# Endocrine functions of bone in mineral metabolism regulation

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**Given the dramatic increase in skeletal size during growth, the need to preserve skeletal mass during adulthood, and the large capacity of bone to store calcium and phosphate, juxtaposed with the essential role of phosphate in energy metabolism and the adverse effects of hyperphosphatemia, it is not surprising that a complex systems biology has evolved that permits cross-talk between bone and other organs to adjust phosphate balance and bone mineralization in response to changing physiological requirements. This review examines the newly discovered signaling pathways involved in the endocrine functions of bone, such as those mediated by the phosphaturic and $1,25(OH)_2D$-regulating hormone FGF23, and the broader systemic effects associated with abnormalities of calcium and phosphate homeostasis.**

## Biological importance of phosphate regulation

Intracellular phosphate is involved in intermediary metabolism and other essential cellular functions (1), whereas extracellular phosphate is necessary for matrix mineralization (2). Since both extremes of hypophosphatemia and hyperphosphatemia have negative effects (1, 3), adaptive mechanisms have evolved to protect organisms from hypophosphatemia and hyperphosphatemia and to coordinate the changing phosphate needs for bone mineralization and phosphate homeostasis. Historically, phosphate homeostasis has been viewed from the perspective of the parathyroid hormone/1,25-dihydroxy vitamin D [PTH/$1,25(OH)_2D$] axis, which regulates both systemic calcium and phosphate homeostasis (Figure 1A). In response to hypocalcemia, the parathyroid gland (PTG) increases the production and secretion of PTH, which targets the renal distal tubule to decrease renal calcium excretion and the proximal tubule to inhibit phosphate reabsorption and to stimulate $1,25(OH)_2D$ production. Action of $1,25(OH)_2D$ on the small intestines increases active calcium and phosphate transport (4). PTH also has direct effects on bone via PTH receptors in osteoblasts, resulting in increased calcium and phosphate efflux from the exchangeable bone fluid compartment (5) and through RANKL-dependent, osteoclast-mediated bone resorption of mineralized bone (6). The direct kidney and bone effects of PTH, along with the concomitant actions of $1,25(OH)_2D$, restore serum calcium levels to normal. The phosphaturic actions of PTH offset vitamin D–mediated gastrointestinal phosphate absorption (4) and PTH-dependent phosphate efflux from bone (7), thereby preventing the development of hyperphosphatemia.

Recently, a novel hormonal cascade involving FGF23 and Klotho has been identified that principally regulates phosphate, vitamin D homeostasis, and mineralization of bone (Figure 1B) (8–13).

## FGF23 and Klotho participation in a bone-kidney axis

FGF23 is a 32-kDa protein with an N-terminal region, containing the FGF-homology domain and a novel 71–amino acid C terminus (8, 9). FGF23 is phylogenetically grouped with FGF19 (mouse FGF15) and FGF21 gene products (8, 10), members of a subfamily of FGFs that act as hormones/systemic factors due to their ability to interact with FGF receptor (FGFR) in the presence of members of the Klotho family of proteins. FGF23 binds to *Klotho* (*KL*), which encodes a type I membrane, β-glycosidase–like protein (11, 12) that is an essential cofactor for FGF23 binding to FGFRs (12–14). In vitro studies indicate that the N-terminal region of FGF23 binds to and activates FGFR1, -3, and -4 at physiological concentrations only in the presence of Klotho, which binds to FGFR and the C terminus of FGF23 to convert the canonical FGFRs to a specific receptor for FGF23 (14–16). This is in contrast with the more typical paracrine/local functions of other FGFs that require extracellular acidic glycosaminoglycans (e.g., heparin) for receptor activation (17).

FGF23 principally functions as a phosphaturic factor (9, 18, 19) and counter-regulatory hormone for $1,25(OH)_2D$ production (20) via binding to KL:FGFR complexes in the kidney (14, 15). Excess FGF23 causes hypophosphatemia via inhibition of solute carrier family 34, member 1–dependent (SLC34A1-dependent) and SLC34A2-dependent phosphate transport (also known as sodium phosphate cotransporter 2 [NPT2a] or NaPi-IIa and NPT2c or NaPi-IIc, respectively). Excess FGF23 also suppresses $1,25(OH)_2D$ via inhibition of 25-hydroxyvitamin D-1α–hydroxylase (CYP27B1) that is converted to $1,25(OH)_2D$ and stimulation of 24-hydroxylase (CYP24) that inactivates $1,25(OH)_2D$ in the proximal tubule of the kidney (9, 18, 21, 22) (Figure 1B). In contrast, deficiency of FGF23 results in the opposite renal phenotype, consisting of hyperphosphatemia and elevated production of $1,25(OH)_2D$ (19, 23–27). In addition, *Fgf23*-null mice have soft-tissue calcifications, severe growth retardation, abnormalities of bone mineralization, and a shortened lifespan (25–27). Inactivating mutations or deletion of *Kl* results in end-organ insensitivity

**Nonstandard abbreviations used:** ADHR, autosomal dominant hypophosphatemic rickets; ARHR, autosomal recessive hypophosphatemic rickets; ASARM, acidic, serine- and aspartic acid–rich motif; BMP1, bone morphogenetic protein 1; DMP1, dentin matrix acidic phosphoprotein 1; FGFR, FGF receptor; HRH, hypophosphatemic rickets and hyperparathyroidism; *KL*, *Klotho*; MEPE, matrix extracellular phosphoglycoprotein with ASARM motif; NPT2, sodium phosphate cotransporter 2; OGD, osteoglophonic dysplasia; $1,25(OH)_2D$, 1,25-dihydroxy vitamin D; PHEX, phosphate-regulating gene with homologies to endopeptidases on the X chromosome; PTG, parathyroid gland; PTH, parathyroid hormone; sFRP4, secreted frizzled-related protein 4; SLC34A1, solute carrier family 34, member 1; XLH, X-linked hypophosphatemic rickets.

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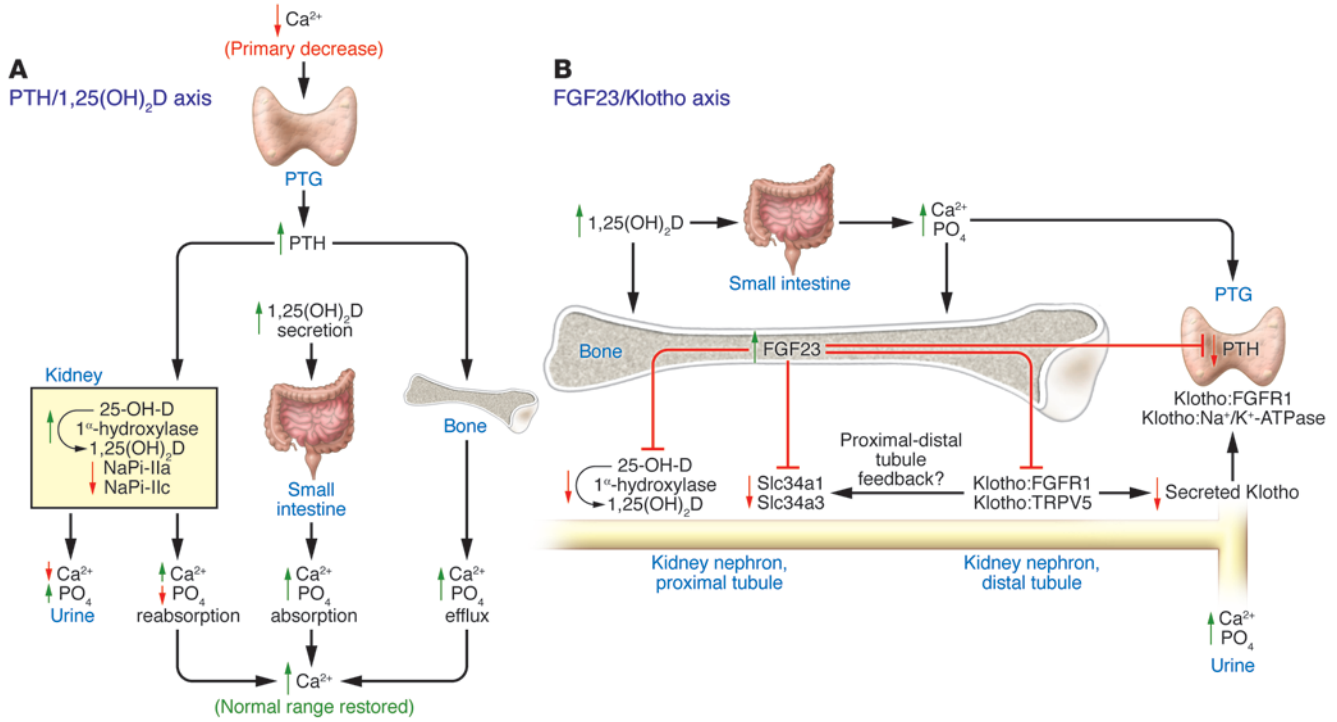


**Figure 1**

Interrelationships among FGF23, PTH, $1,25(OH)_2D$, and Klotho. (**A**) The PTH/$1,25(OH)_2D$ axis. The principal function of the PTH/$1,25(OH)_2D$ axis is to regulate calcium homeostasis. Decrements in serum calcium levels stimulate PTH secretion by the PTG, which targets the kidney to reduce urinary calcium excretion, stimulate $1^{\alpha}$-hydroxylase activity, and enhance the fractional excretion of phosphate ($PO_4$), and targets bone to increase the efflux of calcium and phosphate. The resulting increase in $1,25(OH)_2D$ targets the gastrointestinal tract to increase dietary absorption of calcium, which suppresses PTH. (**B**) The FGF23/Klotho axis. FGF23 produced by bone principally targets the kidney, leading to reductions in serum phosphate and $1,25(OH)_2D$ levels by stimulating the fractional excretion of phosphate and reducing $1^{\alpha}$-hydroxylase activity. The receptor for FGF23 in the kidney is a Klotho:FGFR1 complex located in the distal tubule. There may be a distal-to-proximal feedback mechanism that mediates the effects of FGF23 on the proximal tubule. FGF23 also decreases the kidney expression of Klotho, which diminishes renal tubular calcium reabsorption via its interactions with transient receptor potential cation channel, subfamily V, member 5 (TRPV5). FGF23 may also directly target the PTG to reduce PTH secretion. FGF23 is the principal phosphaturic hormone and may function to counter the hypercalcemic and hyperphosphatemic effects of excess $1,25(OH)_2D$ through reductions in PTH and elevations in FGF23 levels.

to FGF23, which is characterized by hyperphosphatemia, elevated $1,25(OH)_2D$ levels, early mortality, and soft-tissue calcifications (13, 28–30), a phenotype that resembles that of *Fgf23*-null mice (31). In contrast, FGF21 stimulates lipolysis and glucose uptake and FGF19 regulates bile acid secretion via binding to another member of the Klotho family, β-Klotho (32–35).

Klotho also has other functions that are distinct from its role in mediating FGF23 effects (36), including regulation of calcium absorption in the kidney distal convoluted tubule, through stabilizing the membrane expression of the transient receptor potential cation channel, subfamily V, member 5 (36), and regulation of PTH secretion (37), by facilitating the recruitment of an $Na^+/K^+$-ATPase to the parathyroid chief cell membrane (38). A circulating form of Klotho containing the N-terminal extracellular domain is derived either from a separate *Kl* gene transcript generated through alternative transcriptional termination that encodes a secreted protein (39) or by membrane shedding, involving the proteases ADAM10 or ADAM17 (40). Cleavage and secretion of Klotho is regulated by both extracellular calcium fluctuation and insulin. Circulating Klotho has a plethora of functions, including effects on the intracellular insulin/insulin-like growth factor 1–signaling cascade (12, 41) and adipocyte differentiation (42).

## Organs that produce FGF23

FGF23 is mainly produced and secreted by osteocytes in bone (19, 27) (Figure 1B), but it is also expressed in pericyte-like cells surrounding the venous sinuses in the bone marrow, in the ventrolateral thalamic nucleus in the brain and in thymus, and in lymph nodes (19). The relative contribution of these sites to circulating FGF23 levels is not known, and this is an important problem needing investigation. Nevertheless, the high levels of FGF23 expression in osteocytes, the most abundant cell in bone, suggests that the source of circulating FGF23 is mainly bone (19).

## FGF23 target organs

The target organs for FGF23 are theoretically defined by the coexpression of the membrane form of Klotho and FGFR1c, -3c, and -4 (14, 15). Klotho is expressed in high levels in PTGs, kidney, testis, ovary, brain, pituitary, and apical plasma membrane of ependymal cells in the choroid plexus but not in bone, lung, liver, skin, spleen, small intestines, or adrenal glands. The limited pattern of Klotho expression corresponds to the target tissues functionally defined by upregulation of the gene early growth-responsive 1 (*Egr-1*) after administration of FGF23 to mice (14, 15). The kidney, however, is the principal physiologically defined target for FGF23,

in which this circulating factor inhibits phosphate reabsorption and production of $1,25(OH)_2D$ (9, 25) (Figure 1B). The precise segments and the physiologically relevant receptor(s) for FGF23 in the kidney are not entirely clear. Although FGF23 can bind to FGFR3, FGFR4, and FGFR1 in vitro, neither deletion of FGFR3 or FGFR4 impairs the phosphaturic actions of FGF23 in mice, suggesting that the remaining in vitro target, FGFR1, is the physiologically relevant receptor for FGF23 in the kidney (43). Moreover, the highest levels of the FGFR1:Klotho complex are present in the distal tubules, whereas the biological actions of FGF23 are in the proximal tubules (44) (Figure 1B). Ex vivo studies of proximal tubular segments or cell lines have demonstrated variable effects of exogenously added FGF23 to inhibit sodium-dependent phosphate transport (9, 45). Interpretation of these findings are confounded by the use of nonphysiological amounts of FGF23 and the authenticity of the proximal tubular phenotype in cell culture models, which may be contaminated with distal tubular cells and/or may have undergone dedifferentiation (45–49). Alternatively, FGF23 actions on the proximal tubule may be indirect, possibly through FGF23 stimulation of the distal tubule and release of paracrine factors that regulate proximal tubule function (i.e., a distal-to-proximal tubular feedback mechanism). Identification of the tubular segments in the kidney that are the direct target for FGF23 is another important unresolved issue. In addition, FGF23 decreases the expression of Klotho by the kidney, thereby creating complex feedback pathways for regulating phosphate and calcium metabolism (36, 50).

The PTG also expresses FGFR and Klotho and is a target for FGF23 as evidenced by the effects of recombinant FGF23 to stimulate increments in Egr-1 expression in the PTG of mice (14) (Figure 1B), but there are some discrepancies about whether FGF23-dependent pathways stimulate or inhibit PTH secretion (37, 51). Elevated FGF23 levels in human diseases and mouse models are associated with hyperparathyroidism (22, 52), but FGF23-mediated reductions in $1,25(OH)_2D$ production and secondary increments in PTH might explain this association. Conversely, FGF23 has recently been shown to inhibit expression of *PTH* mRNA and secretion of PTH from parathyroid cells (37, 53). Adding to the complexity of this issue is the observation that elevated Klotho levels are associated with hyperparathyroidism in humans, potentially via a direct effect to regulate PTH secretion through its maintenance of cell surface $Na^+/K^+$-ATPase activity (36).

Additional abnormalities are observed in association with FGF23 excess and deficiency, such as rickets/osteomalacia, abnormalities in glucose homeostasis, growth retardation, abnormalities in thymic function, and age-related changes (19, 25, 27), suggesting a broader role for FGF23. The choroid plexus and pituitary gland are potential targets for FGF23, but its function remains unknown. There is also uncertainty regarding whether FGF23 has direct effects on bone or if the unexplained defect in bone mineralization in *Fgf23*-null mice is due to excessive $1,25(OH)_2D$. An indirect effect is supported by the finding of a similar mineralization abnormality associated with high $1,25(OH)_2D$ levels caused by deletion of the 24-hydroxylase, the absence of Klotho expression in bone, and the ability of deletion of the vitamin D receptor to rescue the phenotype of *Fgf23*-null mice (26, 54, 55). However, recent studies indicate that FGF23 may have a direct effect on osteoblasts in vitro (56), but the physiological relevance and specificity of these cell culture findings need to be confirmed in vivo (57). Although additional functions of FGF23 remain to be clarified, the overall principal physiological function of the FGF23/Klotho axis is to regulate phosphate and $1,25(OH)_2D$ homeostasis, with a secondary effect on renal calcium handling (Figure 1B).

## Regulation of FGF23

*Systemic factors regulating FGF23.* There are various systemic factors that regulate circulating FGF23 levels. The feedback signals underlying FGF23 actions as a counter regulatory hormone to offset the effects of excessive $1,25(OH)_2D$ are best defined. In this regard, $1,25(OH)_2D$ directly stimulates FGF23 expression in osteocytes via a vitamin D response element (VDRE) in the *Fgf23* promoter (20) (Figure 1B and Figure 2A). Since FGF23 suppresses $1,25(OH)_2D$ production, $1,25(OH)_2D$ stimulation of FGF23 closes a feedback loop (20). $1,25(OH)_2D$ may also indirectly regulate FGF23 expression. In this regard, selective deletion of the vitamin D receptor (VDR) in cartilage produces an unidentified chondrocyte-derived inhibitor of *FGF23* transcription (58) (Figure 2A). In the setting of excess $1,25(OH)_2D$ and reduced PTH levels, FGF23-mediated phosphaturia helps prevent potential hyperphosphatemia from enhanced $1,25(OH)_2D$-dependent gastrointestinal phosphate absorption and diminished PTH-mediated phosphaturia. Thus, a primary function of FGF23 is to enhance phosphate excretion and suppress $1,25(OH)_2D$ production, which may have evolved to protect an organism from vitamin D intoxication (20).

Given that FGF23 is a "phosphaturic hormone", it is expected to be regulated by serum phosphate. This appears to be the case in chronic kidney disease, in which elevations in FGF23 compensate for the reduced renal clearance of phosphate and the degree of FGF23 elevation correlates with the severity of hyperphosphatemia (59). However, extracellular phosphate does not appear to directly stimulate *FGF23* mRNA levels or *FGF23* gene promoter activity in osteoblastic cultures (20). Although phosphate loading in mice increases FGF23 levels (60), evidence of the importance of dietary phosphate in regulating FGF23 levels in humans is also conflicting (61, 62). Thus, the proximate factors mediating the effects of phosphate on FGF23 production remain unknown. Pathways controlling serum calcium may also regulate FGF23. In this regard, elevated FGF23 level is associated with both low calcium intake in the absence of vitamin D deficiency (63) and with excess PTH in primary hyperparathyroidism; whereas suppression of FGF23 is observed in response to the hypocalcemic hormone calcitonin in patients with tumor-induced osteomalacia (64, 65). However, neither extracellular calcium nor PTH directly stimulate FGF23 promoter activity in osteoblasts (20), suggesting that their effects may also be indirect.

*Local factors regulating FGF23 and bone mineralization.* There are also local bone-derived factors such as phosphate-regulating gene with homologies to endopeptidases on the X chromosome (PHEX), a cell surface endopeptidase also located predominately in osteoblasts and osteocytes (19), which regulates FGF23 production in bone as well as the mineralization of extracellular matrix (20), thereby providing signaling pathways for coordinating bone phosphate accretion with renal phosphate conservation. Deletion of either *Phex* or the gene coding for dentin matrix acidic phosphoprotein 1 (*Dmp1*) in mice results in nearly identical increments in FGF23 production by osteocytes, leading to overlapping phenotypes characterized by hypophosphatemia, aberrant vitamin D metabolism, and rickets/osteomalacia (19, 66). Ablation of FGF23 corrects these abnormalities in both *Phex*- and *Dmp1*-deficient mice (19, 66). Understanding how deficiency in PHEX

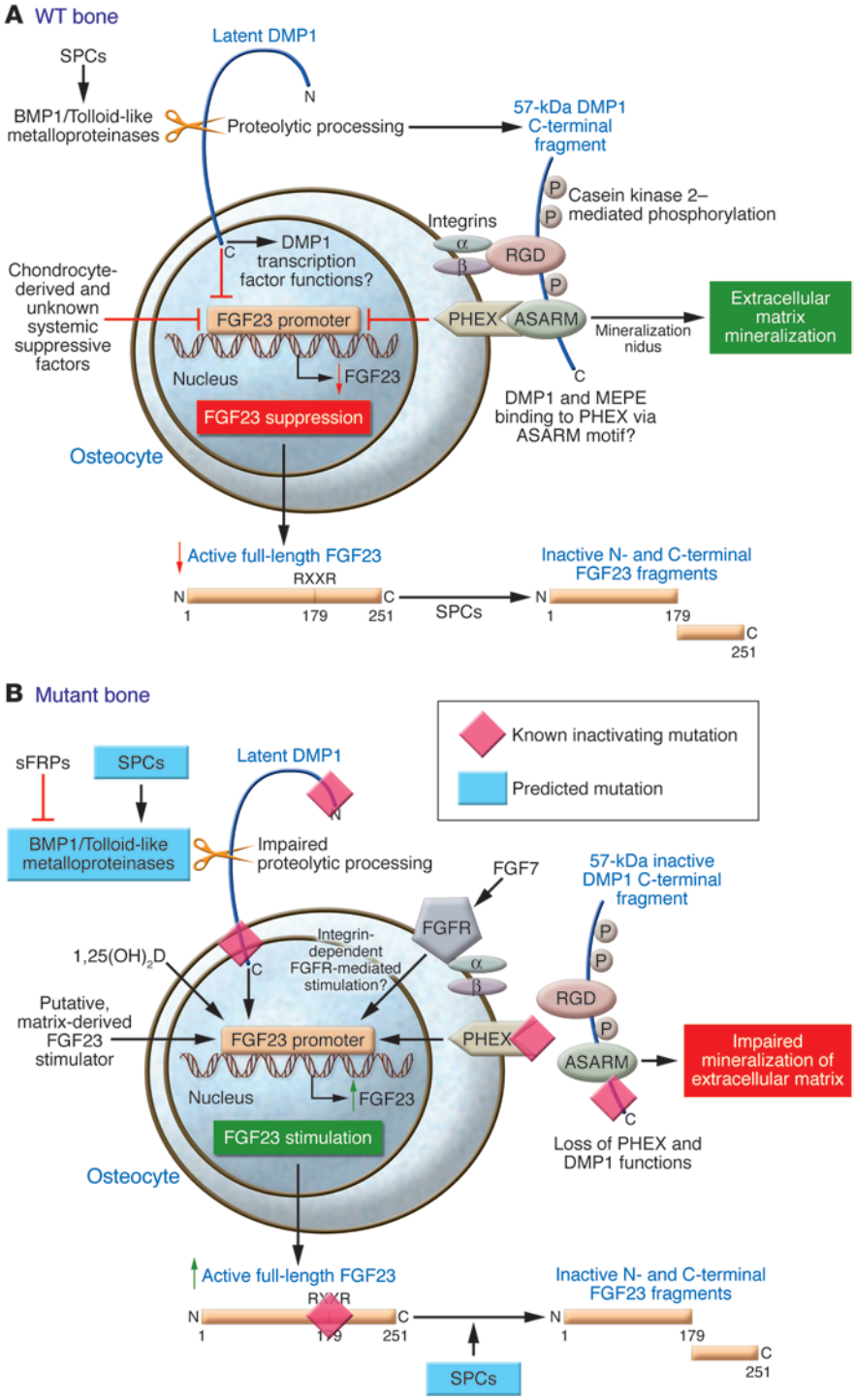


**Figure 2**
Hypothetical model of FGF23 regulation. (**A**) FGF23 regulation in wild-type osteocytes. FGF23 expression in wild-type osteocytes is low due to putative suppressive signals. DMP1 is processed by BMP1/Tolloid-like metalloproteinases to create N- and C-terminal fragments. The model proposes that the C terminus of DMP1 suppresses FGF23 through its binding to PHEX via the ASARM motif and to integrins via the RGD site as well as facilitates mineralization of matrix. In addition, DMP1 is known to have direct transcriptional activities. Putative chondrocyte-derived and unknown systemic factors also suppress FGF23 as described in the text. In addition, FGF23 undergoes posttranslational processing to inactive N- and C-terminal fragments by yet-to-be defined subtilisin-like proprotein convertases (SPCs). (**B**) Potential mutations and pathways leading to increased FGF23 production. DMP1 and PHEX mutations may indirectly regulate FGF23 promoter activity through the accumulation in the extracellular matrix of an unknown FGF23-stimulating factor or through direct effects on osteocyte function. Loss of PHEX or DMP1 might also permit integrin interactions with FGFRs, leading to FGFR-mediated increases in FGF23 production. Other phosphaturic factors, such as sFRPs (by interfering with DMP1 processing), FGF7 (through activation of FGRs), and MEPE (through competition with DMP1 for PHEX binding [not shown]) may stimulate FGF23 through common pathways. In addition, known mutations of FGF23 that prevent degradation as well as theoretical mutations in SPCs that degrade FGF23 and/or indirectly modulate DMP1 processing by BMP1 are shown.

and DMP1 regulates FGF23 expression in osteocytes and impairs bone mineralization is one of the more important issues remaining to be addressed (Figure 2).

PHEX colocalizes with FGF23 in osteocytes. Inactivating PHEX mutations result in increased FGF23 expression in osteocytes as well as phosphate-dependent and -independent defects in bone mineralization. Recent studies indicate that the conditional deletion of *Phex* in the osteoblast lineage in mice is sufficient to reproduce the *Hyp* phenotype, namely hypophosphatemia, characterized by decreased $1,25(OH)_2D$ levels and rickets/osteomalacia, consistent with the central role of the osteocyte in the regulation of FGF23 and phosphate homeostasis (67). Although an initial study suggested that PHEX processes FGF23 (46), subsequent studies have failed to establish PHEX-dependent cleavage of FGF23 (68–70). Moreover, explantation of bone from *Hyp* mice into wild-type mice demonstrates that the high expression of FGF23 in osteocytes is an intrinsic/local effect caused by the loss of PHEX (71). Screening of substrate phage libraries has identi-

fied that PHEX cleaves small peptides, such as the ASARM (acidic, serine- and aspartic acid–rich motif) peptide derived from MEPE (matrix extracellular phosphoglycoprotein with ASARM motif) (72), but the physiologically relevant substrates for PHEX that regulate FGF23 expression and mineralization are not known (69). It is clear, however, that the absence of PHEX is necessary but not sufficient to stimulate FGF23. In this regard, a functional PHEX is missing in osteoblasts of *Hyp* mice, but osteoblasts do not upregulate FGF23 expression until they differentiate into osteocytes embedded in bone matrix (19, 71). The importance of temporal and spatial expression of PHEX is also evidenced by the paradox that restoration of PHEX expression in transgenic mice using heterologous promoters fails to rescue the elevated FGF23 expression (73–75). These observations suggest the possible presence of a matrix-derived FGF23 stimulatory factor that may be inhibited or sequestered by a functional PHEX and somehow released or activated when PHEX is inhibited or mutated (Figure 2B). In addition, in humans, activating mutations of FGFR1 (76) and mutations leading to increments in circulating Klotho levels (51) are associated with elevated FGF23 levels, raising the possibility that these factors may also regulate the osteocyte production of FGF23 (Figure 2B).

A major advance in understanding how extracellular matrix proteins might regulate FGF23 production comes with the discovery that inactivating mutations of DMP1 lead to increased FGF23 in autosomal recessive hypophosphatemic rickets (ARHR) (77). DMP1 is expressed in mineralized tissues, including osteocytes, ameloblasts, and cementoblasts, but is also expressed in nonmineralizing tissues such as brain, salivary gland, liver, muscle, pancreas, and kidney (78). How DMP1 deficiency stimulates FGF23 production is not known, but several possibilities are apparent from our knowledge of DMP1 structure and function (Figure 2B). DMP1 contains an RGD domain for integrin binding, an ASARM peptide (which might allow DMP1 to bind to PHEX), a large number of acidic domains, an N-terminal site for binding to MMP9, sites for casein kinase-2–mediated phosphorylation, and conserved cleavage sites for bone morphogenetic protein 1/Tolloid-like MMPs (BMP1/Tolloid-like MMPs) and cathepsin B (Figure 2). DMP1 exists as a latent protein that is cleaved into 37-kDa and 57-kDa phosphoproteins by BMP1 or cathepsin B (Figure 2A). The highly phosphorylated C-terminal 57-kDa fragment (containing 42 phosphates/mol) likely functions as a nucleator for mineralization. The $NH_2$-terminal fragment from DMP1 is a proteoglycan with a chondroitin sulfate chain attached through Ser74 that also binds to proMMP-9 (79). DMP1 also is localized to the dendrites of embedding osteocytes. Finally, DMP1 has been reported to translocate to the nucleus where it may regulate gene transcription (80).

Although FGF23-dependent hypophosphatemia contributes to impaired mineralization, an intrinsic defect in mineralization of extracellular matrix persists in *Phex*- and *Dmp1*-mutant mice even when phosphate is normalized (71). In *Dmp1*-null mice, the absence of the nucleation and mineral propagation functions of DMP1 likely contribute to the defective mineralization (81, 82). The mechanism for the hypophosphatemia-independent mineralization abnormalities in *Hyp* mice is less well understood, but existing data fails to support a role for DMP1. Rather, *Hyp* bone is characterized by elevated proteolytic activity and the production by osteoblasts of a mineralization inhibitor, referred to as Minhibin (83). In *Hyp* mice, the elevated proteolytic activity is associated with the increased production of an ASARM peptide from MEPE and DMP1, which has the ability to inhibit mineralization (84) and may represent Minhibin (83). Moreover, the administration of cathepsin inhibitors improves bone mineralization in *Hyp* mice, without correcting FGF23 expression or hypophosphatemia, indicating the separate regulation of hypophosphatemia and mineralization by PHEX (85).

Based on our current understanding of PHEX and DMP1 structure and function, a model can be proposed that outlines various possible mechanisms for local regulation of FGF23 expression in osteocytes (Figure 2). The model predicts that in wild-type bone DMP1 and PHEX suppress FGF23 transcription, possibly through sequestration of putative FGF23 stimulating factors or degradation or release of a suppressive factor in the extracellular matrix, or more likely, due to direct effects of DMP1 and PHEX to suppress *FGF23* gene transcription (Figure 2A). PHEX is capable of binding to ASARM motifs in DMP1, potentially sequestering these factors or altering their metabolism or facilitating its binding to integrins. The C terminus of DMP1, which contains RGD integrin-binding and ASARM domains, is sufficient to rescue the phenotype of *Dmp1*-null mice (86), suggesting that this region may hold the key to understanding FGF23 regulation. In contrast, inactivating mutations of PHEX and DMP1 increase FGF23 expression in this model via indirect effects mediated by the production and accumulation in the bone matrix of an unidentified PHEX substrate that acts as an FGF23-stimulating factor or by a direct cellular action of loss-of-function PHEX or DMP1 mutations to regulate osteocyte function, leading to increased FGF23 production (Figure 2B). Impaired processing of DMP1 would also stimulate FGF23, by creating a functional DMP1-deficient state equivalent to *Dmp1*-null mice (Figure 2B). Loss of PHEX or DMP1 is predicted to result in the production or increased bioavailability of an unknown, local, bone-derived "FGF23 stimulating factor" or an intrinsic cellular defect, leading to increased FGF23 levels. FGFR1 and possible paracrine/autocrine effects of other members of the FGF family may regulate FGF23 production as well. A possible candidate ligand for this pathway is FGF2, which is synthesized by osteoblasts, deposited in extracellular matrix, and exerts autocrine or paracrine effects to inhibit osteoblastic differentiation (87). The phosphaturic effects of FGF7 might also be mediated via FGFR stimulation of FGF23 (88). This model also depicts the recent finding that FGF1 binds to integrins to facilitate FGF1 signaling (89). It will be important to investigate in future studies whether the C terminus of DMP1 could potentially interfere with FGF1-integrin interactions via its binding to integrins (Figure 2A), leading to suppression of FGF23 in normal osteocytes (Figure 2A). This model also proposes a common pathway for other putative phosphatonins, such as MEPE and secreted frizzled-related protein 4 (sFRP4), which in this model indirectly modulate FGF23 production (Figure 2B). For example, the phosphaturic actions of MEPE overexpression in this model are caused by binding of the ASARM peptide to PHEX and inhibition of its activity (90, 91). The phosphaturic actions of sFRP4 might be explained by the effect of sFRPs to inhibit BMP1 and the processing of DMP1 leading to elevated FGF23 levels (Figure 2B). Finally, yet-to-be discovered mutations of a subtilisin-like proprotein convertase would also be predicted to lead to FGF23-dependent hypophosphatemia by inhibiting the degradation of this phosphaturic factor. Although many aspects of this model are speculative, it provides a conceptual framework to guide future studies.

**Table 1**
Classification of hypophosphatemic and hyperphosphatemic disorders

| Clinical syndromes | Disease genes | Mechanisms |
|---|---|---|
| **FGF23-dependent hypophosphatemic disorders** | | |
| Hereditary | | |
| XLH | *PHEX* | Increased FGF23 production by osteocytes |
| ADHR | *FGF23* | Decreased FGF23 degradation |
| ARHR | *DMP1* | Increased production of FGF23 by osteocytes |
| OGD | *FGFR1* | Increased production of FGF23/end-organ gain of function? |
| Acquired/Sporadic | | |
| HRH | *KI* | Gain-of-function end-organ responsiveness; direct actions of β-Klotho |
| McCune-Albright syndrome, polyostotic fibrous dysplasia | *GNAS1* | Increased FGF23 production by fibrous lesions in bone |
| Tumor-induced osteomalacia | Possibly *MEPE* and *sFRP4* | Increased FGF23 production by tumors |
| Epidermal nevus syndrome | *FGFR3* | Increased circulating FGF23 due to end-organ gain of function |
| **Primary disorders of renal phosphate reabsorption** | | |
| Hereditary | | |
| Hereditary hypophosphatemic rickets with hypercalciuria | *SLC34A3* | Transporter defect leading to impaired renal tubular phosphate reabsorption and increased $1,25(OH)_2D$ production |
| Autosomal recessive pulmonary alveolar microlithiasis | *SLC34A2* | No hypophosphatemia |
| No human disease–causing mutation identified | *SLC34A1* | Transporter defect leading to impaired renal tubular phosphate reabsorption |
| X-linked recessive hypophosphatemic rickets | *CLCN5* | Defective endocytosis and redistribution of NaPi-II from the plasma membrane to intracellular vesicles; Fanconi-like proteinuria |
| Lowe oculocerebrorenal syndrome | *OCRL* | Defective phosphatidylinositol 4,5-bisphosphate 5-phosphatase–mediated endocytosis of Npt2 and Fanconi-like proteinuria |
| No human disease–causing mutation identified | *NHERF1* | Aberrant Npt2 protein localization to internal sites in the renal proximal tubule cells |
| **Hyperphosphatemic disorders** | | |
| Hereditary/acquired/sporadic | | |
| Tumoral calcinosis | *GALNT3*, *FGF23*, *KLOTHO* | Decreased stability or production of FGF23 or end-organ resistance to FGF23 (loss of Klotho) |

## Role of FGF23 in our understanding of disorders of phosphate homeostasis

Insights into the function and interrelationships between FGF23, Klotho, and sodium-dependent phosphate transporters have clarified the pathogenesis of hereditary and acquired hypophosphatemic disorders (Table 1). These can be broadly classified into FGF23-dependent disorders and primary disorders of proximal renal tubular phosphate transport.

*Diseases of FGF23 excess*. Hereditary hypophosphatemic disorders caused by a primary increase in circulating levels of FGF23 include autosomal dominant hypophosphatemic rickets (ADHR; MIM 193100), autosomal recessive hypophosphatemic rickets (ARHR; MIM 241520), X-linked hypophosphatemic rickets (XLH; MIM 307800), and osteoglophonic dysplasia (OGD; MIM 166250) (Table 1). All have overlapping clinical characteristics, including hypophosphatemia due to renal phosphate wasting and inappropriately normal $1,25(OH)_2D$ levels for the degree of hypophosphatemia and rickets/osteomalacia, and the absence of hypercalciuria. There is a single report of a patient with hypophosphatemic rickets and hyperparathyroidism (HRH; MIM pending) (51). HRH differs from the others disorders described here in that hyperparathyroidism is a predominant feature. The clinical features of ADHR, ARHR, XLH, OGD, and HRH are caused by excess FGF23, but the mechanisms whereby FGF23 levels are increased differ between these disorders. ADHR is caused by mutations (R176Q and R179W) in the RXXR furin-like cleavage domain of FGF23 that impairs proteolytic inactivation of FGF23 (68, 92). ARHR is caused by inactivating mutations in DMP1 (77, 93), a member of the small integrin-binding ligand N-linked glycoprotein (SIBLING) family of extracellular matrix protein that augments mineralization (81). Loss of *DMP1* results in increased transcription of FGF23 by osteocytes (77). XLH is caused by inactivating mutations in *PHEX* (94–96). Loss of *PHEX* also leads to increased expression of FGF23 by osteocytes (70). How loss-of-function *DMP1* and *PHEX* mutations lead to increases in *FGF23* gene transcription is not known (97). OGD is an autosomal dominant bone dysplastic disorder caused by activating mutations in the *FGFR1* gene, suggesting FGFR1 may regulate FGF23 expression in bone and/or the renal handling of phosphate (76). HRH also has elevated FGF23 levels, but the primary genetic abnormality is a translocation causing elevated circulating levels of Klotho (51). How excess Klotho leads to increased FGF23 levels, however, is not clear. Elevated circulating Klotho levels could potentially bind to and stabilize FGF23, target bone to increase FGF23 production, or indirectly stimulate FGF23 secretion via effects on other pathways.

McCune-Albright syndrome (MIM 174800), also called polyostotic fibrous dysplasia (PFD), is caused by a mosaicism for postzygotic activating mutations in the guanine nucleotide binding protein, alpha stimulating gene (*GNAS1*), leading to fibrodysplastic tissue and associated hypophosphatemia and elevated circulating FGF23 levels in some patients (98) (Table 1). Tumor-induced osteomalacia, or oncogenic osteomalacia, is a paraneoplastic syndrome of renal phosphate wasting, aberrant vitamin D metabolism, and osteomalacia that is associated with elevated FGF23 levels (59, 99). The proximate cause of increased FGF23 production is not known, but this disorder is associated with increased levels of MEPE and sFRP4, which may regulate PHEX and DMP1 metabolism, respectively (100) (Figure 2). Linear sebaceous or epidermal nevus syndrome (ENS) is caused by a mosaicism of activating FGFR3 mutations in the human epidermis in some patients (101). A subset of these patients have ipsilateral focal bone disease associated with hypophosphatemic rickets, elevated circulating FGF23 levels, and aberrant $1,25(OH)_2D$ levels, similar to other syndromes caused by elevated FGF23 (102).

Other disorders are characterized by a secondary/adaptive increase in FGF23. For example, FGF23 is markedly increased in early stages of chronic kidney diseases, in which its actions to decrease CYP27B1 and increase CYP24 lead to diminish calcitriol levels and contributes to the development of secondary hyperparathyroidism (103). In addition, increments in FGF23 have been associated with increased mortality in patients with end-stage renal disease, suggesting that it may act as a uremic toxin (104). Anti-FGF23 neutralizing antibodies are being developed that offer a potential way to treat disorders of excessive FGF23 (105); whether these will be clinically useful, however, is uncertain.

*Diseases of FGF23 deficiency*. Hyperphosphatemic familial tumoral calcinosis (HFTC; MIM 211900) is a rare autosomal recessive disorder characterized by hyperphosphatemia, normal or elevated $1,25(OH)_2D$ levels, soft-tissue calcifications, and typically massive lobulated periarticular calcifications levels (106). To date there have been three different mutations identified as leading to either decreased bioactive circulating FGF23 levels or end-organ resistance to FGF23. These include mutations of the genes encoding FGF23 (23), Klotho (29) and GalNAc transferase 3 (GALNT3) (107), a Golgi-associated enzyme that *O*-glycosylates a furin-like convertase recognition sequence in FGF23 (108). Missense mutations of FGF23 impair its secretion, leading to inadequate circulating levels of this phosphaturic factor (23). Mutations of GALNT3 destabilize FGF23, resulting in a low-intact serum FGF23 levels but high levels of biologically inactive C-terminal FGF23 fragments. A mutation in the gene coding for Klotho (H193R) results in decreased Klotho expression and reduced FGF23-Klotho-FGFR complex formation and to end-organ resistance to FGF23 (29).

*FGF23-independent hypophosphatemic disorders: diseases affecting phosphate transporters.* A primary defect in proximal tubular phosphate reabsorption results in elevations of $1,25(OH)_2D$, which increases gastrointestinal calcium absorption and leads to hypercalciuria — two features that distinguish primary renal phosphate wasting disorders from disorders of FGF23 excess. *SLC34A1* and *SLC34A3* encode the sodium-dependent phosphate transporters in the proximal tubule of the kidney. Hereditary hypophosphatemic rickets with hypercalciuria (HHRH; MIM 241530) is caused by inactivating mutations in the *SLC34A3* gene encoding NPT2c or NaPi-IIc (109). Mutations in *SLC34A1*, which encodes the electrogenic NPT2a or NaPi-IIa, have not been unequivocally shown to cause hypophosphatemia in humans. Interestingly, mice with homozygous deletion of *Slc34a1* have mild hypophosphatemia and rickets/osteomalacia (110), possibly due to the compensatory upregulation of SLC34A3 (111). *SLC34A2* encodes NPT2b or NaPi-IIb, which is responsible for transcellular phosphate absorption in the small intestine. *SLC34A2* is expressed mainly in lung and mammary gland and to a lesser extent in intestine, kidney, and prostate (112). Mutations in this gene cause pulmonary alveolar microlithiasis and are not reported to be associated with major abnormalities of serum phosphate (113).

X-linked hypercalciuric nephrolithiasis, or Dent disease (MIM 300009), includes several related forms of hereditary proximal tubulopathy caused by mutations in the chloride channel 5 *(CLCN5)* gene, which encodes a voltage-gated chloride channel and chloride/proton antiporter (114). Phosphaturia results from defective endocytosis and redistribution of SLC34A1 from the plasma membrane to intracellular vesicles and is associated with low–molecular weight proteinuria and hypercalciuria. Lowe oculocerebrorenal syndrome (LOS; MIM 30900) is caused by mutations in the gene oculocerebrorenal syndrome of Lowe (*OCRL1)*, which encodes a phosphatidylinositol 4,5-bisphosphate 5-phosphatase that is also involved in endocytosis. Patients with LOS are characterized as having more pronounced hypophosphatemia/rickets and proximal tubular proteinuria than patients with Dent disease, and LOS has other features such as elevated lactate dehydrogenase and/or creatine kinase levels.

## Conclusion

The study of the regulation and function of the phosphaturic and $1,25(OH)_2D$ regulating factor FGF23 has lead to the recognition that bone is an endocrine organ, in which osteocytes produce FGF23 that participates in a bone-kidney axis, regulating phosphate, vitamin D, and mineral homeostasis. This hormonal axis establishes a new conceptual framework for understanding the pathogenesis, diagnosis, and treatment of hyperphosphatemic and hypophosphatemic disorders. In addition, by continuing to elucidate the complex systems biology surrounding FGF23 regulation and function, we will likely gain novel insights into bone and renal physiology.

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