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Physiological Bone Remodeling: Systemic Regulation and Growth Factor Involvement

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Bone remodeling is essential for adult bone homeostasis. It comprises two phases: bone formation and resorption. The balance between the two phases is crucial for sustaining bone mass and systemic mineral homeostasis. This review highlights recent work on physiological bone remodeling and discusses our knowledge of how systemic and growth factors regulate this process.

The skeleton is an extremely specialized and dynamic organ that undergoes continuous regeneration. The process of bone modeling is responsible for the formation and maintenance of the shape of bone. Bone modeling leads to the acquisition of peak bone mass. It begins in fetal life and continues until skeletal maturity (i.e., epiphyseal plate closure or completion of longitudinal growth of the skeleton). Bone modeling occurs by removal of bone from one site and formation of bone at different sites. The bone regeneration process persists even after skeletal maturity by periodic replacement of old bone with newly formed bone at the same location, called remodeling. Bone remodeling is required for repair of old damaged bone due to daily physical load and prevention of aging effects and its consequences. Impairment in the bone remodeling process often results in the progression to osteoporosis, a major worldwide health concern. The overall process of bone remodeling is a tightly controlled and coordinated process regulated by a number of cell types. The maintenance of physiological bone remodeling and systemic mineral homeostasis requires balance between bone formation and bone resorption (121, 150). In this review, we discuss the recent observations of the role of systemic hormones and growth factor signals in regulation of bone remodeling.

Bone Remodeling

To accomplish normal physiological bone remodeling, the proper coupling of bone formation and bone resorption requires direct communication among different bone cells. Cells of the osteoblast lineage (osteoblasts, osteocytes, and bone-lining cells) and bone-resorbing cells (osteoclasts), together with their precursor cells, are organized in specialized units called bone multicellular units (BMU) (33). Osteoblasts originate from mesenchymal stem cells in the bone marrow stroma and are responsible for bone matrix synthesis and its subsequent mineralization. On the other hand, osteoclasts are large, multinucleated giant cells formed from the fusion of mononuclear progenitors of the monocyte/macrophage in a process termed osteoclastogenesis. The primary purpose of the BMU is to facilitate normal physiological bone remodeling.

There are many factors that can be involved in regulating the precise bone remodeling cycle (Table 1). The remodeling cycle is composed of seven sequential phases, namely, quiescence, activation, resorption, reversal, formation, mineralization, and termination. Activation precedes resorption, which precedes reversal, with termination as the last step (FIGURE 1).

Systemic Regulation of Bone Remodeling

The bone remodeling process is controlled by various local and systemic factors, and their expression and release, in a well organized manner. Calcitonin (CT), parathyroid hormone (PTH), vitamin D3 [1,25$(OH)_2$ vitamin D_3] and estrogen are the major hormonal regulators of osteoclastic bone resorption. Secretion of the first three is driven by the requirement to control the physiological serum calcium level. In addition to systemic hormonal regulation, it has become more and more apparent that growth factors such as IGFs, TGF-β, FGFs, EGF, WNTs, and BMPs play significant roles in regulation of physiological bone remodeling (FIGURE 2). Because of our accumulated and recent knowledge of the critical function of these growth factors in the skeleton, we have been able to turn from classical treatment of skeletal diseases with hormones to manipulation of some of the growth factor pathways to treat diseases such as osteoporosis.

PTH and PTHrP

PTH is a hormone synthesized and secreted by the parathyroid glands. The main function of PTH is to maintain blood calcium homeostasis. In addition, PTH regulates bone mass in an endocrine manner (52). PTH-related peptide (PTHrP) is a genetically

Table 1. *Hormones and factors involved in BMU remodeling sequence*

Remodeling Phases	Hormones and Factors Regulating Bone Remodeling
Activation phase	+, PTH, IGF-1, IL-1, IL-6, PGE_2, Calcitriol, TNF-α −, Estrogen
Osteoclast recruitment and resorption phase	+, RANKL, M-CSF, αvβ3 integrins, IL-1β, IL-1α, TNF-α, retinoic acid, S1P −, OPG, GM-CSF, estrogen, Calcitonin, IL-4, IL-18, TGF-β
Reversal phase	TGF-β, IGF-1, IGF-2, BMPs, PDGF, or FGF
Osteoblast recruitment and formation phase	+, WNTs, BMPs, IGF-1, FGF-2, FGF-18, PDGFs, PTH, $1,25(OH)_2$ vitamin D_3, Runx2, TGF-β, CT-1, ephrin B2 −, PDGFs, glucocorticoids, leptin, pyrophosphate, Sema4D
Termination phase	Feedback signal of normal sclerostin expression

S1P, sphingosine-1-phosphate; CT-1, cardiotrophin-1; Sema4D, semaphorin 4D.

related peptide that can mimic several functions of PTH. While PTH and PTHrP share nearly 70% of homology within the first 13 residues of their amino-terminal domain, the domain essential for their bioactivity (47, 99), cross talk between COOH and NH_2 domains of PTHrP have been reported to trigger signal transduction in mesenchymal stem cells and osteoblast-like cells (23, 60, 177). In contrast to PTH, PTHrP is expressed in almost all tissues and exerts its effect in an autocrine/paracrine manner. PTH1R is the common receptor for both PTH and PTHrP, and is expressed in bone and cartilage cells of the mesenchymal lineage (72, 112, 193). PTHrP regulates the proliferation and differentiation of growth plate chondrocytes, and PTHrP-null mice are characterized by the shortening of the growth plate due to accelerated chondrocyte maturation (100). Targeted disruption of PTHrP in embryonic mice is lethal and causes impaired endochondral bone development (74, 96). PTH1R-null mice ex-

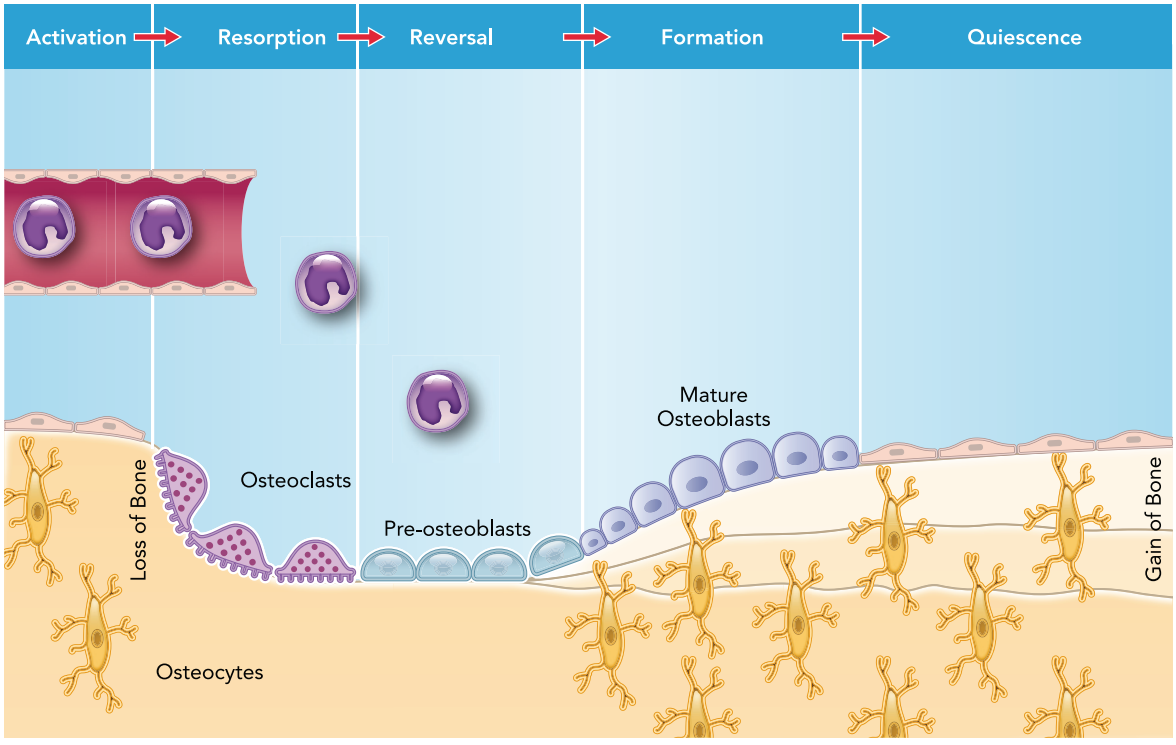


FIGURE 1. **Physiological bone remodeling**
Bone remodeling follows coordination of distinct and sequential phases of the process. The remodeling cycle is composed of six sequential phases, namely, quiescence, activation, resorption, reversal, formation, and termination. Activation precedes resorption, which precedes reversal, with mineralization as the last step. The first stage of bone remodeling involves detection of an initiating remodeling signal, which has usually been described as resorption by osteoclasts. In the resorption phase, osteoblasts respond to signals generated by osteocytes or direct endocrine activation signals, recruiting osteoclast precursors to the remodeling site. The resorption phase is of limited duration depending on level of the stimuli responsible for osteoclast differentiation and activity. It is followed by the reversal phase that is characterized by disappearance of almost all osteoclasts. The formation phase is distinct by complete replacement of osteoclastic cells with osteoblastic cells. The termination signals of bone remodeling include the terminal differentiation of the osteoblast. The resting bone surface environment is maintained until the next wave of remodeling is initiated.

hibit a similar skeletal phenotype. Apart from articular chondrocytes, PTHrP is also expressed by early osteoblastic lineage cells, suggesting a role for PTHrP in regulation of cells in bone (75). PTHrP-overexpressing mice have shorter and thicker limbs. Furthermore, both PTHrP heterozygous-null mice and osteoblast-specific PTHrP knockout mice have decreased bone volume and defective

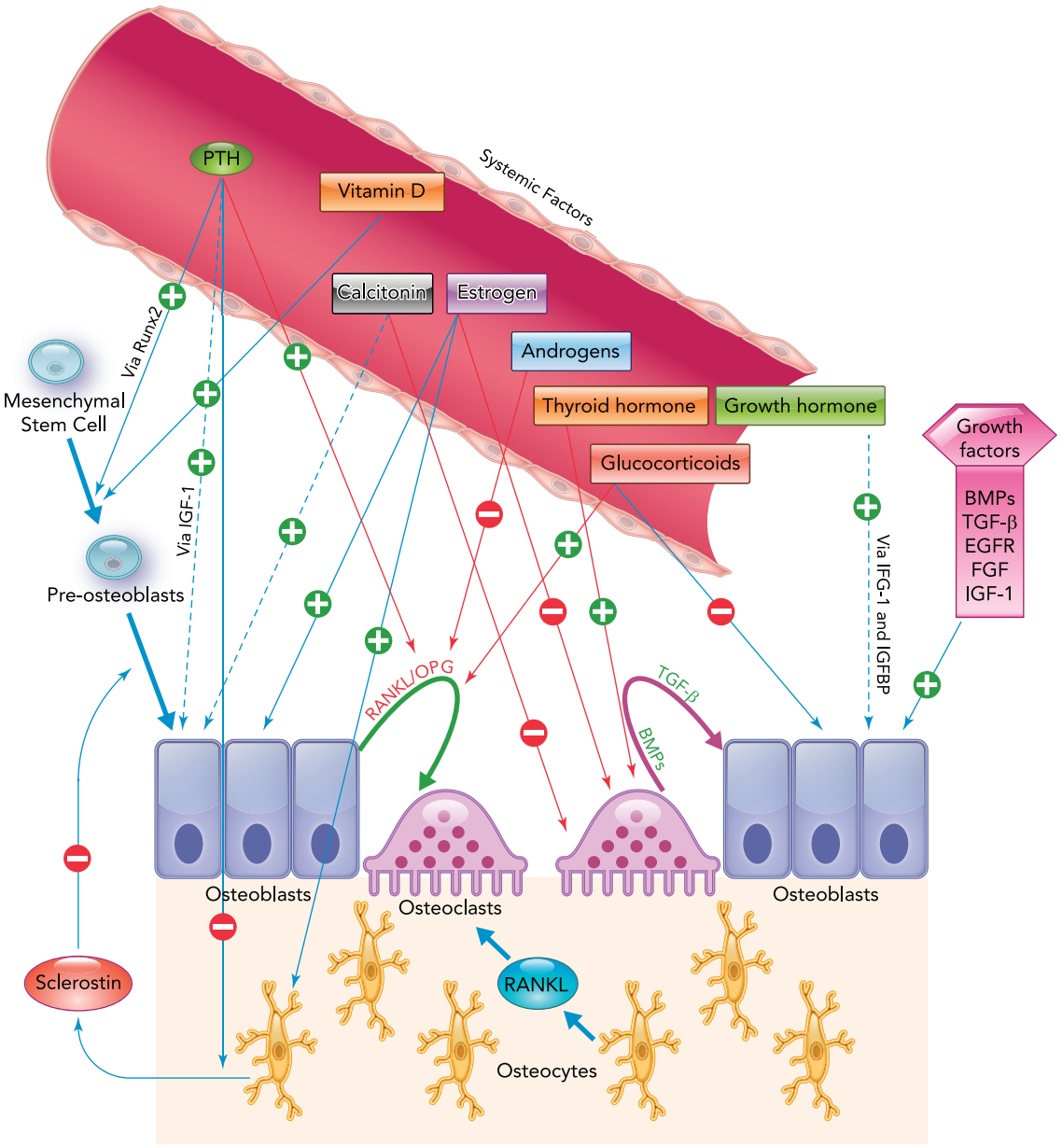


FIGURE 2. **Systemic and growth factor regulation of bone remodeling**
PTH induces differentiation of committed osteoblast precursors, induces RUNX2 expression in osteoblasts, increases osteoblast numbers, and extends osteoblast survival. PTH stimulates the proliferation and differentiation of osteoprogenitors to mature osteoblasts via IGF-1. Along with IGF-I, PTH induces RANKL and MCSF from mature osteoblasts to promote osteoclastogenesis. PTH elevates cAMP levels and inhibits Mef2-stimulated Sost promoter activity, leading to decreased expression of sclerostin and an elevated bone formation rate. $1,25(OH)_2D_3$ stimulates osteoblastogenesis via differentiation of mesenchymal stem cells (MSCs) to osteoblasts. Calcitonin increases osteoblast proliferation and suppresses bone resorption by inhibiting the activity of osteoclasts. Estrogen inhibits bone resorption by directly inducing apoptosis of the bone-resorbing osteoclasts. Androgens can also indirectly inhibit osteoclast activity and bone resorption via effects on osteoblasts/osteocytes and the RANKL/RANK/OPG system. Besides systemic hormonal regulation, other growth factors, such as IGFs, TGF-β, FGFs, EGF, WNTs, and BMPs, also play a significant role in regulation of physiological bone remodeling.

bone, suggesting the anabolic role of PTHrP in bone formation and leading to testing PTHrP analogs for treatment of osteoporosis (103, 124).

PTH can exert both catabolic and anabolic effects on bone (37). It is well established that daily injections of low doses of PTH increase bone mass in animals and humans (36, 127). PTH has no direct effect on osteoclasts. Continuous administration of PTH or PTHrP induces bone resorption indirectly through their actions on osteoblastic cells (151, 176). Several effects of PTH on induction of osteoclast formation are mediated through the osteoblast by stimulation of RANKL and inhibition of OPG mRNA expression (105). It is well recognized that constant high levels of PTH facilitate bone resorption, whereas low and intermittent doses of PTH cause an increase in bone mineral density at the lumbar spine and hip, and promote new bone. PTH's anabolic actions are thought to be via its effects to increase the proliferation and differentiation of osteoblasts in vitro and in vivo (43, 90, 137, 163, 172), decrease osteoblast apoptosis (70, 71, 116), and activate bone-lining cells (38, 102).

A number of investigations into the mechanisms of PTH's anabolic effects have elucidated the role of several growth factor and chemokine pathways. For instance, PTH induces the synthesis of IGF-1 to stimulate osteoblast proliferation and differentiation as well as indirectly stimulate osteoclast activity (17, 111, 181). It was recently shown that PTH-mediated new bone formation is attenuated by Dkk1, an inhibitor of Wnt signaling showing the role of the Wnt pathway in PTH action (57). In addition, activation of the PTH receptor in T lymphocytes has a role in PTH-induced bone formation by increasing Wnt10b synthesis by these cells (11). Mice expressing a constitutively active PTH/PTH-related peptide (PTHrP) receptor (PPR) in osteocytes exhibit increased bone mass and bone remodeling, which is sufficient for inhibiting SOST expression (another Wnt pathway inhibitor) through LRP5-dependent and -independent mechanisms (12, 141, 158). These are some of the many studies showing cross talk between PTH and Wnt signaling is an important mechanism regulating bone formation. Finally, PTH-induced osteoblastic expression of MCP-1 leads to recruitment and differentiation of osteoclast precursors and is required for enhanced bone formation (106, 174). These data show a role for MCP-1 in the anabolic effects of PTH. Thus the complexity and knowledge of PTH and PTHrP regulation of the skeleton has opened up opportunities for new treatment modalities.

1,25(OH)$_2$ Vitamin D$_3$

Vitamin D is essential for normal development and maintenance of the skeleton. Active 1α,25(OH)$_2$D$_3$ plays a central role in calcium and bone homeostasis through binding to the vitamin D receptor (VDR) present mainly in intestine, bone, kidney, and parathyroid gland. VDR knockout mice are phenotypically very similar to those with vitamin D deficiency (108, 194). In contrast to its classical endocrine role, there is accumulating evidence for an autocrine role for vitamin D in bone (5, 6). The enzyme CYP27B1, which converts 25D to the active form 1,25D, promotes osteoblast proliferation and maturation in human osteoblasts in vitro. It has been shown that the expression of *CYP27B1* is dependent on the stage of osteoblast maturation, and expression in vitro is maximal immediately before mineralization reaches its peak (7). Gene deletion of *CYP27B1* in mice caused osteomalacic abnormalities thought to be due largely to hypocalcaemia and hyperparathyroidism as a result lead to cortical porosity (149). In addition, recently it has been shown that 25D metabolism in osteoclast lineage cells appears to promote osteoclast differentiation, ameliorate osteoclast activity, and promote coupling of bone resorption to formation (86, 87). In contrast, chronic 1,25(OH)$_2$D$_3$ treatment causes the OPG gene to become insensitive to repression, thereby facilitating the anabolic phase of the vitamin D effect on bone (89).

It has been recently shown that 1,25(OH)$_2$D$_3$ treatment enhanced mineralization of the osteoblast and expression of osteocyte markers (dentin matrix protein-1 and FGF-23) in induced pluripotent stem (iPS)-derived osteoprogenitors (iPSop cells), suggesting that 1,25(OH)$_2$D$_3$ is a potent promoter of the osteoblast-osteocyte transition (77). In support of this notion, there is also evidence for presence of VDR transcripts in osteocytes and direct activation of 25(OH)D$_3$ in osteocytes (34, 133).

In summary, 1,25(OH)$_2$ vitamin D$_3$ appears to have both endocrine and autocrine actions in bone.

Calcitonin

Calcitonin (CT) is a 32-amino acid hormone that is secreted by the thyroid C cells. Tissue-specific alternative splicing of the calcitonin gene results in the production of another peptide, calcitonin gene-related peptide-1 (CGRP-1). Most of the effects of both peptides are exerted by binding to the calcitonin receptor (CTR) and calcitonin receptor-like receptor, members of the family of GPCRs that are coupled to both PKA and PKC via Gs and Gq, respectively (44, 67, 109). CT mediates its actions through the CTR expressed on osteoclasts (65). Osteoclast-specific deletion of the CTR increased

bone formation by inhibiting the release of sphingosine 1-phosphate (S1P) from osteoclasts (82). CT inhibits both basal and stimulated resorption via directly causing a loss of the ruffled border of osteoclasts and reducing osteoclast numbers over time (27, 28).

Although calcitonin receptors are thought not to be present on osteoblast-like cells, CT is reported to interact with osteoblasts in some systems. CT has been reported to enhance osteoinduction by rhBMP-2 on osteoblasts (144). CT also increases the extracellular level of insulin-like growth factors (IGF-1 and IGF-2) in cultures of human osteoblast-like cells (42). In addition, CT may also prevent osteoblast and osteocyte apoptosis (155). Despite these reports, any effect of CT on osteoblasts remains unclear. Evidence suggests that osteocytes can also express the CTR (54, 148), and its expression declines with age (53). CT mainly acts to complement the function of PTH by counteracting the increased bone resorption caused by PTH (26). Yet, these recent observations may change our view of CT.

Estrogen

Estrogen is the one of the major hormonal regulators of bone metabolism in both women and men. Estrogens attenuate osteoclastogenesis and stimulate osteoclast apoptosis. However, its regulation of bone metabolism is complex. Female mice in which the ERα was specifically deleted in mature osteoclasts using the cathepsin K promoter exhibited decreased bone volume due to an increase in the number of osteoclasts, whereas deletion of ERα in male mice does not alter bone mass. Female mice with ERα deletion in osteoblasts had decreased cancellous (proximal tibia, vertebra, and distal femur) and cortical (tibial midshaft and L5 vertebral cortex) bone mass due to a reduction in osteoblast activity rather than osteoclast number, indicating that ERα in osteoblasts is required for appropriate bone mass (123). Mice with deletion of ERα in mesenchymal progenitors have decreased proliferation and differentiation of periosteal cells due to decreased canonical Wnt signaling, which results in low cortical bone mass (3).

In osteoclasts, estrogen blocks RANKL/M-CSF-induced activator protein-1-dependent transcription through a reduction of c-Jun activity, thus suppressing RANKL-induced osteoclast differentiation (165). In addition, estrogen has also been shown to modulate the production of a number of bone-resorbing cytokines, including IL-1, IL-6, TNF-α, M-CSF, and prostaglandins (4, 85, 117, 145). T cells are an essential mediator of the bone-wasting effects of estrogen deficiency in vivo, and estrogen prevents osteoclastic bone resorption through suppressing the production of TNF-α from T cells (25). Furthermore, estrogen has been shown to inhibit osteoblast apoptosis and to increase osteoblast lifespan via activation of the Src/Shc/ERK signaling pathway (91) and down-regulation of JNK (92).

Taken together, estrogen modulates both osteoclast and osteoblast activities, which subsequently lead to inhibition of bone remodeling, decreased bone resorption, and maintenance of bone formation. Estrogens regulate cortical and trabecular bone mass via osteoblast progenitors and osteoclasts, respectively (3, 118).

Androgens

Estrogen is a well known and intensely studied hormonal regulator of postmenopausal bone health in women. However, androgens also have several beneficial effects on development and maintenance of bone mass in both men and women. Androgen deficiency results in increased bone remodeling and bone loss in men (79). However, some part of the bone loss is due to reduced levels of estrogen, which is derived from aromatization of testosterone (41). Androgens can modulate growth plate maturation and closure, and thus affect longitudinal bone growth. In addition, androgens regulate trabecular and cortical bone mass, and inhibit bone loss (184).

Global deletion of the AR in male mice resulted in high bone turnover, increased resorption, decreased trabecular and cortical bone volume, as well as decreased periosteal bone formation (80). In contrast, mice with conditional deletion of the AR in osteoblasts and osteocytes have normal cortical bone, showing that androgens modulate periosteal bone formation without direct involvement of the mature osteoblast lineage (31, 140, 169). Probably, these effects might result from improvement in physical activity due to stimulatory effects of androgen on muscle mass and strength, which leads to activation of bone-forming sites and stimulation of osteoprogenitors. In contrast, these mice show decreased trabecular bone mass, indicating a role for the AR in trabecular osteoblastic cells. The mechanisms responsible for this positive effect of the AR on trabecular bone mass remain elusive (31, 140, 169).

Recent evidence suggests that androgens can indirectly inhibit osteoclast activity and bone resorption via effects on osteoblasts/osteocytes and the RANKL/RANK/OPG system (46). Androgen increases collagen, type 1 protein and mRNA levels, osteocalcin secretion, and alkaline phosphatase activity in transformed clonal human osteoblastic cells, which leads to their increased differentiation and mineralization (15, 76). As well, suppression of either testosterone or estradiol increases the PTH bone-resorbing effects in men (104), showing the

interplay between the sex steroids and the parathyroid axis. In conclusion, androgens directly and indirectly modulate osteoblast and osteoclast activities, respectively, and may maintain bone mass through both the AR and ERα. Further studies are needed to delineate the role of androgens on the skeleton in each sex.

Thyroid Hormone

The hypothalamic-pituitary-thyroid axis plays a major role in skeletal development, peak bone mass achievement, and regulation of bone turnover (51). Hypothyroidism causes reduction in osteoblast formation and osteoclast resorption, and results in low bone turnover or slowing of the bone remodeling process (9, 134). In contrast, in thyrotoxicosis, there is an increase in osteoblast and osteoclast activity, and an increase in bone turnover with an impaired bone formation cycle, resulting in a remodeling process favoring rapid resorption (45). In adults, hyperthyroidism is associated with increased bone remodeling, reduced bone mineral density (BMD), and increased fracture risk, mainly in postmenopausal women (128, 135).

In vitro, the biologically active derivative of thyroxine, triiodothyronine (T_3), has effects on both osteoblasts and osteoclasts. T_3 has dual actions on osteoblasts: it has been reported that T_3 promotes osteoblast differentiation and inhibits osteoblast proliferation in both osteoblastic cell lines and primary calvarial osteoblasts. In osteoclasts, T_3 stimulates osteoclast activity either directly (73) or indirectly through cytokines (19).

The action of T_3 requires thyroid hormone nuclear receptors (TRs). It has been reported that both TRα and TRβ receptors play a role in mediating TH actions on bone remodeling (18, 128, 131). TRα-deficient mice acquire a higher bone mass than wild-type mice, whereas pharmacological activation of TRβ with GC-1 (a TRβ agonist) spares the skeleton from bone loss, yielding the conclusion that T_3 mediates its effects in bone via TRα (10, 45). Overall, like the other members of the nuclear receptor superfamily, thyroid hormones also have effects on both osteoblast and osteoclast activities, and are essential for bone mineral homeostasis, normal skeletal growth, and maintenance of bone mass.

Glucocorticoids

Glucocorticoids (GCs) can affect physiological bone remodeling via accelerating bone resorption and reducing bone formation (68, 183). Prolonged glucocorticoid therapy or excessive exposure (Cushing's syndrome) decreases bone mineral density (BMD) and may lead to development of glucocorticoid-induced osteoporosis (GIO) (113, 114). Bone deterioration is more apparent in trabecular than in cortical bone in GIO and depends on dose and duration of glucocorticoid exposure (21). In adult bone, functional glucocorticoid receptors (GR) are mainly found in pre-osteoblast/stromal cells and osteoblasts (1). Consistent with these observations, GCs fail to decrease bone formation in osteoblast-specific (Runx2-Cre) GR knockout mice, suggesting that negative effects of GCs on bone formation are mediated through osteoblastic GR (157). Similarly, GCs stimulate osteoclast numbers by suppressing synthesis of OPG and stimulating RANKL synthesis by pre-osteoblast/stromal cells, with the decrease in the OPG-to-RANKL ratio supporting osteoclast differentiation and net bone resorption (61, 84). It has been reported that long-term use of GCs reduces bone formation either via direct inhibition of osteoblast proliferation and differentiation (suppression of Runx2 and type I collagen) or by the augmentation of apoptosis rates of mature osteoblasts and osteocytes (153, 154, 183). In addition, GCs negatively regulate secretion of androgens and estrogens by inhibiting gonadotropin secretion and finally increase bone resorption (152). GCs also induce negative calcium balance by decreasing intestinal calcium absorption and increasing urinary calcium excretion (49). Taken together, direct GC-GR signaling in bone can reduce bone mass as well as through indirectly affecting systemic regulators of bone remodeling.

Growth Hormone

Growth hormone (GH) is a peptide hormone secreted from the pituitary gland under the control of the hypothalamus. It has been reported that both GH-deficient humans and $GHR^{-/-}$ mice have reduced longitudinal bone growth (159, 171). GH supplementation to GH-deficient adults and children has positive skeletal effects, including increases in BMD and bone turnover markers (98). GH, along with its binding protein (GHBP), regulates growth directly through the GH receptor (GHR) and indirectly by stimulating liver and skeletal IGF-1 expression (143). It has been reported that GH stimulates osteoblast proliferation and collagen production either directly (132) and/or indirectly by increasing IGF-1 and IGF binding protein (IGFBP) production (40, 190, 191). Consistent with this, hIGF-1 treatment of $GHR^{-/-}$ mice restored normal bone formation (179). GH also stimulates bone resorption, although it is not clear whether these effects are results of direct GH stimulation or mediated by osteoblastic IGF-1 or IGFBP (138, 168). Finally, administration of GH along with PTH in rats increases bone growth and bone formation, decreases bone resorption, and has a synergistic effect on increasing bone density and bone mass (56). Recent work has shown that GH has

direct effects on the osteocyte, further adding to the complexity of this hormone's actions (110).

Growth Factor Regulation of Bone Remodeling

Bone Morphogenetic Proteins

Bone morphogenetic proteins (BMPs) are members of the TGF-β superfamily and regulate differentiation of bone marrow mesenchymal cells into components of bone, cartilage, or adipose tissue. It has been reported that BMP-2, -4, -5, -6, and -7 all have strong osteogenic capability and that their signaling is essential for bone remodeling and maintenance of bone mass through activation of BMP type 1A and type 1B receptors (97, 125). BMP-mediated signaling increases the expression of Runx2 and Osx, mainly through the Smad and MAPK pathways.

One of the highly studied BMP family members, BMP-2, enormously increases osteocalcin expression, and short-term expression of BMP-2 is sufficient to induce bone formation (64, 139). It has been reported that loss of both BMP-2 and BMP-4 resulted in severe impairment of osteogenesis (8). In addition, BMP-7 upregulated ALP activity and increased mineralization, resulting in increased osteoblast differentiation (164). BMP-2 has osteoinductive properties and improves healing of open tibial fractures (20). BMP-2 has been used extensively in orthopedic procedures, but some of the trials have recently been criticized (20, 22, 55).

Transforming Growth Factor-β

The transforming growth factor (TGF)-β signaling pathway is known to control bone remodeling and maintenance. Compared with wild-type mice, TGF-β1 knockout mice have decreased bone mineral content in the proximal tibial metaphysis and shorter tibiae (48). In contrast, transgenic mice overexpressing TGF-β2 develop osteoporosis in association with increased osteoclastic bone resorption (39). Paradoxically, TGF-β1 stimulates apoptosis of osteoclasts (63). Thus the role of TGF-β appears complex and to involve both the osteoclast and the osteoblast.

TGF-β has dual effects on osteoblasts. TGF-β has been shown to stimulate expression of early osteoblast differentiation and bone matrix proteins by osteoblasts. However, TGF-β inhibits late osteoblast differentiation. TGF-β binds to its receptors (TβRI and II) and activates Smad2 and Smad3 via phosphorylation. Furthermore, phosphorylated Smad3 physically complexes with Runx2. This reduces the transcriptional and osteogenic activity of Runx2 through the involvement of histone deacetylases (HDAC4 and HDAC5) (2).

TGF-β and BMPs show opposite roles in osteoblast differentiation by using different Smad molecules. Endogenous TGF-β opposes BMP signaling by inducing the inhibitory Smads and subsequently inhibits late-stage osteoblast maturation (30). TGF-β also signals with other bone remodeling systems. TGF-β and PTH signaling coordinate each other in regulation of osteoblastogenesis and bone remodeling signaling (156). The TβRII forms an endocytic complex with the PTH1R and phosphorylates the PTH1R cytoplasmic domain, accelerating downregulation of PTH1R signaling (156). In support of this, deletion of TβRII in osteoblasts in mice results in a bone phenotype similar to hyperactivation of the PTH1R (156). TGF-β proteins are present in their latent form in the bone matrix, and osteoclasts are able to release and activate TGF-β from the bone matrix via osteoclastic bone resorption (175). As a result, active TGF-β1 recruits bone mesenchymal stem cells to the bone remodeling area, via phosphorylation of Smad2 and Smad3, leading to coupling of bone resorption with bone formation (175).

Epidermal Growth Factors and Receptor

The epidermal growth factor receptor (EGFR) is a transmembrane glycoprotein. EGFR deficiency leads to early lethality with a few surviving Egfr-null pups displaying craniofacial alterations, delayed primary ossification of the cartilage, and impairment in trabecular bone formation (180). It was recently found that decreasing EGFR expression in pre-osteoblasts and osteoblasts in mice results in reduced osteoblastogenesis and increased bone resorption, finally leading to decreased trabecular and cortical bone (196). Thus osteoblastic EGFR signaling plays an anabolic role in bone metabolism (198). However, all EGF-like ligands inhibit osteoblast differentiation (198). Moreover, they suppress gene expression of osteoblast-specific transcription factors Runx2 and osterix, and osteoblastic markers such as alkaline phosphatase, bone sialoprotein (BSP), and osteocalcin (198). It has been shown that attenuation of EGFR activity in osteoblast lineage cells decreases the number of osteoprogenitor cells, showing that EGFR stimulates osteoprogenitor proliferation and that EGFR signaling is required to maintain the osteoprogenitor population (29, 198). Apart from these actions, EGF and TGF-α stimulate bone resorption in fetal rat long bones and human marrow, suggesting that EGFR signaling also regulates osteoclastogenesis and bone resorption. It has been reported, using osteoblast/osteoclast co-culture, that this occurs by decreasing osteoblastic expression of OPG and by increasing osteoblastic expression of MCP-1 but having no effect on RANKL expression (197).

Fibroblast Growth Factors

Fibroblast growth factors (FGF) belong to a family of heparin-binding growth factors. Basic FGF (FGF-2) is expressed by osteoblastic cells and is an important modulator of cartilage and bone growth and differentiation. Ablation of endogenous FGF-2 in mice decreases trabecular bone and bone formation rates (130). FGF-2 also stimulates the differentiation of mesenchymal bone marrow stromal cells to adipocytes or osteoblasts (120, 186). Endogenous FGF-2 was found to be necessary for the bone anabolic effects of PTH and BMP-2 in mice (136, 161). Another FGF family member, FGF-18, is an essential regulator of the osteogenic differentiation of murine mesenchymal stem cells (58), and FGF-18-deficient mice display delayed ossification, showing an important role of FGF-18 in osteogenesis (142). In contrast, FGFR1 inactivation leads to increased bone mass, indicating that signaling through this receptor negatively controls osteoblast maturation and bone formation in vivo (69). FGFR2 plays a role in bone formation, and deletion of FGFR2 results in atypical function of osteoblasts, decreased proliferation of osteoprogenitor cells, and decreased bone mineral density. Wnt and FGFR signaling act together to control mesenchymal stem cell differentiation, suggesting that cross talk between FGF/FGFR signaling and Wnt-β-catenin signaling regulate osteoblast function (122).

FGF-23 is an important member of the FGF family produced primarily by osteocytes and by osteoblasts that acts on the kidney and the parathyroid gland (119, 162), and has a major role in phosphate homeostasis and signaling between bone and the kidney. The production of FGF-23 by osteocytes is also regulated by systemic hormones, mainly PTH and $1,25(OH)_2$ vitamin D_3 (66, 81, 88, 167). FGF-23 negatively regulates erythropoiesis in peripheral blood and bone marrow in young adult mice (32). Recently, it has been shown that FGF-23 has a direct effect on the differentiation of bone marrow stromal cells (107). The effects of FGF-23 on bone mineralization are thought to be exerted through interrelated physiological pathways associated with phosphate homeostasis (166). However, it has been shown that excess FGF-23 can negatively regulate bone mineralization, and this bone mineralization effect of FGF-23 is independent of systemic phosphate levels (170). We still do not know all of the roles and actions of FGF-23, and since it is the first marker to increase in chronic kidney disease (CKD), the gap in knowledge of this growth factor is an impediment to treatment of CKD (160, 178).

Insulin-like Growth Factor-1

Insulin-like growth factor-1 (IGF-1) is the most abundant growth factor deposited in the bone matrix and stimulates cell proliferation and function, and survival of osteoblasts. The presence of IGF-1 in osteoblasts and osteoclasts suggests its involvement in active bone growth and remodeling (101). The primary function of IGF-1 in the bone matrix is to maintain bone mass and skeletal homeostasis during bone remodeling (185). Mice lacking functional global IGF-1 exhibit severe deficiency in bone formation and a 60% deficit in peak bone mineral density (BMD) (16). Like the TGF-βs, IGF-1 has been implicated in the coupling process through its actions on MSC differentiation (59, 126, 195). Furthermore, IGF-1 promotes osteoclast differentiation, and IGF-1-null mice show high bone mass and impaired bone resorption, suggesting a positive role for IGF-1 in regulation of osteoclast differentiation (182). IGF-1 regulates the expression of RANKL and RANK, and facilitates normal physiological interaction between the osteoblast and the osteoclast (182). Liver is the primary source of serum IGF-1, and serum IGF-1 levels are tightly controlled by pituitary growth hormone (GH), whereas numerous other tissue factors along with GH regulate tissue IGF-1. There are several reports demonstrating the synergistic effects of IGF-1 and PTH on bone remodeling and establishing the involvement of locally produced IGF-1 in the anabolic effects of PTH (188, 189, 192).

WNT and WNT Antagonists

WNT signaling is implicated in proximal-distal outgrowth and dorsoventral limb pattern formation during skeletal development. Genetic studies in human and animal models suggest that the canonical Wnt/β-catenin pathway, together with BMP signaling and Runx2, has a significant vital role in osteoblast differentiation, skeletal development, and bone formation (95, 129). WNT signaling represses mesenchymal stem cell commitment to the chondrogenic and adipogenic lineages and enhances differentiation of osteoblastic lineages (35, 78, 83). Osteoblast and osteocyte WNT-β-catenin signaling has a negative effect on osteoclast differentiation and bone resorption through the increased secretion of osteoprotegerin (50, 62, 93).

Canonical WNT signal transduction involves stabilization of β-catenin by inhibiting GSK-3-β-mediated β-catenin phosphorylation. On the other hand, activation of noncanonical pathways by osteoblast-expressed WNT5a stimulates differentiation of osteoclast precursors and osteoclastogenesis (115). WNT $10b^{-/-}$ mice had decreased trabecular bone and serum markers (13). WNT10b is the best-characterized bone formation-promoting canonical WNT

and can trigger osteoblast Wnt/β-catenin signaling in a paracrine manner (13, 14, 173). It has been reported that WNT6a and WNT10a modulate the fate of mesenchymal cell differentiation to osteoblasts or adipocytes, and thereby control physiological bone remodeling (24). Taken together, WNT signaling has a direct and indirect effect on osteoblast and osteoclast bone cell lineages, leading to an overall increase in bone formation.

Dickkopf family members (Dkk1 and Dkk2) and secreted frizzled-related proteins (Sfrps) are families of extracellular proteins that negatively modulate canonical Wnt signaling (93). Another of these is sclerostin, a secreted glycoprotein encoded by the SOST gene, which binds to LRP5/6 receptors and inhibits BMP- and WNT-stimulated bone formation (94). Neutralizing monoclonal antibodies to Dkk1 and sclerostin (AMG 785) have been developed as new treatments for osteoporosis (146, 147, 187). The anti-sclerostin antibodies, currently in *phase 3* trials, appear to be most promising and may be a novel anabolic therapy for osteoporosis. If these antibodies do not have the drawback of the "anabolic window" of recombinant PTH (1-34 teriparatide), then they may replace the latter as the anabolic treatment agent of choice.

Conclusions

The current understanding of bone remodeling is primarily supported by the experimental results obtained from both in vivo and in vitro research. Many studies have been conducted on the generation and differentiation of different bone cells and involvement of these bone cells along with different stimuli on bone formation and bone remodeling. On the basis of these observations, many hypotheses have been proposed to understand the complex interactions of hormones, growth factors, and their receptors, and their autocrine, paracrine, and endocrine modes of signaling, which regulate physiological bone remodeling. This knowledge has led us from a period of treating metabolic bone diseases by classical endocrinology to one of using the growth factors or their inhibitors to treat these diseases. However, such a complex regulatory system, which involves numerous factors and their interactions, means that understanding of the behavior of the bone system is still fragmentary, and further knowledge is needed to maximize treatment options and to best target the growth factors. ■

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