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FGF23 Synthesis and Activity

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Abstract

Purpose of Review: The phosphaturic hormone FGF23 is produced primarily in osteoblasts/osteocytes and is known to respond to increases in serum phosphate and 1,25$(OH)_2$ vitamin D (1,25D). Novel regulators of FGF23 were recently identified, and may help explain the pathophysiologies of several diseases. This review will focus on recent studies examining the synthesis and actions of FGF23.

Recent Findings: The synthesis of FGF23 in response to 1,25D is similar to other steroid hormone targets, but the cellular responses to phosphate remain largely unknown. The activity of intracellular processing genes control FGF23 glycosylation and phosphorylation, providing critical functions in determining the serum levels of bioactive FGF23. The actions of FGF23 largely occur through its co-receptor αKlotho (KL) under normal circumstances, but FGF23 has KL-independent activity during situations of high concentrations.

Summary: Recent work regarding FGF23 synthesis and bioactivity, as well as considerations for diseases of altered phosphate balance will be reviewed.

Keywords

Fibroblast growth factor-23; FGF23; PTH; vitamin D; phosphate; Klotho; rickets; osteomalacia; GALNT3; FAM20C; PSC3; Furin

Introduction

The mineral ion phosphate is involved in a myriad of critical cellular processes, more notably in part forming the basic structure of nucleic acids, phospho-lipid bilayer membranes and energy metabolism when bound as ADP/ATP. Extracellularly, phosphate is central to the structure of mineralized bone as hydroxyapatite. Within the heterogeneous spectrum of diseases related to low or high blood phosphate, much of the pathogenesis arises in the form of skeletal disease from too little phosphate, and calcifications and vascular disease from the inability to rid the body of phosphate. Control of serum phosphate occurs

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Conflict of Interest

Megan L. Noonan and Kenneth E. White each declare no potential conflicts of interest.

Human and Animal Rights and Informed Consent

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through endocrine actions to regulate absorption from the diet in the gut, reabsorption via the kidneys and long-term storage in the skeleton. When blood phosphate is low, it can be released from the skeleton, which comprises the largest phosphate stores in the body. FGF23, produced in bone, responds to increases in serum phosphate and 1,25D by acting hormonally on the kidneys to return these metabolites to normal. The synthesis and secretion of bioactive, intact FGF23 ('iFGF23') from osteoblasts and osteocytes relies on several intracellular processing genes in the ER-Trans-Golgi network (TGN). The process by which cells 'decide' to produce iFGF23 or proteolytic fragments ('cFGF23', as measured by an ELISA detecting '*C*-terminal' FGF23 epitopes; or also 'Total FGF23') is essentially unknown. Some FGF23 stimulators have been unanticipated, but help to explain the underlying pathophysiology of several phosphate-related disorders and have led to recent clinical trials.

During normal situations of phosphate balance, FGF23 acts through interactions with the FGF23 co-receptor α-Klotho (KL) and fibroblast growth factor receptors (FGFRs) in the kidney. FGF23 can have bioactivity through KL-independent pathways associated with heart and inflammatory anomalies, but data supports that this occurs primarily during situations of aberrant phosphate handling and high circulating FGF23 concentrations. Herein, we will discuss the factors regulating the synthesis of FGF23, as well as implications for novel therapeutic directions based upon a molecular understanding of FGF23 production and bioactivity.

Regulation of FGF23 Synthesis

Full-length FGF23 (32 kDa) is the biologically active form of the protein which can be cleaved into 20 and 12 kDa fragments. The N-terminal region of FGF23 is a conserved FGF-homology domain, whereas the C-terminus comprises a 71-amino acid tail, a region less conserved across the FGF family[1]. Intracellular proteolysis and inactivation of FGF23 occurs at the subtilisin-like proprotein convertase (SPC) site $R_{176}HTR_{179}/S_{180}AE$, (RXXR/SAE motif) that separates the FGF-like domain from the C-terminal tail[1,2]. The *FGF23* mutations, R176Q/W, R179Q/W, which cause Autosomal dominant hypophosphatemic rickets (ADHR) are located within this site[2,3] and lead to resistance to Furin cleavage and stabilization of full-length iFGF23. The control of FGF23 synthesis and downstream bioactivity has been found to respond to a surprisingly broad group of stimuli.

Blood phosphate concentrations:

In vivo, serum iFGF23 increases are associated with elevated blood phosphate; by converse feedback, hypophosphatemia downregulates FGF23 mRNA in bone. However, the precise mechanisms controlling FGF23 production in response to high or low phosphate have yet to be elucidated. Recent work by Bon, *et al.* has attempted to uncover this mechanism, which is described as being mediated through the Type III sodium-phosphate co-transporter Pit-2[4]. When mice were fed a low-phosphate diet, only Pit-2 KO mice had inappropriate induction of FGF23 mRNA and intact protein. This suggests Pit-2 may be responsible, in part, to halt further induction of FGF23 when phosphate has reached normal or low levels. The gene expression of the FGF23 processing enzymes *Fam20c* and *Galnt3* were measured in wild-

type and Pit2-KO mice to test whether the observed increases in iFGF23 were due to reduced cleavage rates, however no changes were detected. The authors recapitulated these findings *ex vivo* and showed that phosphate-dependent secretion of FGF23 was independent of the FGFR/MAPK pathway previously associated with FGF23 production[4] (Table 1). It remains to be determined what pathways are activated through extracellular phosphate and how the *FGF23* promoter is regulated during changes in phosphate. Insight into sites of regulated FGF23 expression have been gained from conditional deletion of a flox-Fgf23 allele in bone using Col2.3-cre (early osteoblasts) and DMP1-cre (late osteoblasts/osteocytes). Both had significantly blunted iFGF23 response to high phosphate diet, showing that at least in part, late-stage osteoblasts/osteocytes must contribute to the production of FGF23 in response to elevated phosphate. When bred onto the *Hyp* background, a mouse model of X-linked hypophosphatemic rickets (XLH) characterized by elevated serum iFGF23, the Col2.3-cre conditional *Fgf23* deletion normalized serum phosphate and improved the bone phenotype[5]. FGF23 mRNA is produced outside of the skeleton, and Onal *et al.* showed expression of FGF23 mRNA in non-osseous tissues including lung, spleen, liver, and intestine[6] but whether these sites are responsive to changes in serum phosphate remains to be determined.

$1,25(OH)_2$ vitamin D:

The first reports of vitamin D regulating FGF23 came from *in vitro* and *in vivo* studies with 1,25D treatment, however this did not explain the mechanism responsible for induction of FGF23[7,8]. Active 1,25D regulates expression of its target genes via a heterodimer complex of the vitamin D receptor (VDR) with the retinoid X receptor (RXR) then binding to vitamin D response elements (VDRE). It was reported that several VDRE exist in or near the *Fgf23* promoter, and through a promoter luciferase assay, 1,25D was shown to directly induce FGF23 mRNA *in vitro*[9]. This finding was confirmed independently in the UMR-106 osteoblastic cell line, showing significant induction of FGF23 mRNA with 1,25D treatment[10]. Further, different inbred mouse strains showed to have varying levels of baseline FGF23 and 1,25D, an important consideration for studying these regulators of mineral homeostasis[11].

More recently, studies have tested the idea of serum calcium regulating FGF23, however it has been a challenge to decipher the direct effects independently of vitamin D or PTH. To account for these confounding factors, mouse models deficient for PTH or vitamin D have been used. It was shown that wild-type, vitamin D-deficient, and PTH-deficient mice fed a high calcium ‘rescue’ diet all showed significant increases in FGF23 mRNA and serum FGF23 which had a significant positive correlation with serum calcium[12] (Table 1). In contrast, it was shown that acute changes in calcium concentration did not affect circulating FGF23 levels, however the consequence of chronic changes in serum calcium are not known[13]. Further, it was reported that when serum calcium levels were decreased, this correlated with reduced circulating FGF23, despite elevated PTH and 1,25D[14]. While this evidence suggests regulation of FGF23 by calcium, a direct mechanism accounting for this regulation has been elusive.

Circulating αKlotho:

The membrane bound co-receptor for FGF23 activity, αKlotho, allows for specific homing of FGF23, as it is expressed in kidney and parathyroid[15], key tissues in phosphate and calcium balance. This receptor can be cleaved to produce a circulating soluble form of Klotho (sKL) that has biological effects (see below). The effects of sKL on phosphate metabolism were previously demonstrated by delivering sKL via adeno-associated virus (AAV) to wild-type mice[16]. With successful targeting and expression in the liver, sKL was shown to markedly increase FGF23 mRNA in bone as well as induce expression of iFGF23. This reduced *Npt2a* and *Cyp27b1* mRNA expression in the kidney. Importantly, it was shown *in vitro* that the FGF23-mediated activity through sKL can only occur with both FGF23 and Klotho present to elicit the proper downstream signaling via ERK. More recently, sKL was used to successfully reduce elevated phosphate potentially via increased iFGF23 in a mouse model of CKD-MBD[17]. Further, delivery of sKL to KL-null mice reduced the prevailing vascular calcifications that occur in this model due to hyperphosphatemia. These findings support that sKL can control FGF23 production, but whether this occurs during normal phosphate handling remains to be studied in depth.

Parathyroid hormone (PTH):

The role of PTH in mineral homeostasis has been well characterized. PTH acts in the kidney to increase 1,25D production for calcium absorption. Interestingly, PTH has been shown to induce FGF23 expression both *in vitro* and *in vivo*[18,19]. More recently, Knab *et al.* defined this further showing that PTH does indeed stimulate expression of cFGF23 and bone FGF23 mRNA, but not the iFGF23 form. Changes in iFGF23 were only seen in mice harboring the ADHR mutation, and were still modest[20]. To begin to understand the mechanisms controlling this regulation, Meir *et al.* showed that the induction of FGF23 via PTH is mediated through the transcription factor Nurr1, of which several potential response elements were identified in the *Fgf23* promoter, though the induction of FGF23 downstream of Nurr1 remains incompletely understood[21].

Effects of iron handling including anemia and hypoxia, inflammation, and erythropoietin (EPO):

Iron handling has come to light as a very potent stimulator of FGF23 (see Table 1). This concept was shown in a study where wild-type and ADHR knock-in mice were fed a low-iron diet to reduce iron stores[22]. Regardless of genotype, mice receiving the low-iron diet had significantly increased serum cFGF23. Importantly, when iFGF23 was measured it was significantly elevated only in mice with the ADHR mutation, revealing that: 1) low iron/hypoxia could drive FGF23 mRNA, and 2) a key mechanism for controlling FGF23 activity was through increased proteolytic cleavage, which was compromised in the ADHR mice[22]. These findings supported a clinical trial to supplement iron in anemic ADHR patients to reduce iFGF23 (ClinicalTrials.gov Identifier: NCT02233322). Related to hypoxia's effects on FGF23 synthesis, responses to inflammation, or the functional anemia from inflammation, have also been shown to stimulate FGF23 synthesis (Table 1). Supporting these interactions are clinical studies demonstrating significant associations between FGF23 and inflammation[23,24]. Further, in patients receiving EPO therapy for anemia, there were

significant elevations in both cFGF23 and iFGF23 over the course of 24 hours, in concert with reduced hepcidin, an iron-lowering hormone. Additionally, EPO injections into wild-type mice drove Fgf23 mRNA expression in bone, as well as elevated cFGF23 and iFGF23, while also uncovering a novel site of FGF23 production in bone marrow progenitor cells[25,26]. A recent study of CKD patients also demonstrated that levels of circulating FGF23 were associated with anemia[27], however the relative proportions of elevated FGF23 due to the manifestations of CKD, including changes in serum phosphate, anemia, and inflammation, in addition to changes in PTH, remains to be determined. Please see the accompanying review article authored by Wheeler and Clinkenbeard for a comprehensive review of the effects of iron handling on FGF23 production.

Control of FGF23 Synthesis via Protein Processing

Control of FGF23 synthesis not only occurs at the mRNA level, but through intracellular mechanisms that determine whether FGF23 remains in the intact bioactive form or is cleaved and inactivated. Determining levels of iFGF23 and cFGF23 in disease can provide valuable information regarding the various FGF23-related syndromes. As described above, FGF23 contains a conserved $_{176}$RXXR$_{179}$/S$_{180}$AE motif, a site that is recognized by Furin, a subtilisin-like proprotein convertase (SPC). When Furin was deleted from human U2OS osteoblastic cells, only iFGF23 was secreted from cells, whereas wild-type cells secreted both iFGF23 and cFGF23 protein, indicating Furin cleaving FGF23[28]. In addition to Furin, it was recently shown that FGF23 may also be proteolytically regulated by plasminogen activators and that PAI-1, a serine protease inhibitor, may block this cleavage, however it remains to be determined if the fragments from this cleavage elicit any downstream biological activity[29].

The intact form of FGF23 is *O*-glycosylated on T$_{178}$ of the SPC site via the GalNActransferase GALNT3[30]. Once glycosylated, this moiety blocks the site from Furin cleavage, promoting the stability of iFGF23[28]. Loss of function mutations in *GALNT3* result in hyperphosphatemic familial tumoral calcinosis (hfTC), characterized by low serum iFGF23 (but markedly elevated cFGF23) leading to hyperphosphatemia and ectopic calcifications. The *Galnt3*-KO mouse phenocopies many aspects of hfTC including low iFGF23 and high cFGF23. To attempt to rescue mineral metabolism in the *Galnt3*-KO, Ichikawa, *et al*, expressed either one or two *Fgf23*-R176Q alleles from the ADHR knock-in mouse on the *Galnt3*-KO background. The *Galnt3*-KO mice with WT *Fgf23* alleles had decreased iFGF23 but marked increases in cFGF23 due to hyperphosphatemia coupled with increased cleavage. Mice with one or both *Fgf23* alleles containing ADHR mutations in the *Galnt3*-KO background had normal serum phosphate. Further, cFGF23 was rescued to normal levels, showing the ADHR stabilizing mutation can rescue *Galnt3* ablation[31]. The robust data associated with these collective studies suggest that targeting the FGF23 RXXR/SAE region to produce stable FGF23 isoforms could be useful pharmacologically to treat hfTC.

The identification of a post-translational mechanism for control of iFGF23 synthesis has led to a more refined understanding of the production of this hormone. FGF23 contains a Ser-x-Glu (SAE) motif directly adjacent to the RXXR cleavage site that is specific for family with

sequence similarity 20 member C (FAM20c) phosphorylation. Loss-of-function mutations in *FAM20c* cause Raine syndrome[32,33], a bone dysplasia that can be fatal. However, some patients are hypophosphatemic due to increased iFGF23. The *Fam20c*-KO mouse recapitulated this phenotype, identifying Fam20c as a novel upstream regulator of circulating iFGF23 concentrations, however the cellular mechanisms shifting the balance of iFGF23/cFGF23 ratios was not understood[34]. *In vitro* studies showed that FGF23 is serine-phosphorylated at the S-A-E site by Fam20c and as a consequence, blocks T_{178} *O*-glycosylation via GALNT3, making FGF23 more susceptible to cleavage[28]. This discovery explained the mechanism for the Raine syndrome phenotype, i.e. iFGF23 was being phosphorylated less efficiently, and could be 'over' stabilized physiologically by GALNT3-mediated glycosylation.

FGF23/FGFR/Klotho complexes and bioactivity

Through genetic gain and loss of function experiments in mice and the phenotypes of patients with diseases of hypo- and hyperphosphatemia, it was clear that FGF23 acts in the kidney to control minute to minute blood phosphate and 1,25D concentrations. These concepts have recently been further expanded into clinical targeting of FGF23 through the approved FGF23 neutralizing antibody Burosumab for the treatment of patients with XLH[35–37]. FGF23 is known to reduce the proximal tubule (PT) apical membrane expression of NPT2a and NPT2c to reduce renal phosphate reabsorption. In a manner converse to PTH, FGF23 down-regulates the renal vitamin D 1-α-hydroxylase (Cyp27b1) and increases expression of the catabolic 25(OH) vitamin D-24-hydroxylase (Cyp24a1). The net activity is to decrease serum phosphate and 1,25D[38]. Thus, in situations of elevated FGF23 (under normal renal function), phosphate is reduced and 1,25D can be either normal, or reduced. The normal levels of serum 1,25D in the setting of increased FGF23 are referred to as 'inappropriately normal' as hypophosphatemia is typically a strong driver of 1,25D through release of FGF23-mediated suppression on the renal vitamin D metabolizing system. Taken together, these bone-kidney feedback loops for FGF23 and overlap of PTH activity are responsible for maintaining the calcium/phosphate product.

FGF23 mediates its high-affinity biological activity through its co-receptor KL. The mature protein KL is produced as at least two distinct species: membrane bound KL (mKL) is a 130 kD single-pass transmembrane protein that has a large extracellular domain and a very short (10 residue) intracellular domain that does not possess signaling capabilities[39]. The extracellular portion of KL has two β-glucosidase like domains that resemble motifs from this deglycosylating enzyme family. The crystal structure of KL revealed that key residues required for β-glucosidase enzymatic activity have been replaced during evolution[40]. Another isoform of KL ('cut KL' or 'cKL', or 'soluble KL' or 'sKL') is also found in the circulation, and arises through the proteolytic processing of mKL on the extracellular membrane surface by ADAM and BACE proteases to release the large extracellular domain[41]. The sKL species can be detected in urine as a qualitative measure of circulating concentrations[42].

Based upon the phenotypic similarities of the *KL*- and *Fgf23*-KO mice, it was found that KL fosters high-affinity FGF23 signaling and bioactivity, and genetic experiments in mice link

FGFR1c as the most likely FGFR associated with renal FGF23 function[43]. However the mechanisms underlying the *in vivo* renal signaling properties of KL-FGF23-FGFR complexes remain somewhat unclear. In this regard, within the kidney, the overwhelming majority of KL localizes to the distal convoluted tubule (DCT)[44,45], whereas it is known that FGF23 has effects on phosphate transport and the vitamin D metabolic enzymes within the proximal tubule (PT)[46,47]. *In vitro* work demonstrated that mKL is capable of initiating FGF23-dependent MAPK signaling when complexed with FGFRs[45]. Interestingly, following short term FGF23 injection, FGF23 signaling via phospho-ERK1/2, was localized to the renal DCT, completely distinct from the PT[45]. A series of recent genetic experiments using conditionally-deleted KL mouse lines has attempted to shed light on the potential spatial separation of KL expression and FGF23 bioactivity.

To begin to understand the role of KL-FGF23 interactions in kidney, Olauson and colleagues used the Ksp (Cadherin 16; or 'Cdh16 or Ksp1.3') –Cre mouse to conditionally delete a floxed-KL allele from the distal nephron[48]. Upon analysis of the derived mice, it was found that the KL-Ksp mice were fertile and had a normal gross phenotype. Interestingly, the mice were hyperphosphatemic with elevated iFGF23, and the mechanism for increased serum phosphate was consistent with elevated PT NPT2a expression. The KL-Ksp mice also had increased VDR expression, suggesting an increased sensitivity to 1,25D as a compensatory mechanism. KL expression in the kidney was variable in the KL-Ksp mice, and a subgroup of mice had normal serum phosphate but elevated FGF23[48]. Collectively, the phenotypes of the DT-deleted KL-Ksp mice supported a DCT-PT axis for FGF23 bioactivity.

Building upon these studies, KL was subsequently deleted from PT using three different PT-Cre mouse lines. The deleter strains included the kidney androgen protein (Kap-Cre), the Slc34a1 (Npt2a)-Cre, and the Pepck (Phosphoenolpyruvate carboxykinase)-Cre[49]. Interestingly, all of the PT-deleted KL mice had mild or no hyperphosphatemia and varied effects on 1,25D production. There was variability in the expression of FGF23, with Pepck-KL mice having significantly elevated serum iFGF23 and the smallest increase in NPT2a, suggesting variability in the localization or strength of the various PT-Cre lines[49]. In another study, a Ndrg1-CreERT2 transgenic mouse, which expresses Cre in the PT upon tamoxifen treatment, was used to delete floxed-KL. These investigators observed a more pronounced effect in vivo with markedly elevated iFGF23 and elevated serum phosphate in the KL-deleted mice[50]. Thus, functional effects downstream of targeted genetic deletions also supported a role of PT-expressed KL.

To address potential intra-PT cross talk between FGF23 and other factors, the Slc34a1-cre was used in line of mice with a genetic background of PT-specific PTH receptor (PTHR1)/KL double-floxed mice. The PT-PTHR-deleted mice had increased FGF23 and PTH, whereas serum phosphate was normal and NPT2a and NPT2c were unchanged[51]. In contrast, the PTPTH1R/KL double KO mice displayed elevated serum phosphate and increased NPT2a and NPT2c, in parallel with increased serum FGF23[51]. Therefore the combined loss of the ability of FGF23 and PTH to down regulate phosphate transporter expression in the PT is consistent with the hypothesis that there exists compensatory and independent hormonal control of PT phosphate handling. Collectively, the aforementioned studies support a role for both PT and DCT KL expression in controlling FGF23 bioactivity.

Although evidence supports DCT KL-regulated sodium[52] and calcium[53,54] control, the primary phenotypes with loss of KL activity appear to predominantly relate to phosphate handling[15]. Whether the targeted cre-expression profiles are precise for each tubule segment, or have low expression in several tubule segments and could influence KL production along the nephron, should be further explored to potentially explain some of the differences observed in the intra-nephron KL deletion studies. Additionally, the derived KL-FGFR1-FGF23 crystal structure supports a role for the sKL form of KL to potentially deliver FGF23 systemically[40], and careful molecular analysis suggests a wider KL expression profile across tissues than was previously realized[55]. Therefore, the role of PT and DCT KL, and whether cross-segment communication for FGF23 bioactivity is required remains to be fully understood.

FGF23 and KL-independent signaling

The overlapping phenotypes of the FGF23- and KL-KO mice revealed the reliance of FGF23 on its co-receptor for full biological activity with regard to phosphate homeostasis. Interestingly, patient data suggested a strong association between elevated circulating FGF23 and cardiovascular outcomes. In the setting of chronically elevated FGF23 levels, as opposed to its activity in KL/FGFR/FGF23 complexes, FGF23 has also been shown to directly interact with FGFRs in a KL-independent manner. *In vivo* studies comprised of direct FGF23 injections into rodent hearts (heart does not express KL under basal conditions), as well as *in vitro* studies with isolated and cultured cardiomyocytes, has shown this effect to be mediated through FGFR4 and NFAT/PLCγ signaling[56]. Indeed, mice with activating mutations in FGFR4 develop cardiac hypertrophy and showed increased NFAT/PLCγ signaling, and in the converse situation, FGFR4-KO mice are resistant to FGF23-mediated cardiohypertrophy[57]. Importantly, the FGF23-dependent cardiohypertrophy was shown to be reversible by modulating serum phosphate through providing a low phosphate diet to mice[58]. Although the actions of FGF23 on heart are direct, many of the underlying pathways, as well as whether FGF23 also influences FGFR expression in cardiac tissue remain to be understood.

In addition to effects on cardiac tissue, elevated FGF23 has been linked to KL-independent roles in inflammation. Recent studies demonstrated that FGFR4 activation by FGF23 increased calcineurin signaling in cultured hepatocytes[59]. FGF23 activity has also been associated with increased production of inflammatory cytokines, including C-reactive protein, which was reduced in mice with deletion of FGFR4[60]. In the same study, administration of an FGFR4 neutralizing antibody reduced synthesis of C-reactive protein in the 5/6 nephrectomy rat model of CKD[60]. Of note, it appears that the levels of FGF23 must be markedly elevated to observe these effects, as recent data show the *Hyp* mouse model of XLH did not appear to show cardiac pathology or markers of inflammation[61], although FGF23 is known to be >5–7 times elevated in this model versus controls. Importantly, genetic background may play a role in the development of heart phenotypes as strains of mice with CKD due to *Col4a3* loss of function mutations vary on their severity when placed on 129 or C57Bl6 genetic backgrounds[62]. Therefore, although KL-independent FGF23 signaling occurs at high FGF23 concentrations, studies are needed to define the threshold concentrations required for these events.

Summary

In summary, significant progress has been made in understanding the molecular mechanisms controlling FGF23 synthesis, including the discovery of somewhat surprising regulators of this hormone. These findings are opening new paths for understanding FGF23 effects in numerous disease states. Further, the post-synthesis processing of FGF23 has emerged as a critical regulator of FGF23 bioactivity and has the potential for identifying novel therapeutic targets.

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References

1. Shimada T et al. Mutant FGF-23 responsible for autosomal dominant hypophosphatemic rickets is resistant to proteolytic cleavage and causes hypophosphatemia in vivo. Endocrinology 143, 3179–3182, doi:10.1210/endo.143.8.8795 (2002). [PubMed: 12130585]

2. ADHR-Consortium. Autosomal dominant hypophosphataemic rickets is associated with mutations in FGF23. Nature genetics 26, 345–348 (2000). [PubMed: 11062477]

3. White KE et al. Autosomal-dominant hypophosphatemic rickets (ADHR) mutations stabilize FGF-23. Kidney Int. 60, 2079–2086, doi:10.1046/j.1523-1755.2001.00064.x (2001). [PubMed: 11737582]

**4. Bon N et al. Phosphate-dependent FGF23 secretion is modulated by PiT2/Slc20a2. Mol Metab 11, 197–204, doi:10.1016/j.molmet.2018.02.007 (2018). [PubMed: 29551636] This reference supports that the phosphate transporter PiT2 is required for producing FGF23 is response to changes in extracellular phosphate.

5. Clinkenbeard EL et al. Conditional Deletion of Murine Fgf23: Interruption of the Normal Skeletal Responses to Phosphate Challenge and Rescue of Genetic Hypophosphatemia. J. Bone Miner. Res 31, 1247–1257, doi:10.1002/jbmr.2792 (2016). [PubMed: 26792657]

**6. Onal M et al. A Novel Distal Enhancer Mediates Inflammation-, PTH-, and Early Onset Murine Kidney Disease-Induced Expression of the Mouse Fgf23 Gene. JBMR plus 2, 32–47, doi:10.1002/jbm4.10023 (2018). [PubMed: 29527594] This citation demonstrates that distal portions of the FGF23 promoter may regulate FGF23 under specific physiological and disease conditions.

7. Kolek OI et al. 1alpha,25-Dihydroxyvitamin D3 upregulates FGF23 gene expression in bone: the final link in a renal-gastrointestinal-skeletal axis that controls phosphate transport. Am. J. Physiol. Gastrointest. Liver Physiol 289, G1036–1042, doi:10.1152/ajpgi.00243.2005 (2005). [PubMed: 16020653]

8. Shimada T et al. Targeted ablation of Fgf23 demonstrates an essential physiological role of FGF23 in phosphate and vitamin D metabolism. J. Clin. Invest 113, 561–568, doi:10.1172/JCI19081 (2004). [PubMed: 14966565]

9. Liu S et al. Fibroblast growth factor 23 is a counter-regulatory phosphaturic hormone for vitamin D. J. Am. Soc. Nephrol 17, 1305–1315, doi:10.1681/ASN.2005111185 (2006). [PubMed: 16597685]

10. Kaneko I et al. FGF23 gene regulation by 1,25-dihydroxyvitamin D: opposing effects in adipocytes and osteocytes. J. Endocrinol 226, 155–166, doi:10.1530/JOE-15-0225 (2015). [PubMed: 26148725]

11. Fleet JC et al. Gene-by-Diet Interactions Affect Serum 1,25-Dihydroxyvitamin D Levels in Male BXD Recombinant Inbred Mice. Endocrinology 157, 470–481, doi:10.1210/en.2015-1786 (2016). [PubMed: 26587785]

12. David V et al. Calcium regulates FGF-23 expression in bone. Endocrinology 154, 4469–4482, doi:10.1210/en.2013-1627 (2013). [PubMed: 24140714]

13. Gravesen E, Mace ML, Hofman-Bang J, Olgaard K & Lewin E Circulating FGF23 levels in response to acute changes in plasma Ca(2+). Calcif. Tissue Int 95, 46–53, doi:10.1007/s00223-014-9861-8 (2014). [PubMed: 24801007]

14. Rodriguez-Ortiz ME et al. Calcium deficiency reduces circulating levels of FGF23. J. Am. Soc. Nephrol 23, 1190–1197, doi:10.1681/ASN.2011101006 (2012). [PubMed: 22581996]

15. Urakawa I et al. Klotho converts canonical FGF receptor into a specific receptor for FGF23. Nature 444, 770–774, doi:10.1038/nature05315 (2006). [PubMed: 17086194]

16. Smith RC et al. Circulating alphaKlotho influences phosphate handling by controlling FGF23 production. J. Clin. Invest 122, 4710–4715, doi:10.1172/JCI64986 (2012). [PubMed: 23187128]

17. Hum JM et al. Chronic Hyperphosphatemia and Vascular Calcification Are Reduced by Stable Delivery of Soluble Klotho. J Am Soc Nephrol 28, 1162–1174, doi:10.1681/ASN.2015111266 (2016). [PubMed: 27837149]

18. Krajisnik T et al. Fibroblast growth factor-23 regulates parathyroid hormone and 1alpha-hydroxylase expression in cultured bovine parathyroid cells. J. Endocrinol 195, 125–131, doi:10.1677/JOE-07-0267 (2007). [PubMed: 17911404]

19. Kawata T et al. Parathyroid hormone regulates fibroblast growth factor-23 in a mouse model of primary hyperparathyroidism. J. Am. Soc. Nephrol 18, 2683–2688, doi:10.1681/ASN.2006070783 (2007). [PubMed: 17855636]

20. Knab VM et al. Acute Parathyroid Hormone Injection Increases C-Terminal but Not Intact Fibroblast Growth Factor 23 Levels. Endocrinology 158, 1130–1139, doi:10.1210/en.2016-1451 (2017). [PubMed: 28324013]

21. Meir T et al. Parathyroid hormone activates the orphan nuclear receptor Nurr1 to induce FGF23 transcription. Kidney Int. 86, 1106–1115, doi:10.1038/ki.2014.215 (2014). [PubMed: 24940803]

22. Farrow EG et al. 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 108, E1146–1155, doi:10.1073/pnas.1110905108 (2011). [PubMed: 22006328]

23. Hanks LJ, Casazza K, Judd SE, Jenny NS & Gutierrez OM Associations of fibroblast growth factor-23 with markers of inflammation, insulin resistance and obesity in adults. PLoS One 10, e0122885, doi:10.1371/journal.pone.0122885 (2015). [PubMed: 25811862]

24. Holecki M et al. Inflammation but not obesity or insulin resistance is associated with increased plasma fibroblast growth factor 23 concentration in the elderly. Clin. Endocrinol. (Oxf.) 82, 900–909, doi:10.1111/cen.12759 (2015). [PubMed: 25736796]

25. Clinkenbeard EL et al. Erythropoietin stimulates murine and human fibroblast growth factor-23, revealing novel roles for bone and bone marrow. Haematologica 102, e427–e430, doi:10.3324/haematol.2017.167882 (2017). [PubMed: 28818868]

26. Hanudel MR et al. Effects of erythropoietin on fibroblast growth factor 23 in mice and humans. Nephrol. Dial. Transplant, doi:10.1093/ndt/gfy189 (2018).

27. Nam KH et al. Circulating Fibroblast Growth Factor-23 Levels are Associated with an Increased Risk of Anemia Development in Patients with Nondialysis Chronic Kidney Disease. Sci. Rep 8, 7294, doi:10.1038/s41598-018-25439-z (2018). [PubMed: 29740119]

28. Tagliabracci VS et al. Dynamic regulation of FGF23 by Fam20C phosphorylation, GalNAc-T3 glycosylation, and furin proteolysis. Proc. Natl. Acad. Sci. U. S. A 111, 5520–5525, doi:10.1073/pnas.1402218111 (2014). [PubMed: 24706917]

29. Eren M et al. PAI-1 is a critical regulator of FGF23 homeostasis. Sci Adv 3, e1603259, doi:10.1126/sciadv.1603259 (2017). [PubMed: 28924605]

30. Kato K et al. Polypeptide GalNAc-transferase T3 and familial tumoral calcinosis. Secretion of fibroblast growth factor 23 requires O-glycosylation. J. Biol. Chem 281, 18370–18377, doi:10.1074/jbc.M602469200 (2006). [PubMed: 16638743]

31. Ichikawa S et al. Genetic rescue of glycosylation-deficient Fgf23 in the Galnt3 knockout mouse. Endocrinology 155, 3891–3898, doi:10.1210/en.2014-1199 (2014). [PubMed: 25051439]

32. Raine J, Winter RM, Davey A & Tucker SM Unknown syndrome: microcephaly, hypoplastic nose, exophthalmos, gum hyperplasia, cleft palate, low set ears, and osteosclerosis. J. Med. Genet 26, 786–788 (1989). [PubMed: 2614802]

33. Simpson MA et al. Mutations in FAM20C are associated with lethal osteosclerotic bone dysplasia (Raine syndrome), highlighting a crucial molecule in bone development. J. Hum. Genet 81, 906–912, doi:10.1086/522240 (2007).
34. Wang X et al. Inactivation of a novel FGF23 regulator, FAM20C, leads to hypophosphatemic rickets in mice. PLoS Genet 8, e1002708, doi:10.1371/journal.pgen.1002708 (2012). [PubMed: 22615579]
35. Carpenter TO et al. Randomized trial of the anti-FGF23 antibody KRN23 in X-linked hypophosphatemia. J Clin Invest 124, 1587–1597, doi:72829 [pii]10.1172/JCI72829 (2014). [PubMed: 24569459]
36. Imel EA et al. Prolonged Correction of Serum Phosphorus in Adults With X-Linked Hypophosphatemia Using Monthly Doses of KRN23. J Clin Endocrinol Metab 100, 2565–2573, doi:10.1210/jc.2015-1551 (2015). [PubMed: 25919461]
37. Carpenter TO et al. Burosumab Therapy in Children with X-Linked Hypophosphatemia. N Engl J Med 378, 1987–1998, doi:10.1056/NEJMoa1714641 (2018). [PubMed: 29791829]
38. Shimada T et al. FGF-23 is a potent regulator of vitamin D metabolism and phosphate homeostasis. J Bone Miner Res 19, 429–435 (2004). [PubMed: 15040831]
39. Matsumura Y et al. Identification of the human klotho gene and its two transcripts encoding membrane and secreted klotho protein. Biochem Biophys Res Commun 242, 626–630 (1998). [PubMed: 9464267]
**40. Chen G et al. alpha-Klotho is a non-enzymatic molecular scaffold for FGF23 hormone signalling. Nature 553, 461–466, doi:10.1038/nature25451 (2018). [PubMed: 29342138] This paper reported the triple crystal structure of FGF23-KL-FGFR1, and tests the idea that the soluble form of KL can mediate FGF23 bioactivity in tissue where KL has limited expression.
41. Imura A et al. Secreted Klotho protein in sera and CSF: implication for post-translational cleavage in release of Klotho protein from cell membrane. FEBS Lett 565, 143–147, doi:10.1016/j.febslet.2004.03.090S0014579304003990 [pii] (2004). [PubMed: 15135068]
42. Hu MC et al. Klotho deficiency causes vascular calcification in chronic kidney disease. J Am Soc Nephrol 22, 124–136, doi:ASN.2009121311 [pii]10.1681/ASN.2009121311 (2011). [PubMed: 21115613]
43. Gattineni J et al. Regulation of renal phosphate transport by FGF23 is mediated by FGFR1 and FGFR4. Am J Physiol Renal Physiol 306, F351–358, doi:10.1152/ajprenal.00232.2013 (2014). [PubMed: 24259513]
44. Kuro-o M et al. Mutation of the mouse klotho gene leads to a syndrome resembling ageing. Nature 390, 45–51 (1997). [PubMed: 9363890]
45. Farrow EG, Davis SI, Summers LJ & White KE Initial FGF23-mediated signaling occurs in the distal convoluted tubule. J Am Soc Nephrol 20, 955–960, doi:ASN.2008070783 [pii]10.1681/ASN.2008070783 (2009). [PubMed: 19357251]
46. Larsson T et al. Transgenic mice expressing fibroblast growth factor 23 under the control of the alpha1(I) collagen promoter exhibit growth retardation, osteomalacia, and disturbed phosphate homeostasis. Endocrinology 145, 3087–3094 (2004). [PubMed: 14988389]
47. Shimada T et al. Cloning and characterization of FGF23 as a causative factor of tumor-induced osteomalacia. Proc Natl Acad Sci U S A 98, 6500–6505 (2001). [PubMed: 11344269]
48. Olauson H et al. Targeted deletion of Klotho in kidney distal tubule disrupts mineral metabolism. J Am Soc Nephrol 23, 1641–1651, doi:ASN.2012010048 [pii]10.1681/ASN.2012010048 (2012). [PubMed: 22878961]
49. Ide N et al. In vivo evidence for a limited role of proximal tubular Klotho in renal phosphate handling. Kidney Int 90, 348–362, doi:10.1016/j.kint.2016.04.009 (2016). [PubMed: 27292223]
50. Takeshita A, Kawakami K, Furushima K, Miyajima M & Sakaguchi K Central role of the proximal tubular alphaKlotho/FGF receptor complex in FGF23-regulated phosphate and vitamin D metabolism. Scientific reports 8, 6917, doi:10.1038/s41598-018-25087-3 (2018). [PubMed: 29720668]
51. Ide N et al. In vivo evidence for an interplay of FGF23/Klotho/PTH axis on the phosphate handling in renal proximal tubules. Am J Physiol Renal Physiol, doi:10.1152/ajprenal.00650.2017 (2018).

52. Andrukhova O et al. FGF23 regulates renal sodium handling and blood pressure. EMBO molecular medicine 6, 744–759, doi:10.1002/emmm.201303716 (2014). [PubMed: 24797667]

53. Chang Q et al. The beta-glucuronidase klotho hydrolyzes and activates the TRPV5 channel. Science 310, 490–493 (2005). [PubMed: 16239475]

54. Andrukhova O et al. FGF23 promotes renal calcium reabsorption through the TRPV5 channel. EMBO J 33, 229–246, doi:embj.201284188 [pii]10.1002/embj.201284188 (2013).

55. Olauson H, Mencke R, Hillebrands JL & Larsson TE Tissue expression and source of circulating alphaKlotho. Bone 100, 19–35, doi:10.1016/j.bone.2017.03.043 (2017). [PubMed: 28323144]

56. Faul C et al. FGF23 induces left ventricular hypertrophy. J Clin Invest 121, 4393–4408, doi:46122 [pii]10.1172/JCI46122 (2011). [PubMed: 21985788]

57. Grabner A et al. Activation of Cardiac Fibroblast Growth Factor Receptor 4 Causes Left Ventricular Hypertrophy. Cell metabolism 22, 1020–1032, doi:10.1016/j.cmet.2015.09.002 (2015). [PubMed: 26437603]

**58. Grabner A et al. FGF23/FGFR4-mediated left ventricular hypertrophy is reversible. Scientific reports 7, 1993, doi:10.1038/s41598-017-02068-6 (2017). [PubMed: 28512310] This paper showed that the cardiac hypertrophy due to high phopshate diet and elevated FGF23 could be reversed by lowering serum phopshate,

59. Czaya B et al. Induction of an Inflammatory Response in Primary Hepatocyte Cultures from Mice. Journal of visualized experiments : JoVE, doi:10.3791/55319 (2017).

60. Singh S et al. Fibroblast growth factor 23 directly targets hepatocytes to promote inflammation in chronic kidney disease. Kidney Int, doi:10.1016/j.kint.2016.05.019 (2016).

61. Liu ES et al. Increased Circulating FGF23 Does Not Lead to Cardiac Hypertrophy in the Male Hyp Mouse Model of XLH. Endocrinology 159, 2165–2172, doi:10.1210/en.2018-00174 (2018). [PubMed: 29635291]

62. Neuburg S et al. Genetic background influences cardiac phenotype in murine chronic kidney disease. Nephrol Dial Transplant 33, 1129–1137, doi:10.1093/ndt/gfx332 (2018). [PubMed: 29309658]

63. Perwad F et al. Dietary and serum phosphorus regulate fibroblast growth factor 23 expression and 1,25-dihydroxyvitamin D metabolism in mice. Endocrinology 146, 5358–5364, doi:en.2005–0777 [pii]10.1210/en.2005-0777 (2005). [PubMed: 16123154]

64. Bon N et al. Phosphate (Pi)-regulated heterodimerization of the high-affinity sodium-dependent Pi transporters PiT1/Slc20a1 and PiT2/Slc20a2 underlies extracellular Pi sensing independently of Pi uptake. J Biol Chem 293, 2102–2114, doi:10.1074/jbc.M117.807339 (2018). [PubMed: 29233890]

65. Clinkenbeard EL et al. Neonatal iron deficiency causes abnormal phosphate metabolism by elevating FGF23 in normal and ADHR mice. J. Bone Miner. Res 29, 361–369, doi:10.1002/jbmr.2049 (2014). [PubMed: 23873717]

66. Zhang Q et al. The hypoxia-inducible factor-1alpha activates ectopic production of fibroblast growth factor 23 in tumor-induced osteomalacia. Bone Res 4, 16011, doi:10.1038/boneres.2016.11 (2016). [PubMed: 27468359]

67. Flamme I, Ellinghaus P, Urrego D & Kruger T FGF23 expression in rodents is directly induced via erythropoietin after inhibition of hypoxia inducible factor proline hydroxylase. PLoS One 12, e0186979, doi:10.1371/journal.pone.0186979 (2017). [PubMed: 29073196]

68. David V et al. Inflammation and functional iron deficiency regulate fibroblast growth factor 23 production. Kidney Int 22, 1020–1032, doi:10.1038/ki.2015.290 (2015).

69. Ito N et al. Regulation of FGF23 expression in IDG-SW3 osteocytes and human bone by pro-inflammatory stimuli. Mol. Cell. Endocrinol 399, 208–218, doi:10.1016/j.mce.2014.10.007 (2015). [PubMed: 25458698]

70. Rabadi S, Udo I, Leaf DE, Waikar S & Christov M Acute blood loss stimulates fibroblast growth factor 23 production. Am J Physiol Renal Physiol, ajprenal 00081 02017, doi:10.1152/ajprenal.00081.2017 (2017).

71. Wolf M, Koch TA & Bregman DB Effects of iron deficiency anemia and its treatment on fibroblast growth factor 23 and phosphate homeostasis in women. J. Bone Miner. Res 28, 1793–1803, doi:10.1002/jbmr.1923 (2013). [PubMed: 23505057]

72. Shimizu Y et al. Hypophosphatemia induced by intravenous administration of saccharated ferric oxide: another form of FGF23-related hypophosphatemia. Bone 45, 814–816, doi:10.1016/j.bone.2009.06.017 (2009). [PubMed: 19555782]

73. Schouten BJ, Hunt PJ, Livesey JH, Frampton CM & Soule SG FGF23 elevation and hypophosphatemia after intravenous iron polymaltose: a prospective study. J Clin Endocrinol Metab 94, 2332–2337, doi:jc.2008–2396 [pii]10.1210/jc.2008-2396 (2009). [PubMed: 19366850]

74. Kobayashi K et al. Regulation of plasma fibroblast growth factor 23 by calcium in primary hyperparathyroidism. Eur J Endocrinol 154, 93–99, doi:154/1/93 [pii10.1530/eje.1.02053 (2006). [PubMed: 16381997]

75. Ben-Dov IZ et al. The parathyroid is a target organ for FGF23 in rats. J Clin Invest 117, 4003–4008, doi:10.1172/JCI32409 (2007). [PubMed: 17992255]

Table 1.

Regulators of FGF23 synthesis as determined by ELISA.

Regulator	iFGF23*	cFGF23	Citations
Elevated phosphate	↑↑		63,64
1,25(OH)2 vitamin D	↑↑		38, 7, 9, 10
Hypoxia (HIF1α)	↑	↑↑	65, 66, 67
Inflammation	↑	↑↑	68, 69
Anemia (could also have influence of hypoxia + EPO)	↑	↑↑↑	22, 65, 70
EPO	↑	↑↑	25, 29
i.v. iron (FCM, iron sucrose)	↑↑		71–73
sKL	↑↑↑		16, 17
PTH	↑	↑↑	74, 20, 75, 18
Calcium	↑		12

* It is assumed that an iFGF23 increase would be paralleled by a corresponding cFGF23 ELISA increase; an elevated cFGF23 indicates a larger effect on cFGF23 than on iFGF23, likely via increased FGF23 synthesis and subsequent enhanced proteolysis.