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FGF23 Production by Osteocytes

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Abstract

Fibroblast Growth Factor 23, FGF23, known to be a regulator of phosphate homeostasis, is made by cells residing in bone, the osteocytes, to target a distant organ, the kidney. Recently, elevated FGF23 has been found systemically and in osteocytes in patients and animal models of chronic kidney disease. Also associations of FGF23 serum level have been found with vascular dysfunction, vascular calcification, and increased risk of cardiovascular disease. In this review we discuss FGF23 expression in osteocytes and the potential means to regulate expression and function of this protein at the osteocyte level.

Keywords

Fibroblast Growth Factor 23; phosphate homeostasis; osteocytes; bone metabolism; cardiovascular disease

Introduction- The Osteocyte and FGF23

Osteocytes are cells embedded in the mineralized bone matrix, connected to each other and cells outside the bone through a bone fluid filled lacunocanalicular system [1] [2]. In the adult skeleton they comprise over 90-95% of all bone cells as compared to osteoblasts which make bone (<5%), and osteoclasts which resorb bone (>1%). Osteocytes, unlike most cells in the body, are long-lived, up to decades within the bone matrix. It has long been thought that the major function of osteocytes is to respond to mechanical strain with biochemical signals that regulate osteoblast and osteoclast activity [3]. However, within recent years it has been discovered that osteocytes are also secretory cells that can target tissues other than bone [4]. These cells are multifunctional acting as mechanosensors and communicators, orchestrators of bone modeling and remodeling, regulators of calcium and phosphate homeostasis and function as endocrine cells sending signals to distant tissues.

This review will focus on the role of osteocytes as endocrine cells through production of Fibroblast Growth Factor 23, FGF23. Since its identification in 2000 in the ventrolateral thalamic nucleus of the brain [5], FGF23 has actually been found to be most highly expressed in bone, predominantly in the osteocyte [6-8]. FGF23 is not normally expressed at high levels in osteocytes but is dramatically increased in osteocytes in patients with hypophosphatemic rickets [9] and in patients with chronic kidney disease, CKD [10].

FGF23 is an important hormone in regulating serum phosphate levels. One mechanism by which FGF23 does this is by reducing expression and/or insertion of the sodium-phosphate transporters (type IIa and IIc) within renal proximal tubular membranes so that more

phosphate is excreted [11]. A second mechanism is by inhibiting renal 1- α -hydroxylase expression (and promotes 24-hydroxylase) which reduces 1,25(OH)₂D levels and therefore reduces the amount of phosphate absorption from the gut and bone [12]. These actions are thought to be the main reasons that hypophosphatemia occurs in certain disease states with elevated FGF23 levels. FGF23 also is involved in altering parathyroid hormone (PTH) levels. Specifically, it has been shown that FGF23 can inhibit parathyroid hormone (PTH) mRNA synthesis in the rat [13]. However, the significance of this in humans especially with CKD is a little more difficult to interpret. FGF23 causes a decrease in 1,25(OH)₂D and a low level of 1,25(OH)₂D can cause an up-regulation of PTH. In the setting of CKD there is a chronically elevated serum phosphate level which induces an increase in both PTH and FGF23. In addition, in hyperparathyroidism secondary to renal failure it has been proposed the parathyroid becomes less sensitive to FGF23 signaling by downregulation of FGFRs and Klotho which further complicates the interaction [14].

FGF23 primarily mediates these actions on the kidney and parathyroid through FGF receptor (FGFR) subtypes 1,3,4 [15-16]. Typically it has been thought that FGF23 also needs the transmembrane co-receptor Klotho to help increase the binding affinity of FGF23 to FGFRs [17-18]. The osteocyte itself expresses FGFRs and low expression of klotho and signaling through FGFRs is thought to be involved in regulation of FGF23 expression as will be discussed later in this review.

Regulation of Release of FGF23 by the Osteocyte

Release of FGF23 is one of the main means whereby young osteocytes and mature osteocytes regulate mineralization and phosphate homeostasis. Osteocytes also appear to regulate FGF23 signaling and bio-mineralization through molecules such as phosphate-regulating gene with homologies to endopeptidases on X chromosome (PHEX), Dentin Matrix Protein-1 (DMP1), and Matrix Extracellular Phospho-glycoprotein, MEPE [19], which are all highly expressed in osteocytes [20-22]. X-linked Hypophosphatemic Rickets, is due to mutations in *Phex* [20]. Autosomal Recessive Hypophosphatemic Rickets is due to mutations in *Dmp1* [7]. PHEX is a metalloendoproteinase with an unclear function, but it clearly plays a role in phosphate homeostasis and bone mineralization. DMP1 is also essential for mineralization and both *Dmp1* and *Phex* expression are markers of early osteocytes. In the absence of either functional *Dmp1* or *Phex*, FGF23 is dramatically elevated in the osteocyte and in the circulation, leading to phosphate excretion by the kidney thereby reducing circulating phosphate resulting in osteomalacia and rickets. Loss of function mutations and knock-out murine models have been made for *Phex*, *Dmp1* and *Mepe* [7, 20, 23-25]. *Dmp1* null mice have a similar phenotype to 'Hyp' mice carrying a *Phex* mutation, that of osteomalacia and rickets due to elevated FGF23 levels in osteocytes [7] whereas the *Mepe* nulls have increased bone mass [25].

The exact mechanism(s) by which DMP1 and PHEX regulate FGF23 are yet to be determined, however, recent data suggests that their inhibitory effects are mediated by FGF receptor (FGFR) signaling in the osteocyte [26]. *Dmp1*-null and Hyp mouse models show enhanced FGFR signaling compared to wild-type control mice and inhibition of FGFR signaling results in a reduction of FGF23 in the bone marrow stromal cells from both mouse models. Martin et al. [26] concluded that likely it was not FGF23 acting back on the FGFR itself but some other FGFR mediated pathway. These findings indicate that FGFRs play a role in endocrine signaling by the osteocyte on organs like the kidney but also play an autocrine/paracrine role on the osteocyte itself.

The effects of MEPE on FGF23 and matrix mineralization are complex. Complexity is due to the release of an acidic serine aspartate-rich MEPE-associated motif (ASARM) from its

C-terminus of MEPE. This 19 amino acid peptide is known to be a potent inhibitor of mineralization *in vivo* [27-28] and absence of this peptide in MEPE-null mice may be the reason these mice have increased bone [25]. Binding of PHEX to MEPE prevents its proteolytic degradation and the release of the ASARM peptide [29] preventing down regulation of FGF23. If the ASARM peptide is generated it binds specifically to PHEX *in vitro* to inhibit PHEX enzymatic activity [9], which results in up-regulation of FGF23 expression. In addition, the phosphorylated MEPE-ASARM peptide itself is a substrate for PHEX, with cleavage of ASARM by PHEX neutralizing its activity and restoring mineralization [30]. This is a very complex regulatory system that requires further study.

In addition to being regulated by DMP1, PHEX, and MEPE, 1,25(OH)₂D induces expression of FGF23 in the osteocyte [31-32], suggesting a negative feedback system. In addition to 1,25(OH)₂D, PTH may directly regulate FGF23 levels. Infusion of PTH in mice resulted in increased FGF23 mRNA expression in the calvaria and increased serum FGF23 [33]. PTH was also shown to up-regulate expression of FGF23 mRNA in UMR106 cells. Rhee and colleagues observed an increase in FGF23 expression in osteocytes in transgenic mice with constitutive activation of the PTH receptor (PTHr1) in osteocytes under control of the DMP1 promoter [34].

Not only is biologically active FGF23 regulated at the gene level, but also at the protein level. FGF23 activity is regulated by extent of glycosylation and proteolytic processing. Intact FGF23 (32-35 kDa) is biologically active, whereas the c-terminal form (15-17 kDa) is inactive, yet both are present in the circulation, emphasizing the importance of assays that can make a distinction between the two forms [35]. The c-terminal form may block the bioactivity of the intact form [36]. Glycosylation of intact FGF23 protects against furin-mediated cleavage to generate the c-terminal form. Therefore relative expression of the glycosyl transferase and protease (thought to be furin) determine the ratio of intact/cleaved FGF23. It also appears that iron levels can regulate the levels of cleaved FGF23, while not affecting intact FGF23 [37]. It is not clear how iron deficiency can increase a product of the intact protein without affecting intact protein levels. Examples of high intact FGF23 include hypophosphatemia and low intact includes familial tumoral calcinosis. In Fibrous Dysplasia, both high intact and high cleaved is detected suggesting that the cleaved form neutralizes the biological activity of the intact FGF23 [38]. Recently it has been shown with renal disease only intact with no cleaved FGF23 is present. As FGF23 is elevated in osteocytes in hypophosphatemic rickets and CKD, it is assumed that little if any of the cleaved form of FGF23 is being generated. To date, it is not known how these processes are being regulated in the osteocyte.

The location of the osteocytes that release FGF23 may be different in different disease states. In mouse models of hereditary hypophosphatemia, FGF23 is typically released by osteocytes of both cortical and trabecular bone [31, 39]. However, differences were found in a study conducted on the *Col4a3* null mouse which is a model of human autosomal-recessive Alport Disease in which there is progressive renal failure [40]. In this study, FGF23 was primarily found to be expressed in trabecular osteocytes. Therefore, these authors concluded that there may be differences in FGF23 release in CKD versus other genetic forms of hypophosphatemia. In addition, Stubbs et al. [40] observed that significant increases in *Fgf23* gene expression in the bone did not occur until later stages of CKD. Since FGF23 becomes elevated very early on in CKD and since the major site of FGF23 production is from the osteocyte, this may suggest that post-transcriptional processing, excretion, or breakdown of FGF23 may be altered during the early stages of CKD.

Bone-Heart Crosstalk

While it is well documented that FGF23 plays an important role as an endocrine agent in regulating phosphate and mineralization through organs such as the kidney and parathyroid, there is new evidence that FGF23 may also be important for targeting other organs such as the heart and vasculature. It is well known that the major cause of morbidity and mortality with CKD is heart disease and recent clinical studies have found an independent association between serum FGF23 levels and left ventricular hypertrophy in CKD patients [41-45]. All four isoforms have been detected in cardiomyocytes [46] [47] which we have also confirmed in preliminary data from isolated mouse ventricular cardiomyocytes in our laboratory. Therefore, it has logically been hypothesized that FGF23 may directly induce cardiac hypertrophy. To date, however, there is only one study that has directly tested this possibility. Faul et al. (2011) observed that 48 hour treatment of 10-100 ng/ml FGF23 increased cell size and markers of cardiac hypertrophy of isolated neonatal rat cardiomyocytes which was blocked by a pan-FGF receptor inhibitor. Furthermore, *in vivo* intramyocardial injections of FGF23 increased left ventricular wall thickness by day 7. Lastly, using a model of CKD (5/6 nephrectomized mouse) that has elevated serum FGF23 they found left ventricular hypertrophy was reduced in those animals that received the pan-FGF receptor inhibitor. Interestingly, while klotho has been detected in the SA node, it has not been found in the ventricles [46, 48]. Therefore, cardiac hypertrophy may be an example of FGF23 acting independent of this co-receptor that was thought to be needed for function. It is possible that FGF23 may activate FGFRs without the need for Klotho when serum FGF23 concentrations are at a high level such as during CKD. In addition, it still remains to be elucidated which subtype of FGFR mediates the cardiac hypertrophic effects or if multiple FGFR subtypes work in concert as has been proposed for FGF23 actions on the kidney [49]. Additional research will be required to fully understand the molecular mechanisms and important consequences for direct FGF23 actions on the myocardium.

There are additional clinical reports of associations of FGF23 serum levels with vascular dysfunction [43-44], vascular calcification [50], and increased risk of cardiovascular disease [51]. While these correlations are likely the result of phosphate handling and progression of disease, it remains to be seen if there is also a direct effect of FGF23 on vascular cells. Taken as a whole, these findings in the cardiovascular system suggest that FGF23 released by osteocytes may be even more complex than a hormone involved in phosphate handling, bone disorders, or a marker of kidney disease.

All these findings have raised the question if FGF23 is a marker or inducer of renal and cardiovascular pathologies [52-53]. This is especially significant during the early stages of kidney dysfunction when FGF23 levels rise while calcium, phosphate, and PTH have not yet significantly changed. FGF23 causes a reduction of 1,25(OH)₂D early on in CKD which could trigger the bone deficiencies observed with the disease. FGF23 could also be altering phosphate transporters in vascular cells predisposing to calcification and vascular dysfunction [54]. And finally, FGF23 could be altering signaling in cardiac myocytes to set the stage for pathological hypertrophy. However, there are many additional questions such as why do other diseases with elevated FGF23 levels (such as X-linked hypophosphatemia and autosomal recessive hypophosphatemic rickets) not present an overt cardiovascular phenotype. It is possible that FGF23 is working in concert with other factors specific in the setting of CKD to cause renal and cardiovascular changes. Recently, it has been hypothesized that disruptions in specific interactions between vitamin D, FGF23, Klotho, and the renin-angiotensin-aldosterone system during CKD contribute to disease progression [55].

Targeted Treatment

If FGF23 is found to directly or indirectly induce these organ changes, this puts the osteocyte release of FGF23 as a prime target for therapeutic intervention in CKD as well as hypophosphatemia. Efforts are underway to target or reduce levels of active FGF23. Treatment with FGF23 antibody restored serum phosphate levels in the Hyp mouse model and corrected the osteomalacic phenotype [56] and treatment of *Dmp-1*-null mice with FGF23 antibody ameliorated the bone defects by increasing levels of circulating phosphate [57]. Since the potential actions of FGF23 may extend to the cardiovascular system and beyond, treatments targeting FGF23 provide significant therapeutic potential, not only for bone disease but potentially for other chronic diseases.

In summary, the osteocyte lacunocanalicular system functions as an endocrine organ regulating phosphate metabolism through the production of FGF23. Understanding the interactions of osteocyte factors and their targets should lead to insight into diseases of hyper and hypophosphatemia, diseases of the cardio-vascular system, and chronic kidney disease.

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References

1. Parfitt AM. The cellular basis of bone turnover and bone loss: a rebuttal of the osteocytic resorption--bone flow theory. *Clin Orthop Relat Res.* 1977; 127:236–247. [PubMed: 912987]
2. Kamioka H, Honjo T, Takano-Yamamoto T. A three-dimensional distribution of osteocyte processes revealed by the combination of confocal laser scanning microscopy and differential interference contrast microscopy. *Bone.* 2001; 28:145–149. [PubMed: 11182371]
3. Lanyon LE. Osteocytes, strain detection, bone modeling and remodeling. *Calcif Tissue Int.* 1993; 53:S102–106. discussion S106–107. [PubMed: 8275362]
4. Bonewald LF. The amazing osteocyte. *J Bone Miner Res.* 2011; 26:229–238. [PubMed: 21254230]
5. Yamashita T, Yoshioka M, Itoh N. Identification of a novel fibroblast growth factor, FGF-23, preferentially expressed in the ventrolateral thalamic nucleus of the brain. *Biochem Biophys Res Commun.* 2000; 277:494–498. [PubMed: 11032749]
6. Shimada T, Kakitani M, Yamazaki Y, Hasegawa H, Takeuchi Y, Fujita T, Fukumoto S, Tomizuka K, Yamashita T. Targeted ablation of *Fgf23* demonstrates an essential physiological role of FGF23 in phosphate and vitamin D metabolism. *J Clin Invest.* 2004; 113:561–568. [PubMed: 14966565]
7. Feng JQ, Ward LM, Liu S, Lu Y, Xie Y, Yuan B, Yu X, Rauch F, Davis SI, Zhang S, Rios H, Drezner MK, Quarles LD, Bonewald LF, White KE. Loss of DMP1 causes rickets and osteomalacia and identifies a role for osteocytes in mineral metabolism. *Nat Genet.* 2006; 38:1310–1315. [PubMed: 17033621]
8. Liu S, Bo Y, Xie Y, Zhou J, Quarles LD, Bonewald L, Feng JQ. Elevated levels of FGF23 in Dentin Matrix Protein 1 (DMP1) Null Mice Potentially Explain Phenotypic Similarities to Hyp Mice. *JBoneMin Res.* 2006; 21(Suppl 1):S51.
9. Liu S, Rowe PS, Vierthaler L, Zhou J, Quarles LD. Phosphorylated acidic serine-aspartate-rich MEPE-associated motif peptide from matrix extracellular phosphoglycoprotein inhibits phosphate regulating gene with homologies to endopeptidases on the X-chromosome enzyme activity. *J Endocrinol.* 2007; 192:261–267. [PubMed: 17210763]
10. Pereira RC, Juppner H, Azucena-Serrano CE, Yadin O, Salusky IB, Wesseling-Perry K. Patterns of FGF-23, DMP1, and MEPE expression in patients with chronic kidney disease. *Bone.* 2009; 45:1161–1168. [PubMed: 19679205]
11. Berndt T, Kumar R. Phosphatonins and the regulation of phosphate homeostasis. *Annu Rev Physiol.* 2007; 69:341–359. [PubMed: 17002592]

12. Shimada T, Hasegawa H, Yamazaki Y, Muto T, Hino R, Takeuchi Y, Fujita T, Nakahara K, Fukumoto S, Yamashita T. FGF-23 is a potent regulator of vitamin D metabolism and phosphate homeostasis. *J Bone Miner Res.* 2004; 19:429–435. [PubMed: 15040831]
13. Ben-Dov IZ, Galitzer H, Lavi-Moshayoff V, Goetz R, Kuro-o M, Mohammadi M, Sirkis R, Naveh-Manly T, Silver J. The parathyroid is a target organ for FGF23 in rats. *J Clin Invest.* 2007; 117:4003–4008. [PubMed: 17992255]
14. Canalejo R, Canalejo A, Martinez-Moreno JM, Rodriguez-Ortiz ME, Estepa JC, Mendoza FJ, Munoz-Castaneda JR, Shalhoub V, Almaden Y, Rodriguez M. FGF23 fails to inhibit uremic parathyroid glands. *J Am Soc Nephrol.* 2010; 21:1125–1135. [PubMed: 20431039]
15. Gattineni J, Bates C, Twombly K, Dwarakanath V, Robinson ML, Goetz R, Mohammadi M, Baum M. FGF23 decreases renal NaPi-2a and NaPi-2c expression and induces hypophosphatemia in vivo predominantly via FGF receptor 1. *Am J Physiol Renal Physiol.* 2009; 297:F282–291. [PubMed: 19515808]
16. Gattineni J, Twombly K, Goetz R, Mohammadi M, Baum M. Regulation of serum 1,25(OH)₂ vitamin D₃ levels by fibroblast growth factor 23 is mediated by FGF receptors 3 and 4. *Am J Physiol Renal Physiol.* 2011; 301:F371–377. [PubMed: 21561999]
17. Urakawa I, Yamazaki Y, Shimada T, Iijima K, Hasegawa H, Okawa K, Fujita T, Fukumoto S, Yamashita T. Klotho converts canonical FGF receptor into a specific receptor for FGF23. *Nature.* 2006; 444:770–774. [PubMed: 17086194]
18. Kuro-o M. Klotho. *Pflugers Arch.* 2010; 459:333–343. [PubMed: 19730882]
19. Bonewald, L. Osteocytes. In: Marcus, R.; F, D.; Nelson, D.; Rosen, C., editors. *Osteoporosis.* Elsevier; 2007. p. 169-190.
20. A gene (PEX) with homologies to endopeptidases is mutated in patients with X-linked hypophosphatemic rickets. The HYP Consortium. *Nat Genet.* 1995; 11:130–136. [PubMed: 7550339]
21. Thompson DL, Sabbagh Y, Tenenhouse HS, Roche PC, Drezner MK, Salisbury JL, Grande JP, Poeschla EM, Kumar R. Ontogeny of Phex/PHEX protein expression in mouse embryo and subcellular localization in osteoblasts. *J Bone Miner Res.* 2002; 17:311–320. [PubMed: 11811562]
22. Nampei A, Hashimoto J, Hayashida K, Tsuboi H, Shi K, Tsuji I, Miyashita H, Yamada T, Matsukawa N, Matsumoto M, Morimoto S, Ogihara T, Ochi T, Yoshikawa H. Matrix extracellular phosphoglycoprotein (MEPE) is highly expressed in osteocytes in human bone. *J Bone Miner Metab.* 2004; 22:176–184. [PubMed: 15108058]
23. Rowe PS, Oudet CL, Francis F, Sinding C, Pannetier S, Econs MJ, Strom TM, Meitinger T, Garabedian M, David A, Macher MA, Questiaux E, Popowska E, Pronicka E, Read AP, Mokrzycki A, Glorieux FH, Drezner MK, Hanauer A, Lehrach H, Goulding JN, O'Riordan JL. Distribution of mutations in the PEX gene in families with X-linked hypophosphataemic rickets (HYP). *Hum Mol Genet.* 1997; 6:539–549. [PubMed: 9097956]
24. Strom TM, Francis F, Lorenz B, Boddich A, Econs MJ, Lehrach H, Meitinger T. Pex gene deletions in Gy and Hyp mice provide mouse models for X-linked hypophosphatemia. *Hum Mol Genet.* 1997; 6:165–171. [PubMed: 9063736]
25. Gowen LC, Petersen DN, Mansolf AL, Qi H, Stock JL, Tkalecic GT, Simmons HA, Crawford DT, Chidsey-Frink KL, Ke HZ, McNeish JD, Brown TA. Targeted disruption of the osteoblast/osteocyte factor 45 gene (OF45) results in increased bone formation and bone mass. *J Biol Chem.* 2003; 278:1998–2007. [PubMed: 12421822]
26. Martin A, Liu S, David V, Li H, Karydis A, Feng JQ, Quarles LD. Bone proteins PHEX and DMP1 regulate fibroblastic growth factor Fgf23 expression in osteocytes through a common pathway involving FGF receptor (FGFR) signaling. *Faseb J.* 2011; 25:2551–2562. [PubMed: 21507898]
27. Rowe PS, Kumagai Y, Gutierrez G, Garrett IR, Blacher R, Rosen D, Cundy J, Navvab S, Chen D, Drezner MK, Quarles LD, Mundy GR. MEPE has the properties of an osteoblastic phosphatonin and minihibin. *Bone.* 2004; 34:303–319. [PubMed: 14962809]
28. Martin A, David V, Laurence JS, Schwarz PM, Lafer EM, Hedge AM, Rowe PS. Degradation of MEPE, DMP1, and release of SIBLING ASARM-peptides (minhibins): ASARM-peptide(s) are

- directly responsible for defective mineralization in HYP. *Endocrinology*. 2008; 149:1757–1772. [PubMed: 18162525]
29. Rowe PS, Garrett IR, Schwarz PM, Carnes DL, Lafer EM, Mundy GR, Gutierrez GE. Surface plasmon resonance (SPR) confirms that MEPE binds to PHEX via the MEPE-ASARM motif: a model for impaired mineralization in X-linked rickets (HYP). *Bone*. 2005; 36:33–46. [PubMed: 15664000]
 30. Addison WN, Nakano Y, Loisel T, Crine P, McKee MD. MEPE-ASARM peptides control extracellular matrix mineralization by binding to hydroxyapatite: an inhibition regulated by PHEX cleavage of ASARM. *J Bone Miner Res*. 2008; 23:1638–1649. [PubMed: 18597632]
 31. Liu S, Zhou J, Tang W, Jiang X, Rowe DW, Quarles LD. Pathogenic role of Fgf23 in Hyp mice. *Am J Physiol Endocrinol Metab*. 2006; 291:E38–49. [PubMed: 16449303]
 32. Woo SM, Rosser J, Dusevich V, Kalajzic I, Bonewald LF. Cell line IDG-SW3 replicates osteoblast-to-late-osteocyte differentiation in vitro and accelerates bone formation in vivo. *J Bone Miner Res*. 2011; 26:2634–2646. [PubMed: 21735478]
 33. Lavi-Moshayoff V, Wasserman G, Meir T, Silver J, Naveh-Many T. PTH increases FGF23 gene expression and mediates the high-FGF23 levels of experimental kidney failure: a bone parathyroid feedback loop. *Am J Physiol Renal Physiol*. 2010; 299:F882–889. [PubMed: 20685823]
 34. Rhee Y, Allen MR, Condon K, Lezcano V, Ronda AC, Galli C, Olivos N, Passeri G, O'Brien CA, Bivi N, Plotkin LI, Bellido T. PTH receptor signaling in osteocytes governs periosteal bone formation and intracortical remodeling. *J Bone Miner Res*. 2011; 26:1035–1046. [PubMed: 21140374]
 35. Shimada T, Muto T, Urakawa I, Yoneya T, Yamazaki Y, Okawa K, Takeuchi Y, Fujita T, Fukumoto S, Yamashita T. Mutant FGF-23 responsible for autosomal dominant hypophosphatemic rickets is resistant to proteolytic cleavage and causes hypophosphatemia in vivo. *Endocrinology*. 2002; 143:3179–3182. [PubMed: 12130585]
 36. Goetz R, Nakada Y, Hu MC, Kurosu H, Wang L, Nakatani T, Shi M, Eliseenkova AV, Razzaque MS, Moe OW, Kuro-o M, Mohammadi M. Isolated C-terminal tail of FGF23 alleviates hypophosphatemia by inhibiting FGF23-FGFR-Klotho complex formation. *Proc Natl Acad Sci U S A*. 2010; 107:407–412. [PubMed: 19966287]
 37. Farrow EG, Yu X, Summers LJ, Davis SI, Fleet JC, Allen MR, Robling AG, Stayrook KR, Jideonwo V, Magers MJ, Garringer HJ, Vidal R, Chan RJ, Goodwin CB, Hui SL, Peacock M, White KE. Iron deficiency drives an autosomal dominant hypophosphatemic rickets (ADHR) phenotype in fibroblast growth factor-23 (Fgf23) knock-in mice. *Proc Natl Acad Sci U S A*. 2011; 108:E1146–1155. [PubMed: 22006328]
 38. Bhattacharyya N, Wiench M, Dumitrescu C, Connolly BM, Bugge TH, Patel HV, Gafni RI, Cherman N, Cho M, Hager GL, Collins MT. Mechanism of FGF23 processing in fibrous dysplasia. *J Bone Miner Res*. 2012; 27:1132–1141. [PubMed: 22247037]
 39. Liu S, Zhou J, Tang W, Menard R, Feng JQ, Quarles LD. Pathogenic role of Fgf23 in Dmp1-null mice. *Am J Physiol Endocrinol Metab*. 2008; 295:E254–261. [PubMed: 18559986]
 40. Stubbs JR, He N, Idiculla A, Gillihan R, Liu S, David V, Hong Y, Quarles LD. Longitudinal evaluation of FGF23 changes and mineral metabolism abnormalities in a mouse model of chronic kidney disease. *J Bone Miner Res*. 2011; 26:1002–1016. [PubMed: 211101002/jbmr.516]
 41. Marsell R, Mirza MA, Mallmin H, Karlsson M, Mellstrom D, Orwoll E, Ohlsson C, Jonsson KB, Ljunggren O, Larsson TE. Relation between fibroblast growth factor-23, body weight and bone mineral density in elderly men. *Osteoporos Int*. 2009; 20:1167–1173. [PubMed: 18974917]
 42. Hsu HJ, Wu MS. Fibroblast growth factor 23: a possible cause of left ventricular hypertrophy in hemodialysis patients. *Am J Med Sci*. 2009; 337:116–122. [PubMed: 19214027]
 43. Mirza MA, Larsson A, Lind L, Larsson TE. Circulating fibroblast growth factor-23 is associated with vascular dysfunction in the community. *Atherosclerosis*. 2009; 205:385–390. [PubMed: 19181315]
 44. Mirza MA, Larsson A, Melhus H, Lind L, Larsson TE. Serum intact FGF23 associate with left ventricular mass, hypertrophy and geometry in an elderly population. *Atherosclerosis*. 2009; 207:546–551. [PubMed: 19524924]

45. Gutierrez OM, Wolf M, Taylor EN. Fibroblast growth factor 23, cardiovascular disease risk factors, and phosphorus intake in the health professionals follow-up study. *Clin J Am Soc Nephrol.* 2011; 6:2871–2878. [PubMed: 22034506]
46. Faul C, Amaral AP, Oskoue B, Hu MC, Sloan A, Isakova T, Gutierrez OM, Aguillon-Prada R, Lincoln J, Hare JM, Mundel P, Morales A, Scialla J, Fischer M, Soliman EZ, Chen J, Go AS, Rosas SE, Nessel L, Townsend RR, Feldman HI, St John Sutton M, Ojo A, Gadegbeku C, Di Marco GS, Reuter S, Kentrup D, Tiemann K, Brand M, Hill JA, Moe OW, Kuro OM, Kusek JW, Keane MG, Wolf M. FGF23 induces left ventricular hypertrophy. *J Clin Invest.* 2011; 121:4393–4408. [PubMed: 21985788]
47. Hughes SE. Differential expression of the fibroblast growth factor receptor (FGFR) multigene family in normal human adult tissues. *J Histochem Cytochem.* 1997; 45:1005–1019. [PubMed: 9212826]
48. Takeshita K, Fujimori T, Kurotaki Y, Honjo H, Tsujikawa H, Yasui K, Lee JK, Kamiya K, Kitaichi K, Yamamoto K, Ito M, Kondo T, Iino S, Inden Y, Hirai M, Murohara T, Kodama I, Nabeshima Y. Sinoatrial node dysfunction and early unexpected death of mice with a defect of klotho gene expression. *Circulation.* 2004; 109:1776–1782. [PubMed: 15037532]
49. Li H, Martin A, David V, Quarles LD. Compound deletion of Fgfr3 and Fgfr4 partially rescues the Hyp mouse phenotype. *Am J Physiol Endocrinol Metab.* 2011; 300:E508–517. [PubMed: 21139072]
50. Desjardins L, Liabeuf S, Renard C, Lenglet A, Lemke HD, Choukroun G, Druke TB, Massy ZA. FGF23 is independently associated with vascular calcification but not bone mineral density in patients at various CKD stages. *Osteoporos Int.* 2012; 23:2017–2025. [PubMed: 22109743]
51. Dalal M, Sun K, Cappola AR, Ferrucci L, Crasto C, Fried LP, Semba RD. Relationship of serum fibroblast growth factor 23 with cardiovascular disease in older community-dwelling women. *Eur J Endocrinol.* 2011; 165:797–803. [PubMed: 21873490]
52. Stubbs JR, Egwuonwu S. Is fibroblast growth factor 23 a harbinger of mortality in CKD? *Pediatr Nephrol.* 2012; 27:697–703. [PubMed: 21390563]
53. Alon US. Clinical practice. Fibroblast growth factor (FGF)23: a new hormone. *Eur J Pediatr.* 2011; 170:545–554. [PubMed: 21193927]
54. Danziger J. The bone-renal axis in early chronic kidney disease: an emerging paradigm. *Nephrol Dial Transplant.* 2008; 23:2733–2737. [PubMed: 18469306]
55. de Borst MH, Vervloet MG, ter Wee PM, Navis G. Cross talk between the renin-angiotensin-aldosterone system and vitamin D-FGF-23-klotho in chronic kidney disease. *J Am Soc Nephrol.* 2011; 22:1603–1609. [PubMed: 21852584]
56. Aono Y, Yamazaki Y, Yasutake J, Kawata T, Hasegawa H, Urakawa I, Fujita T, Wada M, Yamashita T, Fukumoto S, Shimada T. Therapeutic effects of anti-FGF23 antibodies in hypophosphatemic rickets/osteomalacia. *J Bone Miner Res.* 2009; 24:1879–1888. [PubMed: 19419316]
57. Zhang R, Lu Y, Ye L, Yuan B, Yu S, Qin C, Xie Y, Gao T, Drezner MK, Bonewald LF, Feng JQ. Unique roles of phosphorus in endochondral bone formation and osteocyte maturation. *J Bone Miner Res.* 2011; 26:1047–1056. [PubMed: 21542006]

Figure 1.
Examples of osteocytes and their dendrites as revealed by Bodian's staining.

Figure 2. Acid-etched resin embedded cortical bone revealing the osteocyte lacunae and their canalicular system. Note the extensive number of canaliculi reaching the bone surfaces.

Figure 3.
One osteocyte resin filled lacuna and its resin filled canalicular processes as revealed by acid-etched resin embedded bone.