

Off-target effects and adverse outcomes of fibroblast growth factor 23 in chronic kidney disease

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ABSTRACT

Chronic kidney disease (CKD) patients have a high risk of death, especially cardiovascular death. High fibroblast growth factor 23 (FGF23) levels are independently associated with higher risk of death and cardiovascular events in these patients, as well as with a greater risk of severe inflammation. Chronic inflammation contributes to the progression of renal disease, the pathogenesis of cardiovascular disease, and mortality. Moreover, clinically, the CKD-associated defect in the immune system also has a relevant impact on morbidity and mortality. Recent findings showing that excess FGF23 may affect non-traditional, off-target organs independently of α Klotho give support to the potential clinical relevance of the adverse effects of FGF23 in CKD, and even in non-CKD patients. In this review, we provide an update on FGF23 and briefly discuss its future in clinical practice.

Keywords: Chronic kidney disease (CKD), fibroblast growth factor 23 (FGF23), heart hypertrophy, inflammation.

INTRODUCTION

Chronic kidney disease (CKD) is associated with an increased risk of death, cardiovascular events, and hospitalization.^{1,2} These adverse outcomes, however, cannot be fully explained by the presence of known, traditional risk factors.² Indeed, non-traditional risk factors appear to be particularly relevant to patients with CKD. These CKD-associated risks include oxidative stress, endothelial dysfunction, decreased hemoglobin levels, and progression of CKD itself.^{3,4} Other non-traditional risk factors of great interest are the presence of inflammation, which is frequently activated in CKD patients, and abnormalities in bone and mineral metabolism. The regulation of calcium and phosphate was only partially understood until the discovery of fibroblast growth factor 23 (FGF23) around the 2000s.⁵ Meanwhile, however, much attention has been paid to FGF23's multiple actions, which go beyond mineral metabolism and

place FGF23 as a potential clinical marker or mediator associated with adverse outcomes in CKD as well as non-CKD patients.

UNDERSTANDING FGF23'S ACTIONS AND REGULATIONS

FGF23 is one of 22 members of the FGF family of proteins that bind and activate alternatively spliced forms of four tyrosine kinase FGF receptors (FGFR1-4).⁶ Most FGFs are paracrine/autocrine factors or function as intracellular mediators, while FGF23 is an endocrine hormone. In fact, intracellular FGFs exert their functions in a receptor-independent manner while canonical FGFs have a heparin-binding site that is necessary for stable binding to the receptors and local signaling. Endocrine FGFs function as circulating factors owing to their reduced heparin/

heparan sulfate-binding affinity, which prevents them from being captured in the extracellular matrix. On the other hand, this reduced affinity also prevents direct interactions between the FGFs and their receptors.⁷ Endocrine FGFs overcome this handicap by using Klotho proteins instead of heparin-sulfate to enhance receptor binding. FGF23 uses α Klotho as a cofactor,⁸ a molecule that, other than serving as a co-receptor for FGF23, also circulates as an endocrine substance and exerts a multitude of important effects.⁵ Considering the ubiquity of FGFRs and the restricted expression of α Klotho, it seems that the presence of α Klotho governs whether a cell is an FGF23 target.⁹

The primary physiological function of FGF23 is to regulate phosphate homeostasis in the kidneys.¹⁰ FGF23 suppresses phosphate reabsorption by binding FGFR-Klotho complexes, which activates the mitogen-activated protein kinase (MAPK) signaling cascade leading to downregulation of sodium-dependent phosphate co-transport (NaPi-2a) in the proximal tubule.^{11,12} This effect is predominantly mediated by the activation of FGFR1 signaling mechanisms, but also to a lesser extent by activation of FGFR4.^{13,14} In addition, FGF23 suppresses renal synthesis of the active form of vitamin D, 1,25-dihydroxyvitamin D [1,25(OH)₂D] by inhibiting the expression of the activating enzyme 25-dihydroxyvitamin D-1 α -hydroxylase and, simultaneously, stimulating the catabolic enzyme D-24-hydroxylase.¹⁵ FGF23 also reduces the production of parathyroid hormone (PTH) by activating a Klotho-independent calcineurin-mediated signaling pathway in the parathyroid glands.¹⁶

In individuals with normal kidney function, increased circulating FGF23 levels lead to renal phosphate wasting and, subsequently, impaired bone mineralization. In the early stages of reduced renal function, a compensatory increase in FGF23 levels helps to maintain phosphate balance,¹⁷ but at the cost of calcitriol deficiency, secondary hyperparathyroidism, and perhaps Klotho deficiency.¹⁸ In advanced stages of CKD, these compensatory mechanisms become overwhelmed and blood FGF23 concentrations increase drastically over phosphate and PTH levels, eventually peaking during dialysis, thus resulting in a group of abnormalities referred to as CKD-mineral bone disease.¹⁹

FGF23 is predominately secreted by bones (osteocytes and osteoblasts) in response to several physiologic stimuli, including 1,25(OH)₂D, PTH, and

phosphate load.²⁰ However, since an increase in FGF23 production precedes the increase of PTH, and both FGF23 and PTH increase before hyperphosphatemia first appears,¹⁷ the primary stimulus for enhanced FGF23 production in early CKD is still unknown. It is noteworthy that several other factors have been described to directly or indirectly stimulate FGF23 production, including aldosterone, serum calcium, pro-inflammatory cytokines, and parameters of iron metabolism.²⁰ Since FGF23 is a small 32-kDa protein, a reduced glomerular filtration rate may concomitantly reduce renal elimination of FGF23 and contribute to increased blood levels of FGF23.^{13,21} In addition, under pathological circumstances, FGF23 may also be produced by extra-skeletal sources, including the kidneys, cells of the innate immune system (e.g. macrophages), cardiomyocytes, bone marrow, and erythroid cells.²¹⁻²⁵ It is not clear, however, to what extent extra-skeletal-derived FGF23 would add to high circulating FGF23 levels. However, increased local production may also be able to stimulate receptor-mediated signaling,²⁴ thereby acting in a paracrine manner.

Some of the FGF23 synthesized by osteocytes is processed by furin, a proteolytic enzyme, yielding N- and C-terminal protein fragments.^{26,27} This intracellular cleavage abrogates the phosphaturic actions of FGF23 and is considered the most important mechanism of FGF23 regulation.²⁸ The two forms, intact or full-length FGF23 (iFGF23; ~32 kDa) and the C-terminal fragment (cFGF23, ~14 kDa) can be detected in human plasma and exert different biological activities.^{29,30} Commercially available assays for cFGF23 detect both cFGF23 and iFGF23, while the iFGF23 assay detects only the intact hormone. C-terminal FGF23 is less abundant in CKD patients than in control subjects and is found at very low levels in dialysis patients.^{29,31} Excess FGF23 may be caused by a combination of increased FGF23 production and defective cleavage. Thus, with the progression of renal disease, it is conceivable that FGF23 production simply overcomes cleavage.²⁹ C-terminal FGF23 competes with iFGF23 for receptor binding, thereby antagonizing its phosphaturic activity.¹⁴ Indeed, some studies have shown a significant association of iFGF23 – but not cFGF23 – with parameters such as phosphate³² and insulin growth factor,³³ whereas others have demonstrated significant association of cFGF23 – but not iFGF23 – with, for example, iron³⁴ and phosphate.³³ Despite these discrepancies, higher iFGF23 and cFGF23 levels have both been consistently and independently associated with mortality risk and decline in renal function in patients with CKD.³⁵⁻³⁷

■ PATHOLOGICAL FGF23 LEVELS AND OFF-TARGET EFFECTS

Pathologically elevated levels of FGF23 may exert α Klotho-independent effects on non-traditional, off-target organs, such as the heart, liver, and cells of the immune system. These maladaptive effects may thereby contribute to cardiovascular disease, kidney disease progression, and mortality, not only in CKD patients, but also in non-CKD populations (Figure 1).

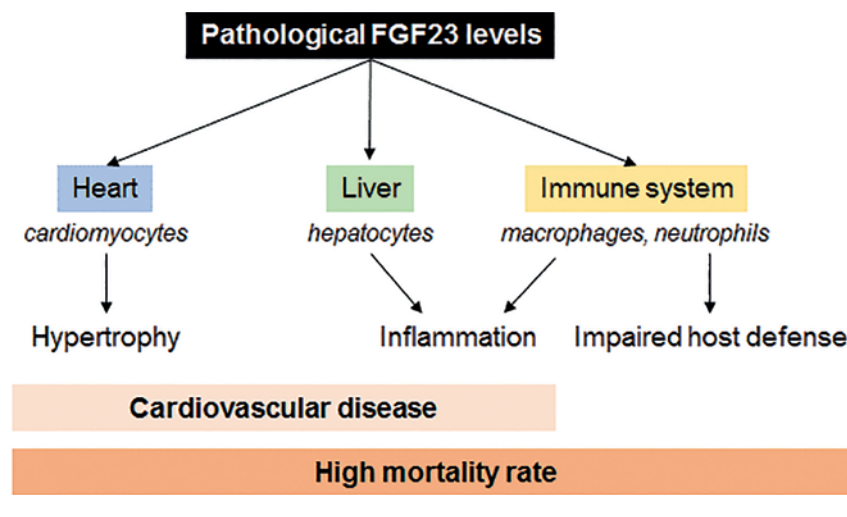
In a groundbreaking study, Faul et al. showed that FGF23 can cause hypertrophic growth of individual cardiac myocytes *in vitro* and induce left ventricular hypertrophy (LVH) in mice via FGFR-dependent and α Klotho-independent activation of the calcineurin-nuclear factor of activated T cells (NFAT) signaling cascade, which is known to mediate pathological cardiac hypertrophy in response to other pathogenic factors.^{38,39} Afterwards, Grabner et al. identified FGFR4 as the cardiac receptor for FGF23.⁴⁰ In proof-of-principle studies, blocking FGFR with a pan-FGFR inhibitor attenuated LVH in a rat model of CKD, despite sustained renal impairment and severe hypertension.^{38,41} These findings support FGFR activation as a potentially modifiable, blood pressure- and volume-independent molecular mechanism of LVH in CKD. However, although these studies demonstrated cardioprotective effects

of FGFR blockade in the absence of any changes in serum phosphate or tissue mineralization,⁴¹ the toxicity of pan-FGFR inhibitors precludes this approach in humans.^{15,42,43} Similarly, monoclonal antibodies that neutralize FGF23 seem to be of limited utility in CKD because complete abrogation of FGF23's effects can cause severe hyperphosphatemia, inducing diffuse vascular calcification and increased mortality.⁴³ The independent effects of FGF23 on the kidneys via FGFR1 and on the heart via FGFR4 hold promise for future clinical therapeutic and drug development, given that selective inhibition of FGFR4⁴⁴ would block the toxic effects of FGF23 in the heart without interfering with the essential effects of FGF23 in the regulation of phosphate homeostasis. Accordingly, Grabner et al. have shown that a specific FGFR4-blocking antibody is able not only to prevent the development of LVH but also to reverse established LVH in rats with CKD.^{40,45}

In fact, FGF23 exhibits off-target effects via FGFR4 not only on the myocardium, but also on the liver. Faul's group demonstrated through a series of *in vitro* and *in vivo* experiments that FGF23 binds to hepatic FGFR4 and, in an α Klotho-independent and NFAT-dependent manner, directly induces hepatic expression and secretion of inflammatory cytokines such as interleukin 6 and C-reactive protein (CRP).⁴⁶ As a proof-of-concept, pharmacologic FGFR4 blockade or calcineurin inhibition reduced hepatic and systemic CRP levels in CKD rats.⁴⁶

Figure 1

FGF23's off-target effects. Excess FGF23 exerts α klotho-independent effects on non-traditional, off-target organs of the cardiovascular and immune systems. These maladaptive effects result in left ventricular hypertrophy, inflammation, and impaired immune function, and support the clinical association of FGF23 with cardiovascular disease and high mortality rates.



Immune cells are also targets of FGF23. In macrophages, FGF23 exerts its functions via FGFR1. FGF23 secreted by pro-inflammatory, classically activated M1 macrophages acts in a paracrine manner to increase TNF α production in uncommitted M0 macrophages. Moreover, FGF23 increases the number of macrophages and inhibits the anti-inflammatory functions of alternatively activated M2 macrophages. As cardiovascular disease and renal failure progression in CKD are associated with macrophage tissue infiltration, it is possible that the production of FGF23 by macrophages contributes to cardiac and renal fibrosis.⁴⁷ Furthermore, the fact that FGF23 regulates important genes involved in inflammation and renal fibrosis (e.g. TGF- β and TNF- α) in murine models of CKD provides a possible mechanistic link between elevated FGF23 and pathways responsible for renal failure progression and cardiovascular diseases.⁴⁸

While on the one hand FGF23 may induce tissue inflammation, on the other hand, it impairs leukocyte/neutrophil activation and recruitment into the inflamed tissue, thereby impairing bacterial clearance and host defense. Mechanistically, FGF23 inhibits integrin activation in neutrophils by signaling through FGFR2 independently of α Klotho. As a proof-of-concept, FGF23 blockade with a neutralizing antibody in a murine model of CKD restored recruitment and host defense in these animals. In ex vivo analysis, distinct impairments in several steps of the leukocyte recruitment cascade were also observed in samples from patients with CKD. Incubation of healthy control leukocytes with FGF23 completely abrogated neutrophil slow rolling, while treatment of patients' leukocytes with an FGFR inhibitor was able to rescue chemokine-induced arrest in these cells.⁴⁹ Clinically, the CKD-associated defect in the immune system has a relevant impact on morbidity and mortality.⁵⁰

Interestingly, the relationship between FGF23 and inflammation seems to be bidirectional. FGF23 induces inflammation, which, in turn, directly or indirectly stimulates FGF23 production.^{46,51,52} The direct effects are mediated by pro-inflammatory cytokines while the indirect effects are associated with alterations of well-known regulators of FGF23 production, including calcium, phosphate, and vitamin D metabolism. However, the major indirect effect of inflammation on FGF23 production seems to be associated with the regulation of iron metabolism. It is well-known that iron levels are reduced in the systemic circulation during inflammatory processes and hypoferrremia leads to increased

FGF23 production.^{20,52} Iron deficiency stimulates fgf23 transcription in osteocytes but does not cause hypophosphatemia in wild-type mice because of increased intracellular degradation of FGF23, which results in elevation of circulating cFGF23 levels but normal iFGF23 levels.⁵³

Such a causal nexus between inflammation and FGF23 production may contribute to the 100- to 1000-fold increase in FGF23 levels frequently found in dialysis patients, as well as to adverse outcomes in CKD patients. Chronic inflammation contributes to the progression of renal disease, the pathogenesis of cardiovascular disease, and mortality.⁵⁴ Consistently, higher FGF23 levels are independently associated with a greater risk of manifesting severe inflammation in patients with CKD,^{46,55,56} as well as with higher risks of death and cardiovascular events in multiple CKD and non-CKD populations.^{35,57-59}

■ FGF23 IN CLINICAL PRACTICE

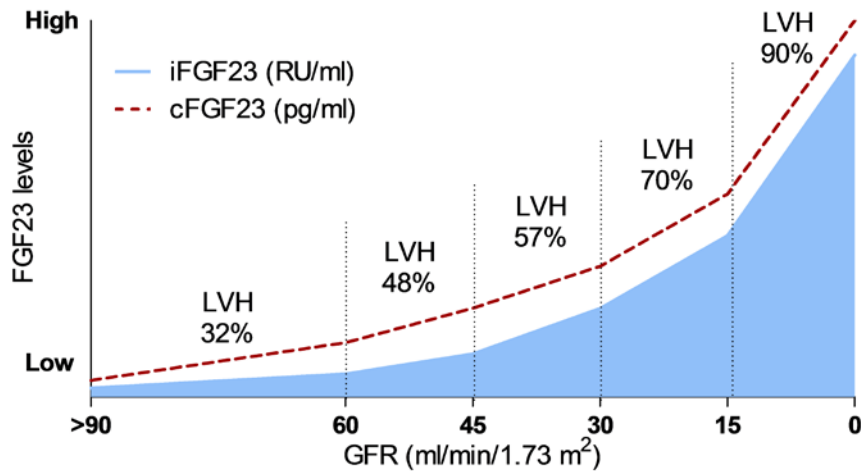
As a potential underlying mechanism for the increased risk of cardiovascular disease and death, epidemiological studies have consistently identified an independent association between elevated FGF23 levels and greater left ventricular mass as well as higher prevalence and incidence of LVH in a variety of populations (Figure 2).^{60,61} In support of the potential clinical relevance of adverse effects of FGF23 on the heart, high FGF23 levels are also independently predictive of higher risk of congestive heart failure events in both CKD and non-CKD patients.^{62,63} Given the strong relationships between LVH, congestive heart failure, and death,^{35,57,58,60-63} it is intriguing to speculate that FGF23 may contribute causally to adverse cardiovascular outcomes in CKD.

FGF23 is elevated in the vast majority of CKD patients. A prospective case-cohort study as part of the Chronic Renal Insufficiency Cohort (CRIC) Study that applied serial FGF23 measurements in patients with moderate to severe CKD showed that FGF23 levels were elevated but remained stable over time in the majority of the studied patients, while smaller subpopulations presented with slowly or rapidly rising FGF23 levels and a dramatically higher risk of death (4.49- and 15.23-fold higher, respectively).⁶⁴

These observations and the mechanistic studies described above raise the question of whether an

Figure 2

Schematic showing changes in plasma FGF23 levels and the prevalence of left ventricular hypertrophy (LVH) during progression of chronic kidney disease. Compiled using data source from Larsson et al.³⁷ and Park et al.¹ iFGF23, intact FGF23; cFGF23, C-terminal FGF23.



intervention to reduce FGF23 levels or inhibit its activity would improve outcomes in patients with CKD. The findings from preclinical studies with various therapies that target FGF23, including FGFR inhibitors, calcitriol/vitamin D,⁶⁵ and anti-FGF23 neutralizing antibodies are supportive,⁴⁹ but the effects of FGF23^{38,41,45} antagonism in CKD are still controversial.⁴³

In humans, cinacalcet therapy lowers circulating FGF23 levels.⁶⁶⁻⁶⁸ In a secondary analysis of the Evaluation of Cinacalcet HCl Therapy to Lower Cardiovascular Events (EVOLVE) trial, Moe et al. showed that about 64% of the patients randomized to cinacalcet presented at least a 30% decrease in FGF23 levels; moreover, this decrease was associated with lower rates of cardiovascular events, including sudden cardiac death, and death. Whether or not these associations are a direct or indirect effect of cinacalcet remains to be clarified. However, these effects seem to be independent of demographic factors, comorbidities, and the cumulative dose of vitamin D sterols. In addition, even though a more pronounced reduction in FGF23 levels was observed in patients with blood levels of calcium, phosphate, and PTH that fell within the recommended range for clinical practice, cinacalcet lowers FGF23 even after accounting for changes in these parameters.^{67,68} In the placebo group, only 28% of the patients achieved such a reduction in FGF23 levels, and this reduction

was not associated with a reduction in cardiovascular end-points.

While on the one hand cinacalcet is able to reduce FGF23, on the other hand, calcimimetics are not routinely available for predialysis patients with CKD. Dietary restriction of phosphate and phosphate binders appears to be a reasonable option to target FGF23. Although controversial, reports on the FGF23-lowering effect of the various types of phosphate-binding therapies favor the use of sevelamer, while calcium-based phosphate binders may not reduce, and may even increase, FGF23 levels.⁶⁹⁻⁷¹

Another possibility is iron therapy. In CKD patients with iron deficiency, switching the phosphate binder sevelamer to an oral iron supplement reduced circulating FGF23 levels independently of phosphate and vitamin D (other phosphate binders and cinacalcet were not changed).⁷² In addition, Wolf et al. have shown that intravenous iron in women with iron deficiency anemia lowers FGF23, whereas carbohydrate moieties in certain iron preparations transiently increase FGF23 by inhibiting its degradation.⁵³ Here, it is worth noting that, concerning iron metabolism/supplementation, there are important differences regarding iFGF23 and cFGF23 levels and associations.^{34,53}

Along these lines, even though FGF23 holds potential as a therapeutic target, data on the extent of its

effects, the potency of the compounds to achieve significant reductions in FGF23 levels in different degrees of renal dysfunction, and the presence of a dose-effect relationship are still missing. Mechanistic aspects of how FGF23 levels increase in different stages of CKD and how these possible therapies lower FGF23 should also be explored in more depth. Furthermore, it is possible that important biological signals and clinical associations are being missed or underestimated owing to differences in iFGF23 and cFGF23 measurements, which strongly indicates the need for the establishment and standardization of reliable assays.

All this knowledge is, however, mandatory to determine whether reductions in FGF23 levels are directly responsible for improved clinical outcomes in CKD patients or if the observed benefits simply rely on better management of modifiers of cardiovascular and/or kidney disease in these patients. Additional specific strategies for controlling FGF23 should be considered and adequately designed clinical trials are still needed to validate FGF23 as a clinical target.

CONCLUSION

FGF23 is an endocrine hormone that uses α Klotho as a cofactor to exert its physiological effects on traditional, on-target organs, such as the kidney and parathyroid glands, thereby regulating phosphate homeostasis and mineral metabolism. In CKD patients, excess FGF23 also exerts α klotho-independent effects on non-traditional, off-target organs, such as the heart, liver, and cells of the immune system. These maladaptive effects result in LVH, inflammation, kidney disease progression, and impaired immune function. Clinically, FGF23 has relevant associations with cardiovascular morbidity and mortality in CKD. Phosphate binders, calcimimetics, and iron therapy have been proposed as possible therapeutic approaches. However, solid evidence regarding the effects of lowering FGF23 on improving outcomes is still missing. In addition, many other open questions should be addressed before FGF23 can be validated as a true clinical target in CKD. FGF23 was identified less than 20 years ago and the next few years are likely to definitively reveal great progress in our understanding of FGF23 in both health and disease.

Disclosure of potential conflicts of interest: none declared.

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