osteoporotic therapy. Bone biopsy may be necessary in the kidney transplant recipient since many experts remain reluctant to use bisphosphonates in patients with adynamic bone disease. Although transiliac crest bone biopsy remains a research tool, more histomorphometric studies would be very helpful in confirming theories of the pathogenesis of transplantation osteoporosis.

Prevention of Transplantation Osteoporosis

The major principles, which have been demonstrated consistently after kidney, liver, heart, lung, and bone marrow transplantation, and which should guide therapy of transplantation osteoporosis are as follows:

- Rates of bone loss are most rapid immediately after transplantation.
- Fractures also occur very early after transplantation, sometimes within only a few weeks of grafting.
- Fragility fractures develop both in patients with low and those with normal pre-transplant BMD.
- Prevention of the rapid bone loss that during the first few months after transplantation is likely to be considerably more effective in reducing the morbidity from fractures than waiting for fractures to occur before initiating therapy.
- Therefore, preventive strategies should be instituted immediately after transplantation both in patients with normal pre-transplant BMD and those with low BMD who are being treated with glucocorticoids (Table 22.6).

Table 22.6 Primary prevention of bone loss in transplant recipients

Measure BMD before or immediately after transplantation and annually for 2 years
Consider pharmacologic therapy in all patients with low bone mass (*T* score between −1.0 and − 2.5) or osteoporosis (*T* score < −2.5)
Endeavor to use the lowest dose of glucocorticoids possible
Consider alternative therapies for rejection
Calcium intake of 1200 mg/d both before and after transplantation
Vitamin D intake of 600–1000 IU, or as needed to maintain serum 25-OHD concentrations above 30 ng/ml (80 nmol/ml)
Physical rehabilitation program both before and after transplantation
Replace gonadal steroids (in men and hypogonadal premenopausal women)
Begin antiresorptive therapy, preferably a bisphosphonate, before transplantation in patients with antecedent osteoporosis or low bone mass
Begin antiresorptive therapy, preferably a bisphosphonate, immediately after transplantation in patients with normal or low bone mass and continue for at least the first year

Table 22.7 Management of the long-term organ transplant recipient

In all patients:
Assess risk factors for osteoporosis
BMD of spine and hip by DXA
Thoracic and lumbar spine radiographs
Calcium intake of 1200 mg/d both before and after transplantation
Vitamin D intake of 600–1000 IU, or as needed to maintain serum 25-OHD concentrations above 30 ng/ml (80 nmol/ml)
Physical rehabilitation program
If BMD testing reveals osteoporosis or there are prevalent vertebral fractures:
Serum electrolytes, BUN creatinine, calcium, parathyroid hormone, 25-hydroxyvitamin D, thyroid function tests
In men, serum total and/or testosterone, with follow-up FSH, and LH if testosterone is low
Urine for calcium and creatinine
Replace gonadal steroids (in hypogonadal men and women, if appropriate)
Begin antiresorptive therapy, preferably a bisphosphonate*

*These recommendations should not be applied to kidney transplant recipients in whom the risk of the adynamic bone lesion is high and benefits of bisphosphonates are controversial

- The long-term transplant recipient with established osteoporosis and/or fractures should not be neglected (Table 22.7).

There are several prospective controlled randomized studies for prevention and treatment of transplantation osteoporosis in the literature, although the quality of these studies varies. The recommendations provided herein are also based upon experience with glucocorticoid-induced osteoporosis and recent guidelines from the American College of Rheumatology [222]. Available therapies of transplantation osteoporosis include antiresorptive drugs (bisphosphonates and denosumab), as well as analogs of vitamin D and gonadal hormone replacement. Since resorption markers increase after transplantation and correlate directly with rates of bone loss, [88] attempts to prevent post-transplantation bone loss, and hopefully fractures, by inhibition of bone resorption are a logical approach.

Bisphosphonates

Bisphosphonates act by inhibiting osteoclastic bone resorption. This class of drugs is most commonly used to treat osteoporosis in postmenopausal women and men. However, they have also been used successfully both to prevent and to prevent glucocorticoid-induced bone loss and bone loss in transplant recipients. Alendronate, risedronate, and zoledronic acid have been approved by the FDA for prevention and treatment of GC-induced osteoporosis. Since transplantation osteoporosis can be considered one form of glucocorticoid-induced osteoporosis and since cyclosporine and tacrolimus-induced bone loss are characterized experimentally by increases in both formation and resorption, bisphosphonates offer considerable hope for prevention of transplantation osteoporosis.

Several [164, 223–235] studies suggest that intravenous bisphosphonates can prevent bone loss and fractures after transplantation. Intravenous pamidronate administered in repeated doses has been shown to prevent bone loss at the LS and FN in kidney, [224, 235] heart, [228, 233] liver, [236] and lung [227, 232] transplant recipients. In a small, open but randomized clinical trial, intravenous pamidronate was administered to kidney transplant recipients at time of grafting and again 1 month later, [223] completely preventing LS and FN bone loss. In contrast, LS BMD fell by 6.4% and FN BMD by 9% in the control subjects. The benefits of this intervention were still apparent 4 years after transplantation, especially at the FN [224]. Coco et al. [235] compared kidney transplant recipients who received intravenous pamidronate at the time of transplantation and at 1, 2, 3, and 6 months afterward, along with calcium and calcitriol, to those treated with calcium and calcitriol alone. There was no bone loss in the patients who received pamidronate, while the other group sustained losses of 4–6%. Bone biopsies performed in a small number of patients after 6 months of therapy, however, revealed a high incidence of adynamic bone disease. Aris et al., in a randomized, controlled but nonblinded trial, demonstrated that intravenous pamidronate (30 mg every 3 months for 2 years) was associated with 8% increases in spine and hip BMD in patients who underwent lung transplantation for cystic fibrosis [227]. However, fracture rates were very high and did not differ between the two treatment groups. A retrospective study suggested that treatment with intravenous pamidronate before and every 3 months after liver transplantation prevented symptomatic vertebral fractures in liver transplant recipients who had osteoporosis before transplantation [237]. In contrast, a more recent prospective study in liver transplant patients found that bone loss at the FN was not prevented with pamidronate, which was given as a single infusion as long as 3 months before grafting. There was no LS bone loss in either group and fracture rates did not differ [236]. In two large prospective studies of patients after allogenic BMT, intravenous pamidronate prevented LS bone loss and reduced proximal femoral bone loss [238, 239]. About 3% of bone loss at the proximal femur still occurred, however, despite doses up to 90 mg one study [239]. The lack of efficacy may be related to a failure of pamidronate to inhibit matrix metalloproteinase (MMP)–mediated bone resorption or to reverse defects in osteoblast function after BMT [240].

Randomized trials with the more potent intravenous bisphosphonates, zoledronic acid and ibandronate, have shown significant protective effects on BMD at 6 and 12 months in recipients of heart, [241] liver, [229, 242, 243] and kidney [230, 234] transplants. Fahrleitner-Pammer et al. reported that in male heart transplant patients, ibandronate prevented bone loss and reduced the risk of vertebral fractures [241]. Crawford et al. administered repeated doses of zoledronic acid before and at 1, 3, and 6 months following liver transplantation, which prevented bone loss at the LS, FN, and total hip (TH), compared with placebo. One year after transplantation, the effects at the FN and TH persisted, but an increase in LS BMD in the placebo group abolished the significant difference at the spine [242]. Bodingbauer et al. investigated 4 mg of zoledronic acid given to a group of liver transplant patients monthly for the first 6 months and then at 9 and 12 monthly after transplantation. With treatment, BMD was stable at the LS and losses were reduced at the FN compared to controls. There was also a reduction in vertebral fractures with zoledronic acid treatment [243]. In another study, Kaemmerer and colleagues treated liver transplant patients treated with 2 mg of intravenous ibandronate every 3 months for 1 year also had stable spine BMD and attenuated hip bone loss compared to controls. Treated subjects had a significant reduction in total number of fractures [244]. Intravenous zoledronic acid (4 mg), given 12 months after BMT, prevented spinal and femoral bone loss [245]. Zoledronic acid has also been shown to increase ex vivo growth of bone marrow CFU-f, perhaps improving osteoblast recovery and increasing osteoblast numbers after BMT.

Clinical trials have also been performed with oral bisphosphonates. In terms of primary prevention of bone loss immediately after transplantation,

several studies have compared alendronate with calcitriol. A randomized trial comparing alendronate (10 mg daily) with calcitriol (0.25 μg twice daily) treatment starting immediately after cardiac transplant found that both regimens prevented bone loss at the lumbar spine and hip 1 year after transplant, compared with a reference group receiving only calcium and vitamin D [23]. Although alendronate and calcitriol were discontinued during the second year after cardiac transplant, BMD remained stable [246]. Kidney transplant patients treated with alendronate (10 mg daily), calcitriol (0.25 μg daily), and calcium carbonate (2 g daily) had marked increases in LS BMD compared to decreases in those who received only calcium and calcitriol [247]. Two recent trials found similar improvements in LS BMD in patients treated with alendronate or risedronate following kidney transplant [248, 249].

Long-term cardiac transplant patients treated with clodronate also had improvements in BMD [250]. A trial of long-term kidney transplant patients who were started on alendronate, calcitriol, and calcium or only calcitriol and calcium approximately 5 years after transplantation, documented significant improvements in LS and FN BMD in the alendronate group. BMD in the other group was stable [251]. Similarly, a retrospective trial in long-term kidney transplant recipients found that bisphosphonate use was associated with preservation of FN BMD [252]. Three recent meta-analyses of bisphosphonate trials in kidney transplant recipients found that bisphosphonates effectively prevented bone loss at the LS and FN [253–255]. In addition, a meta-analysis also demonstrated bisphosphonates use reduced fracture in transplant recipients [255]. In a small randomized trial of long-term kidney transplant recipients that compared alendronate, alfacalcidiol, and alendronate for 1 year, BMD improved at the LS and FN in patients treated with alendronate and alendronate combined with alfacalcidiol. The increase was only significant in the combination alendronate-alfacalcidiol group likely because of inadequate power in this small study [256]. Alendronate has been shown to prevent bone loss after liver transplant as well [182]. In BMT recipients, risedronate given 12 months after BMT improved BMD at the spine and prevented loss at femoral neck [257].

Weekly or monthly dosing regimens [258] are very useful in transplant patients who have many gastrointestinal symptoms and take large numbers of medications. For such patients, the requirement to take oral bisphosphonates first thing in the morning and wait 30–60 min before eating or taking other medications is particularly inconvenient. In two recent studies, weekly alendronate (70 mg) has improved BMD in liver [259] and kidney transplant recipients [260]. Our randomized double-blind, double-dummy active comparator study compared single-dose zoledronic acid and weekly alendronate over 1 year in patients receiving liver or heart transplant. We found that both agents prevent bone loss at hip. In liver transplant patients, both medications increased LS BMD. In contrast, among heart transplant patients, those who received zoledronic acid had increased LS BMD but not those treated with oral alendronate [261].

Although fracture is the most important clinical outcome, very few treatment studies have had adequate statistical power to detect differences in fracture among treated and untreated patients. For this reason, we performed a meta-analysis of randomized controlled clinical trials to determine whether treatment with bisphosphonates or active vitamin D analogs reduced fracture risk in the first year following solid organ transplantation. Treatment with bisphosphonates or vitamin D analogs reduced the number of subjects with fracture (OR 0.50, 95% CI 0.29, 0.83) and number of vertebral fractures (OR 0.24, 95% CI 0.07, 0.78). When bisphosphonate trials were examined separately, there was a reduction in number of subjects with fractures (OR 0.53, 95% CI 0.30, 0.91), but no significant reduction in vertebral fractures (OR 0.34, 95% CI 0.09, 1.24) [255].

Prior to initiation of bisphosphonate treatment, particularly with intravenous agents, it is important to screen for and correct vitamin D deficiency. Bisphosphonates may not be optimally effective in the setting of severe vitamin D deficiency. More importantly, intravenous bisphosphonate treatment can precipitate symp-

tomatic hypocalcemia in patients with severe, unrecognized vitamin D deficiency [262].

At present, bisphosphonates constitute the most promising approach to the prevention of transplantation osteoporosis. As with other forms of therapy, many issues remain to be resolved. These include whether or not they actually prevent fractures, since most studies have been under-powered to address this important issue, the optimal drug and route of administration, whether continuous or intermittent (cyclical) therapy should be used, at what level of renal impairment these drugs should be avoided, whether they are safe in renal transplant recipients with adynamic bone disease and whether they are beneficial in the setting of pediatric transplantation.

Vitamin D and Analogs

Administration of vitamin D or its analogs is often recommended after transplantation [263]. There are several potential mechanisms by which vitamin D and its analogs may influence post-transplantation bone loss. They may overcome GC-induced decreases in intestinal calcium absorption, reduce secondary hyperparathyroidism, promote differentiation of osteoblast precursors into mature cells, or influence the immune system and potentiate the immunosuppressive action of cyclosporine [264–266].

Since most of the observational studies of bone loss after organ transplantation have included at least 400 IU of parent vitamin D in the post-transplant regimen, it is clear that this amount is not sufficient to prevent transplantation osteoporosis. In two recent studies, parent vitamin D, in doses of 800 IU daily [267] or 25,000 IU monthly [24] also did not prevent bone loss after kidney transplantation.

Active forms of vitamin D may be more effective. Calcidiol (25-OHD) prevented bone loss and increased LS BMD after cardiac transplantation [268]. Alfacalcidiol (1-α-OHD) prevented or attenuated bone loss at the LS and FN when given immediately after kidney transplantation [269–271]. Several investigators have studied the effects of calcitriol in transplant recipients. The results have been contradictory, although some studies have found beneficial effects at doses greater than 0.5 μg per day. Sambrook et al. reported that calcitriol (0.5–0.75 mg/d) prevented spine and hip bone loss during the first 6 months after heart or lung transplantation and was as effective as cyclic etidronate [272]. Calcitriol given during the first year after kidney transplantation was associated with an increase in LS, FN, and forearm BMD [50]. In a stratified, placebo-controlled randomized study, heart and lung transplant recipients received calcitriol or placebo for 12 or 24 months after transplantation [273]. While LS bone loss was equivalent between groups, FN bone loss at 24 months was reduced only in the group that received calcitriol for the entire period. Although these results suggest that the protective effects of calcitriol are not sustained after cessation of treatment, we found no bone loss when we discontinued calcitriol after the first post-transplant year [246]. In another study of renal transplant recipients, intermittent calcitriol and calcium prevented TH but not LS bone loss [274]. In contrast, studies of long-term kidney [275] and heart transplant patients [276] have failed to find any benefit of calcitriol. Stempfle et al. found that the addition of a small dose to calcitriol (0.25 μg/d) to calcium supplementation and gonadal steroid replacement offered no benefit with regard to bone loss or fracture prevention after cardiac transplantation [128].

Hypercalcemia and hypercalciuria are the major side effects of therapy of these agents. Either may develop suddenly and at any time during the course of treatment. Thus, frequent urinary and serum monitoring may be required. If hypercalcemia occurs, it must be recognized and reversed promptly because of the adverse effects on renal function and the life-threatening potential of a severely elevated serum calcium concentration. Supplemental calcium and any vitamin D preparations should be discontinued until the calcium normalizes. Although one may be tempted to permanently discontinue pharmacologic doses of vitamin D or its metabolites in view of the necessary serial monitoring and potential dangers,

one might also recommence therapy at a lower dose. However, given the requirement for serial monitoring and the narrow therapeutic window with respect to hypercalcemia and hypercalciuria, we regard pharmacologic doses of vitamin D and its analogs as adjunctive rather than primary therapy for the prevention and treatment of transplantation osteoporosis.

Denosumab

Denosumab is a monoclonal antibody to nuclear factor kappa B ligand that prevents bone resorption by impairing the development, activation, and survival of osteoclasts [277]. It is FDA approved for the treatment of glucocorticoid-induced osteoporosis. Previous studies have shown that denosumab is beneficial to prevent bone loss and lowers fracture risk in postmenopausal women and men with osteoporosis. In a recent randomized, controlled study that involved 795 patients with glucocorticoid-induced osteoporosis, denosumab (60 mg every 6 months) improved BMD at the LS to a greater extent than risedronate (35 mg weekly) at 12 months. Similarly, the improvement in BMD at the TH was greater for denosumab [278].

In another prospective randomized trial, 90 de novo kidney transplant patients were assigned to receive 2 doses, every 6 months, of either denosumab or placebo beginning at 2 weeks postoperatively. After 12 months, denosumab was associated with a 4.6% increase in LS BMD while the placebo group sustained a 0.5% loss in LS BMD [279]. In a subgroup analysis of these patients, denosumab also resulted in increased volumetric BMD and cortical thickness at tibia. With regard to bone strength, micro-finite element analysis showed that bone stiffness increased significantly at the tibia (median difference 5.6%) [280].

An increased risk of urinary tract infections was also reported in the kidney transplant patients who received denosumab treatment. The incidence of other infections was similar between patients treated with denosumab and controls [279]. Unlike bisphosphonates, denosumab is not cleared by the kidney and therefore dose adjustment is not required in CKD setting. However, hypocalcemia can be a serious side effect of denosumab, particularly in patients with CKD [281]. For this reason, serum calcium should be closely monitored in patients with CKD who receive denosumab.

Testosterone

Hypogonadism is common in men with chronic illness. Moreover, the suppressive effects of cyclosporine A and glucocorticoids on the hypothalamic-pituitary-gonadal axis often lower serum testosterone levels. Although testosterone usually normalizes by 6–12 months after transplantation, [132, 133] approximately 25% of men evaluated 1–2 years after transplantation will have biochemical evidence of hypogonadism. Hypogonadism is known to cause osteoporosis in men. Moreover, men with low serum testosterone concentrations have been shown to lose bone more rapidly after cardiac transplantation [132, 133]. Fahrleitner et al. found that hypogonadal men treated with intravenous ibandronate had improved BMD at 1 year if they were treated with testosterone compared with those who were not replaced [282].

In general, men who are truly hypogonadal, with testosterone levels below normal according to the laboratory assay utilized, should be treated with testosterone. Potential benefits of testosterone therapy include increased lean body mass and hemoglobin, and improved BMD. Potential risks include prostatic hypertrophy, abnormal liver enzymes, and acceleration of hyperlipidemia in patients already prone to atherosclerosis from hypertension, diabetes, glucocorticoid, and CsA therapy. Therefore, it is necessary to monitor serum lipids and liver enzymes, and perform regular prostate examinations in men receiving testosterone.

Resistance Exercise

A few small studies have examined the effects of resistance exercise on BMD following heart

[283] and lung [284] transplantation. Resistance exercise led to significant improvements in LS BMD when used alone, and in combination with alendronate. The interpretation of these findings is limited, however, by the extremely small numbers of subjects enrolled and the method used to measure BMD (lateral spine), which is highly variable, leading to a percent change much greater than typically reported.

Summary and Conclusions

There has been tremendous progress in elucidating the natural history and pathogenesis of transplantation osteoporosis. It is now clear that a substantial proportion of candidates for solid organ and bone marrow transplantation already have osteoporosis. Prospective longitudinal studies have provided definitive evidence of rapid bone loss and a high incidence of fragility fractures, particularly during the first post-transplant year. Vertebral fractures occur both in patients with low and those with normal pre-transplant BMD, so that it is impossible to predict fracture risk in the individual patient. Early post-transplantation bone loss (before 6 months) is associated with biochemical evidence of uncoupled bone turnover, with increases in markers of resorption and decreases in markers of formation. Later in the post-transplantation course (after 6 months), concomitant with tapering of glucocorticoid doses, bone formation recovers and the biochemical pattern is more typical of a high-turnover osteoporosis. More recent studies suggest that rates of bone loss and fracture are lower than they were before 1995. However, the rates of bone loss and fracture following transplantation remain unacceptably high. Bisphosphonates are the most consistently effective agents for the prevention and treatment of bone loss in organ transplant recipients. Patients should be assessed before transplantation and receive treatment for prevalent osteoporosis, if present. Primary prevention therapy should be initiated immediately after transplantation, as the majority of bone loss occurs in the first few months after grafting. The lowest possible dosages of glucocorticoid and calcineurin phosphatase inhibitors should be used for immunosuppression. Long-term transplant recipients should be monitored and treated for bone disease as well. With proper vigilance, early diagnosis, and treatment, transplant osteoporosis is a preventable disease.

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Osteoporosis in Premenopausal Women

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Key Points

- Low-trauma fractures occur much less often in premenopausal women than in postmenopausal women. In the absence of other causes of pathological fracture such as malignancy, bone lesion, or osteomalacia, low-trauma fractures can establish the diagnosis of osteoporosis in a premenopausal woman.
- In the context of an ongoing cause of bone loss/increased fracture risk, both the International Osteoporosis Foundation (IOF) and the International Society for Clinical Densitometry (ISCD) provide guidelines for the use of BMD by DXA to define osteoporosis in premenopausal women.
- In healthy premenopausal women without a history of low-trauma fracture or a known cause of bone fragility, low BMD by DXA alone should not be used to diagnose osteoporosis. The relationship between BMD and fracture risk has not been clearly established by longitudinal studies in this population.
- Measurement of BMD by DXA should be performed in young women with a health condition that increases risk for bone loss/fractures as well as in those who have come to medical attention in the context of low-trauma fracture(s).
- The interpretation of BMD should take into consideration the timing of bone mass accrual and physiologic changes associated with pregnancy and lactation.
- The majority of premenopausal women with low-trauma fractures have an identifiable cause of bone fragility; a thorough evaluation is indicated and aims to identify potential causes.
- Management approaches should focus on treatment of underlying causes whenever possible.
- Although pharmacologic therapy is rarely necessary in premenopausal women, those with an ongoing cause of bone loss and those who have had or continue to have major low-trauma

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fractures may require pharmacological intervention.
- Bisphosphonates and teriparatide have been approved for use in premenopausal osteoporosis secondary to glucocorticoid use.
- Few high-quality clinical trials exist to provide guidance on management of premenopausal osteoporosis and there are currently no available data to establish that such medication interventions reduce the risk of future fractures in premenopausal women.

Osteoporosis in Premenopausal Women

Although osteoporosis occurs most commonly after menopause, premenopausal women can also present with low-trauma fractures or low bone mineral density (BMD). The approach to diagnosis and management in this population is different than for postmenopausal women. This chapter will address special considerations for interpretation of BMD in premenopausal women as well as review definitions and epidemiology, and available data regarding etiology, evaluation, and treatment of premenopausal osteoporosis.

Diagnosis of Osteoporosis in Premenopausal Women

Fractures in Premenopausal Women

Osteoporosis is defined as a condition of reduced bone strength and increased risk of fractures. The diagnosis of osteoporosis, describing a condition of bone fragility, is most secure in the context of low-trauma fracture(s). Low BMD is not required to diagnose osteoporosis in the context of low-trauma fracture(s). When there is an unusual fracture, other causes of pathological fracture such as malignancy, bone lesion, and osteomalacia must be ruled out before diagnosing osteoporosis.

Epidemiology

This is a subheading below Fractures in Premenopausal Women Fractures are much less common in premenopausal women compared to postmenopausal women [1–5]. For example, a study of a population in Dorset, England, documented distal radius fracture incidence of 10 per 10,000 population per year for premenopausal women. Incidence rose continuously in women over age 50 years to a peak of 120 per 10,000 population per year in women older than 85 years [4]. However, though rare, premenopausal fracture is a strong independent predictor of future fracture risk. In the Study of Osteoporotic Fractures (SOF), ambulatory white women with a history of premenopausal fractures were 35% more likely to have a fracture during the postmenopausal years compared to women without a history of premenopausal fracture ($p = 0.001$) [2]. The risk was even higher in a retrospective cross-sectional study of 1284 postmenopausal women in New Zealand: self-reported fractures occurring between age 20 and 50 years were associated with a 74% increased risk of fracture after age 50 years [5]. In these and other studies [6, 7], premenopausal fractures remained a significant predictor of postmenopausal fracture risk even after controlling for BMD, estrogen use, and maternal fracture history.

Bone Mineral Density in Premenopausal Women

In postmenopausal women, assessment of BMD by Dual X-ray Absorptiometry (DXA) can be used to diagnose osteoporosis, even in the absence of fractures. Additionally, in postmenopausal women, DXA is a cornerstone of fracture risk prediction models used for therapeutic decision making. DXA is useful in postmenopausal women because of the plethora of longitudinal observational and interventional data correlating BMD by DXA with fracture incidence in this

population. However, there is a dearth of such prospective longitudinal data in premenopausal women. As a result, DXA measurement cannot be used in the same way to diagnose osteoporosis, predict fracture risk, or determine treatment in premenopausal women [8].

Although prospective data relating BMD to fracture risk are lacking in premenopausal women, studies have used cross-sectional data to examine BMD–fracture relationships and have reported lower BMD by DXA in premenopausal women with fractures. Compared to controls without fractures, premenopausal women with Colles fractures had significantly lower BMD at the contralateral radius [9], lumbar spine, and femoral neck [10]. Stress fractures were also associated with lower BMD in female military recruits and athletes compared to controls [11–13]. These studies suggest that there is some relationship between low BMD and fracture in the premenopausal years; however, this relationship is not as well studied as in postmenopausal women. Because of the lack of currently available prospective data relating BMD by DXA to fracture risk, we are not able to employ fracture risk prediction models, such as FRAX, in this population.

Given the lower fracture rates in premenopausal women [2, 4, 5] and lack of prospective studies correlating BMD to fracture incidence, both the International Osteoporosis Foundation (IOF) and the International Society for Clinical Densitometry (ISCD) recommend against using BMD by DXA as the sole guide to diagnosing or treating osteoporosis in this population [14]. Furthermore, premenopausal women should not be routinely screened with DXA for osteoporosis [15, 16]. Measuring BMD is only indicated in premenopausal women with a history of low-trauma fracture and in women with conditions known to cause bone loss or increased fracture risk (addressed later in this chapter).

DXA Interpretation and Definition of Premenopausal Osteoporosis Based on BMD

When BMD by DXA is obtained in premenopausal women, the ISCD recommends comparison of the BMD at the lumbar spine, hip, and forearm to age-matched norms instead of to young premenopausal norms (i.e., use of *Z*-scores instead of *T*-scores) [16]. A *Z*-score of −2.0 or below should be interpreted as "below the expected range for age" and a *Z*-score of more than −2.0 as "within the expected range for age" [16]. The category of "osteopenia," based on *T*-scores, should not be used in premenopausal women.

There are some recommendations that propose exceptions to the use of *Z*-scores in premenopausal women; these pertain to special populations. In young adults who have completed growth and who have an ongoing secondary cause of bone loss or a chronic disorder known to affect bone mass, the IOF recommends using a *T*-score of ≤ -2.5 at the spine or hip to define osteoporosis [17]. In addition, the ISCD recommends the use of *T*-scores for perimenopausal women [16].

Definitions of Premenopausal Osteoporosis Based on BMD

In young women, the ISCD specifies a BMD-based definition of premenopausal osteoporosis as the presence of low BMD for age ($Z \leq -2.0$) together with the presence of risk factors for fracture or secondary causes of osteoporosis [18]. In contrast, IOF recommends the use of *T*-score < -2.5 to define osteoporosis in the context of an ongoing secondary cause [17].

The ISCD [16], IOF [19], and other experts [20–22] recommend against diagnosing young women with osteoporosis based on BMD by DXA alone, without a history of fragility fracture or secondary cause of osteoporosis.

Idiopathic low BMD is a term that can be used to describe low BMD in the absence of a known cause and without a history of low-trauma fracture as an adult [23]. Because fracture risk is unknown, premenopausal women meeting this definition should not be placed into the diagnostic category of "osteoporosis." However, studies have shown abnormal bone microarchitecture in this cohort [23–25].

Using techniques such as high-resolution imaging of bone biopsy samples and high-resolution central and peripheral CT, normally menstruating, healthy premenopausal women with idiopathic low BMD have been found to have deficiencies in bone microarchitecture

including thinner, more widely spaced, and heterogeneously distributed trabeculae and thinner cortices, as well as lower estimated bone strength [23, 24, 26]. These properties were comparable to those in a group of concurrently recruited premenopausal women with fragility fractures and the similarities remained even after correcting for the smaller bone size in the women with idiopathic low areal BMD but no fractures. Caveats of this study are small sample size and possible ascertainment bias. Many in the idiopathic low areal BMD group had a family history of osteoporosis (84%), childhood fractures (26%), or high-trauma adult fractures (16%), which may have affected their enrollment in the study.

In another observational study, constitutionally thin premenopausal women (BMI < 16.5 kg/m^2, physiological menstruation and no known systemic disease or anorexia nervosa) were found to have smaller bone size, lower lumbar and femoral BMD, and diminished breaking strength compared to age-matched women [25].

Although these bone structure studies are small, observational, and may not be generalizable to all premenopausal women, they suggest that having BMD much lower than one's peers is an asymptomatic stage preceding osteoporosis in premenopausal women. However, for several key reasons, low BMD should not be used to make therapeutic decisions in premenopausal women without fractures or a known secondary cause. There are no longitudinal data on the short-term risk of fracture in premenopausal women based on BMD. In addition, fracture risk is much lower in premenopausal women and increases greatly with age. Furthermore, early treatment with osteoporosis medications may commit young women to a higher lifetime cumulative dose of these medications and expose them to the associated risks.

Special Considerations Required for Interpretation of BMD Results in Premenopausal Women

Peak Bone Mass Accrual

Bone mass accrues during growth and young adulthood until peak bone mass is achieved. Age at peak bone mass may differ based upon gender, [27, 28] ethnicity, [29] body size, menarchal age, [30, 31] and skeletal site. The highest rate of bone mass accretion occurs from age 11 to 14 in girls [32] and the rate decreases by 2 years after menarche [27], with at least 90% of peak bone mass acquired by the late teen years [32–34]. Some studies have observed additional bone accrual from age 20 to age 29 years [35]. In addition, peak bone mass accrual may be specific to skeletal site [27], with studies suggesting that achievement of peak bone mass occurs at the proximal femur in women in their twenties and at the spine and forearm around age 30 [36]. The amount of bone in the skeleton at any time depends on whether peak bone mass has been achieved and this must be considered when interpreting BMD measurements in young premenopausal women.

Any process (illness, medication) that perturbs bone mass accrual can lead to a peak bone mass that is below average and a lower BMD by DXA compared to peers. This is also true if a woman is genetically predisposed to achieve a lower peak bone mass. While the attainment of a below-average peak bone mass in a premenopausal woman may result in a lower "bone bank" from which to withdraw in the postmenopausal years, the factors responsible may not persist and may not be ascertainable on evaluation pursued years later. In addition, the effect on bone microarchitecture and strength is uncertain. Furthermore, there are no published data comparing bone quality in women with low peak bone mass due to genetic predisposition to those with secondary causes of bone loss.

Physiologic Changes Associated with Pregnancy and Lactation

Pregnancy and lactation are states of high calcium demand leading to large physiologic changes in bone mass that must be considered when interpreting a woman's BMD around this time. There are rapid, asymptomatic decreases in spine and hip BMD during normal pregnancy/lactation and recovery during weaning.

Calcium Demand

THis is a subheading UNDER Physiologic Changes Associated with Pregnancy and Lactation In humans, calcium demands for the growing fetus are largely met by a dramatic increase in the production of active $1,25(OH)_2$ vitamin D, leading to a doubling of intestinal calcium absorption during pregnancy [37–39]. However, despite these compensatory increases in calcium absorption, bone mass declines by 3–5% over a typical pregnancy [40–42], as evidenced by studies that have measured BMD in women before and after a pregnancy [40, 43], as well as during pregnancy using techniques such as distal radius pQCT and heel ultrasound [44, 45].

This physiology and calcium metabolism changes drastically during lactation. Compensatory intestinal calcium hyper-absorption ceases while calcium demands for breast milk production increase to 300–400 mg/day. To meet this demand, PTHrP, secreted by the mammary glands, mobilizes calcium from the lactating woman's skeleton [46]. Several studies have documented consistent and large declines in bone mass during lactation. Longitudinal studies of areal BMD by DXA document losses at the lumbar spine of 3–10% and at the hip of 2–4% over the first 6 months of lactation [40, 47–53]. The amount of bone loss during lactation is more pronounced with longer durations of lactation and postpartum amenorrhea [47–49]. In addition, changes in serum bone turnover marker levels are associated with duration of lactation [50]. The total bone loss of 1–3% *per month* during lactation is substantially more rapid than the 1–3% *per year* that occurs immediately after menopause. However, fractures during this period are extremely rare.

Postpartum Bone Recovery

This is also a subheading UNDER Physiologic Changes assoc with Pregnancy and Lactation Recovery of bone mass has been documented, often after the 6-month postpartum time-point, even in the setting of continued lactation [47, 49, 51]. Milk production and calcium requirements decline at this time as the infant begins to eat solid food. Menses return at 6–8 months postpartum on average [48, 50]. Recovery has been related to duration of lactation and return of menses [50].

Both human and rodent studies demonstrate that recovery of bone mass after weaning is site specific, with full recovery at the spine, but only partial (or delayed) recovery at the femur [47, 49, 54–56]. However, current human studies of bone mass recovery only include 12–20 months of follow-up postpartum. Longer-term follow-up may further elucidate recovery at different sites.

Effect of Parity and Lactation on Postmenopausal BMD and Fracture Risk

Although both human and animal studies suggest that some areas of the skeleton may not recover completely after pregnancy and lactation-related losses, the majority of epidemiological studies in humans suggest that the net effect of the loss and regain of bone mass during and after lactation does not affect postmenopausal bone mass or long-term fracture risk. In a number of studies, pregnancy and lactation history have been associated with a decreased risk of osteoporosis or fracture [41, 57–69]. Furthermore, conditions such as grand multiparity, repeated pregnancy and lactation without a recovery interval, and extended lactation (even in the context of subsequent pregnancy) have NOT been associated with lower BMD by DXA in cross-sectional comparison to nulliparous premenopausal controls [70, 71].

In addition, a recently published study has examined these issues in a very large dataset, assessing incident fracture rates over a mean of 7.9 years in 92,980 multiracial US women in the Women's Health Initiative observational study on whom pregnancy and lactation history was available [72]. In models adjusted for factors such as years since menopause, family history of fracture, BMI, estrogen use, calcium and vitamin D supplementation, there was no significant association of hip fracture incidence with age at first birth, number of pregnancies, age at first breastfeeding, number of children breastfed, and total duration of breastfeeding. Breastfeeding for at least 1 month was associated with a 16% *lower* risk of hip fracture compared to never breastfeeding (HR 0.84; 95%CI: 0.73–0.98). However, total duration of breast-

feeding was not associated with fracture risk. In addition, a recent systematic review found an overall positive effect of parity on bone mineral density and specifically on BMD at the total hip [73].

Studies in some regions of the world, however, show different results [74–76]. A Turkish and Chinese study found significant inverse associations between duration of lactation and postmenopausal BMD [74, 75]. A study of the Mexican mestizo population showed that breastfeeding for 24 months or more was significantly associated with postmenopausal osteopenia/osteoporosis. These varying results may be due to differences in nutrition and socioeconomic factors between studied populations.

Pregnancy and Lactation Associated Osteoporosis

Pregnancy and lactation associated osteoporosis (PLO) is a severe early presentation of osteoporosis in which young women experience low-trauma or spontaneous fractures, most commonly vertebral fractures, during late pregnancy or lactation [41, 77–79].

The majority of cases occur in primigravid women [79, 80]. Prominent symptoms include severe back pain and height loss. Most women are otherwise healthy, with no known predisposing condition [42, 79, 81–84]. BMD before pregnancy is almost always unknown since there would have been no indication to measure it. BMD by DXA at presentation is generally extremely low [78, 81–92] with *Z*-scores often reported at less than −3.0.

Given the rarity of PLO, natural history of the condition is not well delineated. A few case reports and small case series have reported data over several years of follow-up in untreated women after the incident fracture [78, 79, 88]. Several reports document recovery of bone density postpartum, similar to that seen in healthy women [78, 83, 88, 93]. However, subsequent fracture risk appears to be quite high. In a prospective study in which 107 PLO women at a single center were followed for a median of 6 years after initial fracture, 24% had subsequent fractures [80]. Number of fractures at diagnosis correlated modestly with future fracture risk ($r = 0.56$, $p = 0.003$). Among 30 women with subsequent pregnancy, 20% reported disease recurrence (fracture associated with pregnancy), even though 76% had received therapy with bisphosphonates or teriparatide [80]. Case reports have documented PLO patients who had recurrent fractures [77, 82, 88] but also those who did not have recurrent fractures during subsequent pregnancies (in most cases without lactation) [78, 79, 88, 94, 95].

Evaluation of Premenopausal Women with Low-Trauma Fracture and/or Low BMD

Primary Osteoporosis Diagnosed in Adulthood

Several conditions associated with abnormal skeletal development and manifestations of bone fragility in childhood can have widely variable clinical presentations and degrees of severity. In rare instances, these conditions may lead to symptoms and/or diagnosis in early adulthood rather than childhood. Such conditions include osteogenesis imperfecta [96], hypophosphatasia (associated with osteomalacia), osteoporosis-pseudoglioma syndrome [97, 98]/LRP5 mutations [99], and Marfan and Ehlers-Danlos syndromes.

Age at presentation, severity of disease, and other clinical features may lead to evaluation for such primary causes of osteoporosis/fracture.

Secondary Causes of Osteoporosis in Premenopausal Women

The majority of premenopausal women with low-trauma fractures have an identifiable cause of bone fragility. Causes may include conditions interfering with bone mass accrual or ones leading to ongoing bone loss after peak bone mass accrual. In a Minnesota population study of men and women 20–44 years old with osteoporosis, 90% were found to have a sec-

ondary cause [100]. At tertiary care referral centers, 48–53% of cases of premenopausal osteoporosis had secondary causes [101–103]. Lower rates of identifiable secondary causes at tertiary care centers may be because a greater number of unexplained cases are referred to the specialists.

The most common secondary causes of premenopausal osteoporosis include prolonged glucocorticoid exposure, estrogen deficiency, gastrointestinal illnesses causing malabsorption, hyperthyroidism, and other medication exposure. Table 23.1 provides a list of categories of potential secondary causes and specific disorders within each category. Some conditions such as anorexia nervosa and inflammatory bowel disease cause osteoporosis through multifactorial mechanisms (i.e., malnutrition, estrogen deficiency, other hormone abnormalities, inflammation).

Table 23.1 Causes of osteoporosis in premenopausal women

Any disease affecting bone mass accrual during puberty and adolescence.
Medications (not all have been studied in premenopausal populations)
Glucocorticoids.
Calcineurin inhibitors (e.g., cyclosporine).
Antiepileptic drugs (particularly cytochrome P450 inducers such as carbamazepine and phenytoin).
Chemotherapeutic drugs (particularly high-dose methotrexate).
Heparin: Unfractionated heparin is associated with both BMD loss and increased fracture risk [104–108]. Low molecular weight heparin is not known to increase fracture risk but exposure for >3 months is associated with decreases in BMD [109–111].
Proton pump inhibitors.
Excess vitamin A intake.
Thiazoledinediones.
Estrogen deficiency
Pituitary diseases and hypothalamic amenorrhea
Medications leading to suppression of ovulation and amenorrhea
GnRH agonists (when used to suppress ovulation)
Depot medroxyprogesterone acetate (DMPA)
Chemotherapy leading to amenorrhea
Anorexia nervosa (osteoporosis in this condition is likely to be related to several hormonal and nutritional abnormalities)
Other endocrinopathies and abnormalities of calcium metabolism
Cortisol excess/Cushing syndrome
Hyperthyroidism
Primary hyperparathyroidism
Primary Hypercalciuria
Gastrointestinal/nutritional
Significant vitamin D, calcium, and/or other nutrient deficiency
Gastrointestinal malabsorption
Celiac disease
Inflammatory bowel disease
Cystic fibrosis
Postoperative states (e.g., roux-en-Y gastric bypass)
Inflammatory conditions
Rheumatoid arthritis
SLE
Connective tissue diseases/Primary osteoporosis
Osteogenesis imperfecta
Ehlers-Danlos syndrome
Marfan syndrome
Other conditions:
Renal osteodystrophy
Liver disease (particularly cholestatic liver disease)
Alcohol use disorder
HIV infection and/or medications
Gaucher disease
Mastocytosis
Hereditary hemochromatosis
Thalassemia major
Diabetes (Type 1 and 2)

It is important to perform a thorough workup for secondary causes of premenopausal osteoporosis because treatment of many of these conditions has been associated with gains in BMD. Although data specific to premenopausal women is not available in each case, these remediable conditions include hypercortisolism (endogenous and iatrogenic), hyperparathyroidism [112], celiac disease [113–115], estrogen deficiency, hypercalciuria, [116] and Crohn's disease [117].

Evaluation of Premenopausal Women with Low-Trauma Fracture and/or Low BMD

Evaluation of low-trauma fracture and/or low BMD in premenopausal women should begin with a detailed history and physical exam, which often reveal a secondary cause, and may rarely

reveal a primary cause. The history should encompass questions related to the common causes of low-trauma fractures including childhood and adult illnesses, medication exposures, GI symptoms, diet and exercise history, nephrolithiasis and family history of fractures, osteoporosis or nephrolithiasis. It should also include a menstrual history and a complete pregnancy and lactation history. Some common secondary etiologies of osteoporosis may have manifestations detectable on physical examination, for example, eating disorders, hyperthyroidism, hypercortisolism, and malabsorption. In addition, features of connective tissue diseases/primary forms of osteoporosis, such as joint hyperlaxity, blue sclerae, and dentogenesis imperfecta, should be sought in the physical exam. Following the history and exam, a basic laboratory evaluation should be conducted with additional laboratory assays pursued as indicated (see Table 23.2). The initial laboratory evaluation could include an electrolyte panel, complete blood count, 25-OH vitamin D (severe deficiency may prompt an investigation for osteomalacia), liver function tests including alkaline phosphatase, TSH, PTH, and 24 hour urine calcium and creatinine as well as cortisol if clinically indicated. Like the history and physical exam, the objective is to identify common underlying disorders leading to fractures or low BMD. Abnormalities on initial evaluation can often guide additional testing.

In addition, in young women with fragility fractures, obtaining serial DXAs can identify whether there is ongoing bone loss in addition to possible suboptimal bone accrual. If BMD continues to decline, secondary causes should be aggressively sought out and intervened upon if possible.

Table 23.2 Laboratory evaluation

Initial laboratory evaluation
Electrolytes including creatinine and estimated GFR
Complete blood count
Serum calcium, phosphate
Serum albumin, transaminases, total alkaline phosphatase
Serum TSH
Serum PTH
Serum 25-hydroxyvitamin D
24 h urinary calcium and creatinine
Additional laboratory evaluation
Estradiol, LH, FSH, prolactin
Bone-specific alkaline phosphatase
24 h urinary free cortisol (or dexamethasone suppression test)
Iron studies (Iron/TIBC, ferritin)
Celiac screen (serum serologies)
Serum/urine protein electrophoresis
ESR or CRP
Vitamin A/retinol level
Specific testing for rare conditions such as osteogenesis imperfecta, Gaucher disease, hypophosphatasia, or mastocytosis, if clinically indicated
Bone turnover markers (C-telopeptide, N-telopeptide, bone-specific alkaline phosphatase, osteocalcin)
Invasive testing
Transiliac crest bone biopsy

Bone Turnover Markers

Bone turnover markers such as C-telopeptide, N-telopeptide, bone-specific alkaline phosphatase and osteocalcin have limited utility in premenopausal women because elevations may be due to various processes. Elevations may indicate active bone modeling in young adulthood, ongoing bone loss, or bone remodeling after a fracture.

Bone Biopsy

In rare cases, a trans-iliac crest bone biopsy may be useful in elucidating the mechanism of fragility fractures or low BMD. Bone biopsy reveals the microarchitectural features of bone. In addition, tetracycline labeling allows for calculation of bone formation rate and other dynamic parameters. Bone biopsy can help differentiate between different kinds of renal osteodystrophy, rule out osteomalacia, and uncover rare causes of bone fragility involving bone marrow changes, such as Gaucher disease or mastocytosis.

Idiopathic Osteoporosis (IOP)

If no known etiology of low-trauma fracture is uncovered after thorough investigation, premenopausal women with such fracture(s) are defined as having idiopathic osteoporosis (IOP). The mean age at diagnosis of IOP is 35 years. Fractures can manifest as a single low-trauma fracture of the hip, spine, or long bone or as multiple fractures (vertebral and nonvertebral) occurring over 5–15 years [100, 102, 118]. Women presenting

with IOP are predominantly Caucasian and often have a family history of osteoporosis [100, 102, 119]. Compared with controls, these women have been found to have lower weight and height in some studies [118, 120, 121].

Premenopausal women with IOP exhibit measurable microarchitectural deficiencies in comparison to controls. As assessed by both high-resolution peripheral quantitative computed tomography (HRpQCT)[24] and bone biopsy [23], premenopausal women with IOP have lower volumetric BMD, fewer and heterogeneously distributed trabeculae, greater trabecular separation, thinner cortices, and reduced bone stiffness assessed by finite element analysis. Even after controlling for bone size, age, and BMI, these differences remain [26].

Etiology of osteoporosis in these normally menstruating women has not been clearly related to estrogen exposure. One study has found lower follicular phase estradiol levels in IOP women [120], though this was not seen in other studies [118, 121].

Tissue-level bone remodeling rate is quite variable in IOP, suggesting diverse pathogeneses of fracture in this population [23]. A subgroup of IOP women with low bone formation rate had the most severe microarchitectural deficits. IGF-1 levels were elevated in this low-turnover group, suggesting the possibility of osteoblast resistance to IGF-1. Although frank hypercalciuria was considered a secondary cause and would have led to exclusion from this study of idiopathic osteoporosis, a high bone remodeling group had high serum $1,25(OH)_2D$ and mildly elevated 24 hour urinary calcium, a pattern resembling idiopathic hypercalciuria [23].

Treatment Considerations for Premenopausal Women with Low-Trauma Fractures and/or Low BMD

Little data exist to guide treatment of women with premenopausal osteoporosis and low BMD. Some lifestyle modifications (described below) are appropriate for all women with low BMD, but additional pharmacologic therapy is only recommended in a subset of patients, namely, those with a history of fragility fractures, as well as some women with low bone mass and an ongoing secondary cause of osteoporosis that cannot be mitigated.

Treatment Considerations

Idiopathic Low Bone Mineral Density

In premenopausal women with isolated low BMD, no fractures, and no known secondary cause after thorough evaluation, pharmacological therapy is rarely indicated. Although these women may have bone microarchitectural abnormalities underlying their low BMD, [23, 24] currently available data suggest that they usually have stable BMD, [122] and a low short-term risk of fracture. BMD should be measured at 1–2-year intervals to identify women with declining BMD. Follow-up measurements may provide guidance as to the necessity of continued evaluation for secondary causes, or therapy if ongoing losses are documented, particularly in the context of extremely low BMD (e.g., Z-score < -3).

Premenopausal Women with IOP and History of Fractures

In premenopausal women with a history of low-trauma fracture, and no known cause found after extensive evaluation, the use of medications to decrease fracture risk should be considered on a case-by-case basis. In our opinion, the use of such medications should be reserved for women with a history of major fractures of the spine or hip, multiple fractures, and/or declining BMD. Few data are available to delineate the specific risks or benefits of osteoporosis medications in women with IOP.

Premenopausal Women with Low BMD or Fractures Related to a Known Secondary Cause

In premenopausal women with low BMD or low-trauma fractures and a known secondary cause of osteoporosis, the first goal of management should

be to address the underlying cause: estrogen replacement for those with estrogen deficiency, gluten-free diet for celiac disease [113–115], nutritional rehabilitation and weight gain for anorexia nervosa [123], parathyroidectomy for primary hyperparathyroidism [112], and management of hypercortisolism [124]. These cited studies evaluate premenopausal patients to varied degrees. Although thiazides are used for idiopathic hypercalciuria, and appear to have beneficial effects on BMD in men [116], few data are available in young women. In some women, it may not be possible to eliminate the cause, such as those with primary osteoporosis (e.g., osteogenesis imperfecta) or those requiring long-term glucocorticoids. Thus, pharmacological therapy may be necessary. Options for treatment are reviewed below.

Treatment Options

Lifestyle Modifications

For all patients, it is appropriate to recommend adequate weight-bearing exercise [125] as well as lifestyle modifications such as smoking cessation and avoidance of excess alcohol. Exercise has been shown to lead to improvements in BMD in premenopausal women [126]. There is debate about the appropriate amount of calcium and vitamin D to recommend to premenopausal patients. The 2011 guidelines from the Institute of Medicine [127] recommend a total of 1000 mg of calcium (from diet and supplements) and 600 IU of vitamin D for premenopausal women. The more recent US Preventive Services Task Force (USPSTF) recommendations on calcium and vitamin D supplementation conclude that there is currently insufficient evidence to assess the risks and benefits of supplementation for primary fracture prevention in premenopausal women [128]. Ultimately, recommendations should be tailored to the individual based upon evaluation of calcium metabolism. Exercise recommendations must also be tailored to the individual patient, since excessive exercise in premenopausal women may lead to weight loss and/or hypothalamic amenorrhea, exacerbating low bone density.

Combination Oral Contraceptives

Replacement of estrogen in premenopausal women who are estrogen deficient may have beneficial effects on bone mass [129–131], although oral reproductive hormone replacement has been shown to be ineffective in most studies examining bone mass in anorexia nervosa, a more complex condition [123, 130, 132]. Studies of oral contraceptives in healthy premenopausal women without known preexisting estrogen deficiency have examined varying estrogen doses in populations of different ages. The majority of these studies show no effect of oral contraceptives on bone mass [130, 133, 134] or fracture risk [135]; however, some have documented an adverse effect of low-dose (<30 μg ethinyl estradiol) oral contraceptives on bone mass in young women [136–138]. A large case-control study from the UK showed that women without fractures were significantly more likely to have used oral contraception, [139] but there is inconclusive evidence for a causative role of oral contraceptives in decreasing fracture risk [135, 140].

Selective Estrogen Receptor Modulators

Selective estrogen receptor modulators (SERMS), such as raloxifene, should not be used to treat bone loss in menstruating women since they block estrogen action on bone and lead to further bone loss [141]. Similar effects have been noted with tamoxifen [142].

Bisphosphonates

Bisphosphonates have been shown to improve BMD in premenopausal women with several different conditions including in breast cancer treatment-induced bone loss, anorexia nervosa, and glucocorticoid-induced bone loss [77, 143–147]. Additionally, bisphosphonates have been shown to improve BMD in young adults with conditions such as cystic fibrosis, osteogenesis imperfecta, and thalassemia [148–150], but premenopausal women have not been specifically studied. Two bisphosphonates, alendronate and risedronate, have been approved by the United States Food and Drug Administration

(US FDA) for use in premenopausal women receiving glucocorticoids and a discussion specific to the case of glucocorticoid-induced osteoporosis is provided below. However, even though trials show favorable short-term BMD outcomes, fracture data are rarely available and long-term risks in premenopausal women are unknown.

The choice to prescribe bisphosphonates in younger women who may have many years of medication exposure must take into account our increasing concerns about the potential risks of long-term use of these agents, including osteonecrosis of the jaw and atypical subtrochanteric femoral fractures [151]. In young women, plans for duration of bisphosphonate use must be discussed as part of the process of initiation of this therapy, and the goal should be for the shortest possible duration of bisphosphonate use.

Bisphosphonates carry a Category C rating for safety in pregnancy from the US FDA because they accumulate in the skeleton, cross the placenta, and accumulate in the fetal skeleton in a rat model, and have been reported to cause toxic effects in pregnant rats [152]. Human data on bisphosphonates in pregnancy are largely anecdotal. Although one report documented transient neonatal hypocalcemia and bilateral talipes equinovarus in two neonates [153], several other reports have documented no adverse maternal and fetal outcomes [77, 154, 155]. It is recommended that effective contraception be utilized during bisphosphonate use, and it must be recognized that the potential for adverse effects from bisphosphonates remains even after these medications are discontinued, as they can remain in the skeleton for years. With these known risks, bisphosphonates should be used with caution in women who may have future pregnancies.

Denosumab

Denosumab is a RANK ligand inhibitor that is currently FDA approved for the treatment of osteoporosis only in postmenopausal women and men. The efficacy and safety of this medication have not been defined in premenopausal women.

Human PTH(1-34)/Teriparatide

Teriparatide or PTH(1-34), a recombinant form of PTH, has been FDA-approved for the treatment of osteoporosis in postmenopausal women and men who are at high risk for fracture, as well as in patients with sustained systemic glucocorticoid therapy (at least 5 mg prednisone per day) who have osteoporosis, and who are at high risk for fracture. This medication has been studied in premenopausal women, with evidence of BMD improvements when used to treat patients with glucocorticoid-induced osteoporosis, anorexia nervosa, pregnancy and lactation-associated osteoporosis, and idiopathic osteoporosis [156–159].In a placebo-controlled study of 21 women with anorexia nervosa and T-score ≤ -2.5 (mean age 47 years), 10 women treated with teriparatide had significant improvements in spine BMD at 6 months, compared to 11 women who received placebo (spine BMD increase 6.0% vs. 0.2%; $p < 0.01$) [156].

In patients with glucocorticoid-induced osteoporosis, premenopausal women treated with teriparatide have greater improvements in lumbar spine BMD compared to those treated with alendronate (see discussion of glucocorticoid-induced osteoporosis in premenopausal women, below) [158].

In young women treated with the GnRH analogue nafarelin to produce ovarian suppression for the treatment of endometriosis, spine BMD declined by 4.9%, while those treated with PTH(1-34) 40 μg daily together with nafarelin had an increase of 2.1% ($p < 0.001$) [160]. It is not clear whether these results would apply to premenopausal women with osteoporosis and normal gonadal status.

In an observational study of teriparatide 20 μg daily in 21 premenopausal women with IOP, BMD increased by 10.8% at the lumbar spine and 6.2% at the total hip (both $p < 0.001$) after 18–24 months of treatment [159]. However, a small subset (4 of the 21) had little or no increase in BMD and were considered nonresponders. Nonresponders had markedly lower tissue-level baseline bone formation rate. Additionally, an examination of bone turnover markers over the course of treatment in these

women suggested that the nonresponders lacked evidence of the "anabolic window" (an increase in bone formation that precedes the increase in bone resorption) that usually characterizes response to this osteo-anabolic agent [159]. While the resorption marker C-telopeptide rose comparably in responders and nonresponders, the peak rise in the bone formation marker PINP (N-terminal propeptides of procollagen type 1) was both blunted and delayed in the nonresponders. In a randomized, double-blind placebo-controlled single switch-over trial of teriparatide 20 μg daily in 41 premenopausal women with IOP, subjects randomized to teriparatide during the first 6 months gained significantly more BMD at the spine than those receiving placebo (5.5 ± 4.2% vs. 1.8 ± 4.0%; $p = 0.01$) [161]. As in the prior observational study, response was quite variable, and some women did not respond to teriparatide [161].

As is the case with bisphosphonates, there are no available data to demonstrate that teriparatide reduces fracture risk in these populations.

Because the long-term effects of teriparatide in young women are not known, use of this medication should be reserved for those at highest risk for fracture or those who are experiencing recurrent fractures. In young women less than 25 years of age, documentation of fused epiphyses is recommended prior to consideration of teriparatide treatment, since continued bone growth is considered a contraindication to use of this medication.

Bone mass declines after cessation of teriparatide in postmenopausal women who do not receive an antiresorptive [162, 163]. Hormone replacement therapy appears to prevent bone loss after cessation of PTH treatment in postmenopausal women [164, 165]. However, normal premenopausal estrogen levels in women with IOP appear insufficient to prevent bone loss after teriparatide cessation. Over 1–2 years after teriparatide discontinuation, BMD declined by 4.8 ± 4.3% (p <0.001) in untreated premenopausal women with IOP [166], suggesting that antiresorptive therapy will be required to prevent bone loss after teriparatide cessation, even in women with no known cause of bone loss.

PTHrP (1-34)/Abaloparatide

Abaloparatide is an analogue of PTHrP currently FDA approved for the treatment of osteoporosis in postmenopausal women at high risk for fracture. There are no data on the efficacy or safety of abaloparatide in premenopausal women. The current label states that "TYMLOS is not indicated for use in females of reproductive potential."

Additional Specific Clinical Scenarios

Pregnancy and Lactation-Associated Osteoporosis

PLO is a rare but serious condition, and the majority of data regarding management comes from case reports and small uncontrolled studies. BMD gains, even up to 10–20% [47, 49, 83, 88], are expected as part of the natural recovery of BMD losses associated with pregnancy/lactation. Thus, in the absence of placebo-controlled trials, it is hard to assess the contribution of medical interventions versus the natural course of BMD improvements postpartum.

Bisphosphonates have a potential role in the management of PLO, after delivery and weaning, as demonstrated by several case reports that have documented improvements in BMD after bisphosphonate use [42, 89, 90, 167, 168]. In one uncontrolled study, five women with PLO-associated vertebral fractures who were treated with bisphosphonates within 2 years of presentation demonstrated a 23% spinal BMD improvement at 24 months [77]. Given the absence of RCTs using bisphosphonates, and the potential safety risks of bisphosphonates in premenopausal women, the recommendation for bisphosphonates remains unclear.

Uncontrolled case reports have also shown that teriparatide can improve BMD in patients with PLO [81, 82, 86, 169]. One observational study of 27 women with PLO and multiple fractures demonstrated improvements in lumbar spine BMD at 12 months in patients who were treated with teriparatide (16 ± 7%), which was a greater BMD improvement than that seen in the five women in the study who chose not to receive treatment (8 ± 7%) [157]. This study provides the

first comparison to an untreated group; results support an augmentation of natural BMD recovery associated with teriparatide treatment. Studies in postmenopausal and premenopausal women have shown BMD declines after cessation of teriparatide, necessitating follow-up consolidation therapy [170]. It is not clear whether a similar approach is needed in women with PLO.

Osteogenesis Imperfecta in Adults

Osteogenesis imperfecta (OI) is a heterogeneous disorder with widely variable disease severity. Although most affected patients are diagnosed in childhood, fractures leading to diagnosis of OI can occur in young adulthood. Fewer data are available to guide treatment in adults with OI than in children with OI. Studies have been small, and thus do not definitively address fracture endpoints [150].

Bisphosphonates have been evaluated in randomized and non-randomized studies in adult OI patients [171–177]. Both oral and IV bisphosphonates have been consistently associated with increases in both spine and hip BMD. However, only one study has documented a reduction in fracture rate in adults, and only in a subgroup with types iii/iv OI [172].

In a randomized controlled trial including 79 adult (predominantly type 1) OI patients, teriparatide treatment over 18 months was associated with significant increases in spine and hip BMD, but no difference was seen in self-reported fractures [178]. In a recent multicenter randomized trial comparing teriparatide to the aminobisphosphonate neridronate in adult OI patients, teriparatide treatment was associated with larger BMD gains and a trend for reduced fractures during follow-up ($p = 0.1$) [179].

Hypophosphatasia in Adults

Hypophosphatasia is also a very heterogeneous disorder with widely variable disease severity. In adults, hypophosphatasia, an inborn error of metabolism due to mutation of the gene-encoding tissue nonspecific alkaline phosphatase, can present as osteomalacia, chondrocalcinosis, and/or stress fractures. Some case reports document potential risks of bisphosphonate treatment in these subjects [180], and some case reports document efficacy of teriparatide [181, 182]. Enzyme treatment for hypophosphatasia is now FDA approved (asfotase alfa), but there are no currently available guidelines for treatment in adults [183].

Glucocorticoid-Induced Osteoporosis (GIOP)

Glucocorticoids can lead to decreased reproductive hormone production and menstrual irregularities. Thus, it is reasonable to consider estrogen replacement as an initial management step in those with hypogonadism in the setting of glucocorticoid exposure.

For patients on long-term steroids, the 2017 American College of Rheumatology Guideline for Prevention and Treatment of Osteoporosis [184] recommends risk stratification of patients based on history of fractures, age, amount, and duration of glucocorticoid use. Given that duration of steroid use, and steroid dosing, can both be factors in propensity for bone loss, general principles include minimizing patients' steroid exposure by using the smallest dose possible for the shortest duration, and treatment with calcium and vitamin D supplementation [185]. Due to the potential for glucocorticoid interference with vitamin D absorption, patients taking glucocorticoids may require higher doses of vitamin D than the average population [186, 187].

Among bisphosphonates, alendronate and risedronate have been approved by the United States Food and Drug Administration (US FDA) for use in premenopausal women receiving glucocorticoids. However, relatively few premenopausal women participated in the relevant large registration trials for bisphosphonates in glucocorticoid-induced osteoporosis and none of the premenopausal women in those trials fractured [188–190]. A few studies have specifically evaluated premenopausal women with autoimmune and connective tissue diseases, and have demonstrated protective effects of intermittent cyclical etidronate and oral pamidronate [145, 146]. Guidelines from the American College of Rheumatology suggest that treatment be considered for glucocorticoid-induced osteoporosis in premenopausal

women at moderate to high fracture risk: those with prior osteoporotic fracture, or those with very low BMD (*Z*-score < −3) or very rapid bone loss AND continuing glucocorticoid treatment at ≥7.5 mg of prednisone or equivalent per day for ≥6 months [185]. Treatment with oral bisphosphonates or teriparatide are preferred in this population, with consideration of longer-acting medications only in special circumstances [185].

As discussed above, teriparatide has been examined in patients with glucocorticoid-induced osteoporosis. In an 18-month randomized double-blind trial comparing teriparatide to alendronate in over 400 men and women with osteoporosis (ages 22–89 years) and glucocorticoid exposure ≥5 mg/day prednisone equivalent for ≥3 months, teriparatide 20 μg daily increased BMD and reduced vertebral fracture incidence to a greater extent than alendronate [191]. In a prespecified analysis of that study, Langdahl et al. compared the effects of alendronate and teriparatide on BMD according to gender and menopausal status [192]. In the 62 premenopausal women included, mean percent increases from baseline in lumbar spine BMD were significantly greater in the teriparatide than in the alendronate group (7.0% vs. 0.7%, p <0.001), similar to the findings in men and postmenopausal women. No new vertebral fractures were seen in either premenopausal treatment group, and there was no significant difference in the number of patients with nonvertebral fractures. As noted above, fractures were also absent in the premenopausal women in short-term clinical trials comparing alendronate or risedronate versus placebo in women on glucocorticoids [189, 190].

Summary and Conclusions

Most premenopausal women with low-trauma fracture(s) or low BMD have a known secondary cause of osteoporosis or bone loss. Women who present with unexplained fractures or low BMD should have a thorough clinical and laboratory evaluation to search for secondary causes of fractures and/or bone loss. The focus of management should be treatment of the underlying cause, where possible. Although pharmacologic therapy is rarely necessary in premenopausal women, those with an ongoing cause of bone loss and those who have had or continue to have major low-trauma fractures may require pharmacological intervention, such as bisphosphonates or teriparatide. Few high-quality clinical trials exist to provide guidance and there are no data that such intervention actually reduces the risk of future fractures.

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24 Safety Considerations for Osteoporosis Therapies

Lianne Tile and Angela M. Cheung

Key Points

- Osteoporosis medications are well tolerated and safe for the majority of patients.
- There are some rare serious adverse effects, such as osteonecrosis of the jaw and atypical femur fractures.
- Risks and benefits of treatment and optimal duration of treatment should be weighed for each patient when considering osteoporosis medications.

Introduction

Osteoporosis and resulting osteoporotic fractures are responsible for significant morbidity, excess mortality, and health care costs in both men and women in the developed world [1]. Medical therapy for osteoporosis has been shown in multiple randomized controlled trials to reduce the risk of vertebral, nonvertebral, and hip fractures, and in some studies has been found to improve survival. Although the overall benefit-to-risk ratio of osteoporosis medications remains very favorable, there have been concerns raised over the past decade about the safety of these treatments [2–6]. This chapter reviews the safety considerations for medications used to treat osteoporosis, and proposes an approach to decision making regarding the duration of use of osteoporosis medications.

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Antiresorptive Therapies

Antiresorptive medications, specifically the aminobisphosphonates and the RANK ligand inhibitor denosumab, are the most commonly used first-line medications for the treatment of osteoporosis and reduction of fracture risk. These medications are generally well tolerated, and have few short-term risks. Although the initial randomized controlled trials of oral and intravenous (IV) bisphosphonates and subcutaneous denosumab indicated a favorable safety profile, post-marketing surveillance has subsequently raised concerns about serious risks associated with long-term use of these medications, specifically atypical femur fractures (AFFs) and osteonecrosis of the jaw (ONJ).

Bisphosphonates

Aminobisphosphonates (alendronate, risedronate, ibandronate, IV zoledronic acid) have been in widespread use to treat osteoporosis and

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reduce the risk of low-trauma fractures since 1995, when alendronate was first approved for use. They remain first-line therapies in all major clinical practice guidelines for the treatment of osteoporosis, as they have been shown to reduce the risk for vertebral, nonvertebral, and hip fractures in postmenopausal women, men, and in glucocorticoid-induced osteoporosis. Initial studies documented adverse effects such as musculoskeletal pain, acute phase reaction, esophagitis, and GI upset. Subsequent to their widespread adoption for treatment of osteoporosis, two rare but serious adverse events have been documented: atypical femur fracture and osteonecrosis of the jaw. These risks of treatment have received widespread attention in the medical literature and in the lay media, and have led physicians and patients to question and even discontinue treatment, leading to an increasing care gap in osteoporosis. Safety concerns with these medications have led to questions about the optimal approach to the use of bisphosphonate therapy and the optimal duration of treatment.

Atypical Femur Fracture

AFFs are stress fractures originating in the lateral shaft of the femur. These fractures have been documented in patients with and without bisphosphonate treatment. They were initially described in a case report in 1978, when Richardson and colleagues reported on unusual fractures associated with osteoporosis in premenopausal women [7]. This was before highly potent bisphosphonates were in use. In 1985, Orwoll and McClung described a similar type of stress fracture in patients with low bone turnover osteoporosis [8]. After bisphosphonates were introduced in the mid-1990s for the prevention and treatment of osteoporosis, there was widespread adoption of these medications. In 2005, Odvina and colleagues, in a case series, documented nine patients who had spontaneous non-spinal fractures while taking alendronate, and four of those were femur fractures [9]. These authors concluded that their findings raise the possibility that severe suppression of bone turnover leading to fracture may develop during long-term alendronate therapy. In 2011 Wang and Bhattacharyya published an analysis of typical and atypical femur fractures associated with bisphosphonate use in the United States in the decade after aminobisphosphonates were introduced. They estimated that for every 100 or so reductions in typical osteoporotic femur fractures, there was an increase of one subtrochanteric fragility fracture, now called an AFF [10]. Similarly, Meier and colleagues described a 47% reduction in classic fractures, but a significantly increased risk of AFFs of 10.7% per year, with bisphosphonate use [11]. They found that longer duration of bisphosphonate treatment was associated with higher risk of AFFs.

Subsequent epidemiologic studies have confirmed the association between bisphosphonates and AFFs, and have helped to clarify the incidence of these rare complications. Data from the Institute for Clinical Evaluative Sciences in Ontario, Canada estimated an incidence of AFF of 1–2 per thousand patient years after 6–7 years of bisphosphonate treatment [12]. Dell, based on data from Kaiser Permanente in California, estimated risk of 1 in 1000 patient years after 8–9.9 years of continuous bisphosphonate use [13]. This study also demonstrated a dramatic increase in risk after 8 years of continuous bisphosphonate use as compared to a shorter duration of treatment. Subsequently, a systematic review concluded that bisphosphonate exposure was associated with an increased risk of AFFs, with an adjusted relative risk of 1.70 [14].

Although bisphosphonate treatment is associated with AFFs, many people who take long-term bisphosphonates do not suffer AFFs. In order to use bisphosphonates as safely as possible, there is interest in identifying risk factors for the development of AFFs. Observational studies have documented a significantly increased risk of AFF in women in comparison with men. AFFs appear to be more common in Asian women than white women, and this may be related to differences in the shape of the femur including varus hip angle, bowleg deformity, and small femoral shaft diameter [15–18]. Other risk factors identified include the use of multiple antiresorptive medications, low vitamin D levels, glucocorticoid use, rheumatoid arthritis, and younger age at initiation of bisphosphonate treatment [16].

The pathophysiology of AFFs remains poorly understood. Antiresorptive medications, which suppress bone remodeling, may result in accumulation of micro-damage that is not repaired, thus leading to the development of stress fractures. Bisphosphonates have an impact on bone material properties including collagen and advanced glycation end products, and long-term use results in increased tissue mineral density, which may enable crack propagation after development of a stress fracture [19]. Differences in hip and lower limb geometry may play a role in the development of AFFs, and in particular may determine where these stress fractures occur in the femur. Recent studies have identified genetic risk factors for AFFs in a subset of patients. In a systematic review published in 2017, a number of monogenetic bone disorders were found to be associated with AFFs [20]. These include hypophosphatasia [21], X-linked hypophosphatemia, pycnodysostosis, osteopetrosis, X-linked osteoporosis, and osteogenesis imperfecta. Some patients, in particular those with osteogenesis imperfecta, also had bisphosphonate exposure [20]. There is postulated to be a drug–gene interaction that may predispose certain patients to AFFs with prolonged bisphosphonate use. Further research is necessary to identify those patients who are at higher risk, so that they can be managed appropriately and followed closely for the development of stress fractures.

After the initial emergence of reports about AFFs, the ASBMR convened a task force. The first task force report, with a case definition for AFF, was published in 2010 [22]. The conclusion of this report was that AFFs are fundamentally different from common osteoporotic femur fractures, and strongly suggest a distinct pathogenesis. A second task force report was published in 2014 [23] (Table 24.1). The revised case definition defined AFFs as occurring below the lesser trochanter and above the supracondylar flare, and pathologic fractures were excluded. This definition included criteria for incomplete as well as complete AFFs. Patients have to meet four out of five major criteria. Minor features were also identified, but not required for the case definition. These minor features highlight that prodromal symptoms, specific radiographic findings, and bilateral fractures are commonly found in patients with AFFs, and these can often be identified prior to a complete fracture occurring.

Table 24.1 2013 ASBMR Case Definition for Atypical Femur Fractures

To satisfy the case definition of AFF, the fracture must be located along the femoral diaphysis from just distal to the lesser trochanter to just proximal to the supracondylar flare.
In addition, at least four of five major features must be present. None of the minor features is required but these have sometimes been associated with these fractures.
Major features[a]
The fracture is associated with minimal or no trauma, as in a fall from a standing height or less.
The fracture line originates at the lateral cortex and is substantially transverse in its orientation, although it may become oblique as it progresses medially across the femur.
Complete fractures extend through both cortices and may be associated with a medial spike; incomplete fractures involve only the lateral cortex.
The fracture is noncomminuted **or minimally comminuted.**
Localized periosteal or endosteal thickening of the lateral cortex is present at the fracture site ("beaking" or "flaring")
Minor features
Generalized increase in cortical thickness of the **femoral diaphyses**
Unilateral or bilateral prodromal symptoms such as dull or aching pain in the groin or thigh
Bilateral **incomplete or complete femoral diaphysis fractures**
Delayed **fracture** healing

Reprinted from Shane et al. [23]. With permission from American Society for Bone and Mineral Research

Bold font indicates changes from the original 2010 ASBMR case definition

ASBMR American Society for Bone and Mineral Research, *AFF* atypical femur fracture

[a]**Excludes** fractures of the femoral neck, intertrochanteric fractures with spiral subtrochanteric extension, periprosthetic fractures, and pathological fractures associated with primary or metastatic bone tumors and miscellaneous bone diseases (e.g., Paget's disease, fibrous dysplasia)

In up to two-thirds of patients with AFF, there is evidence for bilateral AFFs [22–24]. The second fracture is often incomplete, as it is usually recognized after the diagnosis of the initial AFF. Small studies examining strategies for screening for incomplete AFF have concluded

that, with screening, the incidence of incomplete AFFs may be as high as 1–2 per 100 patients who have been treated with bisphosphonates for 3–5 or more years [24]. Screening patients with long-term bisphosphonate use and leg symptoms showed a similar incidence of AFFs. Screening protocols for patients on long-term antiresorptive medication have been proposed, recommending that patients taking bisphosphonates be monitored for thigh pain and other leg symptoms, and if present, imaging to look for stress fractures should be considered. Plain radiographs or full femur imaging using DXA can identify focal cortical thickening of the lateral cortex, and may show a stress fracture line [24–26]. If there are concerning symptoms in the context of antiresorptive medication use, and no significant abnormalities are seen on initial imaging studies, bone scan will accurately identify stress changes. MRI can also be done as a next step, to assess for marrow edema indicative of fracture [27]. If there is a lucent line on X-ray, a CT scan should be considered to determine the depth of the lucent line through the cortex and the extent of the line around the circumference of the femur. This information is helpful in decision making about the need for prophylactic surgery versus medical therapy.

Recommended management for AFFs is outlined in the ASBMR task force report [23]. Calcium and vitamin D should be continued. Antiresorptive therapy, including bisphosphonates and denosumab, should be stopped. Patients with AFFs should be investigated for underlying metabolic bone diseases, including rare diseases that have been associated with AFF such as hypophosphatasia. Complete AFFs need to be surgically repaired with an intramedullary rod, as other methods of fixation are often not successful. Patients with incomplete AFFs can be considered for prophylactic IM nailing, depending on leg symptoms, extent of the fracture, and patient preference. Medical therapy for patients with AFFs includes anabolic bone medications such as teriparatide. Teriparatide is effective in improving bone mineral density and reducing fracture risk in patients with osteoporosis and high fracture risk, but the data suggesting that it promotes healing of atypical fracture are limited [27–30]. Nonetheless, it is the recommended treatment in those with AFFs, particularly in those at high osteoporotic fracture risk. Treatment is generally recommended for 24 months, and it is unclear whether patients should restart antiresorptive therapy afterward. Those with atypical femur stress fractures should be counselled to avoid repetitive stress to the lower limbs, but they will benefit from muscle strengthening via nonresistance or low-resistance-type exercises.

AFF is a dreaded complication of antiresorptive medication use. The absolute risk of AFF is low, but incomplete AFFs appear to be much more common. The risk of AFF is increased with antiresorptive medication use and duration of use, but there also appears to be increased susceptibility in certain patients. Incomplete AFFs can be identified in many cases prior to a complete fracture, so it is important to have an index of suspicion in patients who may be at risk. As stated in the current guidelines, osteoporosis drug treatment needs to be offered to patients according to fracture risk, and ongoing need for antiresorptive drug therapies should be reassessed after 3–5 years of continuous use [31]. Despite this, it is still not clear what the best strategy is to reduce the risk of AFF in patients with osteoporosis [3, 32–35] (Fig. 24.1).

Osteonecrosis of the Jaw

Osteonecrosis of the jaw (ONJ), as defined in the first ASBMR task force report in 2007 [36], is an area of exposed bone in the maxillofacial region that does not heal within 8 weeks in an individual who has been exposed to an antiresorptive agent, and has not had radiation therapy. These patients often have symptoms of inflammation in the oral mucosa, pain, ulceration, and tooth mobility. ONJ was first described in association with use of high-dose IV bisphosphonate therapy in oncology patients. The dose of bisphosphonates in that context is much higher than that used for the treatment of osteoporosis. Patients who develop ONJ often have additional risk factors including corticosteroid use, radiation, and chemotherapy. ONJ is also seen in patients taking antiresorptive medication who have major oral surgery, periodontal disease, diabetes, and glucocorticoid use. ONJ is slow to heal and has

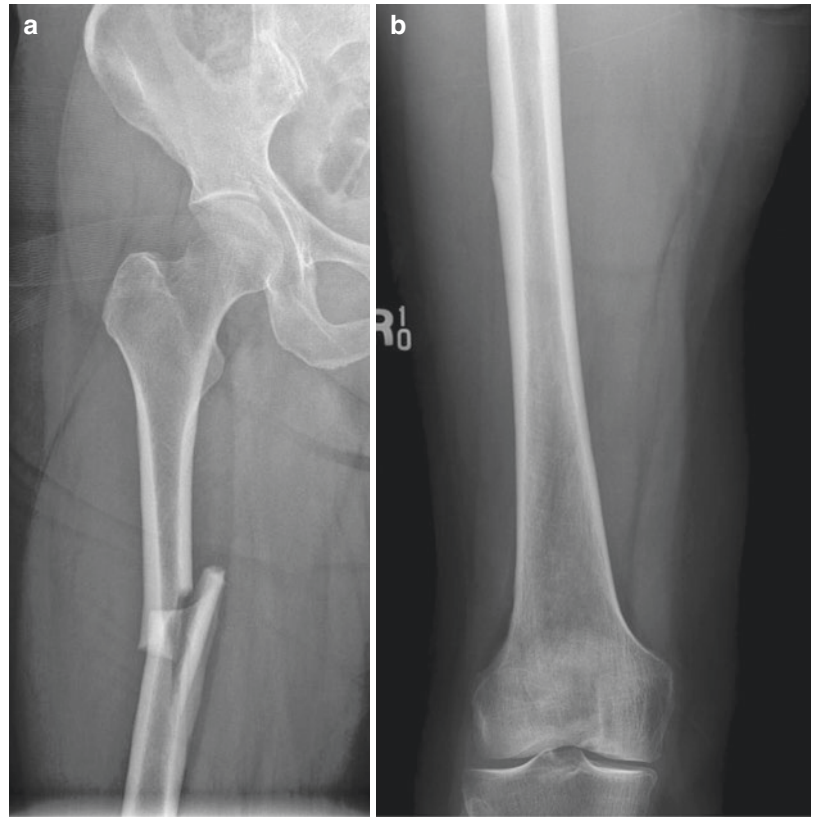

Fig. 24.1 Radiographs of Atypical Femur Fractures (**a**) Complete mid-diaphyseal AFF (**b**) Incomplete mid-diaphyseal AFF (with focal cortical thickening and a lucent line in the middle, i.e., beaking)

significant morbidity, and generally requires management by an oral surgeon.

Osteonecrosis of the jaw is rare, with an estimated incidence of 1 in 10,000–100,000 in patients with osteoporosis treated with standard dose antiresorptive medications. In the oncology population receiving intravenous bisphosphonates, the incidence of ON J may be as high as 1–15% [37]. Maintaining good oral hygiene is important in patients being treated with antiresorptive medications, and major dental surgery should ideally be done before these medications are started. The risk of ONJ may be lower with use of antimicrobial mouth rinses and antibiotics before oral surgery. There is no strong evidence that stopping antiresorptive medication prior to dental surgery will reduce the risk of developing ONJ [37] (Fig. 24.2).

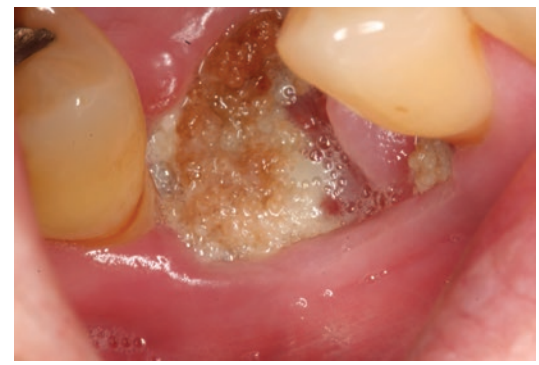

Fig. 24.2 Photograph showing an area of bone exposure in a patient with bisphosphonate-related ONJ. (Courtesy of Coronation Dental Specialty Group. Retrieved from Wikimedia Commons: https://commons.wikimedia.org/wiki/File:Stage_2_MRONJ.jpg. With permission from Creative Commons License 4.0: https://creativecommons.org/licenses/by-sa/4.0/deed.en)

Gastrointestinal Adverse Events

Oral bisphosphonates are associated with gastrointestinal adverse reactions in 1–7% of patients [38]. These include abdominal pain, GERD, dyspepsia, nausea, constipation, and diarrhea. Gastritis is reported in less than 1% of those taking these medications. Esophagitis and esophageal ulceration are also uncommon, although this

remains a clinical concern in patients with underlying esophageal disease, esophageal varices, or connective tissue disease associated with esophageal dysmotility. As oral bisphosphonate medications can directly irritate the esophageal and gastric mucosa, taking these medications as directed is critical. The delayed release formulation of risedronate, which can be taken with food, is associated with a lower risk of GI adverse effects. When bisphosphonates are taken correctly, the risk of serious GI complications remains low.

Esophageal Cancer

The possible association between use of oral bisphosphonates and esophageal cancer was first reported in 2009 [39]. This was followed by a 2010 database study from the UK that reported an increase in the risk of esophageal cancer from one case per 1000 to two cases per 1000 patients after 5 years of bisphosphonate use [40]. However, subsequent studies have not confirmed this association [41].

Acute Phase Reaction

Acute phase response has been observed primarily in patients treated with intravenous bisphosphonates. The reaction is characterized by low-grade fever, myalgias, arthralgias, and bone pain. It is typically self-limited, lasting up to 2–3 days, and is less common with subsequent infusions. It is felt to be due to a release of cytokines with bisphosphonate infusion, resulting in an inflammatory response. Acute phase reaction is uncommon with oral bisphosphonates.

Musculoskeletal Pain

Musculoskeletal pain including bone, joint, and muscle pain is reported in up to 6% of patients taking bisphosphonates [38]. This is not related to an inflammatory response, as in the acute phase reaction, and is not typically severe enough to limit taking these medications.

There is an extremely rare type of musculoskeletal pain with oral and intravenous bisphosphonate therapy, where the pain is severe and debilitating [42]. Biochemical analyses including muscle enzymes are often normal. Stopping bisphosphonate will reverse this adverse effect, although the recovery can be slow and incomplete. This rare idiosyncratic reaction to bisphosphonates is poorly understood. In contrast to the acute phase reaction and the more common type of musculoskeletal pain that occurs soon after initiating therapy, this severe idiosyncratic reaction can occur days, weeks, or months after initiating therapy.

Ocular Complications

Ocular complications are a rare but potentially serious adverse drug reaction with bisphosphonates [43]. There are reports of conjunctivitis, uveitis, episcleritis, and scleritis with oral as well as intravenous bisphosphonate use. Following intravenous bisphosphonate use, the incidence of uveitis and episcleritis was reported as 1% in one study. The incidence is well below 1% with oral bisphosphonates. These patients present with red, painful eyes, and require ophthalmologic evaluation and prompt discontinuation of bisphosphonates. Inflammatory ocular complications respond to topical treatment with corticosteroids, and if bisphosphonates are discontinued, there are no long-term visual sequelae reported.

Atrial Fibrillation

An association between bisphosphonate use and atrial fibrillation was first reported in the HORIZON trial of IV zoledronic acid versus placebo [44]. This association was also observed in a study of alendronate [45]. Subsequently, a meta-analysis in 2012 found no association between bisphosphonate use and development of atrial fibrillation [46, 47].

Hypocalcemia

Intravenous bisphosphonates are standard treatment for hypercalcemia due to malignancy or metabolic disease. Intravenous bisphosphonates used for the treatment of osteoporosis do not usually cause clinically significant hypocalcemia in patients with normal renal function who are calcium and vitamin D replete. Hypocalcemia with oral bisphosphonates is even more uncommon.

Chronic Kidney Disease

There are few studies examining the use of bisphosphonates in patients with chronic kidney disease [48]. These medications are renally excreted, and they are not recommended for use in patients who have GFR of less than 30 ml/min. There can be deterioration in kidney function with use of IV zoledronic acid in those with CKD, so hydration prior to bisphosphonate infusion and close monitoring is recommended. Bisphosphonates should be avoided in patients with end-stage renal disease, especially those who have low bone turnover, as further suppression of bone turnover with bisphosphonates can be harmful.

Approach to Duration of Treatment

Patients on bisphosphonates should be reassessed for fracture risk after 3–5 years of bisphosphonate therapy (3 years for intravenous bisphosphonates and 5 years for oral bisphosphonates). If fracture risk is not high, they should be given a drug holiday of 1–5 years, because bisphosphonates stay in bone and may have a sustained effect on BMD and fracture risk [31]. Duration of drug holiday depends on the duration of prior therapy, as well as the type of medication used. Alendronate and zoledronic acid have a more sustained effect in bone, whereas risedronate may be associated with a faster loss of BMD after discontinuation. For patients at high fracture risk, they should not have a drug holiday; instead they should continue on drug therapy, either with an antiresorptive or an anabolic medication [31].

Denosumab

Denosumab, an inhibitor of RANK ligand, is a highly potent antiresorptive medication that has been shown in randomized controlled trials to be effective in increasing BMD and reducing risk of vertebral, hip, and nonvertebral fracture [49]. This medication, given as a subcutaneous injection once every 6 months, is generally well tolerated. In the FREEDOM study and the subsequent extension, the risk of serious adverse events was similar between placebo and denosumab-treated participants [50–52], although arthralgias were reported more frequently with denosumab (not statistically significant). Hypocalcemia is a risk with denosumab, particularly when used at high dose. There was initial concern about increased risk for infection, but further studies have not shown significant risk. Other uncommon adverse reactions reported with denosumab included hypertension, peripheral edema, skin rash [53], and risk of allergy or anaphylaxis. Although the FREEDOM trial did not report ONJ or AFFs, follow-up studies and post-marketing surveillance show that these long-term risks with antiresorptive therapy are a concern with denosumab.

Atypical Femur Fractures

AFFs have been reported in patients treated with denosumab. The underlying pathophysiology and risk factors are felt to be similar to AFFs occurring in patients treated with bisphosphonates [54, 55]. In the FREEDOM trial extension, the incidence of AFFs was low in those treated with long-term denosumab, with only one case seen in each group in the 10-year follow-up [52]. The true incidence of AFFs in patients on denosumab remains unclear, as many patients who develop AFFs while on denosumab have previously been treated with bisphosphonates. Nonetheless, AFFs have been reported in those taking denosumab who have had no prior treatment with other osteoporosis medications.

Management of AFFs in patients who have received denosumab is the same as with bisphosphonate therapy (see above), as outlined in the ASBMR task force report [22, 23]. Denosumab should be stopped if an AFF is identified, and should be avoided in any patient who has had an AFF. There may be a risk of bone loss with stopping denosumab, but transition to bisphosphonates for 6–12 months to avoid rapid bone loss is contraindicated in this situation.

Osteonecrosis of the Jaw

Osteonecrosis of the jaw was not seen in the original FREEDOM study, but in the long-term follow-up studies [50, 51] and in post-marketing surveillance, ONJ has been reported in those being treated with denosumab for osteoporosis.

ONJ, however, is much more common in patients treated with high-dose denosumab in the context of cancer treatment. In patients taking denosumab for osteoporosis, those who developed ONJ often had additional risk factors including prior bisphosphonate use, invasive dental procedures, glucocorticoid use, or chemotherapy. Invasive dental procedures should ideally be done before starting this medication, or 6 months after the last dose.

The management of ONJ associated with denosumab use involves the same measures as with bisphosphonate-related ONJ [37]. Patients who have had ONJ should not receive further denosumab.

Hypocalcemia

Denosumab is a rapidly acting and highly potent antiresorptive medication. There were no reports of serious hypocalcemia in the FREEDOM trial, but subsequently denosumab has been associated with severe and even life-threatening hypocalcemia. This is particularly in patients receiving high doses of denosumab for oncology indications. In patients treated with denosumab for osteoporosis, symptomatic hypocalcemia has been reported, but it is generally mild and transient [5]. Those with insufficient vitamin D levels or low bone turnover at baseline are at higher risk for developing hypocalcemia. Although denosumab is not renally excreted, and initial studies suggested that it was safe in those with chronic kidney disease, it should be used with caution in this population, as they are at higher risk for developing hypocalcemia. Serum calcium and 25-hydroxy vitamin D levels must be normal prior to initiating denosumab, and for those at higher risk for developing hypocalcemia, calcium should be monitored prior to each injection.

Infection

Denosumab is an inhibitor of RANK ligand, which is a member of the TNF family. There was initial concern about increased risk for developing infection when treated with this medication. Subsequent studies in patients with osteoporosis, as well as in patients taking glucocorticoids and receiving biologic or other immunosuppressive therapies, have not demonstrated a higher risk for infection [56]. The FREEDOM trial reported an increased risk of cellulitis in patients being treated with denosumab compared to placebo. Further data from the FREEDOM extension study did not show that this was an ongoing risk [50], and denosumab has subsequently been used in patients taking immunosuppressive drugs without significantly increased incidence of infection [56].

Risk with Stopping: Multiple Vertebral Compression Fractures

Denosumab causes potent suppression of bone turnover, but it is reversible and the effect wears off quickly after 6 months. Several case series of multiple vertebral compression fractures after discontinuation of denosumab therapy have drawn attention to this topic [57–59]. Those who have had prior vertebral fractures, greater total hip BMD loss while off treatment, and longer off-treatment duration are at increased risk for vertebral fractures after stopping treatment [60]. Prior bisphosphonate therapy does not offer protection against this rare phenomenon [61]. Because of these reports, the European Calcified Tissue Society (ECTS) have issued a position statement stating that discontinuation of denosumab should be followed by a bisphosphonate [62].

Approach to Duration of Therapy

Denosumab has been shown to reduce vertebral, nonvertebral, and hip fractures in postmenopausal women with osteoporosis [49]. Its effect on fracture reduction is sustained for up to 10 years of therapy, as seen in the FREEDOM extension study [51]. Thus, for patients at high risk of fractures, denosumab therapy should be continued. With treatment, patients may continue to gain BMD and reduce their fracture risk. If patients are no longer at high risk for fractures and discontinuation of therapy is being considered, discontinuation of denosumab should be followed by a bisphosphonate for 6–12 months to reduce the rapid rise in bone turnover, and thus reduce the risk of multiple vertebral fractures [62]. Oral bisphosphonates may be better at reducing bone loss than IV zoledronic acid [63, 64].

Hormone Replacement Therapy

Estrogen treatment, with or without progesterone, is effective in inhibiting bone resorption and maintaining bone formation consistent with a premenopausal state. In the Women's Health Initiative, estrogen significantly reduced the incidence of new vertebral, nonvertebral, and hip fractures in postmenopausal women, although that risk then increased when HRT was stopped [65]. The low-dose estrogens that are now in more common use have been shown to increase BMD, though fracture data are not available. Although HRT remains an effective treatment for osteoporosis, concerns about longer-term risks have led to HRT no longer being recommended as a first-line therapy for osteoporosis in the absence of significant menopausal symptoms, as safer and more effective alternatives are available [31].

In the Women's Health Initiative, systemic estrogen and progesterone therapy was associated with a statistically significant increase in risk for breast cancer, stroke, and thromboembolic events. Combined estrogen and progesterone therapy showed a higher risk of breast cancer than estrogen therapy alone [66]. The risk for coronary artery disease and cardiac events was not increased. Estrogen alone increases the risk for endometrial cancer, but this is not seen in those treated with combined estrogen and progestin.

Recent guidelines, including those from the National Institute for Health and Care Excellence (NICE) [67], point to the efficacy and safety of using estrogen in women around the time of menopause. HRT is recommended to relieve the symptoms of menopause; and these guidelines state that in otherwise healthy women less than 60 years old, the benefits of HRT outweighs the risks of therapy. The North American Menopause Society (NAMS) suggests using HRT for the shortest effective time period to manage menopausal symptoms, primarily because of the observed excess risk for breast cancer [68]. The NAMS guidelines recommend against prescribing hormone therapy for chronic disease prevention, but also acknowledge that extended dose hormone therapy might be appropriate in symptomatic postmenopausal women, or for the prevention of osteoporosis, if alternative therapies are not tolerated. Careful assessment of benefit and risk is important when considering the use of HRT for the management of osteoporosis.

Raloxifene

Raloxifene, a selective estrogen receptor modulator, inhibits bone resorption, increases BMD, and decreases the risk of vertebral fractures in postmenopausal women [69, 70]. It has not been shown to decrease nonvertebral or hip fractures. It is considered a second-line therapy for osteoporosis treatment in postmenopausal women in several clinical practice guidelines, as the fracture reduction data are not as robust as with other osteoporosis medications [31]. Long-term use of raloxifene has also been shown to decrease the risk of estrogen receptor positive breast cancer in women at high risk for developing breast cancer [71].

There is a risk for worsening of vasomotor symptoms in women taking raloxifene. Raloxifene significantly increases the risk of thromboembolic complications, similar to HRT, and should be avoided in those with a history of VTE [72, 73]. Raloxifene should be stopped during a period of expected immobilization such as surgery, or a long trans-Pacific flight. There is no cardiac protective effect with raloxifene, and in the Raloxifene Use for The Heart (RUTH) trial, there was an increased risk of stroke deaths in a population of women who were older and at higher risk for cardiovascular disease [74]. Raloxifene has not been associated with AFFs or ONJ [75], so this may be an option for management of osteoporosis in this context.

Bone Formation Therapies

Teriparatide

As opposed to the antiresorptive therapies, teriparatide is an anabolic medication that works primarily to increase bone formation, with a lesser effect on bone resorption especially initially. It is effective in reducing the risk of vertebral and

nonvertebral fractures in postmenopausal women, and in reducing vertebral fractures in those taking glucocorticoid therapy [76, 77]. Teriparatide is given as a self-administered daily injection. This medication is generally well tolerated, with similar incidence of serious adverse events compared with placebo in randomized controlled trials [5]. The most frequently reported symptoms include dizziness, postural hypotension, headache, nausea, and leg cramps. It is recommended that it is taken before bedtime because of risk of hypotension. As teriparatide is recombinant PTH, it should not be used in those with hyperparathyroidism. Transient hypercalcemia is seen in up to 11% of patients post dose. Persistent hypercalcemia is less common, and should be monitored for with blood tests measuring calcium 1 month after starting this medication. Some patients will require a reduction in calcium supplementation, and rarely a dose reduction in teriparatide. Hypercalciuria is seen in up to 10% of treated patients, with no increased risk of clinical adverse events in clinical trials.

Teriparatide is approved for 2 years of use because by that point the increase in bone resorption markers catch up to the increase in bone formation markers, usually considered as the closing of the therapeutic window. There was also concern regarding carcinogenic risk with long-term use. There is a black box warning, based on increased risk of osteosarcoma in rats that were treated with very high doses of teriparatide throughout their life span. In post-marketing surveillance, an increased risk of osteosarcoma has not been seen, despite widespread use of this medication. Nonetheless, it is recommended that teriparatide be avoided in patients at increased risk for developing osteosarcoma, including those with Paget's disease of the bone, prior radiation therapy, history of bone tumors, and in young people with open growth plates [5].

Abaloparatide

Abaloparatide, an analog of PTHrP, is an anabolic medication that has been shown to increase spine and hip BMD and reduce the risk of vertebral and nonvertebral fractures [78, 79]. This medication is approved for use for up to 2 years in the United States, though it is not yet available in Canada, Europe, or Asia. Orthostatic hypotension, dizziness, nausea, and headache were the most common adverse reactions seen in clinical trials. Hypercalcemia has been reported, though the incidence appears to be lower than with teriparatide. Increased uric acid and hypercalciuria were also reported.

Similar to teriparatide, abaloparatide has been associated with an increase in osteosarcoma in animal studies using large doses and long durations of treatment. Abaloparatide should, therefore, be avoided in patients at increased risk for osteosarcoma, as above.

Romosozumab

Romosozumab is a sclerostin inhibitor, and is a new class of bone formation therapy. It was recently approved in Japan, Thailand, the United States, and Canada for 12 months of use. In the placebo-controlled FRAME trial, romosozumab was found to reduce vertebral fractures and clinical fractures (nonvertebral plus symptomatic vertebral fractures) in postmenopausal women with osteoporosis [80]. There was one case of AFF and two cases of ONJ in the romosozumab group out of approximately 3300 women. In the head-to-head comparison of romosozumab and alendronate in the ARCH trial, romosozumab followed by alendronate significantly reduces vertebral, nonvertebral, clinical, and hip fractures when compared to alendronate alone [81]. There were cases of ONJ (one in each group of approximately 2000 women each) and AFF (two in the romosozumab to alendronate group and four in the alendronate to alendronate group) observed during the study period. There was also an imbalance of cardiovascular events with increased risks of myocardial infarction, stroke, and cardiovascular death observed in the romosozumab group. Thus, the Federation Drug Administration (FDA) in the United States recommends not giving romosozumab to patients who have had recent myocardial infarction or stroke in the pre-

ceding year. If a patient develops myocardial infarction or stroke during therapy, romosozumab should be discontinued as well.

Romosozumab is a reversible therapy. BMD gains with romosozumab can be lost with discontinuation of therapy. Thus, romosozumab should be followed by an antiresorptive therapy such as a bisphosphonate or denosumab.

Communication About Risks

Despite the safety considerations discussed in this chapter, it is important to emphasize that medications that are currently available for the treatment of osteoporosis and reduction in fracture risk remain effective and safe. There is evidence that the care gap in the treatment of osteoporosis is growing, and it is important that patients not be denied effective treatment for reduction of fractures [82]. Studies have demonstrated that appropriate use of osteoporosis medications in patients who are at high risk for fracture, if clinicians are mindful of issues around safety and duration of therapy, will minimize risk and maximize benefit.

Communicating risks and benefits appropriately to patients is critical in order to minimize the care gap in the treatment of osteoporosis. This can be successfully achieved by following the core principles of risk communication: anticipation, preparation, and practice [83]. As health care providers, we have to anticipate the concerns that our patients will have, know the data regarding the risks and benefits, and practice what we are going to say to our patients when they raise their concerns. It is important to remember that our patients want to know that we care, before they care about what we know. So it is important to show caring and empathy in our communication of risks and benefits. It is also helpful to put the risks and benefits into perspective, as patients often focus more on the negative rather than on the positive. For patients at high fracture risk, the benefits of therapy far outweigh the risks, and excellent communication can help ensure that they are treated with effective, evidence-based medical therapy for osteoporosis.

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Genetic Determinants and Pharmacogenetics of Osteoporosis and Osteoporotic Fracture

Yi-Hsiang Hsu, Xue Xu, and Sohyun Jeong

Key Points

- BMD is considered as one of the highly heritable disease-associated quantitative traits.
- GWAS have identified ~604 GWAS loci that are associated with bone-health-related phenotypes; including DXA-derived area BMD; qCT-derived volumetric BMD; estimated BMD (eBMD) and heel bone properties from heel quantitative ultrasound; bone geometry, shape, and microstructure; osteoporotic fractures and nonunion; pediatric bone density; serum vitamin D and other serum bone biomarkers.
- Since the majority of the GWAS findings are in the non-coding regions, as well as due to the linkage disequilibrium (LD) among SNPs, it is not always a trivial task to identify targeted genes affected by associated SNPs.
- Pharmacogenetic studies in the area of osteoporosis therapeutics remain quite preliminary. Additional efforts are needed to understand molecular mechanisms of drug action to their targets, to systematically identify genetic determinants of treatment response and to develop practical approaches to preemptive pharmacogenetics implementation toward clinical practice that include patients' genetic profiles especially for the severe adverse drug reactions (ADRs).
- With whole genome sequencing, undiscovered less-common, rare and private coding and non-coding variants, as well as structural variations that have larger effect sizes are likely important and may explain the remaining missing heritability that is not explained by current GWAS findings.

Overview

Osteoporosis affects more than 28 million people in the United States. The lifetime risk for osteoporosis-related morbidity is higher than a woman's combined risk for breast cancer, endometrial cancer, and ovarian cancer. Health care expenditures for osteoporotic patients in the United States are more than 13 billion dollars/year. Thus, identifying risks that are important to

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bone health will improve the understanding of the pathophysiology of osteoporosis and osteoporotic fractures. Among a few well-known risks of osteoporosis and osteoporotic fractures, family history is perhaps the strongest risk factor of osteoporosis, suggesting a strong genetic basis [1–3]. Estimated from twins and siblings, the narrow-sense heritability h^2 of bone mineral density (BMD) is >0.50 [1–3]. Heritability is a measure of how well differences in people's genes account for differences in their phenotypes in the study population. The narrow-sense heritability h^2 is the ratio of additive genetic variance to the total phenotypic variance. Heritability estimates range from zero to one. A wide range of h^2 ranging from 0.924 (autism) to 0.038 (lipoma) from 149 selected common/complex disorders/phenotypes has been reported recently [4]. Compared to these estimated h^2, BMD is considered as one of the highly heritable disease-associated quantitative traits.

In the past 10 years, studying of genetics of complex diseases has seen a meteoric rise in scope. In contrast to Mendelian diseases, usually caused by a mutation in a single gene, common diseases and disease-associated quantitative traits such as osteoporotic fractures and BMD do not segregate in a Mendelian manner within families and are influenced by multiple genetic and environmental factors [5]. The feasibility of carrying out genome-wide association studies (GWAS) on common variants has led to rapid progress in the field of complex-disease genetics; and provided valuable information about the contribution of genetic variants to phenotypes [6]. The design of a typical GWAS involves multiple stages, including (1) genome-wide discovery stage(s), which rely on association analyses of hundreds of thousands of genotyped SNPs or millions of imputed SNPs; (2) replication stage(s), which replicate top associated SNPs in an independent sample or samples; and (3) functional annotation and/or functional validation in cellular and/or animal models. The GWAS study designs and rationales have been reviewed previously [5]. Such study design provides robust association results and avoids potential false-positive findings. One of the major advantages is that GWAS approach interrogates the whole genome to search for associations without any prior assumptions about the underlying cause of a disease or the genetic architecture of a trait.

The objective of treating osteoporotic patients is to reduce the risk of fracture. The conventional treatment options for osteoporosis can be classified into two groups: anti-resorptives and anabolic drugs [7]. Although several osteoporotic medications have shown to be effective in reducing the risk of fracture in postmenopausal women and are recommended as the first-line therapies for patients with osteoporosis, they do not eliminate the possibility of fracture. The response to these anti-osteoporotic therapies is highly variable among individuals [8, 9], and such variability may also be partly determined by genetic factors. Pharmacogenetics is the study of the genetic variation between individuals that affects their response to drugs as well as treatment-related adverse effects, such as osteonecrosis of the jaw (ONJ) and atypical femoral fractures (AFF). Personal genetic profiles could help clinicians to predict individual drug response and prescribe the right drug and dose, thereby optimizing efficacy and avoiding risk of adverse effects. In this chapter, we review and discuss findings from GWAS along with pharmacogenetics studies of osteoporotic treatments.

Findings from GWAS

Previously, we and others have performed GWAS and GWAS meta-analyses on bone-health-related phenotypes; including DXA-derived area BMD [10–31]; qCT-derived volumetric BMD [32, 33]; estimated BMD (eBMD) [34–36] and heel bone properties [37–39] from heel quantitative ultrasound; bone geometry, shape, and microstructure [40, 41]; osteoporotic fractures and nonunion [42–45]; pediatric bone density [46–48]; serum vitamin D [49–53] and other serum bone biomarkers [54–57] (Fig. 25.1). A detailed summary of GWAS loci on bone-health-relevant phenotypes published before 2013 can be found in our previously published review article [5]. More than 5200 GWAS SNPs (p-values $<5 \times 10^{-8}$) genome-widely associated with bone-health-relevant phenotypes were discovered. These

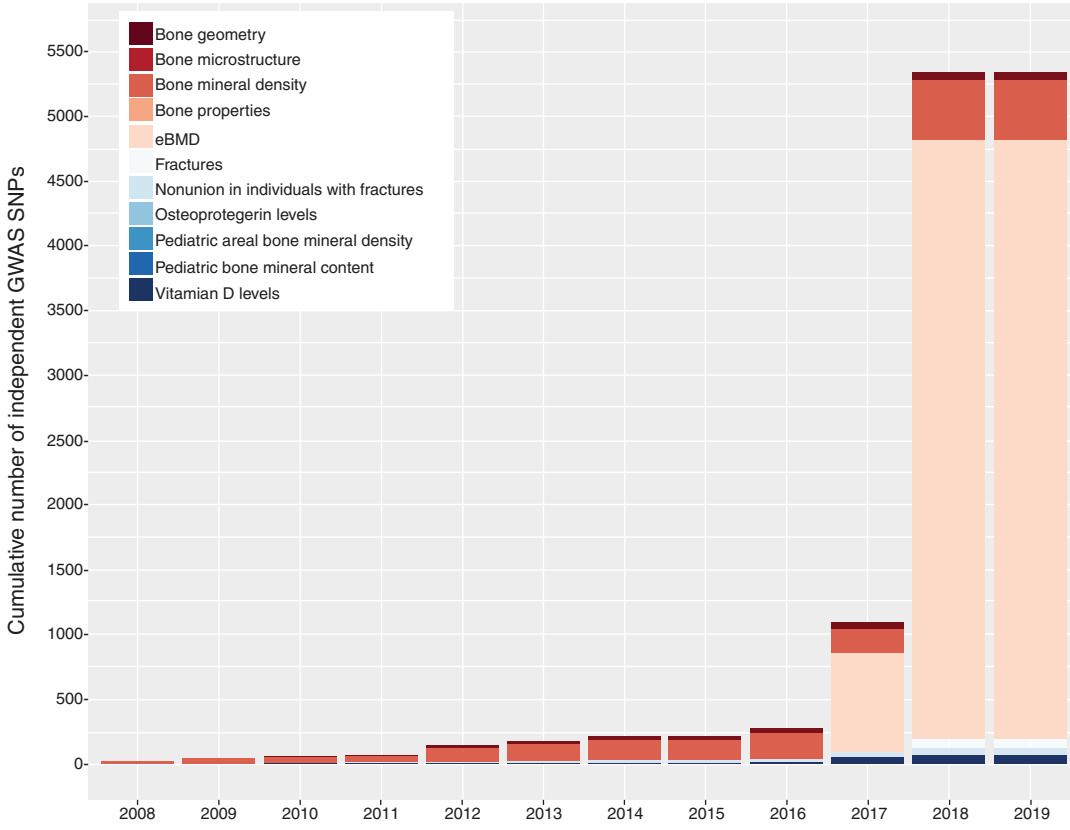

Fig. 25.1 Cumulative number of GWAS independent SNPs (association *p*-values $<5 \times 10^{-8}$) associated with skeletal relevant phenotypes

GWAS SNPs were further clustered into 604 independent GWAS signals (genomic regions). The mapped candidate genes (based on their physical location in the genome) for these 604 GWAS loci can be found at the NHGRI-EBI GWAS catalog (https://www.ebi.ac.uk/gwas). Among 604 GWAS signals, the association signals at the chr7q31.31 locus have been reported to be associated with DXA-derived BMD at multiple skeletal sites in adults and children; qCT-derived vBMD, eBMD from heel quantitative ultrasound as well as fractures. There are three potential candidate genes (*CPED1*, *WNT16*, and *FAM3C)* physically located in the chr7q31.31 locus; thus, we named this GWAS locus "*CPED1-WNT16-FAM3C*" locus. Most GWAS were conducted by analyzing lumbar spine (LS) and femur neck (FN) BMD derived from dual energy X-ray absorptiometry (DXA), which is typically measured in the clinic and is the most common tool for measuring BMD. So far, the largest GWAS analysis (published in 2015) on DXA-derived BMD involved ~33,236 adult Caucasians and only identified 59 GWAS loci [18]. Only a few BMD GWAS studies have been conducted in non-Caucasian participants with relatively smaller sample size. Choi et al. [23] studied the genetic variants that associated with LS and FN BMD in East Asians, and found a new BMD locus specific to East Asians at chr1q23 mapped to *UHMK1* gene. Taylor et al. [24] found another BMD locus specific to African-American women on *SVILA* gene ($n = 8155$). However, no independent replication was conducted.

In addition to LS and FN BMD, GWAS analyses on DXA-derived BMD at different skeletal sites have also been conducted. To identify genetic variants associated with forearm BMD, a GWAS meta-analysis with 5866 European descent Caucasians was conducted and identified genome-widely associated SNPs in the introns of *MEF2C* [25]. With a relatively larger sample size ($N = 8143$)

[18], we also identified a novel low-frequency non-coding variant with large effects on forearm BMD near *WNT16* gene (rs148771817, minor allele frequency (MAF) = 1.1%, effect size (β coefficient in the regression model) = 0.39 SD). The MAF is the distribution of the minor allele for a single nucleotide polymorphism in the study population. The MAF may be different across different ethnicities or race groups. Kemp et al. [26] found strong genetic correlations (r_g = 0.43~0.78) among upper limbs (UL-BMD), lower limbs (LL-BMD), and skull (SK-BMD) derived from total-body DXA in a GWAS analysis with ~4890 participants recruited by the Avon Longitudinal Study of Parents and their Children (ALSPAC). In particular, variants at the *CPED1* gene exerted a larger influence on SK-BMD and UL-BMD when compared to LL-BMD, while variants at the *WNT16* gene influenced UL-BMD to a greater degree when compared to SK- and LL-BMD. Medina-Gomez et al. [27] conducted a GWAS meta-analysis on total body (TB) BMD in 66,628 individuals stratified by five age strata, each spanning 15 years. A total of 76 GWAS loci were identified. Among them, 35 loci were not reported to be genome-widely associated with DXA-derived BMD before; but these loci were nominally associated ($p < 0.05$) with either LS or FN BMD in the same effect direction as in our previously published DXA-derived GWAS meta-analysis [18]. These TB BMD GWAS loci were enriched in osteoclast differentiation, TGF-β signaling pathway, regulation of cell growth, SMAD proteins, and musculoskeletal system relevant pathways.

Volumetric BMD (vBMD) derived from quantitative computed tomography (QCT) is a more accurate estimation of bone density and considered as the "true" bone density comparing to the bone density derived from DXA, which is a projective area bone density. QCT measures bone volume in three dimensions at any skeletal site and estimates bone density as the true volumetric bone density. We performed GWAS meta-analysis (n = 15,275) of lumbar spine vBMD and identified five GWAS loci associated with increased vertebral vBMD [33]. Among them, *SLC1A3* locus on chr5p13 associated with increased trabecular vBMD (effect size = 0.22), and lower risk of fracture (OR = 0.75). The *SLC1A3* locus is a novel GWAS locus that was not identified by DXA-derived area BMD before, suggesting genetic contribution of vBMD may not be comprehensively captured by DXA-derived area BMD. The functional involvement of the *SLC1A3* gene will need to be further studied.

eBMD DXA-derived area BMD or qCT-derived volumetric BMD are not always available in many large-scale cohort and observation studies, such as the UK Biobank. Instead, eBMD is an estimated BMD from heel quantitative ultrasound at the heel calcaneus. eBMD has been demonstrated as a strong predictor of fracture risks and contributes to risk assessment over and above DXA-derived BMD at the hip [58, 59]. eBMD is moderately correlated with DXA-derived BMD at the hip and spine (Pearson's correlation coefficient r = 0.4–0.6) [60]. In addition, these two phenotypes have moderate to high genetic concordances in terms of their genome-wide significant loci (r = 0.69 for lumbar spine and 0.64 for femoral neck) [34], suggesting that parts of underlying biological properties of these two traits may be shared. Thus, eBMD is considered as an alternative measurement of the DXA-derived BMD. As showed in Fig. 25.1, most bone-health-relevant GWAS loci (518 eBMD loci) were identified by two recent genome-wide association analyses on *eBMD* in ~426 K subjects from the UK Biobank [35, 36]. The mapped candidate genes for these 518 eBMD loci can be found at the NHGRI-EBI GWAS catalog (https://www.ebi.ac.uk/gwas/). Among these 518 eBMD GWAS loci, 13 of them are also genome-widely associated with bone fractures. These loci include *SLC8A1, FGFRL1, RSPO3, CCDC170, AQP1, STARD3NL, WNT16, MBL2, LRP5, TMEM135, SOST, FAM210A,* and *LINC01700* gene loci. As expected, a strong negative correlation ($r = -0.77$) was observed between effect size for eBMD (y-axis) and risks of fracture at all skeletal sites (x-axis) for SNPs that reached genome-wide significance for both eBMD and fracture phenotypes (Fig. 25.2) [32]. eBMD heritability, which is eBMD variance in study subjects that can be explained by GWAS SNPs, ranged from 20% to 40% in the UK Biobank study. Although 518 GWAS loci was identified for eBMD, these

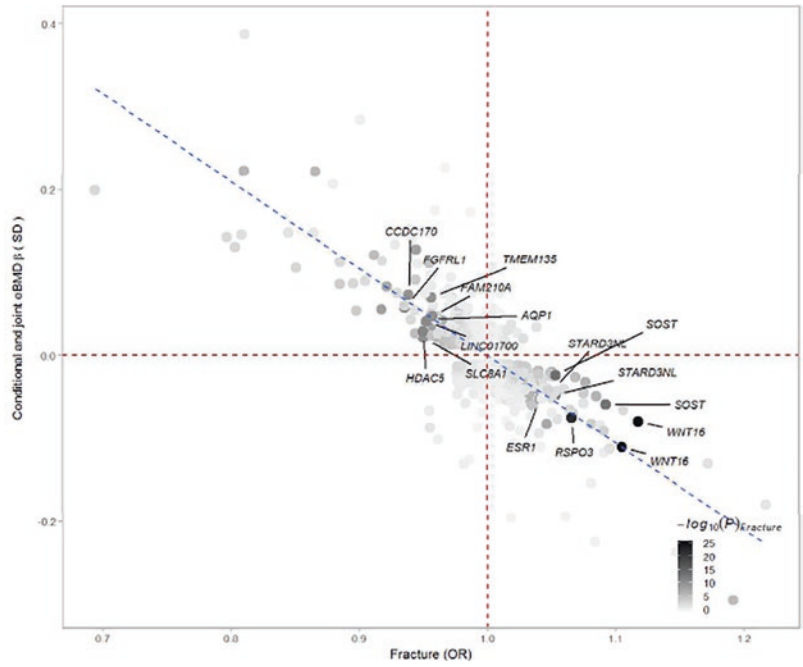

Fig. 25.2 Effect size for eBMD (y-axis) and fractures from the UK Biobank Study. For SNPs that are genome-widely associated with both eBMD and fracture at all sites phenotypes. (Reprinted from Morris et al. [36]. With permission from Springer Nature)

eBMD loci only explain less than half of eBMD variation, suggesting the effect size of these GWAS loci are moderate. As shown in Fig. 25.3, the average absolute conditional effect sizes for genome-wide associated SNPs with minor allele frequencies <1%, 1–5%, ≥ 5% were 0.14, 0.04, and 0.02 SD, respectively. Given DXA-derived BMD is being measured in the UK Biobank subjects, further analyses on comparing GWAS findings between these two phenotypes will better characterize the similarity and difference of their genetic architectures.

Heel bone properties In addition to eBMD, GWAS were also performed on speed of sound (SOS), broadband ultrasound attenuation (BUA), and stiffness index (SI) derived from heel quantitative ultrasound [37–39]. Among them, two studies were conducted in East Asian populations and identified *GLDN* GWAS locus in the Korean population (n = 9158) [38] as well as *CPED1-WNT16, SMG6,* and *LOC10050636-TMEM135* GWAS loci in the Taiwanese and Korean populations (n = 10,697) [39].

Bone geometry Characteristics of a bone's size and shape strongly influence its biomechanical strength and affect skeletal fragility. Bone geometry influences the ability of the bone to resist mechanical loads; and affects fracture risks. Baird et al. [40] identified eight novel loci of hip shape derived from DXA scans in 15,934 individuals. These loci included rs2158915 at 17q24.3, rs1243579 at 14q32.13, rs10743612 at 12p11.22, rs73197346 at 21q22.12, rs59341143 at 4p15.33, rs6537291 at 4q31.21, rs1966265 near *FGFR4* and rs1885245 near *ASTN2*. We performed GWAS on hip geometry [41], including femoral neck length, neck-shaft angle, femoral neck width, and femoral neck section modulus derived from DXA scans in 18,719 adult Caucasians. We identified *IRX1-ADAMTS16*, *FGFR4*, *CCDC91*, and *LRP5-PPP6R3* loci. These loci explained 12–22% of hip geometry heritability.

Fracture is the major consequence of osteoporosis. Although low BMD is the primary risk factor of osteoporotic fractures, studying genetic determinants of osteoporotic fractures itself may identify genetic risk factors that affect biological pathways that beyond bone density. Unlike BMD, the heritability of fracture risk is moderate with h^2 ~30% [61]. We recently reported the largest

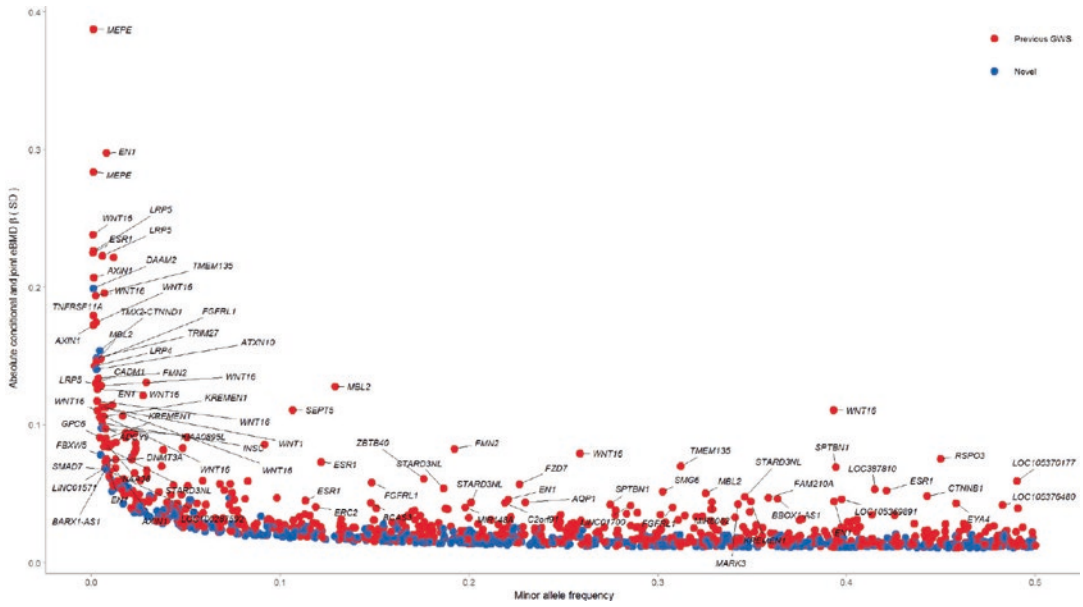

Fig. 25.3 Absolute effect size (y-axis) and minor allele frequency (x-axis) for 1103 eBMD GWAS SNPs. Red dots represent SNPs at previously reported DXA-derived LS or FN BMD loci. Blue dots represent SNPs at novel eBMD loci. The named gene is the mapped in that locus. (Reprinted from Morris et al. [36]. With permission from Springer Nature)

GWAS meta-analysis on fractures, including a discovery set of 37,857 fracture cases and 227,116 controls; with replication in up to 147,200 fracture cases and 150,085 controls [44]. Fracture cases were defined as individuals (>18 years old) who had fractures at any skeletal site confirmed by medical, radiological, or questionnaire reports. We identified 15 fracture GWAS loci, including *SPTBN1, CTNNB1, RSPO3, ESR1, WNT16, C7orf76-SHFM1, STARD3NL, FUBP3, RPS6KA5, MBL2-DKK1, LRP5, GRB10, SOST-DUSP3-MEOX1, FAM210A, RNMT* and *ETS2* gene loci. All 15 fracture GWAS loci have been reported to be associated with BMD from previous BMD GWAS studies. Alonso et al. [42] conducted a GWAS in 1553 postmenopausal women with clinical vertebral fractures and identified a novel locus near *FBLN7* gene at chr2q13. The *FBLN7* locus was associated with an increased risk of clinical vertebral fractures (OR = 1.75, 95%CI = 1.45–2.12, per risk allele) and was independent of bone density. Hwang et al. [43] conducted a GWAS meta-analysis on low-trauma fractures at hip, wrist, humerus, rib, pelvis, or vertebra skeletal sites in East Asian participants aged ≥40 years. The *MECOM* locus was identified and associated with an increased risk of osteoporotic fracture. Nonunion is a clinically significant complication of fracture. Nonunion, or delayed union, following fractures is a failure of healing to occur over the expected time course. Nonunion is considered common and estimated to occur in 5–10% of fractures [62]. McCoy Jr. et al. [45] conducted a GWAS on nonunion in 1760 individuals of Northern European ancestry with upper or lower extremity fracture, including 131 with nonunion, derived from electronic health records. The study identified one novel GWAS loci in the Calcyon (*CALY*) gene. However, independent replication is needed to further validate this finding.

Pediatric bone density The bone remodeling process occurs throughout life and becomes dominant by the time that bone reaches its peak mass (typically by the early 20s). Remodeling continues throughout life so that most of the adult skeleton is replaced about every 10 years. On the other hand, during childhood and adolescence bones are under the modeling process. This process allows individual bones to grow in size and to shift in space. To study genetic determinants of pediatric bone density, Chesi et al. [46] performed

GWAS on DXA-derived areal BMD and BMC at the distal radius in 1399 children. Two GWAS loci were identified, including the well-known *CPED1-WNT16-FAM3C* locus in females as well as a novel locus at chr9p21.3 with the nearest gene flanking each side being *MIR31HG* and *MTAP* genes. In addition, sex also plays an important role in determining loss of peak bone mass during lifetime (30–50% in women versus 20–30% in men) and osteoporotic risk. A follow-up study in the same study participants was conducted by Chesi et al. [48] and GWAS were performed on DXA-derived areal BMD and BMC at the LS and FN skeletal sites in 933 healthy Caucasian children ($N = 933$ for discovery GWAS and $N = 486$ for replication). They identified *IZUMO3 and RBFOX1* GWAS loci associated with LS BMD; and *TBPL2* GWAS locus associated with FN BMD. The *IZUMO3* GWAS locus was male-specific ($p = 1.2 \times 10^{-8}$) and no association with LS BMD was found in female ($p = 0.89$). A significant Het test ($p = 2.1 \times 10^{-4}$) suggests SNP-by-sex interaction and sex (male)-modified genetic effects to affect LS BMD. Thus, studying genetic determinants of the pediatric bone density may most likely identify bone modeling and bone growth genes. On the other hand, studying genetic determinants of bone density in adult may identify genetic determinants of bone remodeling and bone loss.

Vitamin D insufficiency, affecting approximately 40% of the general population in developed countries, is found to be associated with musculoskeletal consequences [63]. Although vitamin D may have beneficial effects on bone health, meta-analyses of vitamin D trials show no effects on bone density or fracture risk [64]. GWAS meta-analyses have identified several GWAS loci, including *CYP2R1, GC, NADSYN1/DHCR7, CYP24A1, SEC23A,* and *AMDHD1* loci that are associated with serum 25-hydroxyvitamin D concentrations in European populations [49, 50]. Sapkota et al. [52] reported *FOXA2* and *SSTR4* loci associated with serum vitamin D deficiency in 3538 South Asian Indians. Hong et al. [53] reported *KIF4B, ANO6/ARID2*, and *HTR2A* loci associated with 25-hydroxyvitamin D concentrations in 8541 African-Americans and 3485 Hispanic Americans. Given conflicting results reported regarding the beneficial effect of vitamin D intake and fracture risk, we performed a Mendelian randomization analysis using summary statistics from the largest fracture GWAS meta-analysis to inference causal relation between serum vitamin D level and fracture risk [44]. Mendelian randomization approach is a way to perform causal inference between "exposure" and "outcomes" via using genetic variants as instrumental variables. In this case, serum 25-hydroxyvitamin D concentration is the "exposure" and fracture risk is the "outcome." We utilized published GWAS loci and variants of serum 25-hydroxyvitamin D concentration as the "instrumental variables." As a positive control, Mendelian randomization analyses showed a clear effect of BMD on fracture risk. One SD decrease in genetically determined BMD of the femoral neck was associated with a 55% increase in fracture risk (OR = 1.55; 95%CI = 1.48~1.63; $P = 1.5 \times 10^{-68}$), suggesting robustness of such approach to inference causal relations. Mendelian randomization analyses agreed with the findings from clinical trials and showed no evidence for serum vitamin D's effect on fracture. Vitamin D levels assessed by use of 25- hydroxyvitamin D concentration variants were not linearly associated with increased fracture risk (OR = 0.84 per SD of vitamin D decreased, 95% CI = 0.70~1.02, $P = 0.07$). Given our Mendelian randomization work only examined a linear relation between vitamin D levels and fracture risk, further analyses are needed with a threshold dependent relation—that is, effects that could be present only at very low levels of vitamin D.

Functional Enrichment on GWAS Findings

Functional characterization of common variants linked to skeletal phenotypes remains a major challenge. To better characterize GWAS findings, we investigated functional enrichment of the mapped protein-coding genes in GWAS loci. *Gene* function information was obtained from gene ontology database (http://geneontology.org/). As shown in Table 25.1, GWAS mapped genes are enriched in key biological pathways of skeleton, including skeletal system development,

Table 25.1 The functional enrichment of the mapped GWAS genes

Gene Ontology (GO) Term	Genes	Count	FDR-P Value
Skeletal system development	*CYP24A1, LTBP3, MMP9, PRRX1, MMP2, CTNNB1, HOXD11, HOXC6, TNFRSF11B, HOXC9, TNFRSF11A, COL11A1, WWOX, GHR, IDUA, SATB2, TBX15, ARID5B, MGP, MEPE, MMP14, PTHLH, EYA1, NAB1, COL1A2, FOXC1, COL1A1, ALPL, ACHE, HOXA11, SOX5, SOX6, BCL2, PKD1, AXIN2, RUNX2, PAPSS2, BMP4, BMP2, TBX3, TBX4, DMP1, TGFBR2, SMAD3, EN1, GAS1, FRZB, HDAC4, SOST, TNFSF11, TRPS1, DLX5, ETS2, SP7, BMPR1B, BMP5*	56	6.83E-15
Positive regulation of macromolecule metabolic process-biosynthetic process	*DLC1, E2F1, MEF2C, EVX1, AURKAIP1, THRB, FOXK1, FOXA2, STAT5A, PPARG, FOXO1, GJA1, CTNNB1, TGFB2, ZBTB38, WNT1, SMARCD3, ATG7, PDGFC, RARB, YAP1, ITCH, SERTAD2, GHR, ANAPC1, IRS2, SATB2, ANAPC4, ESR1, ARID1A, ARID1B, MECOM, IRS1, ARHGEF11, OSM, PSMA1, CCND1, EYA1, VEGFA, FOXC1, SMARCAD1, GLIS2, CSF1, SOX5, SOX6, TCF7L2, TCF7L1, LIF, PSMB3, BCL2, NFAT5, CD4, TCF4, RUNX1, RUNX2, AXIN1, ZNF423, APC, BMP4, KLF6, BMP2, IKZF2, SMAD9, LMX1B, TBX3, KLF12, SMAD7, MAFB, CREBBP, SMAD3, TEAD1, CREB5, AFF1, ISL1, KAT5, HDAC5, HDAC4, PSMD13, CSRNP3, MEOX1, ETS2, EBF1, HIVEP3, MTOR, KLF2, NFIC, KLF4*	87	2.47E-09
Limb development and morphogenesis	*HOXA11, DICER1, PRRX1, HOXD11, CTNNB1, CYP26B1, FBXW4, FBN2, RARB, IDUA, TBX3, FTO, TBX4, EN1, MBNL1, GAS1, MECOM, DKK1, MEOX2, DLX5, PSEN2, LRP6, PTCH1, BMPR1B, LRP5*	25	3.82E-08
Wnt receptor signaling pathway	*WNT16, NDP, TCF7L2, TCF7L1, CTNNB1, WNT1, WNT4, MACF1, RSPO3, FBXW4, RSPO2, AXIN2, APC, AXIN1, TLE3, CELSR2, FRZB, FZD7, WNT2B, CCND1, WNT7B, DKK1, NXN, KREMEN1, SFRP4, LRP6, CSNK1G3, LRP5*	28	6.62E-08
Embryonic limb morphogenesis	*TBX3, HOXA11, DICER1, TBX4, FTO, PRRX1, EN1, GAS1, MBNL1, MECOM, CTNNB1, HOXD11, DKK1, DLX5, FBXW4, PSEN2, CYP26B1, LRP6, PTCH1, RARB, FBN2, LRP5*	22	3.41E-07
Regulation of RNA metabolic process	*MEF2C, THRB, STAT5A, RBM5, FOXO1, REST, HOXD11, CTNNB1, IGHMBP2, HOXC6, TBPL2, WNT1, FLI1, HOXC9, SMARCD3, RARB, SATB2, RREB1, MTA1, ZHX3, ARID1A, ARID1B, MECOM, FOXN3, RAB18, ZNF783, VEGFA, NFE2L1, ERC1, ZNF436, LITAF, HOXA11, SOX5, SOX6, MEIS1, LIF, FOXQ1, MUSK, TCF4, RUNX1, RUNX2, LHX9, BMP4, DNMT3A, KLF6, BMP2, SMAD9, IKZF2, KLF12, KLF9, SMAD7, MAFB, CREBBP, KLF11, TEAD1, SMAD3, EN1, CELSR2, KAT5, MED13L, HDAC5, HDAC4, TULP4, SEBOX, CSRNP3, ETS2, TRPS1, DLX5, EBF1, ATF7, JAZF1, RFX2, RBPJ, ZFHX3, KLF4, RERE, E2F1, PPARD, EVX1, FOXK1, FOXA2, PPARG, FOXK2, PRRX1, ZKSCAN5, ZBTB38, BARX1, MKX, YAP1, PDE8A, SERTAD2, TBX15, ARID5B, TLE3, ESR1, RUNX1T1, FOSB, MBNL1, SPEN, HMGA2, ZNF689, ARHGEF11, OSM, NAB1, FOXC1, ZNF484, SUPT3H, SBNO2, IRX5, IRX1, ZNF75A, NFIX, TCF7L2, TCF7L1, POLR2A, ZFP36L2, XBP1, NFAT5, PEX14, CHD4, NFATC1, ERG, FOXL1, TBX3, LMX1B, TBX4, TRIM27, CREB5, AFF1, ISL1, RPS6KA5, PKNOX2, MEOX2, MEOX1, ZNF460, SP7, NFIC, NFIA*	138	6.21E-07

Table 25.1 (continued)

Gene Ontology (GO) Term	Genes	Count	FDR-P Value
Positive regulation of transcription, DNA-dependent	*E2F1, MEF2C, EVX1, FOXA2, FOXK1, THRB, STAT5A, PPARG, FOXO1, CTNNB1, ZBTB38, WNT1, SMARCD3, YAP1, RARB, SERTAD2, SATB2, ARID1A, ARID1B, MECOM, ARHGEF11, OSM, VEGFA, FOXC1, SOX5, SOX6, TCF7L2, TCF7L1, LIF, NFAT5, RUNX1, TCF4, RUNX2, BMP4, KLF6, BMP2, IKZF2, TBX3, KLF12, LMX1B, MAFB, CREBBP, SMAD3, TEAD1, CREB5, AFF1, KAT5, ISL1, HDAC5, HDAC4, CSRNP3, MEOX1, ETS2, NFIC, KLF4*	55	7.22E-07
Cell fate commitment	*FGFR4, EVX1, FOXA2, HOXA11, PPARG, SOX5, JAG1, SOX6, TCF7L2, CTNNB1, HOXD11, TGFB2, WNT1, BCL2, CYP26B1, FRS2, RUNX2, BMP4, BMP2, GAS1, ISL1, KDR, EYA1, EYA2, SALL1, PSEN2, KLF4*	27	1.01E-06
Tube morphogenesis and development	*DLC1, FGFR4, FOXA2, HOXA11, DICER1, LGR4, HOXD11, CTNNB1, RGMA, WNT4, CD44, DHCR7, BCL2, HS6ST1, RARB, HHIP, BMP4, BMP2, TBX3, TBX4, TGFBR2, MGP, CELSR1, MMP14, BCL2L11, KDR, PTHLH, EYA1, SALL1, VEGFA, PSEN2, FOXC1, PTCH1*	33	8.16E-06
Skeletal system morphogenesis	*SATB2, TBX15, HOXA11, ARID5B, TBX4, TGFBR2, PRRX1, MGP, GAS1, MMP2, HOXD11, PTHLH, EYA1, HOXC9, NAB1, PKD1, COL1A1, BMPR1B, COL11A1, RUNX2, WWOX, IDUA, GHR*	23	8.35E-06
Ossification	*BMP4, CYP24A1, SATB2, BMP2, ACHE, DMP1, SMAD3, MGP, MMP14, MMP2, PTHLH, TNFRSF11A, TNFSF11, SOST, BCL2, NAB1, FOXC1, SP7, COL1A1, AXIN2, RUNX2, WWOX, BMP5*	23	1.40E-05
Cartilage development	*BMP4, SATB2, BMP2, HOXA11, PRRX1, SOX5, MGP, SOX6, HOXD11, PKD1, COL1A1, BMPR1B, COL11A1, RUNX2, BMP5, GHR*	16	1.57E-03
Response to hormone stimulus	*ALPL, ARSB, DHH, TACR3, STAT5A, IGFBP7, ADCY5, PPARG, FOXO1, PDE3B, AQP1, TGFB2, CPN1, CTNNB1, TNFRSF11B, PRKAR2A, BCL2, GNG7, GHR, BMP4, KCNMA1, IRS2, GNRH1, TGFBR2, ESR1, MGP, CRIPAK, MMP14, IRS1, HDAC5, GRB10, CCND1, ADCY9, MC4R, TGFBR3, PTCH1, MTOR, COL1A1, RGS9*	39	2.99E-03
Enzyme-linked receptor protein signaling pathway	*FGFR4, LTBP3, STAT5A, FOXO1, GREM2, TGFB2, LIF, MUSK, DGKD, CD4, PDGFC, NRG1, FRS2, GNG7, GHR, BMP4, PTPRJ, IRS2, BMP2, PTPRD, SMAD9, SMAD7, ARID5B, TGFBR2, AXL, SMAD3, IRS1, KDR, RPS6KA5, EPHA4, GRB10, VEGFA, COL1A2, SPTBN1, TGFBR3, FOXC1, BMPR1B*	37	3.83E-03
Osteoblast differentiation	*PTHLH, BMP4, CYP24A1, SATB2, ACHE, BMP2, SMAD3, SP7, COL1A1, RUNX2, WWOX*	11	2.36E-02

skeletal system morphogenesis, limb development and morphogenesis, embryonic limb morphogenesis, Wnt/β-catenin receptor signaling pathways, ossification, cartilage development, response to hormone stimulus, and osteoblast differentiation. In addition to the skeletal biology pathways, GWAS mapped genes are also enriched in positive regulation of macromolecule biosynthetic process, regulation of RNA metabolic process, DNA-dependent positive regulation of transcription, cell fate commitment, tube morphogenesis and development, and enzyme-linked receptor protein signaling pathway. Other biological functions, although not enriched in GWAS findings, may be specific to certain GWAS mapped genes. These functional pathways include

regulation of osteoclast differentiation (*CALCA, TNFSF11, CSF1, CTNNB1, APC* genes), regulation of chondrocyte differentiation, and endochondral bone morphogenesis (*PTHLH, BMP4, COL1A1, CTNNB1, GHR, HOXA11, HOXD11, NAB1, THRB, RUNX2*), response to steroid hormone stimulus (*ALPL, ARSB, BCL2, ESR1, PPARG, PTCH1, RGS9, TGFBR2, TGFB2*), regulation of mesenchymal cell proliferation (*IRS2, VEGFA, TGFBR2, PRRX1, GAS1, IRS1, KDR*), regulation of muscle development (*HDAC5, BMP4, HDAC4, MUSK, TBX3, BCL2, TGFBR2, SMAD3, NRG1, LUC7L, ZFHX3*), metanephros development (*BMP4, EYA1, BMP2, CD44, SALL1, BCL2, HOXA11, FOXC1, RARB, HOXD11*), cell-matrix adhesion (*DLC1, EPDR1, ITGA1, ITGB5, VTN, ITGB2, BCL2L11, CTNNB1, CD44, BCL2, FBLN5, PKD1, THBS3*), response to mechanical stimulus (*BMP4, BMP2, SLC1A3, TGFBR2, PKD1, MGP, PTCH1, COL1A1, MMP14, COL11A1*), pathway-restricted SMAD protein phosphorylation (*BMP2, SMAD7, TGFBR2, TGFBR3, TGFB2*), regulation of peptidyl-serine phosphorylation (*OSM, LIF, SMAD7, BCL2, AXIN1*) and carbohydrate homeostasis (*GCKR, PPARG, PDE3B, PTCH1, BAD, CACNA1C, IRS1, TCF7L2* genes). GWAS findings provide significant numbers of novel hypotheses and may elucidate the functional implications for skeletal metabolism that will bring new insights into skeletal biology.

Characterizing Novel Functions from GWAS Mapped Genes

GWAS identified genetic variants associated with bone-health-relevant phenotypes. Although, such approach provides an unbiased hypothesis-free approach to screen the genetic determinants of traits/phenotypes across the whole genome, the simple statistical signals do not provide the much-needed functional implication to predict the underlying biological processes involved in disease pathophysiology. Functional studies, including experiments in cellular and animal models will need to be conducted to further characterize the functional involvement of these associated variants and their targeted genes. Although 604 GWAS loci have been identified, only a handful of loci/genes have been characterized with regard to their functional impact on bone health. The limitations are due to low-throughput, time-consuming wet-lab experiments as well as the difficulty to identify targeted genes affected by associated variants. Since the majority of the GWAS findings are in the non-coding regions, as well as due to the linkage disequilibrium (LD) among SNPs, it is not always a trivial task to identify targeted genes affected by associated SNPs. The current approach to map candidate genes to GWAS loci is to map the nearest protein-coding gene near the most significantly associated SNP in each GWAS locus. Given there may be multiple independent associated SNPs in each GWAS locus as well as more than one potential biological candidate gene(s) in each GWAS locus, multiple protein-coding genes may be mapped as targeted candidate genes affected by associated SNPs in each GWAS locus.

In addition to well-known and well-studied genes functioning in the OPG/RANK/RANKL pathway and Wnt/β-catenin signaling pathways, the following are a few examples of potential novel biology discovered from GWAS studies with functional experiments.

EN1 encodes the engrailed homologs 1 and is one of the homeobox-containing genes. The human engrailed homologs 1 and 2 encode homeodomain-containing proteins and have been implicated in the control of pattern formation during development of the central nervous system. In addition, EN1 has been shown to be involved in Wnt signaling interaction with Dkk1 [65]. Studies of calvarial bone development and fracture healing of long bones in mice have shown that perinatal $En1^{-/-}$ mutants display osteopenia and enhanced skull bone resorption, whereas in normal adult mice En1 is up-regulated in the bone callus post fracture [66]. The *EN1* locus harbors multiple non-coding variants associated with both increased LS and FN BMD. The *EN1* locus is also associated with reduced fracture risks with ORs ranging from 0.85 to 0.98 per minor allele of different SNPs [18]. To investigate the functional role of *EN1* in

bone metabolism, we generated *En1* knockout mice [66]. Most $En1^{-/-}$ animals die soon after birth, we generated $En1^{Cre/flox}$ self-deleted En1 (sdEn1) conditional mutants. We found that mutants have lower trabecular bone volume fraction (BV/TV), trabecular number (Tb.N), and trabecular thickness (Tb.Th) estimated by μCT in both the lumbar L5 vertebrae (Fig. 25.4)

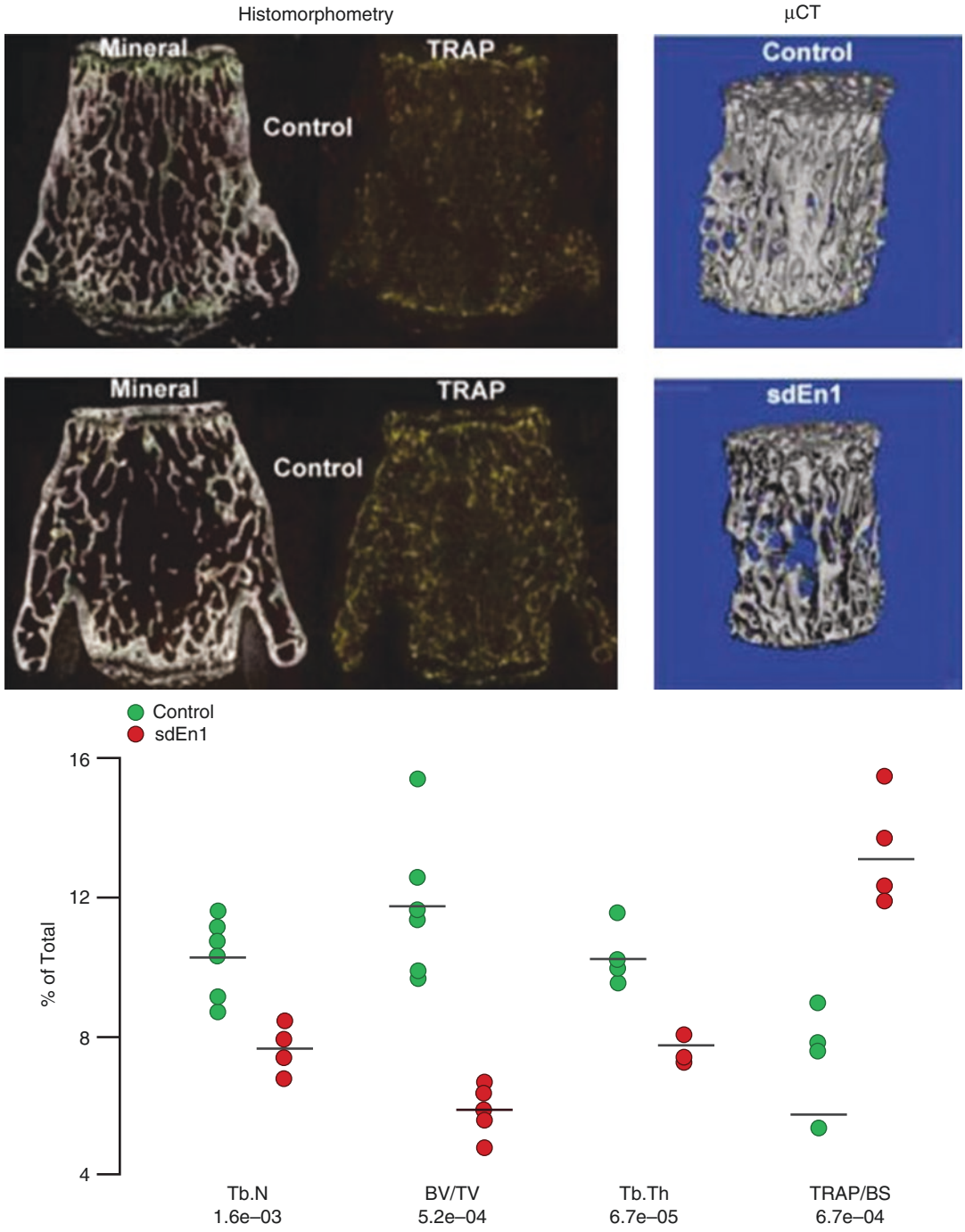

Fig. 25.4 Bone microarchitecture at lumbar L5 vertebrae estimated by μCT in the $En1^{Cre/flox}$ self-deleted En1 (sdEn1) conditional mutants. (Reprinted from Zheng et al. [18]. With permission from Springer Nature)

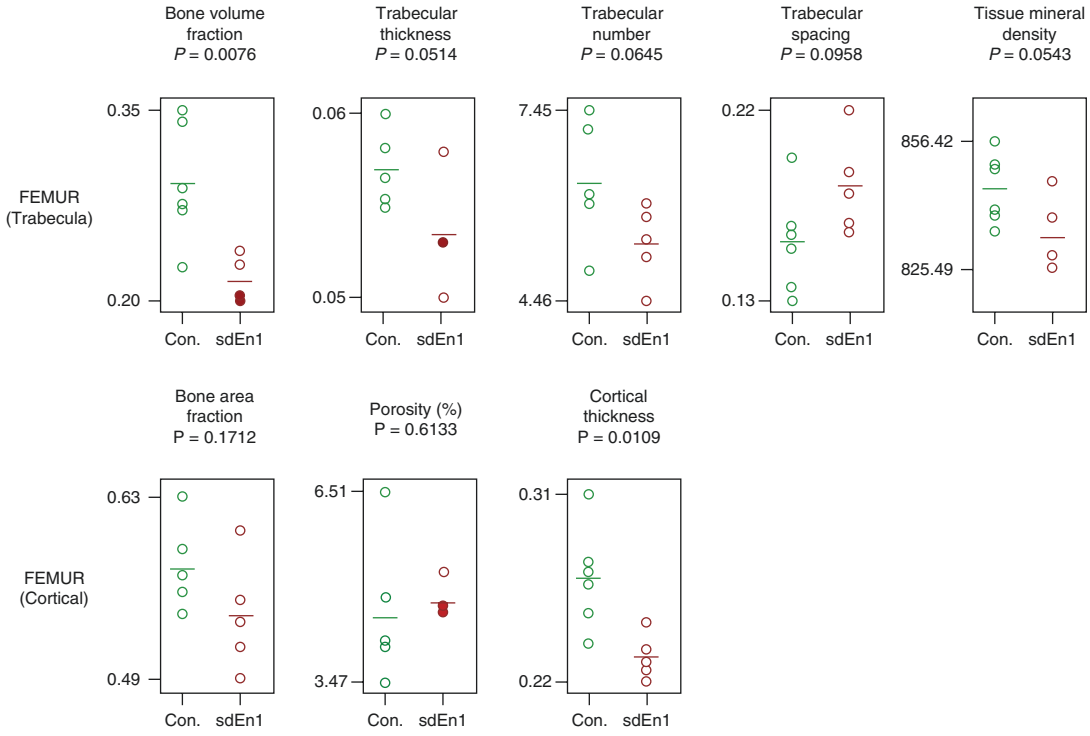

Fig. 25.5 Bone microarchitecture at femur estimated by μCT in the *En1*$^{Cre/flox}$ self-deleted En1 (sdEn1) conditional mutants. (Reprinted from Zheng et al. [18]. With permission from Springer Nature)

and the femur (Fig. 25.5) as compared to littermate controls. Histomorphometry images of L5 vertebrae (Fig. 25.4 *Left*) showed decreased trabecular bone volume and increased bone surface area occupied by osteoclast cells (TRAP/BS) when comparing *En1*$^{Cre/flox}$ mutants to the *En1*$^{flox/+}$ control mice. A decrease in femoral cortical thickness was also observed (Fig. 25.5). These findings suggest that *En1* plays an important role in bone physiology. We hypothesized the potential mechanism for the low bone mass might be an increase in osteoclastic activity induced by *En1* null osteogenic cells. This then initiated the expected coupled increase in mineralizing bone formation mediated by an increased number of osteogenic cells and thus conformed to a high-turnover, osteoporosis-like phenotype. Further experiments are needed to validate this hypothesis.

GPC6 encodes a member of the glycosylphosphatidylinositol-anchored, membrane-bound heparan sulfate proteoglycan protein family that is involved in cellular growth control and differentiation. The heparan sulfate proteoglycans attached to the GPC6 core protein regulate Wnt signaling pathways [67] and may involve in bone formation and mineralization. *Gpc6*$^{-/-}$mice had femurs and vertebrae that were shorter than the wild type. *Gpc6*$^{-/-}$ mice also had increased femoral bone mineral content and increased cortical thickness. The biomechanical consequence of these structural abnormalities was an increase in yield load, which reflects an increased material elasticity [34]. In addition, loss of function (LoF) mutations in *GPC6* result in omodysplasia-1 (MIM 258315), a rare autosomal recessive skeletal dysplasia characterized by short-limbed dwarfism with craniofacial dysmorphism, suggesting a role for *GPC6* in skeletal biology [68].

DAAM2 (dishevelled associated activator of morphogenesis 2) is a key regulator of the Wnt signaling pathway, which is required for various processes during development [69], such as

dorsal patterning, determination of left/right symmetry, or myelination in the central nervous system. DAAM2 acts downstream of Wnt ligands and upstream of beta-catenin (CTNNB1) and it is required for canonical Wnt signaling pathway during patterning in the dorsal spinal cord by promoting the aggregation of Disheveled (Dvl) complexes, thereby clustering and formation of Wnt receptor signalosomes and potentiating Wnt activity. CRISPR–Cas9-mediated knockouts of *DAAM2* in osteoblast cells lines resulted in a marked reduction in inducible mineralization [36]. Despite normal trabecular and cortical bone density as well as minimal changes in bone morphology and mineral content in *Daam2* KO mice, we found marked reduction in bone strength, especially increased porosity, suggesting that *Daam2* KO mice is not simply a result of abnormal bone turnover, but also a consequence of increased porosity and impaired bone composition and structure [36].

Pharmacogenetics of Osteoporosis Treatments

As shown in Table 25.2, current FDA-approved anti-resorptives include bisphosphonates (BPs) (e.g., alendronate, risedronate, ibandronate, and zoledronic acid), calcitonin, estrogen agonist/antagonist (raloxifene), estrogens and/or hormone therapy, tissue-selective estrogen complex (conjugated estrogens/bazedoxifene), parathyroid hormone 1–34(teriparatide, abaloparatide), and the receptor activator of nuclear factor kappa-B (RANK) ligand inhibitor denosumab [70, 71] that decreases bone resorption [72]. Anabolic agents, such as teriparatide and abaloparatide, are human parathyroid hormone analogs and promote the production of new bone [73]. Romosozumab, a newly approved monoclonal antibody, blocks the effects of sclerostin secreted by osteoclasts and works mainly by increasing new bone formation, which has been added to osteoporosis treatment options recently [74]. According to the 2016

Table 25.2 FDA-approved drugs for postmenopausal women and/or men at high risk of fracture with osteoporosis

Drug category	Drug name	Drug target	Target Gene	Drug Action
Bisphosphonates	Alendronate Risedronate Zoledronic acid Ibandronate	Farnesyl pyrophosphate synthase (FPS/FDPS)	*FDPS*	Inhibitor
	Zoledronic acid	Geranylgeranyl pyrophosphate synthase(GGPS)	*GGPS1*	Inhibitor
Selective estrogen receptor modulators (SERMs)	Raloxifene	Estrogen receptor alpha (ERα) Estrogen receptor beta (ERβ)	*ESR1* *ESR2*	Agonist
	Bazedoxifene			Agonist/antagonist
Estrogens	Estradiol (E2 or 17β-estradiol)	ERα, ERβ	*ESR1, ESR2*	Agonist
Calcitonin-like protein family	Calcitonin	Alpha-actinin-1	*ACTN1*	Incorporation into and destabilization
Parathyroid hormone (PTH)/parathyroid hormone–related protein (PTHRP) analogs	Teriparatide	PTH/PTHRP receptor	*PTH1R*	Agonist
	Abaloparatide		*PTH1R*	Agonist
Monoclonal antibody	Denosumab	Tumor necrosis factor(TNF) ligand superfamily member 11	*TNFSF11*	Neutralizing antibody
	Romosozumab	Sclerostin	*SOST*	Neutralizing antibody

Based on data from Refs. [75, 121]

American Association of Clinical Endocrinologists/American College of Endocrinology (AACE/ACE) guidelines, first-line treatments for postmenopausal osteoporosis patients at high risk of osteoporotic fracture include alendronate, risedronate, zoledronic acid, and denosumab. For those who cannot use oral therapies and are at high risk of fractures, use of teriparatide, denosumab, or zoledronic acid is recommended [75].

In the context of pharmacogenetics of osteoporosis treatments, dozens of association studies for anti-resorptives have been published, but no GWAS analysis is conducted so far. Most of these pharmacogenetics studies have investigated associations between drug response and well-studied candidate genes of BMD, for example, SNPs in *VDR*, *ERα*, *ERβ*, *COL1A1*, *OPG*, and *LRP5* genes in subjects received hormone replacement therapy (HRT) [76–83], SERMs [84, 85], or bisphosphonates [86–91]. HRT response was also studied in the Wnt/β-catenin signaling pathways, including L*RP5*, *FZD6*, *AXIN2, APC*, and *TCF1* genes [92]. The study characteristics and findings are summarized and described in Table 25.3. Overall, the genetic polymorphisms in *VDR* gene (TT genotype at the *TaqI* polymorphism) [80, 81], *ERα gene* (PP genotype at the *PvuII* polymorphism) [77–79, 82, 85], and *COL1A1 gene (*SS genotype at the *Sp1* polymorphism) [83, 88] contributed to increased response in terms of BMD increase after treatments; but *VDR gene (*bb genotype at the *BsmI* polymorphism) [86, 87] presented nonresponse to anti-resorptive treatments. On the other hand, pharmacogenetic studies on genes other than *VDR*, *ER α*, and *COL1A1* genes are controversial and need additional studies to replicate their findings.

Among several known adverse drug reactions (ADRs) of osteoporosis treatments, genetic associations were examined in rare but severe ADRs, including osteonecrosis of the jaw (ONJ) and atypical fracture of the femoral bone (AFF). These are well-known ADRs associated with long-term bisphosphonate use [93]. The incidence of ONJ in patients with osteoporosis treatment is low at 0.1%, while the incidence in cancer patients treated with high doses of intravenous bisphosphonates is much higher at 3–10% [94]. A few genes have been reported to be associated with higher risks of the ONJ. These genes are *CYP2C8* [95–97], *COL1A1* [98, 99], *RANK* [98, 99], *MMPs* [98–100], *OPG* [98, 99], *OPN* [98, 99], *FDPS* [101], *and RBMS3* [102]. With relatively small sample size and limited replications, these findings will need to be further validated [103].

AFF has been observed for subjects that received 4+ years of bisphosphonate treatment. The risk of developing AFF decreased rapidly after cessation of treatment [104–106]. Kharazmi et al. [107] conducted a GWAS analysis regarding bisphosphonate-associated AFF in 51 cases and population-based controls (n = 4891) and found no association with genome-wide significance. With small sample size and lack of statistical power, Kharamzi et al. concluded that their study found no evidence of a common genetic predisposition for bisphosphonate-associated atypical femoral fracture. Roca-Ayats et al. [108] performed whole-exome sequencing on six AFF (including one family with three sisters and three unrelated additional patients) all treated with BPs for more than 5 years and three controls (three subjects treated with BPs for more than 6 years but without AFFs). A total of 37 rare and potentially pathogenic variants (in 34 genes) shared by the three sisters and/or three unrelated additional patients, were identified, such as the p.Asp188Tyr mutation in the *GGPS1* gene; p.Arg98Trp and p.Ser216Cys in the *CYP1A1* gene, p.Arg97Gln in the *MVD* gene as well as rare coding mutations in *SYDE2, NGEF, COG4,* and *FN1* genes. The Asp188 in the *GGPS1* gene is located in an active site of the geranylgeranyl pyrophosphate (GGPP) synthase, which is involved in the binding of the substrate via a magnesium salt bridge. Functional validation found that p.Asp188Tyr markedly reduced GGPP synthase activity in shRNA-mediated knockdown of GGPS1 in both mouse calvarial and mouse macrophage cells lines [109]. Loss of GGPPS function resulted in defective osteoblast and osteoclast activity [109]. Thus, the p.Asp188Tyr mutation in the *GGPS1* gene may play a role in bone fragility in these patients, exacerbated by BP treatment.

Table 25.3 Pharmacogenetic efficacy studies on osteoporosis treatments

Author	Drug	Subjects (*n*, age)	Ethnicity	Gene/SNPs	Association to Drug Use
Kurabayashi et al. (1999) [80]	HRT	82 women, 40–64 years	Japanese (Asian)	*ERα; PvuII, XbaI, VDR; TaqI, ApaI, FokI*	TT genotype (*TaqI*); higher increase of spinal BMD
Salmén et al. (2000) [79]	HRT	331 PMO; 47–56 years	Finnish (Caucasian)	*ERα; PvuII*	P allele: protective against the risk of fracture
Giguère et al. (2000) [82]	HRT	425 PMO; 42–85 years	French-Canadian (Caucasian)	*VDR; BsmI* *ERα; PvuII*	Combined VDR-bb/ESR-PP; higher heel stiffness index (BMD)
Ongphiphadh-anakul et al. (2000) [78]	HRT	124 PMO; N/A	Thai (Asian)	*ERα; PvuII*	P allele: higher increase of spinal BMD
Kurabayashi et al. (2004) [81]	Longitudinal HRT (≥3 years)	81 women; 40–64 years	Japanese (Asian)	*ERα; PvuII, XbaI, VDR; TaqI, ApaI, FokI*	TT genotype (*TaqI*); Observed higher increase of spinal BMD at 1 year disappeared after longer than 2 years of treatment
Yahata et al. (2005) [76]	HRT	84 PMO; 40–64 years	Japanese (Asian)	*ERα; 18 intronic SNPs*	IVS6 + 14,144 GG genotype: higher increase of spinal BMD
Rapuri et al. (2006) [77]	HRT	489 PMO; 65–77 years	USA (NA)	*ERα; PvuII, XbaI*	PP (*PvuII*) and XX (*XbaI*) genotypes: higher increase of total body, spinal, and femoral BMD
Simsek et al. (2008) [83]	HRT	111 PMO; 46–54 years	Turkish (Caucasian)	*COL1A1; Sp1*	SS genotype: higher increase of spinal and femoral BMD
Kim et al. (2011) [92]	HRT	308 PMO 48–60 years	Korean (Asian)	Wnt signaling pathway gene; *LRP5,FZD6, AXIN2,APC,TCF 1*	*LRP5* c.266A > G and c.3893C > T: higher risk of non-response to HRT
Kim et al. (2016) [122]	HRT	509 PMO;	Korean	Period gene; SNPs in *PER1, PER2, PER3,* VNTR in *PER3*	*PER1* c.2884C > G; higher risk of non-response to HRT
Palomba et al. (2003) [84]	Raloxifene	75PMO; N/A	Italian (Caucasian)	*VDR; BsmI*	BB genotype: higher increase of spinal BMD
Heilberg et al. (2005) [85]	Raloxifene	28 PMO on hemodialysis; N/A	Brazilian (NA)	*ERα; PvuII, XbaI*	PP (*PvuII*) and XX (*XbaI*) genotypes: better lumbar spine BMD response
Palomba et al. (2005) [87]	Alendronate/HRT/ raloxifene	1100 PMO; N/A	Italian (Caucasian)	*VDR; BsmI*	bb genotype: lower increase of spinal BMD
Marc et al. (1999) [86]	Etidronate	24 PMO; 56–73 years	Slovenian (Caucasian)	*VDR; BsmI*	bb genotype: lower increase of spinal BMD

(continued)

Table 25.3 (continued)

Author	Drug	Subjects (*n*, age)	Ethnicity	Gene/SNPs	Association to Drug Use
Qureshi et al. (2002) [88]	Etidronate	136 peri-menopausal women; N/A	UK (N/A)	*COL1A1; Sp1*	SS genotype: higher increase of femoral BMD
Arko et al. (2002) [89]	Alendronate	79 PMO; 65.2 ± 6.7 years	Slovenian (Caucasian)	*ERβ; RsaI*	*RsaI*; No significant Association with BMD
Marini et al. (2008) [123]	Amino-bisphosphonate	234 PMO; 59.94 ± 7.44 years	Danish (Caucasian)	*FDPS rs2297480*	rs2297480 CC genotype; decreased response of bone turnover markers
Kruk et al. (2009) [90]	Risedronate	249 osteo-porotic men; 36–83 years	Mostly Caucasian	*LRP5; V667M and A1330V*	No significant association with BMD changes
Wang et al. (2009) [91]	Alendronate	80 PMO; 64.2 ± 7.7 years	Chinese (Asian)	*OPG; A163G, T245G, T950C*	Genotypes AA (A163G) and TT (T245G) show a better therapeutic response
Olmos et al. (2010) [124]	Alendronate, risedronate, raloxifene	191 PMO; 65 ± 8 years	Spaniards (Spanish)	*FDPS*	rs2297480 or rs11264359; associated with hip BMD change
Choi et al. (2010) [125]	Alendronate, risedronate	144 osteoporotic women; 61.5 ± 9.9 years	Korean (Asian)	*FDPS* *GGSP1*	*GGPS1 -8188A* ins/del; associated with femoral neck BMD change
Han et al. (2016) [126]	Alendronate	639 PMO; N/A	Chinese (Asian)	*6 tag SNPs of GGPPS*	No correlation was found

PMO: postmenopausal women with osteoporosis

Pharmacogenetic studies in the area of osteoporosis therapeutics remain quite preliminary. Additional efforts are needed to understand molecular mechanisms of drug action to their targets, to systematically identify genetic determinants of treatment response [110] and to develop practical approaches to preemptive pharmacogenetics implementation toward clinical practice that include patients' genetic profiles especially for the severe ADRs. These approaches could advance precision medicine for osteoporosis treatments and contribute to patients' quality of life.

Future Directions

The discovery of underlying genetic determinants will expand our understanding of biological mechanisms that control skeletal integrity. Furthermore, this work will create opportunities for novel diagnostics and drug targets to support a personalized approach to treating osteoporosis and prevent osteoporotic fracture [111]. With increased availability of genome-wide genotyping, sample size of GWAS analyses on bone-health-relevant phenotype increased dramatically. Figure 25.6 graphically displays this linear relationship between sample size and the number of identified independent GWAS associated SNPs. Thus, as the sample sizes grow, we expect to identify more GWAS associated SNPs. On the other hand, although GWAS have made strides in identifying loci that point to underlying disease pathophysiology, such GWAS approach has inherent limitations. To date, we have identified 604 loci associated with bone-health-relevant phenotypes, yet GWAS-identified loci explained only 20–40%, a small fraction, of the genetic variance of bone-health-relevant phenotypes. This is the so-called missing-heritability phenomenon [112, 113] and simply increasing sample size will not lead to the discovery of all the missing heritability by current GWAS approach, which is focused on common variants. Undiscovered, less common, rare and private coding and non-coding variants, as well as structural variations that have larger effect sizes are likely important; and may explain the remaining missing heritability. As an example, a small-scale whole-exome sequencing (WES) and whole-genome sequencing (WGS) among Icelandic people revealed loss of function mutations in *LGR4* [28], *COL1A2* [29], and *PTCH1* [30] associated with extreme low BMD. Although

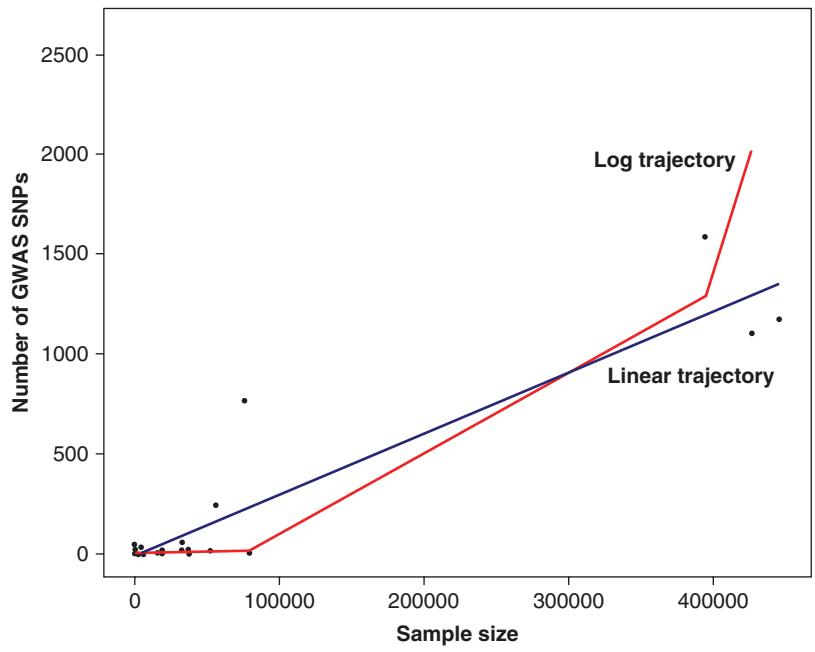

Fig. 25.6 Relation between sample size and number of identified independent GWAS associated SNPs (association p-values $<5 \times 10^{-8}$)

WES has identified functional coding variants for complex phenotypes, but less than 5% of trait-associated SNPs from GWAS lie in coding regions [114]. If this proportion is representative of the distribution of truly causal variants, WGS will be required to discover variants located in intronic, intergenic, and promoter regions. Recent studies [115–118] have established the potential for whole-genome sequencing (WGS) to provide comprehensive enumeration of sequence variation necessary for the detection of functional alleles that provide important clues to disease pathophysiology. The Trans-Omics for Precision Medicine (TOPMed) Project [119], sponsored by the National Institutes of Health's National Heart, Lung and Blood Institute (NHLBI), is the largest population-based whole-genome sequencing project for common/complex disorders/phenotypes. As of June 2019, over 120,000 deeply sequenced whole genomes have been done in subjects with different ethnic/ancestral genetic background. We and others are utilizing this resource to identify less common and rare variants that are associated with BMD and fracture phenotypes [120]. The TOPMed project is part of a broader Precision Medicine Initiative. In addition to whole-genome sequencing (WGS), the project is also measuring other omics, including metabolic profiles, protein and RNA expression pattern data with other phenomics, environmental, and clinical data. This resource will offer the opportunity to integrate different "omics" together to understand pathophysiology of diseases as well as develop better therapeutic approaches.

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