FGF23 from bench to bedside

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Kovesdy CP, Quarles LD. FGF23 from bench to bedside. Am J Physiol Renal Physiol 310: F1168–F1174, 2016. First published February 10, 2016; doi:10.1152/ajprenal.00606.2015.—There is a strong association between elevated circulating fibroblast growth factor-23 (FGF23) levels and adverse outcomes in patients with chronic kidney disease (CKD) of all stages. Initially discovered as a regulator of phosphate and vitamin D homeostasis, FGF23 has now been implicated in several pathophysiological mechanisms that may negatively impact the cardiovascular and renal systems. FGF23 is purported to have direct (off-target) effects in the myocardium, as well as canonical effects on FGF receptor/α-klotho receptor complexes in the kidney to activate the renin-angiotensin-aldosterone system, modulate soluble α-klotho levels, and increase sodium retention, to cause left ventricular hypertrophy (LVH). Conversely, FGF23 could be an innocent bystander produced in response to chronic inflammation or other processes associated with CKD that cause LVH and adverse cardiovascular outcomes. Further exploration of these complex mechanisms is needed before modulation of FGF23 can become a legitimate clinical target in CKD.

MORTALITY IS EXTREMELY HIGH in patients with chronic kidney disease (CKD) and end-stage renal disease (ESRD) (27, 52). Abnormalities in bone and mineral metabolism are among the nontraditional risk factors that have been proposed as potential explanations for this excess mortality in patients with CKD and ESRD. Fibroblast growth factor-23 (FGF23), a phosphaturic hormone that suppresses 1,25(OH)2 vitamin D [1,25(OH)2D] production by the kidney, has recently emerged as one of the most powerful predictors of adverse outcomes in patients with CKD (24, 46, 53) and ESRD (37). Circulating FGF23 concentrations increase early in the course of CKD and achieve levels that are several hundred times the normal range in advanced CKD and ESRD (45). The magnitude of the risk imparted by elevated FGF23 levels is disproportionately greater compared with other components of bone-mineral metabolism (37), suggesting that high FGF23 levels may have effects that are independent from its primary role to regulate phosphorus and vitamin D homeostasis (58). There is a gap in our knowledge regarding the mechanisms leading to increased FGF23 in CKD, but emerging evidence suggests that FGF23 production is not directly regulated by serum phosphorus, but may be stimulated by several unexpected pathophysiological mechanisms, such as activation of the inflammatory system and the renin-angiotensin-aldosterone system (RAAS) (57). While elevations in FGF23 have an adaptive physiological role, there is controversy regarding whether FGF23 also has maladaptive pathological effects in advanced renal failure.

Physiology of FGF23

Members of the family of fibroblast growth factors have diverse biological activities, including roles in angiogenesis, mitogenesis, cellular differentiation, development, cell migration, and tissue injury repair (48). The family consists of 22 polypeptides divided according to their mechanisms of action into intracellular, paracrine, and hormone subgroups (49). FGF23 belongs to the latter group (along with FGF19 and -21) and has hormone-like characteristics imparted by a unique COOH-terminus that interacts with α-klotho, an obligate coreceptor required for FGF23 binding to FGF receptors (FGFRs) in target tissues (47, 124).

FGF23 is predominantly produced in osteocytes and osteoblasts, but it is also expressed in small quantities in the venous sinuses of the bone, ventrolateral thalamic nuclei of the brain, thymus, and lymph nodes (66, 69). The primary physiological actions of FGF23 are mediated through the FGFR-α-klotho complex in the renal tubules and include inhibition of proximal tubular phosphate reabsorption via suppression of the sodium phosphate transporter, and reductions in circulating levels of 1,25(OH)2D through inhibition of cytochrome P-450 (Cyp) 27b1 to decrease its production and stimulation of Cyp24A1 to increase its degradation (95). Other renal actions of FGF23 include stimulation of distal tubular sodium and calcium reabsorption (4, 5) and suppression of α-klotho and angiotensin-converting enzyme (ACE) 2 transcription in the kidney (10, 45). Although FGF23 can also suppress parathyroid hormone (PTH) secretion (10, 45), PTH concentrations are typically increased in most disease states characterized by elevated FGF23 levels and PTH, along with calcium (122, 123), and 1,25(OH)2D, is an important stimulator of FGF23 production (57).

Primary FGF23 overproduction is characterized by hypophosphatemia, urinary phosphate wasting, abnormally low 1,25(OH)2D level for the degree of hypophosphatemia, and rickets or osteomalacia, as described in disorders such as X-linked hypophosphatemic rickets, tumor-induced osteomalacia, and fibrous dysplasia (94, 107). On the other hand, FGF23 deficiency is characterized by tumoral calcinosis, caused by hyperphosphatemia and elevated circulating 1,25(OH)2D levels (11).

FGF23 gene expression in bone is regulated by multiple factors, including 1,25(OH)2D, calcium, PTH, paracrine FGFs, sympathetic nervous system, bone mineralization (75), lepton, estrogen, and glucocorticoids (74, 113), and factors affecting
oxidative stress/iron metabolism (108). The main physiological regulator of FGF23 production, however, appears to be 1,25(OH)_{2}D, which stimulates FGF23 and creates a negative feedback loop regulating 1,25(OH)_{2}D production (68). Exposure to high-phosphate diets also increases FGF23 concentrations in mice, and, although serum phosphate positively correlates with circulating FGF23 in ESRD, direct regulation of FGF23 production by extracellular phosphate has been difficult to demonstrate, and phosphate restriction has minimal effects to lower FGF23 levels (44, 113). The roles of the other factors that regulate FGF23 are less well defined. Excessive secretion of FGF23 by the diseased kidney has also been suggested in polycystic kidney disease, possibly reflecting end-organ resistance to FGF23 (104), but the exact mechanisms of increased FGF23 in CKD remain to be determined. FGF23 degradation also regulates its biological activity; intact FGF23 is cleaved by COOH-terminal fragments (67). Recent studies indicate that FGF23 is cleaved by a furin proprotein convertase into inactive NH_{2}- and COOH-terminal fragments (67). Whether COOH-terminal FGF23 has inhibitory effects on the actions of intact FGF23 is controversial (28).

**FGF23 in CKD: Mechanisms of Adverse Effects**

FGF23 levels increase early in the course of CKD (45), and elevated FGF23 levels are associated with significantly worse outcomes in both predialysis CKD and in ESRD (7, 24, 37, 46, 53). While it is possible that the above associations are merely another manifestation of CKD-mineral and bone disorder’s (MBD) global effect on various pathological processes, there are reasons to believe the FGF23’s physiological roles and its involvement in disease processes may extend beyond the realm of CKD-MBD.

**Left ventricular hypertrophy.** Left ventricular hypertrophy (LVH) is associated with increased risk of sudden cardiac death and progression to heart failure (62, 111). LVH has been described in 46–74% of patients with non-dialysis-dependent CKD and ESRD (26, 32, 61, 90) and is associated with increased mortality in both populations (25, 54, 65, 92, 128). In patients with kidney disease, LVH develops as a result of a confluence of various factors, which can be broadly categorized as afterload-dependent (arterial resistance), preload-dependent (volume overload, anemia, and arteriovenous fistulas in dialysis patients), and non-preload- or afterload-dependent (various humoral effects, including hyperparathyroidism, hyperphosphatemia, hyperhomocysteinemia, cytokine aberrations, hyperaldosteronism, and vitamin D deficiency) (1–3, 32, 39, 61, 72, 103, 110) factors. An additional factor in the latter group could be FGF23, which has been associated with the presence of LVH by echocardiography in the elderly (80) and in patients with CKD (22, 36, 106).

The mechanisms explaining the association of FGF23 with LVH have been a matter of debate. The administration of recombinant FGF23 to mice results in increased blood pressure and LVH (4), and mild degrees of LVH and hypertension are observed in patients with X-linked hypophosphatemia who have mutations leading to increased circulating FGF23 concentrations (84). Recent studies have suggested that FGF23 has a direct effect on cardiac myocytes independent of intermediates such as elevated blood pressure (22, 31). Such an effect is controversial because cardiac myocytes do not express α-klotho, which is necessary for FGF23 to exert its physiological effects (59). FGFR4 knockout mice, interestingly, are resistant to high-phosphate diet-induced LVH, and pharmacological amounts of FGF23 can activate FGFR4 signaling in vitro (31). FGFR4, however, is the physiological receptor for FGF19 and is involved in regulation of bile acid production by the liver (89). Moreover, polymorphisms of FGFR4 are associated with various cancers (77). The absence of an association with these phenotypes and FGF23 raises questions about the role of FGFR4 in mediating the pathological effects of FGF23. Moreover, the related hormonal FGF21 is also increased in CKD and is not associated with LVH (115). Most importantly, there are no studies showing that reductions of FGF23, either by its genetic ablation or treatment with blocking antibodies, prolong survival or prevent LVH in CKD. These findings raise the possibility that the association between FGF23 and adverse outcomes may not be mediated by direct action of FGFRs in the heart.

FGF23 could also be involved in LVH indirectly via humoral pathways through its classic FGFR/α-klotho mechanism, such as the activation of the RAAS (19), or it could be an innocent bystander produced as a result of pathways responsible for the development of pathological LVH, such as inflammation (vide infra).

**Renin-angiotensin-aldosterone system.** The RAAS plays a pivotal role in maintaining vascular tone, optimal salt and water homeostasis, and normal cardiac output in humans. Overactivity of the RAAS has been linked to a multitude of pathological processes, including left ventricular hypertrophy and heart failure. Both angiotensin II and aldosterone directly stimulate collagen synthesis, and angiotensin II inhibits matrix metalloproteinase 1 and hence causes myocardial fibrosis (12, 13, 112). Furthermore, the RAAS has also been suggested to trigger myocardial and vascular inflammation, leading to perivascular myocardial fibrosis and the development and progression of diastolic dysfunction (99), and LVH can be abolished by medications inhibiting the RAAS (14, 70), leading to improved clinical outcomes independent of blood pressure reduction (78, 93).

A relatively recent advance in the field of RAAS research has been the discovery of angiotensin-converting enzyme-2 (ACE2), a homolog of the ACE enzyme, which cleaves angiotensin II to generate angiotensin-(1–7) (Fig. 1) (20, 109). ACE2 is a functional component of the renin-angiotensin system (RAS) that counteracts the effects of angiotensin II and participates in blood pressure regulation (35) and normal heart (18) and endothelial (96, 118) function. States of ACE2 insufficiency have been linked to the development of atherosclerosis (71), congestive heart failure, cardiac hypertrophy and myocardial fibrosis (63, 87, 126), renal oxidative stress, inflammation and fibrosis (127), and the development of kidney disease (86, 88, 117). In experimental settings, ACE2 overexpression results in the amelioration of left ventricular remodeling and left ventricular dysfunction (125) and inhibits hypoxia-induced collagen production by fibroblasts (33), and ACE2 delivered to heart muscle by a lentiviral vector was shown to inhibit angiostatin II-induced cardiac hypertrophy and fibrosis in experimental animals (43). Furthermore, the product of ACE2 [angiotensin-(1–7)] was shown to have antibibiotic and antiinflammatory effects on cardiac fibroblasts (50) and prevent angio-
FGF23 represents an important physiological regulatory mechanism; hence, completely abolishing its effects may cause more harm than good. Neutralization of the effect of FGF23 with monoclonal FGF23 antibody in rats with CKD led to the development of hyperphosphatemia, aortic calcification, and increased mortality, even though the rats experienced resolution of secondary hyperparathyroidism, increased 1,25(OH)\(_2\)D levels, increased serum calcium, and normalization of bone structure and turnover rate (100). Other studies employing pharmacological inhibition of FGF23 did not show increased mortality in experimental animals (6, 116, 120), perhaps suggesting that the level of FGF23 blockade may be important, and the goal should be modulation, rather than abrogation, of its effects. Small clinical trials conducted in patients with CKD and ESRD suggested that prolonged dietary phosphate restriction combined with a phosphate binder may lower FGF23 levels (30, 44, 82, 97, 102).

Medications used in the treatment of CKD-MBD such as phosphate binders (29, 55, 85, 121) and cinacalcet hydrochloride (17, 23, 56, 114) have been shown to lower FGF23 levels (30, 44, 82, 97, 102).

Soluble klotho. Klotho protein exists as both membrane-bound klotho (functioning as a coreceptor for FGF23) and soluble (secreted) klotho, which has enzymatic activity (16, 59). Soluble klotho can be produced from increased gene transcription of the alternatively spliced secreted isoform or from ectodomain shedding of the membrane extracellular domain of full-length klotho (74). Effects of soluble klotho include FGF23-independent modulation of the sodium phosphate cotransporter (41), increased calcium reabsorption in the kidney (15), antiaging effects by suppression of tyrosine phosphorylation of insulin and insulin-like growth factor-I (IGF-I), leading to downregulation of IGF-I signaling (60), and inhibition of vascular calcification (64). Soluble klotho also protects the heart against cardiac hypertrophy and remodeling by downregulation of transient receptor potential cation channel, subfamily C, member 6 channels (119). Klotho-deficient mice have been shown to develop pathological cardiac hypertrophy and remodeling (40, 119). In CKD patients soluble α-klotho levels decrease as FGF23 levels increase (42, 91, 101). FGF23 may cause downregulation of soluble α-klotho (45), which could represent another indirect effect of FGF23 on the heart contributing to LVH and adverse outcomes in CKD.

Sodium metabolism. FGF23 has recently been shown to stimulate sodium retention in the distal renal tubules. In mouse models of FGF23 and α-klotho deficiency, absorption of sodium in the distal tubules was reduced, whereas mice injected with recombinant FGF23 or those with elevated endogenous FGF23 had increased sodium reabsorption (4). The effect of FGF23 on sodium reabsorption was mediated by an increase in the membrane abundance of sodium chloride cotransporter (NCC) in the distal tubules. Increased renal sodium retention and volume expansion led to hypertension and LVH. The NCC inhibitor chlorothiazide was shown to prevent this effect when it was given with recombinant FGF23 (4), suggesting yet another possible alternative explanation for FGF23’s effects on the heart.

Clinical Perspective

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Medications used in the treatment of CKD-MBD such as phosphate binders (29, 55, 85, 121) and cinacalcet hydrochloride (17, 23, 56, 114) have been shown to lower FGF23 levels in patients with CKD and ESRD. In a post hoc secondary analysis of the EVOLVE trial, patients treated with cinacalcet...
who achieved a reduction in FGFR23 showed a nominally significant improvement in cardiac outcomes (81). It remains unclear if these effects can translate to improved clinical outcomes. Given the complexity of the pathophysiology underlying the putative negative effects of FGFR23, more detailed exploration of the effects of FGFR23 modulation will be necessary before its widescale application in clinical practice.

Conclusions

FGFR23 has emerged as an important predictor of cardiovascular risk in patients with kidney disease. It is less clear whether or not FGFR23 should be regarded as a pathological causative factor for these outcomes. Although there is some evidence that FGFR23 could potentially affect the cardiovascular system through direct (“off-target”) effects and greater evidence for several indirect effects (such as its actions on soluble klotho and activation of the RAS), there is no evidence that inhibition of FGFR23 offers a survival advantage. Conversely, the association between FGFR23 and adverse outcomes could represent epiphenomena, or an innocent bystander of other factors in CKD that actually cause the adverse cardiovascular effects. More studies are needed to determine if lowering of FGFR23 by various measures could be used as a therapeutic intervention in CKD and ESRD.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

C.P.K. prepared figures; C.P.K. drafted manuscript; C.P.K. and L.D.Q. approved final version of manuscript; L.D.Q. edited and revised manuscript.

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