

FGF23 and PTH—double agents at the heart of CKD

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The changes in mineral metabolism in chronic kidney disease (CKD) are well established as being major contributors to the morbidity and mortality of CKD patients, both early and established. The known factors involved were considered to be phosphate (P), calcium, parathyroid hormone (PTH), vitamin D and metabolic acidosis. A number of epidemiological studies have shown that high P associates with mortality; but more recently, the field has been invigorated by a new player, namely fibroblast growth factor 23 (FGF23). We shall review the exciting developments in research on FGF23 and its interplay with other factors that are known to be altered in CKD, particularly P and calcium, PTH and vitamin D. In particular, we will consider new findings on the effect of FGF23 on the heart.

Phosphorus metabolism in CKD

One of us (E.S.) showed in 1966 that there is a control system that governs P excretion in uremia. This system appears to be designed to preserve normal P concentrations in the plasma as long as possible, and it expresses itself by a progressive increase in P excretion per nephron as the number of nephrons diminishes. The efferent limb of this control system was PTH. In experiments in dogs with experimental uremia and regulated phosphorus intake when glomerular filtration rate (GFR) diminished due to nephron destruction, P excretion decreases transiently and transient hyperphosphatemia ensues [1, 2]. The latter would result in reciprocal hypocalcemia. They hypothesized that the decrease in ionized calcium rather than the elevation of P *per se* stimulates the parathyroid glands to increase the rate of hormone secretion. This hypothesis remains the state of the art and has been enhanced by studies showing an independent effect of phosphate on PTH gene expression and secretion independent of changes in ionized calcium and serum calcitriol [3–5]. The effect of changes in phosphate on parathyroid gland function demands an intact parathyroid gland architecture in that *in vitro* they were present only in intact glands or tissue slices but not in isolated cells. Changes in dietary phosphorus also regulated parathyroid cell proliferation [6, 7]. The

cellular mechanisms involved in these responses are still not defined. Prokaryotes such as bacteria and unicellular eukaryotes such as yeast also respond by changing the expression of a large number of genes in response to growth in media with different phosphorus concentrations and their sensing mechanisms have yet to be defined [8].

At the level of the kidney, the response of the renal P transporters, NaPi2a and c, to changes in serum P is well defined. In response to a low P, there is a rapid transfer of the NaPi transporters to the brush border membrane, followed by increased NaPi messenger RNA (mRNA) levels and NaPi protein synthesis [9–12]. The Na⁺-Pi cotransporter PiT-2 (SLC20A2) is also expressed in the apical membrane of rat renal proximal tubules and regulated by dietary phosphorus [13]. All of these result in the increased uptake of filtered P. These effects occur both *in vitro* and *in vivo*. Therefore, renal P conservation is regulated directly by the kidney as well as by secondary changes in serum PTH.

PTH acts at the level of the proximal renal tubule via the PTHR1 expressed at the basolateral and apical membrane to activate Gs α /PKA and Gq/PLC/PKC, respectively [14]. Activation of both signaling pathways leads to internalization of the Na⁺-Pi cotransporters NaPi2a and c, resulting in renal P wasting and hypophosphatemia [9].

In addition, there is compelling evidence for changes in oral phosphorus being recognized by a small intestine factor which then acts directly on the kidney to increase urinary P excretion [15]. Identification of the intestinal phosphaturic factor remains elusive. There is another factor involved in renal P and vitamin D homeostasis, namely FGF23.

FGF23 and klotho

The recent discovery that osteocytes and osteoblasts are the principal source for FGF23 identifies the bone not only as the major reservoir for calcium and phosphorus but as an endocrine organ that communicates with other organs involved in mineral homeostasis [16, 17]. FGF23 acts on its receptor complex, klotho-FGFR1, in the kidney to increase P excretion and to decrease calcitriol synthesis [14, 18–20].

This action of FGF23 allows bone to respond to P mobilization and changes in bone turnover by secreting FGF23 and thereby removing the excess of extracellular P as well as protecting the organism from vitamin D effects to increase the intestinal absorption of calcium and phosphorus.

The classical endocrine action of FGF23 is dependent upon its binding and activation of the klotho-FGFR1 complex. Klotho exists as a transmembrane form as well as a secreted form either due to alternative RNA splicing or the product of the α -secretase, sheddases, ADAM (a disintegrin and metalloprotease) 10 and 17 and β -secretase, β -APP (amyloid precursor protein) cleaving enzyme 1 [21]. Klotho transcript is highest in the kidney and parathyroid gland, but it is also expressed in the brain, heart, pancreatic islet β -cell and placenta with lesser abundance.

The presence of both FGF23 and PTH are necessary for normal P homeostasis

FGF23 and PTH both act on the kidney independently to inhibit the activity of the Na-Pi cotransporters 2a and 2c [14]. Excess of either humoral factor leads to an enhanced phosphaturia with increased calcium reabsorption [22], but only PTH leads to an increase in serum calcium. This is because the PTH receptor, PTH1R, is expressed in bone and kidney unlike klotho that is only expressed in the kidney. Furthermore, PTH stimulates calcitriol synthesis that further increases serum calcium and FGF23 has the opposed effect on vitamin D and calcium. It is of interest why the excess of PTH is not compensated for by a decrease in FGF23 and in turn, why an increase in FGF23 is not compensated for by a decrease in PTH. This is also intriguing in the opposite situation, where both hypoparathyroidism and tumoral calcinosis are characterized by hyperphosphatemia. In hypoparathyroidism, there is a modest increase in serum FGF23, which correlates with the hyperphosphatemia but these patients also receive calcitriol that would itself increase FGF23 [23, 24]. Why is there no profound increase in serum FGF23 to maintain phosphorus homeostasis? Similarly, in tumoral calcinosis where FGF23 levels are undetectable, why is there no dramatic increase in serum PTH to correct the serum P [25]? The answers are not clear; particularly, when one considers that there is a well-defined PTH-FGF23 feedback loop, which is described below. Contributing factors may be changes in concentrations of serum calcitriol and of course any small change in serum Ca^{2+} to which the parathyroid is exquisitely sensitive. FGF23 decreases and PTH increases serum calcitriol. FGF23 decreases serum PTH in the physiological state and PTH increases serum FGF23. Lanske's laboratory showed that mice with deletion of the genes for either FGF23 or klotho still respond to administered PTH with a significant phosphaturia (Figure 1) [26]. So, PTH can regulate serum P without FGF23 but the responses in disease states are often not as clear-cut as one would expect. Bone remodeling is particularly important in regulating FGF23 secretion and this topic is discussed below [27]. What is clear is that FGF23 and PTH are both necessary for normal P homeostasis and PTH remains the prime factor for calcium homeostasis.

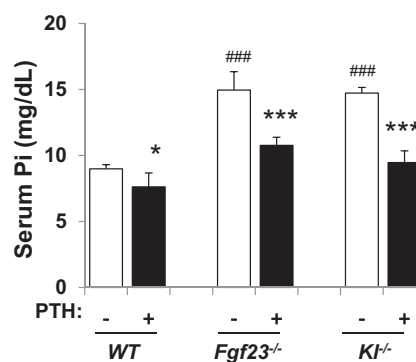


Fig. 1. FGF23/klotho signaling is not essential for the phosphaturic action of PTH. Injection of PTH decreases serum P levels in wild type (WT) mice and mice with genetic deletion of FGF23 (Fgf23^{-/-}) and klotho (K1^{-/-}) indicating that the phosphaturic action of PTH in mice is independent of FGF23/klotho [26]. Reproduced with permission of the *Journal of Bone and Mineral Research*.

The FGF and FGFR family

The FGF family is one of the largest growth factor families, consisting of 23 members sharing 13–71% sequence similarity in mammals [28]. FGFs possess a large range of activities in embryonic development and physiological functions in the adult. In the embryo, FGFs often signal across mesenchymal–epithelial boundaries, where they regulate organogenesis and pattern formation. In the adult, FGFs play important roles in regulating homeostasis, wound healing and tissue repair. Unregulated expression of FGFs can cause cancer. FGFs bind and activate alternatively spliced forms of four tyrosine kinase FGF receptors (FGFRs 1–4). The spatial and temporal expression patterns of FGFs and FGFRs and the ability of specific ligand–receptor pairs to actively signal are important factors regulating FGF activity in a variety of biological processes [29]. FGF signaling activity is regulated by the binding specificity of ligands and receptors and is modulated by extrinsic cofactors such as heparan sulfate proteoglycans [28, 30]. Each FGFR contains an extracellular ligand-binding domain, a single transmembrane domain and an intracellular tyrosine kinase domain. The extracellular ligand-binding domain of the FGFR contains two or three Ig-like domains. Alternative RNA splicing that utilizes one of two unique exons results in two different versions of Ig-like domain III (referred to as domains IIIb and IIIc) in FGFRs 1–3. This alternative splicing is an essential determinant of ligand-binding specificity. The IIIb ('b') and IIIc ('c') splice forms are regulated in a tissue-specific manner, such that the b isoform is restricted to epithelial lineages and the c isoform is preferentially expressed in mesenchymal lineages [28].

Regulation of FGF23 synthesis and secretion

Healthy individuals injected with PTH (1-34) develop hypophosphatemia and elevated calcitriol levels, along with an increase of serum FGF23 [31], suggesting that calcitriol or PTH are important regulators of FGF23 synthesis independent of the serum P concentration. However, increased circulating FGF23 levels were likewise reported in some

patients with hypoparathyroidism [23], suggesting that the hyperphosphatemia observed under these conditions can modulate FGF23 despite low or normal calcitriol levels [32]. P-independent regulation of FGF23 by calcitriol may also explain why FGF23 is unable to compensate in patients with hyper- or hypoparathyroidism, i.e. conditions in which calcitriol is elevated to inappropriately stimulate or decreased to inappropriately reduce FGF23 levels, respectively [32, 33]. Overexpression of high molecular weight FGF2 increases FGF23/FGFR/KLOTHO signaling to downregulate NPT2a, causing P wasting, osteomalacia and decreased bone mineral density [34]. Microarray gene expression analysis showed that canonical activation of FGFR pathways by addition of recombinant FGF1 and FGF2 to osteoblast cultures stimulated FGF23 promoter activity [35] and selective inhibition of FGFR blocks FGF23 transcription in bone suggesting that FGFR signaling is essential for FGF23 expression in bone [36, 37]. Moreover, PHEX and DMP1 control a common pathway regulating bone mineralization and FGF23 production, the latter involving activation of the FGFR signaling in osteocytes [37].

A FGF23-PTH endocrine loop

FGF23 acts on its receptor klotho-FGFR1 in the parathyroid as part of a bone-parathyroid axis whereby FGF23 decreases PTH gene expression, secretion and parathyroid cell proliferation [38, 39]. In turn, PTH acts on bone cells both *in vivo* and *in vitro* to increase FGF23 expression [40, 41]. Furthermore, Lavi-Moshayoff *et al.* [40] showed that PTH is necessary for the high FGF23 levels of early kidney failure due to an adenine high-phosphorus diet. Parathyroidectomy before the diet totally prevented the 5-fold increase in FGF23 levels in kidney failure rats (Figure 2). Moreover, parathyroidectomy of early kidney failure rats corrected their high FGF23 levels (Figure 2) [40]. Therefore, in early kidney failure, the high FGF23 levels are dependent

on the high PTH levels. PTH infusion for 3 days to mice with normal renal function increased serum FGF23 and calvaria FGF23 mRNA levels. To demonstrate a direct effect of PTH on FGF23, they added PTH to rat osteoblast-like UMR106 cells [40]. PTH increased FGF23 mRNA levels (4-fold) and this effect was mimicked by a PKA activator, forskolin. They showed that the PTH increase in FGF23 expression involves the PKA and Wnt pathways. Importantly, hemodialysis patients treated with the calcimimetic cinacalcet had a pronounced decrease not only in serum PTH, calcium and phosphorus but also serum FGF23 [42] as did total parathyroidectomy [43]. The effect of PTH on FGF23 completes a bone-parathyroid endocrine feedback loop. Importantly, secondary hyperparathyroidism is essential for the high FGF23 levels in early CKD.

In CKD, serum levels of both FGF23 and PTH are increased implying resistance of the parathyroid to FGF23, which we and others have shown is due to downregulation of klotho-FGFR1 in the parathyroid [44–46]. There is a similar downregulation of klotho in the kidneys of rats with experimental CKD [47] and interestingly, the administration of klotho has been shown to improve renal function by binding to TGFβ in the kidney [48]. The increase in FGF23 in early CKD may be responsible, at least in part, for the early decrease in calcitriol levels in CKD patients.

Lopez *et al.* [41] showed that renal klotho protein expression was decreased in parathyroidectomized rats and the administration of calcitriol led to an increase not only in serum calcium but also normalized the klotho expression with no change in serum P. These results suggest that calcitriol or calcium regulate renal klotho and emphasize the tight interactions among the minerals, their hormones and receptors.

FGF23, klotho and the cardiovascular system

The FGF23 renal coreceptor klotho has been shown to be an early biomarker for CKD in man and mouse, and klotho

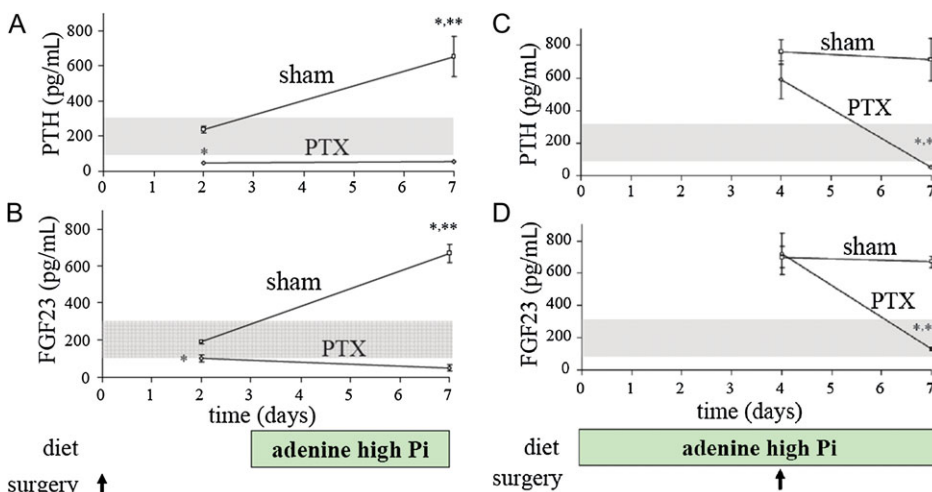


Fig. 2. Parathyroidectomy (PTX) prevents and corrects the increased FGF23 levels of adenine high Pi-induced early kidney failure [40]. PTX before the adenine high-Pi diet (A–B). Rats underwent PTX or sham operation. At Day 3, they were fed the adenine diet for four additional days as indicated below the graphs. Blood was sampled at Days 2 and 7 and results are shown for PTH (above) and FGF23 (below). Adenine diet before PTX (C–D). Rats were fed an adenine high-Pi diet for seven days and at Day 4, underwent PTX or sham operation as indicated below the graphs. Reproduced with permission of the *American Journal of Physiology*.

deficiency contributes to the soft tissue calcification in CKD [49]. Soluble klotho ameliorates vascular calcification by enhancing phosphaturia, preserving glomerular filtration and directly inhibiting P uptake by vascular smooth muscle [49]. FGF23 has now been shown to be not only associated with an increased mortality in patients with CKD but also to directly cause left ventricular hypertrophy (LVH) [50].

Klotho expression and action on blood vessels

Klotho is not only an essential component of the FGF23 receptor complex but also has functions of its own. These are now starting to be unraveled. In human and experimental CKD, there is less klotho expression in the kidney and the parathyroid as well as lower levels in the serum and urine [49]. Correction of tissue klotho levels in mice with experimental CKD, either by administration of klotho protein or the generation of transgenic mice expressing the klotho gene, improves not only renal function but also the vascular calcification evident in the CKD mice. The CKD mice had a 10-fold increase in serum PTH, which was decreased to control levels in the transgenic klotho CKD mice [49]. This last result emphasizes the importance of klotho in the parathyroid in mediating a decrease in serum PTH after the ligand FGF23 and now alone. Klotho is a phosphaturic substance in its own right independent of FGF23 [51]. The beneficial effect of klotho on vascular calcification was a result of more than its effect on renal function and phosphatemia, suggesting a direct effect of

klotho on the vasculature. *In vitro*, klotho suppressed sodium-dependent uptake of P and mineralization induced by high P and preserved differentiation in vascular smooth muscle cells (VSMC) [49]. Klotho inhibits Na-coupled P cotransport when directly added to cultured proximal tubule-like cells and in cell-free brush border membrane vesicles (BBMV) without FGF23 [49]. Therefore, klotho opposes soft tissue calcification in CKD. Klotho ameliorates vascular calcification by enhancing phosphaturia, preserving glomerular filtration and directly inhibiting P uptake by VSMC [48]. In addition, klotho proteins interact with the VEGFR-2/TRPC-1 complex in the surface of endothelial cells to maintain endothelial integrity(c) [52]. So these studies suggest that klotho exerts a beneficial effect in other cells of the vascular wall besides VSMC.

Serum FGF23 levels are associated with increased mortality in dialysis patients

Serum FGF23 levels increase markedly as GFR decreases reaching >10 000-fold increases in dialysis patients. Levels of FGF23 directly correlate with mortality in these patients [53–55]. The order of magnitude of the increase in mortality risk in association with high serum FGF23 levels is by a factor of 5–6, whereas the increase in mortality risk in association with high serum phosphorus is only by a factor of 1.3–2. Moreover, the increased mortality risk with high FGF23 levels was essentially independent of increased phosphorus levels. The high levels of FGF23 are related to hyperphosphatemia, vitamin D therapy and secondary

A cascade of factors contributing to CKD-MBD

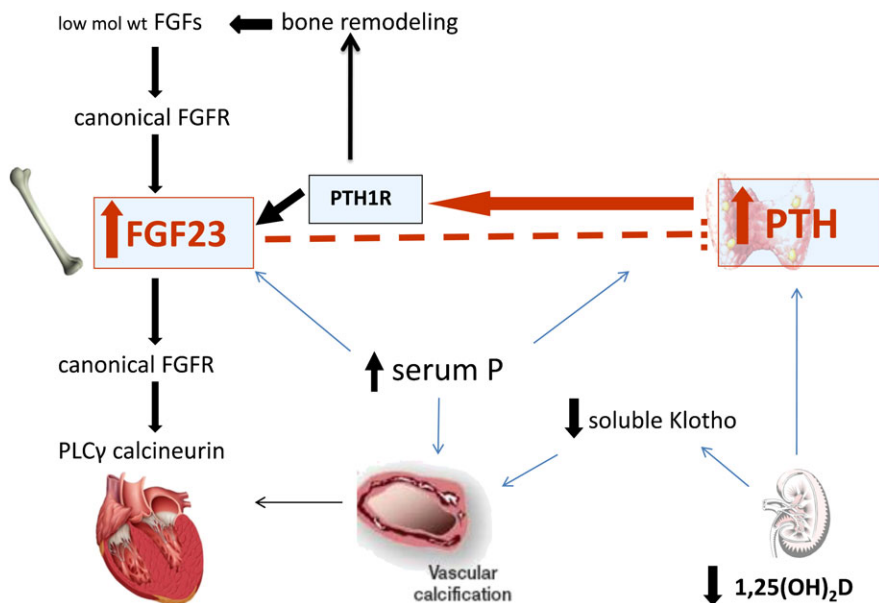


Fig. 3. A cascade of factors contributing to CKD-MBD. In advanced CKD dietary, the phosphorus load is tropic to both PTH and FGF23 and FGF23 itself acts to decrease serum calcitriol that is a further stimulus to PTH secretion. FGF23 normally acts to decrease PTH but not in CKD where its receptor is downregulated in both the parathyroid and kidneys. PTH acts directly on bone cells to increase FGF23 expression and secretion and is essential for the high FGF23 levels of CKD. The high PTH also leads to enhanced bone remodeling thereby releasing low-molecular weight FGFs that act on canonical FGFRs to increase FGF23. The high FGF23 levels act on FGFRs in the heart activating PLC γ calcineurin contributing to the LVH in these patients. The high serum P and the low levels of soluble klotho are factors involved in the vascular calcification of these patients.

hyperparathyroidism. How FGF23 causes or is related to the increased mortality has been enigmatic but new work sheds light on the subject.

FGF23 directly induces LVH [50]

Patients with CKD have a remarkably high prevalence of LVH. There are a number of factors that contribute to the LVH and recently it has been shown by a combination of epidemiological, *in vitro* and *in vivo*, studies that FGF23 directly induces LVH [50]. FGF23 levels were measured in baseline plasma samples from 3000 individuals who underwent echocardiography 1 year later as part of the prospective Chronic Renal Insufficiency Cohort (CRIC) Study. Elevated FGF23 levels were independently associated with LVH in patients with varying degrees of CKD. Moreover, elevated FGF23 levels at baseline were associated with increased future risk of new-onset LVH. They then compared the response of isolated neonatal rat ventricular cardiomyocytes (NRVMs) to 48 h of treatment with FGF23 versus FGF2, the prototypical FGF. FGF23-induced hypertrophy of isolated NRVMs via FGFR activation but through a klotho-independent pathway. They then showed that the PLC γ -calcineurin-NFAT axis is the dominant signaling pathway of FGF23-mediated hypertrophy of NRVMs and not the MAPK pathway, which is activated by FGF23 activation of klotho-FGFR1. *In vivo*, FGF23 directly injected into the myocardium or given intravenously led to LVH in rodents and the administration of an FGF-receptor blocker to rats with 5/6 nephrectomy-induced kidney failure attenuated LVH. Therefore, these studies have provided solid evidence for an effect of FGF23 to directly induce LVH (Figure 3).

We should not forget that FGF23 is by no means the only factor that is altered in CKD where there are direct effects on the myocardium. For instance, PTH directly affects rat myocardial cells, causes early death of cells by increasing calcium entry into heart cells [56] and has long been considered a uremic toxin [57]. There is renewed interest in the contribution of vitamin D deficiency to heart disease and the effects of hyperphosphatemia, hypertension, volume overload and changes in lipoproteins are among a myriad of other real or potential uremic toxins which are well studied and beyond the scope of this review. But there is now substantial epidemiological and laboratory data that FGF23 is of prime importance as a cause of myocardial damage.

Unanswered questions

There are still many more questions to be answered. For instance, the proof provided that klotho is not expressed in the myocardium was only shown for klotho mRNA. It needs to be shown that the protein is also not present due to translation from an alternatively transcribed form of klotho. The *in vitro* studies are convincing but klotho is expressed in blood vessels and it is possible that the *in vivo* results reflect an effect of FGF23 on klotho in blood vessels, which then affects the myocardium. For that matter, klotho is strongly expressed in the sinoatrial node of the heart [58]. Was that relevant here? Which FGFR binds

FGF23 in the myocardium and what cofactors such as heparin compounds are needed for binding?

Whatever it is, Faul *et al.* have opened up an exciting new avenue of research in the field of CKD-MBD and for that we are grateful. Targeting FGF23 by decreasing the serum PTH in patients with secondary hyperparathyroidism [42] as well as maintaining a serum P in target range and a more judicious use of vitamin D analogues seem prudent advice for our patients at this stage of the game. FGF23 antibodies have been developed and they may one day become tools in the management of CKD patients.

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