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Commentary

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Kidney to bone via bedside to bench...and back?

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The rapid rise in circulating fibroblast growth factor 23 (FGF23) associated with kidney injury results in calcitriol deficiency, altered calcium homeostasis, and secondary hyperparathyroidism, and may contribute to cardiovascular complications and death. However, the mechanisms of increased FGF23 in states of kidney injury remain unclear. In this issue of the *JCI*, Simic et al. screened plasma taken from the renal vein of patients undergoing cardiac catheterization and identified glycerol-3-phosphate (G-3-P) as the most significant correlate of simultaneous arterial FGF23 levels. When G-3-P was administered to mice, FGF23 production increased in bone. In a series of elegant mouse studies, the authors discovered a pathway linking increased G-3-P to increased FGF23 via increases in lysophosphatidic acid (LPA), which activates the LPA receptor 1 in FGF23-secreting cells in the bone and bone marrow. Although the authors present human data that broadly support the results from the mouse models, further research is needed to determine whether targeting the G-3-P/FGF23 pathway has the potential to modify FGF23-related complications in the clinic.

FGF23 in kidney disease

Elevation of circulating fibroblast growth factor 23 (FGF23) levels is an early, progressive, and pervasive complication of chronic kidney disease (CKD). Rising FGF23 levels in CKD promotes the renal phosphate excretion that helps delay the onset of hyperphosphatemia but incurs substantial compensatory costs, including calcitriol deficiency, altered calcium homeostasis, and secondary hyperparathyroidism (1, 2). More ominously, secondary FGF23 excess in patients with CKD may contribute causally to their exceptionally high risks of pathological left ventricular remodeling, atrial fibrillation, heart failure, anemia, inflammation, infection, and death (3–9). Although much has been learned in recent years about the effects of FGF23, a pressing question in the field remains unanswered: What causes the initial elevation of FGF23 in early CKD that precedes the onset of hyperphosphatemia, other alterations in mineral metabolism, and even overt reductions in the glomerular filtration rate?

FGF23 levels also increase dramatically and almost immediately after the onset of different forms of acute kidney injury (AKI) (10, 11). This exquisite sensitivity of FGF23 to kidney injury raises the possibility that the injured kidney releases factors that directly stimulate FGF23 production. In an elegant report in this issue of the *JCI*, Simic et al. leverage this premise to generate intriguing findings about a kidney/bone axis that regulates FGF23 production (12). Using proteomics and metabolomics as agnostic discovery platforms, the authors screened renal venous blood from patients undergoing cardiac catheterization to identify proteins and metabolites that correlate with simultaneous arterial FGF23 levels. Increased renal venous glycerol-3-phosphate (G-3-P) was the only one of more than 5000 candidate molecules that achieved proteome-wide or metabolome-wide significance ($P < 9.6 \times 10^{-6}$). The ratio of its renal venous to arterial concentrations confirmed that the kidney was producing G-3-P and adding it to the circulation.

Animal and human models

Armed with G-3-P as a potential kidney-derived FGF23 secretagogue, Simic and colleagues shifted their translational attention to the laboratory. Exogenous administration of G-3-P to WT mice stimulated transcription and translation of FGF23 in bone and bone marrow, and the resulting increase in FGF23 induced phosphaturia, lowered serum phosphate, and suppressed calcitriol levels. These effects, which are hallmarks of FGF23 excess in the setting of normal kidney function, support the physiological relevance of G-3-P stimulation of FGF23 production. In further experiments, the authors demonstrated that circulating G-3-P is converted to lysophosphatidic acid (LPA) by G-3-P acyltransferase isoform 2 (GPAT2) locally in bone and bone marrow, where LPA binds the LPA receptor 1 (LPAR1) on FGF23-secreting cells to stimulate FGF23 production. This mechanistic pathway was confirmed by global pharmacologic inhibition of GPAT, specific shRNA knockdown of GPAT2 in cultured osteoblasts, and genetic deletion of *Lpar1* in mice, each of which prevented the G-3-P–mediated increases in FGF23 that were observed in the controls (12).

To investigate the pathophysiological relevance of the G-3-P/GPAT2/LPA/LPAR1/FGF23 axis in states of kidney injury, the authors turned to animal and human models of AKI. In response to bilateral kidney ischemia-reperfusion injury in mice, kidney tissue and plasma levels of G-3-P rose, consistent with the known effects of ischemia to shift energy metabolism toward increased glycolytic flux. Recapitulating the results from animals without kidney injury, G-3-P stimulated FGF23 production in the ani-

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mals with AKI via LPA and LPAR1 (12). It would have been interesting if the authors had repeated the experiment with unilateral ischemia-reperfusion injury to test whether unilateral increases in renal G-3-P production are sufficient to stimulate FGF23 expression in a situation in which the functioning contralateral kidney would avert AKI.

In a parallel case-control study, G-3-P levels rose more in 13 patients who developed AKI after cardiac surgery than in the 13 control patients who did not develop AKI. Among the patients with AKI, G-3-P correlated, albeit modestly, with concomitant FGF23 levels. Although broadly supportive of the animal data, the small clinical study has major limitations that preclude detailed inference to human AKI, including small sample size, differences between patients and control subjects in baseline kidney function and FGF23 levels, and perfunctory correlation analyses with no adjustment for these or other potential confounders (12). Translation of the pathophysiological link between kidney ischemia, excess renal production of G-3-P, and increased FGF23 secretion back to human AKI will require larger, appropriately analyzed studies.

G-3-P is an intermediary metabolite at the hub of glycolysis, lipogenesis, and oxidative phosphorylation. Simic et al. add to the growing body of evidence that suggests that G-3-P regulates many cellular functions beyond its role as a substrate for glycolysis and fatty acid metabolism, including insulin secretion, gluconeogenesis, insulin sensitivity, fat synthesis and storage, cell proliferation and survival, and, now, production of FGF23 (12, 13). Likewise, G-3-P can now be added to the list of FGF23-regulating metabolic factors including insulin, insulin-like growth factor 1, and AMP-activated protein kinase, which inhibit FGF23 expression (14, 15), and adipokines such as leptin and plasminogen activator inhibitor 1, which stimulate FGF23 (16, 17). These multiple avenues of crosstalk between FGF23 and metabolism raise the question of whether FGF23 itself exerts currently undiscovered metabolic effects as part of a classic negative endocrine feedback loop. Although Simic et al. reported that FGF23 failed to suppress G-3-P expression in isolated proximal tubule cells, this does not preclude the possibility that other kidney cells could manifest a G-3-P response to FGF23, or that FGF23 could directly exert metabolic effects in the kidney and other organs through intermediaries other than G-3-P (12). Future studies should use metabolomics and other tools to investigate the potential effects of FGF23 on energy production and utilization in a variety of end organs.

Cellular origins of FGF23

Simic et al. shed new light on the cellular origins of FGF23, which is a topic of significant controversy in the field (12). In their initial patient study, renal vein FGF23 itself did not emerge as a correlate of arterial FGF23, despite its presence on the aptamer-based SOMAscan proteomics platform (12, 18). This finding argues against ectopic renal production as a significant source of FGF23, at least in this older population with some degree of kidney dysfunction and high suspicion of cardiovascular disease. Larger clinical research studies should investigate G-3-P and FGF23 in more diverse populations across the full spectrum of kidney function, including patients with and without cardiovascular disease.

Besides the kidney, G-3-P administration also did not increase *Fgf23* mRNA expression in multiple other organs in which ectopic FGF23 production had been invoked, including skeletal muscle, liver, heart, and lung. Only bone and nonhematopoietic bone marrow stromal cells showed increased *Fgf23* mRNA expression following G-3-P treatment, the latter confirmed in mice with osteocyte-specific deletion of *Fgf23* and in bone marrow cells that were isolated, sorted, and treated ex vivo with G-3-P (12). It is important to note that these findings do not exclude the possibility that FGF23 is produced outside of bone in response to other stimuli, such as calcitriol, a high-phosphate diet, inflammation, iron deficiency, and CKD.

Magnitude of G-3-P effect

One gap in the Simic paper (12) is the lack of benchmarking of the G-3-P effect on FGF23 expression relative to other established stimuli. Previous studies reported that iron deficiency, IL-1β, calcitriol, high dietary phosphate load, and CKD increased *Fgf23* mRNA expression by approximately 2-fold to 12-fold (19–22). Although different protocols render side-to-side, between-study comparisons less conclusive than direct within-study comparisons, the 4-fold to 5-fold increases in *Fgf23* mRNA expression induced by G-3-P in bone and bone marrow fall within the range of effect of other established stimuli that are known to be biologically and clinically relevant.

It is especially interesting that G-3-P stimulated *Fgf23* transcription and increased secretion of full-length FGF23 into the circulation. This contrasts the effects of inflammation and iron deficiency that simultaneously increase *FGF23* transcription and posttranslation cleavage of FGF23 so that concentrations of C-terminal FGF23 fragments, but not full-length FGF23, rise in the circulation (23). This important finding suggests currently unknown effects of G-3-P on the multistep regulation of FGF23 in osteocytes and bone marrow stromal cells that justify further research. Reminiscent of the effects of calcitriol, which also raises full-length FGF23, it is noteworthy that the FGF23-stimulating effects of the G-3-P/GPAT2/LPA/LPAR1 pathway ultimately require calcitriol-VDR activation as a permissive cofactor. Further investigation that contrasts the effects of the G-3-P and calcitriol axes versus the iron and inflammation axes could shed important new light on the coordinated regulation of FGF23 production and cleavage.

Clinical relevance

The exciting findings of Simic et al. (12) raise a number of important clinical questions. Is G-3-P–mediated FGF23 elevation limited to AKI or also relevant to CKD, in which there is far more evidence that FGF23 contributes to poor clinical outcomes? Likewise, does G-3-P contribute causally to FGF23 elevation in heart failure, which is the other most common cause of secondary FGF23 excess besides CKD? It is currently unknown whether FGF23 elevation in heart failure is driven by concomitant reductions in kidney function or is intrinsic to heart failure itself. Given that heart failure causes diffuse end-organ ischemia, perhaps elevated G-3-P, from the kidney or other ischemic organs, is a fundamental mechanism of secondary FGF23 excess in

heart failure. Finally, will manipulating the G-3-P/GPAT2/LPA/LPAR1 axis have therapeutic benefits in AKI, CKD, heart failure, or related conditions? Complete abrogation of FGF23 using neutralizing antibodies causes severe hyperphosphatemia, metastatic calcification, and accelerated mortality in rodent models of CKD (24), suggesting that targeting the G-3-P/GPAT2/LPA/LPAR1 axis to similarly lower FGF23 in states of secondary FGF23 excess may also be unsafe. Selective targeting of pathogenic FGF receptors that avoids hyperphosphatemia might be expected to mitigate adverse effects of targeting secondary FGF23 excess, but has yet to be tested (25). All of these questions should be the subject of future studies that determine whether the impressive data presented by Simic et al. (12) translate from the laboratory to clinical relevance and, ultimately, clinical benefits for patients.

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