Hormonal Regulation of Bone Mineral Homeostasis

Simply stated, regulation of bone mineral homeostasis refers to controlling the intra- and extra-cellular levels of two ions—calcium and phosphate—with three hormones: parathyroid hormone (PTH), the active metabolite of vitamin D 1,25 dihydroxyvitamin D (1,25(OH)2D3), and fibroblast growth factor 23 (FGF23) acting on three target tissues, i.e. bone, intestine, and kidney (see Figure 1). This simple conceptual framework only partially reflects the true situation. Other ions are involved: pH, sodium, potassium, magnesium, chloride, bicarbonate, and sulfate all alter the cellular handling of calcium and phosphate. Likewise, other hormones including calcitonin, prolactin, glucocorticoid hormones, growth hormone, insulin, insulin-like growth factors (IGFs), and a large number of cytokines contribute in important ways to the regulation of bone mineral homeostasis. Finally, we now recognize that a large number of tissues other than bone, intestine, and kidney are target tissues for the calciotropic hormones in ways that contribute to bone mineral homeostasis. However, in this short article I will focus on the interactions among PTH, 1,25(OH)2D3, and FGF23 as they regulate calcium and phosphate levels through actions on bone, intestine, and kidney.

Integration of Hormone Action at the Tissue Level

Figure 1 introduces the major hormones PTH, 1,25(OH)2D3, and FGF23 regulating serum calcium and phosphate levels through their actions on bone, intestine, and kidney. PTH is secreted by the parathyroid glands. Vitamin D is produced from 7-dehydrocholesterol in the skin by a photochemical reaction involving ultraviolet B radiation (UVB) (sun is the natural source of UVB). The liver, among other tissues—including the skin—converts vitamin D to the major circulating form 25 hydroxyvitamin D (25OHD). The kidney is the major source of 1,25(OH)2D3 for the circulation, although a variety of tissues, including a number of epithelial and immunoregulatory cells, possess the enzyme CYP27B1, which is capable of converting 25OHD to 1,25(OH)2D3. FGF23 is thought to originate primarily from osteoblasts and osteocytes, although the source of this relative newcomer to the list of calcitropic hormones is still under active investigation and other cells express it. Calcium and phosphate enter the blood from the intestine, are excreted by the kidney, and are stored in the body principally in bone. In order to maintain homeostasis, the net absorption of calcium and phosphate by the intestine must be precisely balanced by net excretion of these ions by the kidney.

Absorption of these ions by the gut is not a continuous process, but depends on dietary intake. The efficiency with which absorption occurs for a given dietary load is the regulated variable. Glomerular filtration of these ions by the kidney is relatively constant and dependent on overall renal function, so the control takes place in adjusting the efficiency with which these ions are reabsorbed from the glomerular filtrate as it passes through the proximal tubule, thick and thin limbs of Henle’s loops (TALH), distal tubule, and collecting ducts. Bone provides the major buffer for maintaining relatively constant blood levels of these ions. This is achieved by balancing bone formation, which deposits these ions in bone with bone resorption, which releases these ions to the blood stream. Although each tissue has distinct mechanisms and molecules by which it contributes to bone mineral homeostasis, some common themes are found, at least at the level of protein families. Therefore, TRPV6 (dominant in intestine) and TRPV5 (dominant in kidney) are highly homologous calcium channels in the apical membranes of their respective epithelial cells and play critical roles in calcium absorption (intestine) and reabsorption (kidney), respectively. Similarly, calbindins are a family of homologous proteins initially thought to play important roles in the intracellular transport of calcium through the epithelia of the intestine and kidney, with different calbindins in these different tissues, although recent data suggest their role in transcellular transport may be limited. Different but homologous plasma membrane calcium adenosine triphosphatases (ATPases), calcium pumps (PMCA), reside in the basolateral membranes of the epithelia of the intestine and kidney to transport the calcium out of the cell and into the bloodstream. Finally, different but homologous sodium/phosphate co-transporters reside in the apical membranes of the epithelia of the intestine and kidney to regulate phosphate absorption and reabsorption.

Little is known about the role, if any, of these protein families in bone. The expression and function of these proteins are highly regulated, in particular by the calciotropic hormones. Not surprisingly, there are sensors in these tissues that, along with hormonal regulation, control these processes. The best studied of these sensors is the calcium-sensing receptor found in the parathyroid gland, bone, intestine, and kidney, among other tissues. A sensor for phosphate is less clearly demonstrated, but probably exists in some or all of these tissues. Various hormones act on the tissues by different mechanisms, as I will discuss below. However,
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CYP27B1 expression is not regulated by PTH, at least not directly. On the other hand, the keratinocyte and macrophage do not have PTH receptors, and so their effects are well co-ordinated to ensure an increased supply of bone minerals during periods of growth, steady-state levels during middle life, and gradual loss during aging.

Hormonal and Ionic Feedback Loops

Figure 2 shows the feedback loops that operate among the calciotropic hormones. PTH is the major regulator of 1,25(OH)2D3 production by the kidney. This regulation appears to be genomic and mediated by protein kinase A phosphorylation of transcription factors that act on the proximal region of the CYP27B1 promoter. Non-renal sites of 1,25(OH)2D3 production such as the keratinocyte and macrophage do not have PTH receptors, and so their CYP27B1 expression is not regulated by PTH, at least not directly. On the other hand, PTH production is inhibited by 1,25(OH)2D3, again at the transcriptional level. The relationship between FGF23 and 1,25(OH)2D3 is the reverse, with FGF23 inhibiting CYP27B1 activity and 1,25(OH)2D3 stimulating FGF23 production. FGF23 requires both specific FGF receptors (FGFR1c, 3c, and 4) and Klotho as a co-receptor. To my knowledge, regulation of CYP27B1 expression by FGF23 outside the kidney has not been reported, limited perhaps by the lack of Klotho in these tissues. However, the parathyroid gland expresses both FGFRs and Klotho, and recent studies indicate that FGF23 inhibits PTH production and secretion. PTH may stimulate FGF23 secretion in that serum levels of these hormones correlate in models of hyper-parathyroidism, but a direct regulation by PTH of FGF23 expression has not been established. In addition to the hormonal feedback loops are the feedback loops involving calcium and phosphate (see Figure 3). Both calcium and phosphate may have direct actions to suppress CYP27B1 activity in the kidney. However, their major influences are likely to be indirect: calcium suppressing CYP27B1 expression by suppressing PTH production and secretion, and phosphate suppressing CYP27B1 expression by stimulating FGF23 production and secretion. Phosphate may also stimulate PTH secretion directly, but this action is less established than the inhibitory effects of calcium on PTH secretion.

Intestinal Absorption of Calcium and Phosphate

1,25(OH)2D3 stimulates intestinal calcium and phosphate absorption, and acts through both genomic and non-genomic mechanisms to achieve this regulation. The mechanism for calcium absorption has been better studied than that of phosphate absorption. Both transcellular and paracellular pathways are involved in calcium absorption, but much of what we know about vitamin D regulation focuses on the transcellular process. Calcium transport through the intestinal epithelial cell involves a three-step process: getting calcium into the cell, moving it through the cell without initiating toxic events, and discharging the calcium into the bloodstream against a steep electrochemical gradient. The calcium channel TRPV6 is instrumental to gating calcium flux into the cell, and this process may be regulated by calmodulin and facilitated by the major calmodulin-binding protein in the microvillus: brush border myosin I. Calcium transport through the cell is facilitated by calbindins that have been found in endocytic vesicles within the cytoplasm, a process that would keep intracellular levels of calcium from rising to toxic levels, although intestinal calcium transport is not dependent on calbindins, as noted earlier. The removal of calcium at the basolateral membrane is mediated by sodium/calcium exchangers and calcium ATPase. The expression of TRPV6, calbindin, and PMCA has been shown to be induced by 1,25(OH)2D3. The mechanism by which 1,25(OH)2D3 regulates phosphate transport is less clear, and phosphate absorption appears to be less dependent on vitamin D status than calcium absorption. Low-phosphate diets increase intestinal calcium transport and the expression of the apical sodium/phosphate co-transporter NaPi-IIb, which may promote phosphate transport into the epithelial cell, but this adaptation also occurs in vitamin D receptor (VDR)- and CYP27B1-null mice. The site most sensitive to 1,25(OH)2D3-stimulated calcium transport is the duodenum, whereas the jejunum is the site most sensitive to 1,25(OH)2D3-stimulated phosphate transport.

Renal Reabsorption of Calcium and Phosphate

Just as 1,25(OH)2D3 is the principal regulator of intestinal calcium and phosphate absorption, PTH and FGF23 are the principal regulators of renal reabsorption of calcium (PTH) and phosphate (PTH and FGF23), although the vitamin D metabolites are likely to play some role. Given the comparable proteins in the distal tubule for calcium reabsorption that exist for intestinal calcium absorption, it is quite surprising that it has been difficult to demonstrate a direct role for 1,25(OH)2D3 in renal calcium reabsorption in vivo. PTH reduces the glomerular filtration rate (GFR) and so reduces the filtered load.
of calcium, stimulates active calcium transport in the TALH, and increases calcium flux into the epithelial cells of the distal tubule through TRPV5 calcium channels. The calcium-sensing receptor also plays an important role in the renal handling of calcium by responding to increased calcium with inhibition of calcium reabsorption in the TALH and promoting diuresis via inhibition of the water channel aquaporin 2. Control of phosphate reabsorption occurs primarily in the proximal tubule, and the major regulation is focused on the expression and function of the sodium/phosphate co-transporter NaPi-llα. PTH and FGF23 both suppress NaPi-llα and so inhibit phosphate reabsorption. Dietary levels of phosphate have been shown to modulate renal phosphate reabsorption independent of PTH, but whether phosphate has a direct action on phosphate reabsorption or whether it works primarily through FGF23 has not been settled.

Regulation of Bone Remodeling
The regulation of bone remodeling by PTH, 1,25(OH)2D3, and FGF23 is complex. The major cells in bone responsible for bone remodeling are the osteoblasts that form new bone, the osteoclasts that resorb bone, and the osteocytes that may be the prime environmental sensors that direct where and how much bone should be formed and resorbed. Although osteoblasts contain VDR and can be shown to respond to 1,25(OH)2D3, at least in vitro, studies with mice (and humans) null for or containing inactivating mutations in the VDR or CYP27B1 suggest that as long as adequate levels of calcium and phosphate in the blood are maintained, bone formation is relatively unimpaired in the absence of 1,25(OH)2D3 or its receptor (VDR). This may be a simplistic interpretation, but certainly rickets can be prevented in such circumstances by providing diets high in calcium, phosphate, and lactose to enhance non-vitamin-D-dependentcalcium and phosphate absorption. Osteoblasts are not the only cells in which 1,25(OH)2D3 and calcium play complementary roles, and one may interpret these results to indicate that at least in bone 1,25(OH)2D3 plays a modulating role to facilitate the actions of calcium, phosphate, and other hormones.

The actions of PTH are no less complex. Continuously high levels of PTH lead to bone resorption, and in vitro its actions result in decreased osteoblast proliferation and differentiation. The stimulation of bone resorption can be understood by the ability of PTH to induce receptor activator of nuclear factor kappa B ligand (RANKL) (as can 1,25(OH)2D3), a major molecule produced by osteoblasts, which by interacting with RANK in osteoclasts and their precursors increases both osteoclast number and activity. PTH also inhibits osteoprotegerin (OPG), a RANK decoy that blocks RANKL function. Less clear is how PTH stimulates bone formation.

Recently, two not mutually exclusive possibilities have emerged. PTH has been found to inhibit sclerostin (SOST) expression in osteocytes. The gene product of SOST is SOST, which is an inhibitor of the wnt pathway. Activation of the wnt signaling pathway underlies the high-bone-mass phenotype found in individuals with specific mutations in LRPS, a co-receptor for the wnt receptor frizzled, rendering it less susceptible to inhibition by SOST and Dkk-1. Wnt signaling can lead to osteoprogenitor proliferation via activation of b-catenin. Therefore, PTH, by inhibiting the activator, activates the wnt pathway and so increases osteoblast numbers. A second mechanism involves IGFs. IGF-I (or IGF-II) stimulates osteoblast proliferation and differentiation and blocks their apoptosis. IGF-I is also required for osteoclast formation and activity. PTH stimulates IGF-I production by osteoblasts. Mice null for IGF-I or its receptor (IGF-IR) have a markedly blunted response to PTH with respect to bone formation (or osteoclast formation). At this point, it is not known whether the IGF signaling pathway and the wnt signaling pathway overlap in mediating the anabolic actions of PTH, but it is likely that they do.

Elevated levels of FGF23 as they occur in various genetic and tumor-induced forms of hypophosphatemic rickets lead to osteomalacia, whereas lack of circulating FGF23 results in hyperphosphatemia and tumoral calcinosis. What is not clear is whether FGF23 has a direct action on bone to block normal bone formation/mineralization or whether its actions are indirect via its ability to inhibit 1,25(OH)2D3 production and phosphate reabsorption by the kidney. DMP1 is a protein almost exclusively produced by osteocytes and is thought to be involved in the mineralization of bone. Mutations in DMP1 lead to rickets and osteomalacia with high FGF23 levels, suggesting that DMP1 may inhibit FGF23 production by bone, but these findings are also consistent with the possibility that FGF23 reciprocates by stimulating DMP1 production to complete the feedback loop. This possibility has not been explored.

Conclusions
The calciotropic hormones interact not only among themselves but also with the minerals they are regulating in their role as regulators of bone mineral homeostasis. Although numerous target tissues are involved, as are numerous ions and hormones, the most important are PTH, 1,25(OH)2D3, and FGF23 acting on bone, intestine, and kidney to regulate blood levels of calcium and phosphate. Regulation entails control of how much comes into the body from the diet, how much leaves the body through the kidney, and how much is stored and released from bone. Both feed-forward and feedback mechanisms are involved. The mechanisms regulating calcium and phosphate flux in one tissue differ in detail if not in kind from those in other tissues. Different hormones act on different tissues. Nevertheless, it is quite apparent that the different hormones, ions, and tissues involved communicate with each other, sending and receiving clear messages to ensure the precise regulation of these important minerals.
1. van de Graaf SJ, Bindels RJ, Hoenderop JG, Physiology of epithelial Ca\(^2\+) transport, Rev Physiol Biochem Pharmacol, 2007; 158:7–78.


By the Same Author

### Non-classic Actions of Vitamin D

**Bikle D**

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Vitamin D receptors (VDRs) are found in most tissues, not just those participating in the classic actions of vitamin D such as bone, gut, and kidney. These non-classic tissues are, therefore, potential targets for the active metabolite of vitamin D, 1,25(OH)\(_2\)D. Furthermore, many of these tissues also contain the enzyme CYP27B1, which is capable of producing 1,25(OH)\(_2\)D from the circulating form of vitamin D, 25(OH)D. This review is intended to highlight the actions of 1,25(OH)\(_2\)D in several of these tissues, but starts with a review of vitamin D production, metabolism, and molecular mechanism.

Medline was searched for articles describing actions of 1,25(OH)\(_2\)D on parathyroid hormone and insulin secretion, immune responses, keratinocytes, and cancer. Vitamin D production in the skin provides an efficient source of vitamin D. Subsequent metabolism to 1,25(OH)\(_2\)D within non-renal tissues differs from that in the kidney. Although VDRs mediate the actions of 1,25(OH)\(_2\)D, regulation of transcriptional activity is cell-specific. 1,25(OH)\(_2\)D inhibits PTH secretion but promotes insulin secretion, inhibits adaptive immunity but promotes innate immunity, and inhibits cell proliferation but stimulates their differentiation.

The non-classic actions of vitamin D are cell-specific and provide a number of potential new clinical applications for 1,25(OH)\(_2\)D3 and its analogs. However, the use of vitamin D metabolites and analogs for these applications remains limited by the classic actions of vitamin D leading to hypercalcemia and hypercalcuria.

### Role of IGF-I Signaling in Regulating Osteoclastogenesis

**Wang Y, Bikle D. et al.**

*J Bone Miner Res, 2006;21(9):1350–58.*

Although IGF-I has been clearly identified as an important growth factor in regulating osteoblast function, information regarding its role in osteoclastogenesis is limited. Our study was designed to analyze the role of IGF-I in modulating osteoclastogenesis using IGF-I knockout mice (IGF-I\(^{-/-}\)). Trabecular bone volume (BV/TV), osteoclast number, and morphology of IGF-I\(^{-/-}\) or wildtype mice (IGF-I\(^{+/+}\)) were evaluated in vivo by histological analysis. Osteoclast precursors from these mice were cultured in the presence of RANKL and macrophage-colony stimulating factor (M-CSF) or co-cultured with stromal/osteoblastic cells from either genotype. Osteoclast formation was assessed by measuring the number of multinucleated TRACP+ cells and pit formation. The mRNA levels of osteoclast regulation markers were determined by quantitative RT-PCR. In vivo, IGF-I\(^{-/-}\) mice have higher BV/TV and fewer (76% of IGF-I\(^{+/+}\)) smaller osteoclasts with fewer nuclei. In vitro, in the presence of RANKL and M-CSF, osteoclast number (55%) of IGF-I\(^{-/-}\)mice were significantly fewer and smaller than that from the IGF-I\(^{+/+}\) mice. IGF-I (10ng/ml) increased the size, number (2.6-fold), and function (resorptive area, 2.7-fold) of osteoclasts in cultures from IGF-I\(^{+/+}\) mice, with weaker stimulation in cultures from IGF-I\(^{-/-}\) mice. In co-cultures of IGF-I\(^{-/-}\) osteoblasts with IGF-I\(^{-/-}\) osteoclast precursors, or IGF-I\(^{+/+}\) osteoblasts with IGF-I\(^{-/-}\) osteoclast precursors, the number of osteoclasts formed was only 11 and 48%, respectively, of that from co-cultures of IGF-I\(^{+/+}\) osteoblasts and IGF-I\(^{-/-}\) osteoclast precursors. In the long bones from IGF-I\(^{-/-}\) mice, mRNA levels of RANKL, RANK, M-CSF, and c-fms were 55, 33, 60, and 35% of that from IGF-I\(^{+/+}\) mice, respectively. Our results indicate that IGF-I regulates osteoclastogenesis by promoting their differentiation.

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