New Insights Into the FGF23-Klotho Axis

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Summary: Abnormal mineral metabolism is a hallmark in patients with advanced chronic kidney disease (CKD). Hyperphosphatemia, and the homeostatic mechanisms controlling phosphate metabolism, have received particular attention over the past decade. The phosphate-regulating hormone fibroblast growth factor-23 (FGF23) was discovered through studies of rare hypophosphatemic disorders, whereas Klotho, which subsequently turned out to be a co-receptor for FGF23, was identified in a mouse model showing hyperphosphatemia and multiple aging-like traits. The FGF23–Klotho endocrine axis is a pivotal regulator of mineral metabolism. In CKD, early onset of Klotho deficiency contributes to renal FGF23 resistance and a maladaptive increase in circulating FGF23. FGF23 is an early biomarker of renal injury and increased FGF23 predicts adverse clinical outcomes, in particular cardiovascular disease. A paradigm of FGF23 excess and Klotho deficiency is proposed, in which FGF23 preferentially stimulates left ventricular hypertrophy, and loss of Klotho augments fibrosis, endothelial dysfunction, and vascular calcification. The clinical benefit of FGF23 and Klotho measurements remain uncertain, nevertheless, the FGF23–Klotho axis is a solid candidate for a novel diagnostic and therapeutic target in CKD.

Keywords: Fibroblast growth factor-23, FGF-23, calcium, phosphate, vitamin D, parathyroid hormone, chronic kidney disease

The existence of novel phosphate-regulating hormones (phosphatonin) was predicted several decades ago based on observations in patients with hereditary and acquired disorders of hypophosphatemia caused by excessive renal phosphate wasting. Autosomal-dominant hypophosphatemic rickets (ADHR) (online mendelian inheritance in man 193100), X-linked hypophosphatemic rickets (XLH) (online mendelian inheritance in man 307800), and tumor-induced osteomalacia (TIO) are 3 model diseases that supported the presence of a humoral factor(s) causing phosphaturia independent of parathyroid hormone (PTH). White et al. identified the FGF23 gene by positional cloning techniques and unraveled activating FGF23 mutations as the causative factor of ADHR as a result of stabilization of its encoded protein. In parallel, Shimada et al isolated FGF23 as a differentially expressed transcript in a surgically resected TIO tumor and showed that systemic administration of recombinant FGF23 recapitulated the TIO phenotype of hypophosphatemia, inappropriately low 1,25-dihydroxyvitamin D levels, and severe rickets/osteomalacia (which are also the principal features in ADHR and XLH). Subsequently, it was shown that XLH patients, who carry a large set of inactivating mutations in a membrane-bound metalloproteinase gene termed PHEX, show high FGF23 expression in bone, which produces a phenotype virtually indistinguishable from ADHR. Collectively, FGF23 was the long sought-after phosphatonin that unified the underlying pathophysiology of renal phosphate wasting in ADHR, XLH, and TIO.

Type I, membrane-bound, α-Klotho (referred to as Klotho) was discovered in 1997 by Kuro-o et al, who, by a random insertional mutation approach in mice, identified hypomorph Klotho alleles owing to disruptions in its gene promoter region. Downstream conse-
quences of low Klotho transcription were numerous aging-like phenotypic traits including growth retardation, shortened lifespan, skeletal abnormalities including osteomalacia, and disseminated calcifications in soft tissues and vasculature. Notably, a human homozygous inactivating mutation in Klotho translates into a similar biochemical profile and ectopic calcifications, part of a syndrome termed tumoral calcinosis. Klotho mice show a procalcific biochemical profile of hyperphosphatemia, hypervitaminosis D, and hypercalcemia. Correction of these biochemical abnormalities by genetic or dietary approaches ameliorates their phenotype, corroborating an instrumental role of disturbed mineral metabolism in the underlying pathophysiology associated with Klotho deficiency.

FGF23 AND KLOTHO CHARACTERISTICS AND FUNCTION

FGF23 characteristics and function

The FGF23 gene consists of 3 exons and encodes a 251-amino acid protein of approximately 32 to 36 kDa, depending on glycosylation status. The N-terminus contains a signal peptide for secretion, confirming that FGF23 is an atypical member of the FGF family that circulates in blood with endocrine, rather than autocrine or paracrine, effects. A schematic view of FGF23 is shown in Figure 1A. Low affinity for certain heparins also enables FGF23 to be released from the cell surface into the blood. Bone is the dominant source of FGF23, in particular osteocytes, and, to lesser extent, osteoblasts. FGF23 may have important local functions in bone such as inhibition of osteoid mineralization and modulation of mesenchymal stem cell differentiation, but these functions have been characterized incompletely and are not discussed in this review.

The key target organ for FGF23 is the kidney, where it acts directly on renal tubules to reduce phosphate reabsorption by decreasing the brush-border membrane abundance of the type II sodium-dependent phosphate (Na/Pi) cotransporter (NPT2)a and NPT2c. The principle of intracellular FGF23 signaling is FGF23 receptor (FGFR) dimerization and activation of their intrinsic FGFR tyrosine kinase activity by transphosphorylation. Major substrates of FGFR kinase are FGFR substrate 2α (which stimulates renin-angiotensin

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**Figure 1.** (A) FGF23 and the principal site of intracellular proteolytic cleavage that impacts the ratio of intact versus secreted proteins. (B) Klotho isoforms representing full-length membrane Klotho that are also a co-receptor for FGF23, and the 2 soluble isoforms derived from membrane shedding or by secretion of a truncated (alternatively spliced) Klotho variant. (C) The principle of intact versus C-terminal FGF23 measurements is based on assay epitope detection in relation to the proteolytic site. KL, Klotho.
system—mitogen-activated protein kinase and phospholipase C λ1 (which simulates protein kinase C and calcineurin/nuclear factor of activated T-cells signaling).\textsuperscript{13} Internalization of these transporters reduces tubular reabsorption of phosphate from the glomerular filtrate to blood, translating into higher fractional excretion of phosphate and subsequently decreasing serum phosphate levels. Furthermore, FGF23 decreases systemic phosphate exposure indirectly through modulation of vitamin D metabolism by reducing the conversion of native 25-hydroxyvitamin D to active 1,25-dihydroxyvitamin D, and by enhancing the degradation pathway of vitamin D. This is achieved by a direct regulation of the renal vitamin D—regulating enzymes CYP27B1 and CYP24A1, respectively.\textsuperscript{14} The phosphate-lowering effect of FGF23 in the intestine is accomplished by attenuated active phosphate absorption through the vitamin D–regulated phosphate co-transporter NPT2b. A third effect of FGF23 is to inhibit synthesis and secretion of PTH from the parathyroid glands.\textsuperscript{15,16} Thus, by direct and indirect means, FGF23 reduces serum phosphate levels (and also calcium levels) through its concerted action on the kidney and parathyroid glands.

Klotho characteristics and function

Klotho function appears more complex at first sight. Membrane-bound Klotho is a 130-kDa protein containing 2 large IgG-like domains (KL1 and KL2), with a short single-pass transmembrane and intracellular domain in its C-terminus, and serves as a facilitator of signal transduction for FGF23. Unlike most other FGFs, which are dependent on interactions with various heparins for FGF-receptor binding, Klotho creates a high-affinity tertiary FGF23/FGF-receptor/Klotho complex. The role of Klotho as a co-receptor for FGF23 initially was reported by Urakawa et al.\textsuperscript{17} A predicted functional overlap of FGF23 and Klotho arose from the phenotypic similarities of Klotho mice and those reported for Fgf23 null mice. Importantly, Klotho mice have increased blood FGF23 in the magnitude of a thousand-fold or higher, corroborating Klotho as an obligate co-receptor for FGF23. Olauson et al\textsuperscript{18} further showed the principle of renal FGF23 resistance in 2 mouse models harboring cell-specific/tissue-specific deletions of Klotho. A partial deletion of Klotho in renal distal tubules generated a linear relationship between residual renal Klotho, serum phosphate, and FGF23 levels, albeit a marked threshold effect was noted: mice with less than 30% remnant renal Klotho had an exponential increase in serum FGF23 incommensurate to the magnitude of hyperphosphatemia and parallel changes in mineral metabolism. This indicates a critical level of Klotho to maintain FGF23 signaling properties and that FGF23 end-organ resistance promotes its expression independently of mineral metabolism. Another mouse model with a complete deletion of Klotho throughout the nephron fully recapitulated the biochemical and aging phenotypes of Klotho mice, thus unraveling the kidney as the principal organ mediating systemic Klotho effects.\textsuperscript{19}

Klotho also exists in 2 circulating isoforms: a full-length, 130-kDa protein shed from the cell membrane by proteolytic cleavage, a process mediated at least partially by ADAM metallopeptidase domain (ADAM) 10 and ADAM17, and a truncated, alternatively spliced, secreted isoform of approximately 60 kDa containing only 1 of the 2 IgG-like domains.\textsuperscript{20,21} In the kidney, the shedded 130-kDa Klotho isoform can stimulate phosphate excretion independently of FGF23 by NPT2a internalization.\textsuperscript{22} Soluble Klotho also plays a direct role in renal handling of calcium by altering the sugar moiety of the transient receptor potential vanilloid type 5 (TRPV5) channel. It mediates hydrolysis of the terminal sialic acids, leading to retention of TRPV5 in the cell membrane and escape from cellular internalization.\textsuperscript{23} Membrane-anchored TRPV5 stimulates the reabsorption of calcium and reduces calcium excretion. Importantly, a widely expressed protein termed Memo associates with the FGFR signalosome and is warranted for optimal FGFR signal transduction. Memo knockout mice resemble FGF23 and Klotho mice, albeit with some important differences, including the absence of hyperphosphatemia, suggesting that shedded Klotho activity may preserve normophosphatemia in the absence of FGF23 signaling.\textsuperscript{24}

Finally, soluble Klotho isoforms act as modulators of signaling pathways, including those for insulin/insulin-like growth factor 1, Wnt, and transforming growth factor-β.\textsuperscript{25–27} These functions may be critical elements for the progression of chronic kidney disease (CKD). The principal isoforms of Klotho are shown in Figure 1B.

ASSAYS FOR CIRCULATING FGF23 AND KLOTHO

Circulating FGF23 can be quantified from serum or plasma by commercially available antibody-based assays (enzyme-linked immunosorbent assays [ELISAs]). Biologically active intact FGF23 has an estimated half-life of 20 to 60 minutes and is cleaved enzymatically into inactive C-terminal and N-terminal fragments.\textsuperscript{28} As shown in Figure 1C, there are 2 principal types of assays: one that only recognizes intact FGF23, and one that detects both intact FGF23 and C-terminal fragments. In most CKD studies there appears to be a good correlation between intact and C-terminal FGF23, supporting the finding that intact
FGF23 is the predominant form in blood and that the assays are largely interchangeable. On the other hand, some comparative studies have indicated that the proportion of intact FGF23 may be higher in CKD patients than in healthy individuals. This is in contrast to many other peptide hormones and may be related to uremia-induced alterations in proteins important for FGF23 stability, including enzymes modulating its sugar moieties such as GALNT3 and FAM20C, and the recently reported plasminogen activator inhibitor-1.

Recent findings also have suggested that iron status substantially could influence the ratio between intact and C-terminal FGF23. Approximated reference values for the 2 most commonly used FGF23 kits (from Kainos, Tokyo, Japan and Immutopics, San Clemente, CA, USA) are shown in Table 1 and typically is in the range of 10 to 100 pg/mL (ie, similar as for many other circulating hormones).

In contrast to FGF23, validated methods to measure soluble Klotho currently are lacking. A sandwich ELISA was established in 2010 (Immuno-Biological Laboratories, Minneapolis, MN, USA), and the first reports showed a gradual decrease in circulating Klotho with increasing age. Data on circulating Klotho in CKD are conflicting. A few smaller studies have shown a decrease in circulating Klotho paralleling CKD progression, however, the correlations were weak and driven mainly by a few outliers. In contrast, Seiler et al showed that soluble Klotho was not associated with renal function in a larger study of 312 patients with various stages of CKD. By using an alternative approach, Hu et al showed an early and drastic decrease in Klotho concentrations in serum and urine from patients with CKD. The reasons for these discrepancies currently remain unknown.

**REGULATION OF FGF23 AND KLOTHO**

A central aspect of FGF23 and Klotho is the regulation of their expression and the apparent disconnect between tissue level and concentration in blood. FGF23 is regulated by several feedback loops consistent with endocrine systems: it reduces serum phosphate, 1,25-dihydroxyvitamin D, and PTH levels, and these factors stimulate FGF23 expression in bone. Active vitamin D is the most potent regulator of FGF23 and acts directly on FGF23 gene transcription by binding a vitamin D–responsive element in the gene promoter region. PTH stimulates FGF23 expression through protein kinase A– and Wnt-dependent
mechanisms in the presence of PTH receptors. By contrast, it remains uncertain how phosphate regulates FGF23. In CKD, FGF23 is increased before the onset of hyperphosphatemia, indicating that a high serum phosphate level per se is unlikely the sole trigger of FGF23 synthesis and secretion. There is also an apparent dissociation between its transcript and protein levels, as evidenced in murine CKD models displaying high serum FGF23 levels in the absence of increased transcripts in bone. Another hypothesis is that intestinal absorption of phosphate is integral for FGF23 secretion, however, this mechanism appears to play a limited role because dietary phosphate restriction and/or the use of phosphate-binding therapies have a relatively limited impact on reducing serum FGF23 in healthy subjects as well as in CKD patients. A third intriguing hypothesis is that the absolute filtered phosphate load per nephron is the key stimulus for FGF23, phosphate-driven FGF23 expression. An emerging paradigm is that a permissive threshold directly or indirectly, modulates FGF23 expression.

Consistent with the notion that extracellular calcium, rather than phosphate or calcium alone. This is consistent with the observation that the absolute filtered phosphate load per nephron is the key stimulus for FGF23. This could explain FGF23 increases in individuals with mild to moderate CKD when serum phosphate level is kept at a normal level owing to a compensatory increase in fractional excretion of phosphate. Regardless, it is elusive how the bone is capable of sensing fluctuations in serum phosphate and/or filtered phosphate load per nephron. We speculate that bone cells may harbor phosphate-sensing properties that influence FGF23 secretion by modification of post-translational pathways involved in its secretory function. There are several known mutations in FGF23, as well as in proteins involved in its post-translational modification, that elicit defective intracellular trafficking or proteolytic cleavage of FGF23. The end result is an altered ratio of intact versus fragmented FGF23 in blood that ultimately defines its systemic biological activity.

Observational data support that FGF23 is associated more strongly with the calcium phosphate product rather than phosphate or calcium alone. This is consistent with the notion that extracellular calcium, directly or indirectly, modulates FGF23 expression. An emerging paradigm is that a permissive threshold for serum calcium is required for FGF23 expression and may be mediated by FGF23-driven FGF23 expression.

Several other factors such as leptin, estrogen, and calcitonin were reported to regulate FGF23, likely by mechanisms involved in altered bone turnover rate. Similarly, several drugs such as bisphosphonates alter FGF23 expression without concomitant changes in serum minerals, presumably through a similar and indirect mechanism. Endogenous ligands for FGF receptors in bone also could stimulate FGF23 expression. As mentioned previously, reduced renal expression of Klotho is an important determinant of serum FGF23, but the underlying mechanism(s) remains elusive. Somewhat paradoxical, systemic overexpression of Klotho strongly increases circulating FGF23, suggesting that soluble Klotho may interfere directly with local FGF23 regulation in bone.

Recent studies have suggested that certain preparations of intravenous iron can induce transient increases in FGF23 and subsequent hypophosphatemia. In this regard, Farrow et al showed that mice with an ADHR mutation (making FGF23 resistant to proteolytic cleavage) fed a low-iron diet suffered from hypophosphatemia and osteomalacia/rickets, as explained by high intact FGF23 levels, whereas wild-type mice fed the same diet had normal intact, but increased, C-terminal FGF23 levels, and remained normophosphatemic. This corroborates the finding that iron may be a direct or indirect transcriptional regulator of FGF23 and that intracellular processing of FGF23 is a critical determinant of its systemic activity.

Klotho regulation is also a matter of debate. The number of studies examining its regulation in vivo has been limited because quantification of membrane-bound Klotho requires invasive methods. In physiology, activators of the vitamin D receptor effectively stimulate Klotho expression by binding vitamin D-responsive elements in the gene promoter. Ionized calcium and phosphate on the other hand decreases Klotho expression through as yet unknown mechanisms. Furthermore, FGF23 dose-dependently down-regulates Klotho in kidney and parathyroid glands. In CKD, the reduction of Klotho likely is multifactorial because inflammation, oxidative stress, and uremic toxins all have been shown to suppress Klotho. Epigenetic mechanisms may play an important role given that uremic serum induces hypermethylation of the Klotho gene promoter.

**FGF23 AND KLOTHO IN CKD**

CKD is characterized by a progressive loss of nephrons, which is paralleled by a decrease in the glomerular filtration rate (GFR). Larsson et al initially showed a strong negative association between GFR and FGF23, which was confirmed in a large set of follow-up studies. The increase in FGF23 precedes other overt changes in mineral metabolism with a majority of CKD subjects having abnormal FGF23 levels when their GFR is less than 60 mL/min. Studies in acute kidney injury (AKI) also have suggested that FGF23 is an early and rapid biomarker of renal damage. Tissue Klotho on the other hand appears equally sensitive to fluctuations in GFR, with a reduction that dynamically coincides with FGF23 increases. In opposite to FGF23, Klotho expression is reduced rapidly in AKI and speculatively is involved in the pathologic transition from AKI to CKD. There is essentially a linear relationship between GFR and FGF23 in mild to moderate CKD, however, this relationship becomes exponential in advanced CKD,
with many dialysis patients reaching thousand-fold increases in FGF23 concentrations. The reason(s) for extreme increases in FGF23 levels remains uncertain, however, as shown in Klotho mouse models, may be attributed to its renal resistance.

The dynamics of FGF23 in CKD are attributed to its established physiological action of protecting against phosphate overload and, when relevant, vitamin D toxicity. Thus, FGF23 increases to counteract hyperphosphatemia at the expense of decreasing 1,25-dihydroxyvitamin D. Additional triggers for FGF23 in late CKD are secondary hyperparathyroidism (SHPT) and Klotho deficiency, the latter induced by factors in the uremic environment such as inflammatory cytokines and mediators of oxidative stress. FGF23 further exacerbates Klotho deficiency by its direct suppression, leading to an accelerated and self-fueled vicious circle of FGF23 excess and Klotho deficiency that may drive renal and cardiovascular complications (Fig. 2).

FGF23 AND KLOTHO IN SECONDARY HYPERPARATHYROIDISM

SHPT is a cardinal complication in CKD. It is defined primarily by growth of the parathyroid glands and enhanced secretion of PTH, ultimately causing parathyroid hyperplasia and the development of multinodular adenomas. An important note is that SHPT should not be relegated to a biochemical abnormality but rather put in the context of a long-lasting dynamic interplay between parathyroid glands, bone, and the cardiovascular system throughout the course of CKD. The principal stimuli for SHPT traditionally are considered to be hypocalcemia, vitamin D deficiency, and hyperphosphatemia, but FGF23–Klotho also are modulators of SHPT.

Klotho is expressed at high levels in parathyroid glands. It first was shown by Bon-Dov and Krajisnik et al\textsuperscript{15,16} that the parathyroid is a target for FGF23 and that FGF23 directly suppresses \textit{PTH} gene transcription as well as protein secretion. One report proposed that Klotho itself is essential for mediating PTH secretion during hypocalcemia.\textsuperscript{63} These findings have not been reproduced, although a patient with a chromosomal translocation leading to aberrant Klotho overexpression developed severe SHPT.\textsuperscript{64} An initial controversy was the unabated parallel increase in PTH and FGF23 in CKD with consideration that FGF23 directly suppresses PTH. There are several potential explanations for this. Vitamin D deficiency, often followed by a relative hypocalcemia as a result of diminished intestinal calcium absorption, overrides the inhibitory action of FGF23 on PTH to preserve calcium balance. This hypothesis was substantiated by a study in which injections with FGF23 antibodies were given to uremic rats. The neutralization of FGF23 prevented the decrease in 1,25-dihydroxyvitamin D and the development of SHPT, but simultaneously resulted in hyperphosphatemia, hypercalcemia, and increased vascular calcification.\textsuperscript{65} Second, during progression of SHPT the parathyroid glands gradually become unresponsive to FGF23. Until recently, this was considered a result of decreasing parathyroid Klotho levels.\textsuperscript{66} However, Olauson et al\textsuperscript{67} showed that the deletion of parathyroid Klotho in mice did not enhance the susceptibility to SHPT in renal failure, which is explained by the presence of a Klotho-independent calcineurin–FGF23 signaling pathway. Parathyroid FGF23 resistance in uremia is more likely to be explained by FGF-receptor deficiency or other as yet unidentified mechanisms. Another potentially important implication of this novel FGF23/calcineurin-mediated suppression of PTH is that the combination of low parathyroid Klotho and treatment with calcineurin inhibitors in renal transplant recipients may aggravate pre-existing SHPT. Comparison of SHPT in this population of patients using calcineurin inhibitors versus other immunosuppressive regimens is an important topic for further investigation.

FGF23, KLOTHO, AND CLINICAL OUTCOMES

Because FGF23 is measured reliably in serum or plasma, a wealth of epidemiologic studies have explored cross-sectional and longitudinal relationships between FGF23 and clinical outcomes. A summary of key clinical studies assessing the relationship between FGF23 and longitudinal outcomes is shown in Table 2. The outcomes of these studies unequivocally point to FGF23 as a robust marker of disease surrogate markers as well as hard clinical end points including mortality and cardiovascular events. Such relationships have been confirmed in CKD patients as well as in the community-dwelling adults and in populations enriched for pre-existing cardiovascular disease. In CKD, higher FGF23 also is associated with more specific renal end points such as time to renal replacement therapy, CKD progression rate, and graft survival in renal transplant recipients. The consistency of these associations across various strata of kidney function, geographic regions, and in widely heterogeneous cohorts is striking and has ignited intense efforts to elucidate if clinical FGF23 measurements in CKD would provide added value, and whether FGF23 is a marker or mediator of cardiovascular disease and CKD-related complications.

The latter question is difficult to answer because epidemiologic studies by definition cannot establish causality and because of the high likelihood of residual confounding that is attributed to the dynamic complexity of mineral metabolism. However, a key study by Faul et al\textsuperscript{68} identified FGF23 as a direct stimulator of
### Table 2. Key Longitudinal Studies of FGF23 and Outcome: Mortality, CV Outcomes, and Renal Outcomes

<table>
<thead>
<tr>
<th>Study</th>
<th>Population</th>
<th>Sample Size (n)</th>
<th>Median Follow-Up Period (y)</th>
<th>Main Outcome(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fliser et al, 2007</td>
<td>CKD stages 2-5 (MMKD study)</td>
<td>177</td>
<td>4.4</td>
<td>C-terminal and intact FGF23 predict dsCr and initiation of RRT</td>
</tr>
<tr>
<td>Gutierrez et al, 2008</td>
<td>Incident HD patients (ArMORR study)</td>
<td>10,044</td>
<td>1.0</td>
<td>C-terminal FGF23 predicts all-cause mortality</td>
</tr>
<tr>
<td>Jean et al, 2009</td>
<td>Prevalent HD patients</td>
<td>219</td>
<td>2.0</td>
<td>C-terminal FGF23 predicts all-cause mortality and CV calcification</td>
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<tr>
<td>Olauson et al, 2010</td>
<td>Incident HD patients (MIA study)</td>
<td>229</td>
<td>1.9</td>
<td>Intact FGF23 predicts all-cause mortality only in men with a history of CVD</td>
</tr>
<tr>
<td>Parker et al, 2010</td>
<td>Patients with stable coronary heart disease, 22% had eGFR &lt; 60 mL/min/1.73 m² (Heart and Soul study)</td>
<td>833</td>
<td>6.0</td>
<td>C-terminal FGF23 predicts all-cause mortality and CV events</td>
</tr>
<tr>
<td>Isakova et al, 2011</td>
<td>CKD stages 2-4, mean eGFR 43 mL/min/1.73 m² (CRIC study)</td>
<td>3,579</td>
<td>3.5</td>
<td>C-terminal FGF23 predicts increased all-cause mortality</td>
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<td>Kendrick et al, 2011</td>
<td>CKD stages 4-5 ND, mean eGFR 18 mL/min/1.73 m² (HOST study)</td>
<td>1,099</td>
<td>2.9</td>
<td>C-terminal FGF23 predicts all-cause mortality, CV events, and initiation of RRT</td>
</tr>
<tr>
<td>Titan et al, 2011</td>
<td>Diabetic CKD with proteinuria, mean eGFR 48 mL/min/1.73 m²</td>
<td>55</td>
<td>4.0</td>
<td>Intact FGF23 predicts composite of dsCr, initiation of RRT, and death</td>
</tr>
<tr>
<td>Wolf et al, 2011</td>
<td>Renal transplant recipients, mean eGFR 51 mL/min/1.73 m²</td>
<td>984</td>
<td>3.0</td>
<td>C-terminal FGF23 predicts all-cause mortality and allograft loss</td>
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<td>Lundberg et al, 2012</td>
<td>IgA nephropathy, CKD stages 1-4, mean eGFR 71 mL/min/1.73 m²</td>
<td>180</td>
<td>4.6</td>
<td>Intact FGF23 predicts rate of GFR decrease and dsCr</td>
</tr>
<tr>
<td>Faul et al, 2011</td>
<td>CKD stages 2-4 (CRIC study subanalysis)</td>
<td>411</td>
<td>2.9</td>
<td>C-terminal FGF23 predicts incident LVH</td>
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<td>Ix et al, 2012</td>
<td>Community-dwelling adults ≥ 65 y, mean eGFR 71 mL/min/1.73 m²</td>
<td>3,107</td>
<td>10.5</td>
<td>C-terminal FGF23 predicts all-cause mortality and incident HF</td>
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<td>Baia et al, 2013</td>
<td>Renal transplant recipients, mean eGFR 47 mL/min/1.73 m²</td>
<td>593</td>
<td>6.1</td>
<td>C-terminal FGF23 predicts CV and all-cause mortality</td>
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<td>Årnlöv et al, 2013</td>
<td>Community-dwelling elderly men, mean eGFR 74 mL/min/1.73 m² (ULSAM study)</td>
<td>727</td>
<td>9.7</td>
<td>Intact FGF23 predicts CV and all-cause mortality</td>
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<td>Årnlöv et al, 2013</td>
<td>Community-dwelling adults, mean eGFR 80 mL/min/1.73 m² (PIVUS study)</td>
<td>973</td>
<td>5.1</td>
<td>Intact FGF23 predicts a MACE composite</td>
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<td>Floege et al, 2014</td>
<td>Prevalent HD patients with SHPT (EVOLVE study post hoc)</td>
<td>3,883</td>
<td>5.3</td>
<td>C-terminal FGF23 predicts all-cause mortality</td>
</tr>
<tr>
<td>Udell et al, 2014</td>
<td>Stable ischemic heart disease (PEACE study post hoc)</td>
<td>3,627</td>
<td>5.2</td>
<td>C-terminal FGF23 predicts CV death and incident HF and identifies patients with greater benefit of ACE-inhibitor therapy</td>
</tr>
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</table>

Abbreviations: ACE, angiotensin-converting enzyme; ArMORR, Accelerated Mortality on Renal Replacement study; CV, cardiovascular; CRIC, Chronic Renal Insufficiency Cohort Study; dsCr, doubling of serum creatinine; eGFR, estimated glomerular filtration rate; HD, hemodialysis; HF, heart failure; HOST, Homocysteine in End-stage and Advanced Kidney Disease study; MACE, major cardiovascular event; MIA, Malnutrition, inflammation and atherosclerosis study; MMKD, Mild to Moderate Kidney Disease Study; ND, nondialysis; PEACE, Prevention of events with angiotensin-converting enzyme inhibition study; PIVUS, Prospective Investigation of the Vasculature in Uppsala Seniors Study; RRT, renal replacement therapy; ULSAM, Uppsala Longitudinal Study of Adult Men Study.
myocardial growth and left ventricular hypertrophy (LVH) independent of Klotho. A similar signaling mechanism exists in parathyroid glands, and another study found that FGF23 modified vascular reactive oxygen species production and vasoconstriction. The clinical relevance of the direct effect of FGF23 on cardiac hypertrophy remains uncertain. The link between FGF23 and LVH also could be explained by alternate mechanisms such as FGF23 regulation of renal sodium uptake and subsequent volume-driven LVH. Furthermore, direct neutralization of FGF23 in a CKD rat model did not attenuate LVH, although the potential benefit of FGF23 inhibition may have been offset by aggravated hyperphosphatemia and hypercalcemia.

Current epidemiologic data provide a justifiable rationale to explore the benefits of FGF23-lowering treatment, however, such strategies face significant challenges. Dietary restriction of foods with high-phosphate bioavailability and/or content may be important, with the caveat that dietary prescriptions commonly are associated with poor compliance. Current phosphate-lowering therapies, such as non-calcium-containing phosphate binders, cause a relatively modest reduction of FGF23, which may not translate into a clinically relevant benefit in terms of FGF23 reduction. The magnitude of FGF23 lowering by using calcimimetics is overall modest yet variable, and could be promising for some patients, although calcimimetic treatment currently is restricted to the dialysis population. Treatment with C-terminal FGF23 fragments, which has been reported to antagonize the full-length active protein, was suggested as a treatment option in CKD, although proof of this concept remains to be evaluated. Direct neutralization of FGF23 is unlikely to be successful in patients with residual urine production given that CKD rats treated with neutralizing FGF23 antibodies experienced accelerated vascular calcification and mortality owing to aggravated hyperphosphatemia and hypercalcemia. Furthermore, in dialysis patients the high rate of FGF23 production is unlikely to be antagonized efficiently by sporadic antibody injections. Specific FGFR inhibitors are currently in clinical development for other indications and may be a feasible strategy to mitigate FGF23 toxicity.

Because of the aforementioned methodologic challenges in measuring soluble Klotho there are only a handful of studies examining the longitudinal association between circulating Klotho and clinical outcomes. In a study by Seiler et al, FGF23 but not Klotho predicted death or initiation of renal replacement therapy. Similarly, FGF23 but not Klotho were associated with left ventricular ejection fraction and left ventricular mass in a study of 100 cardiology inpatients. At present, soluble Klotho does not appear to be a useful clinical biomarker in CKD.

**SHOULD FGF23 BE MEASURED IN THE CLINICAL SETTING?**

A clear rationale for FGF23 measurement in clinical practice currently is lacking. There are fundamental gaps in our knowledge about its regulation and organ-specific consequences at different stages of CKD. Objective FGF23 thresholds that predict clinically relevant outcomes are lacking and it is uncertain how measurement of FGF23 would provide therapeutic guidance. Additional studies that specifically explore the clinical utility of FGF23 measurement are warranted.

**CURRENT CONTROVERSIES IN FGF23 AND KLOTHO BIOLOGY**

The exact mechanism(s) by which FGF23 promotes renal phosphate wasting has not yet been elucidated fully. Klotho expression largely is restricted to renal distal tubules, whereas the phosphate transporters NPT2a and NPT2c are localized exclusively in proximal tubules. There are 2 principal explanations for this. First, some studies have reported a low Klotho expression in the proximal tubules as well, which may be sufficient to mediate FGF23 signaling. The second hypothesis proposes a distal-to-proximal tubular mechanism (ie, FGF23 binds the FGFR–Klotho receptor complex in the distal tubules), causing the release of an as yet unidentified factor that acts on the proximal tubules, followed by down-regulation and internalization of NPT2a and NPT2c. In support of the latter theory, activated phospho-ERK1/2, an early marker of mitogen-activated protein kinase signaling, was activated exclusively in Klotho-expressing cells in the distal tubules after intravenous FGF23 delivery. Furthermore, mice with a distal tubuli-specific deletion of Klotho develop hyperphosphatemia in the face of increased systemic FGF23, also favoring the hypothesis that distal tubular Klotho is a prerequisite for FGF23-mediated control of renal phosphate metabolism. Generation of proximal tubuli-specific Klotho knockout mice could shed further light on this complex set of issues.

Current epidemiologic evidence links FGF23 to cardiovascular dysfunction, including endothelial dysfunction, arterial stiffness defined by increased pulse-wave velocity, atherosclerosis, and vascular calcification. The contribution of FGF23 and Klotho to these pathologic alterations in the cardiovascular system is under debate. In 2012, Lim et al showed that FGF23 attenuated vascular calcification in isolated human vascular smooth muscle cells in a Klotho-dependent manner. Furthermore, they reported that uremic factors promote vascular Klotho deficiency, explaining the lack of anticalcific effects by FGF23 in CKD. Although some subsequent studies have confirmed the expression of Klotho in the arterial wall and the direct effects of FGF23 on the vasculature, other
investigators could not confirm arterial Klotho expression and direct effects of FGF23 on vascular function or calcification.\textsuperscript{97,98} These discrepancies potentially could be explained by differences in antibody specificity and tissue preparation, and warrant further investigation. The role of Klotho on vascular function is discussed more carefully in another article in this issue.

Another unresolved issue is the role of soluble Klotho in CKD. Several reports have indicate that soluble Klotho protects vascular integrity and directly inhibits phosphate-driven vascular calcification.\textsuperscript{81,69} This reconciles with the theory that lack of systemic Klotho in CKD may accelerate vascular dysfunction and calcification. In the original article identifying Klotho, the phenotype of hypomorph Klotho mice essentially was rescued by crossing them with mice overexpressing Klotho.\textsuperscript{4} Serum biochemistries were not reported in the rescued mice but these studies nevertheless indicated that circulating Klotho plays a role in the prevention of vascular complications. A current enigma is the apparent disconnect between tissue and circulating Klotho level. A recent article by Lindberg et al showed that the kidney is the principal source of soluble Klotho, and that its renal expression closely reflects its concentration in the blood (J Am Soc Nephrol 2014, in press). This was substantiated by an article from Sakam et al\textsuperscript{62} that showed that soluble Klotho closely correlated to renal tissue Klotho in 239 patients who had undergone a renal biopsy. Nevertheless, a number of studies have reported unchanged circulating Klotho levels in patients with impaired renal function, even in dialysis patients, as measured by ELISA techniques. Future studies should determine whether this is owing to differential shedding and clearance of soluble Klotho in CKD, the possibility that uremic factors may interact with Klotho analysis, or other unidentified causes.

CONCLUSIONS

FGF23 and Klotho are mutually and independently critical regulators of mineral metabolism in health and disease. With decreasing kidney function, CKD becomes a state of excess FGF23 and Klotho deficiency, with FGF23 being an important prognostic marker for CKD-related complications including cardiovascular disease and mortality. Recent data have supported a causal role of disrupted FGF23–Klotho expression for the onset and aggravation of such complications. Assessment of FGF23 and Klotho as modifiable risk factors in CKD and their potential roles as clinical prognostic markers are still ahead.

REFERENCES


