Inflammation and functional iron deficiency regulate fibroblast growth factor 23 production

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Circulating levels of fibroblast growth factor 23 (FGF23) are elevated in patients with chronic kidney disease (CKD), but the mechanisms are poorly understood. Here we tested whether inflammation and iron deficiency regulate FGF23. In wild-type mice, acute inflammation induced by single injections of heat-killed *Brucella abortus* or interleukin-1β (IL-1β) decreased serum iron within 6 h, and was accompanied by significant increases in osseous *Fgf23* mRNA expression and serum levels of C-terminal FGF23, but no changes in intact FGF23. Chronic inflammation induced by repeated bacteria or IL-1β injections decreased serum iron, increased osseous *Fgf23* mRNA, and serum C-terminal FGF23, but modestly increased biologically active, intact FGF23 serum levels. Chronic iron deficiency mimicked chronic inflammation. Increased osseous FGF23 cleavage rather than a prolonged half-life of C-terminal FGF23 fragments accounted for the elevated C-terminal FGF23 but near-normal intact FGF23 levels in inflammation. IL-1β injection increased *Fgf23* mRNA and C-terminal FGF23 levels similarly in wild-type and Col4a3^{ko} mice with CKD but markedly increased intact FGF23 levels only in the CKD mice. Inflammation increased *Fgf23* transcription by activating Hif1α signaling. Thus, inflammation and iron deficiency stimulate FGF23 production. Simultaneous upregulation of FGF23 cleavage in osteocytes maintains near-normal levels of biologically active, intact circulating FGF23, whereas downregulated or impaired FGF23 cleavage may contribute to elevated intact serum FGF23 in CKD.

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Fibroblast growth factor 23 (FGF23) is a bone-derived hormone that is essential for maintaining normal phosphate and vitamin D homeostasis.[1] FGF23 inhibits renal phosphate reabsorption by downregulating expression of the sodium phosphate cotransporters NPT2a and NPT2c and decreases circulating 1,25-dihydroxyvitamin D_3 levels by inhibiting renal expression of CYP27B1 (1-α-hydroxylase) and stimulating expression of CYP24A1 (24-hydroxylase).[2,3] FGF23 levels rise progressively in chronic kidney disease (CKD), and higher levels are strongly associated with increased risks of CKD progression, cardiovascular events, and mortality.[4–9] Experimental data suggesting that FGF23 may contribute causally to certain cardiovascular complications of CKD[10] emphasize the therapeutic importance of defining the molecular mechanisms that stimulate FGF23 production beginning early in the course of CKD.

FGF23 is regulated by an incompletely understood interplay between local bone factors that modulate turnover and mineralization[11–13] and systemic factors that control mineral metabolism.[14,15] High levels of parathyroid hormone (PTH), 1,25-dihydroxyvitamin D_3, phosphate, and calcium stimulate FGF23 production[14–16] but cannot adequately explain the increases in FGF23 levels in early CKD. Indeed, FGF23 elevations usually antedate hyperparathyroidism and hyperphosphatemia,[17] and CKD is characterized by low levels of 1,25 dihydroxyvitamin D_3 and calcium rather than high levels.

Iron is a newly described regulator of FGF23 production. Animal and human studies demonstrate that iron deficiency stimulates *Fgf23* transcription, which is counterbalanced by commensurately increased cleavage of newly synthesized FGF23 within healthy osteocytes.[18] This results in high circulating concentrations of FGF23 fragments that can be detected with C-terminal FGF23 (cFGF23) assays but normal serum phosphate levels because the levels of intact, biologically active FGF23, measured by intact FGF23 (iFGF23) assays, remain normal.

As CKD progresses, total FGF23 levels rise and the proportion of circulating cFGF23 fragments relative to intact hormone decreases, perhaps because FGF23 cleavage is

impaired in CKD.[19,20] In the setting of reduced cleavage of newly synthesized FGF23, factors that activate FGF23 transcription, such as iron deficiency, would be expected to increase circulating levels of intact hormone, as occurs in autosomal dominant hypophosphatemic rickets, which is the prototype disease of impaired FGF23 cleavage.[18] 'Functional' iron deficiency is a consequence of chronic inflammation in which iron sequestration in the reticuloendothelial system decreases the amount of iron available for erythropoiesis, despite adequate total body iron stores. Given the high prevalence of functional iron deficiency in CKD,[21] and the association between chronic inflammation and elevated FGF23 levels observed in cross-sectional studies of CKD patients,[22,23] we hypothesized that inflammation and functional iron deficiency might be novel, interrelated mechanisms of increased FGF23 production that may contribute to elevated FGF23 levels in CKD.

RESULTS

Iron deficiency regulates FGF23

We fed 3-week-old wild-type mice a low iron diet for 3 weeks. By 6 weeks of age, the mice developed iron deficiency, marked by decreased serum iron and ferritin levels ($P<0.05$) compared with mice fed the control diet (Figure 1a and b). Iron deficiency resulted in significantly increased serum cFGF23 levels (Figure 1c) and significantly increased osseous expression of *Fgf23* mRNA (Figure 1e) and protein (Figure 1f), but *Fgf23* mRNA expression was not induced in the kidney. As shown in the previous studies of young mice,[24] iron deficiency also significantly increased iFGF23 (Figure 1d). Consistent with increased iFGF23, renal expression of *Npt2c* and *Cyp27b1* decreased significantly, and *Cyp24a1* expression (Figure 1g) increased significantly resulting in lower $1,25(OH)_2D$ levels (Table 1). Urinary phosphate excretion tended to increase, although this trend did not reach significance, and serum phosphate levels were unchanged. Bone expression of both the osteoblastic marker *Sp7*, which encodes osterix, and the osteoclastic marker *Ctsk*, which encodes cathepsin K, decreased significantly (Figure 1g), consistent with decreased bone turnover.

Acute inflammation induces functional iron deficiency and increases FGF23 production

To determine whether inflammation regulates FGF23, we studied the *Brucella abortus* (BA) mouse model[25] that develops acute and chronic inflammation starting at 3 h and lasting through 14 days after a single intraperitoneal injection of heat-killed bacteria.[26] Six hours after injection, BA significantly decreased serum iron (Figure 2a) and increased ferritin levels compared with controls (Figure 2b), consistent with the acute phase inflammatory reaction.[27,28] Serum cFGF23 levels rose significantly compared with controls, concomitant with a significant eightfold elevation in bone expression of *Fgf23* mRNA, but iFGF23 levels were unchanged (Figure 2c and e). Analysis of femoral bone protein extracts demonstrated evidence of increased FGF23 production and cleavage compared with control (Figure 2f). Consistent with the lack of increase in iFGF23 levels, there were no differences in renal mRNA expression of *Cyp24a1*, *Npt2a*, or *Npt2c* (Figure 2g). As shown previously,[29–31] acute inflammation resulted in significantly increased PTH levels, *Cyp27b1* expression (Figure 2g and Table 1), and bone mRNA expression of *Sp7* and *Ctsk* (Figure 2g), consistent with increased bone turnover.[32]

We also investigated FGF23 regulation in response to interleukin-1β (IL-1β) injection, which is an intensely proinflammatory cytokine[33] and an established cause of inflammation-induced, functional iron deficiency.[34] Six hours after a single injection of IL-1β, serum iron decreased and ferritin increased significantly (Figure 2h–i). Longitudinal evaluation during 6 h following IL-1β injection demonstrated that serum cFGF23 progressively increased, but iFGF23 was unchanged (Figure 2j–k). Bone expression of *Fgf23* mRNA and protein increased significantly (Figure 2l–m). Renal *Fgf23* mRNA expression also increased significantly in response to IL-1β, but the effect was modest compared with the marked increase in bone (Figure 2l).

IL-1β significantly increased renal expression of *Cyp27b1* and *Npt2a* (Figure 2n), but the change in serum phosphate levels did not reach significance, likely because of increased renal phosphate excretion induced by significantly higher PTH levels (Table 1). Similar to the BA model, IL-1β significantly increased osseous mRNA expression of markers of bone remodeling, including *Sp7*, *Ctsk*, and *Bglap*, which encodes osteocalcin (Figure 2n).

Functional iron deficiency in the absence of inflammation increases FGF23 production

Hepcidin is an important molecular mediator of functional iron deficiency. Hepcidin is produced by the liver in response to inflammation, and it increases iron sequestration and decreases gastrointestinal iron absorption.[35,36] To induce a state of functional iron deficiency without superimposed inflammation, we administered 1 μg/g exogenous hepcidin to wild-type mice. Six hours after hepcidin injection, serum cFGF23 levels and bone expression of *Fgf23* mRNA increased significantly, whereas iFGF23 levels remained unchanged (Supplementary Figure S1 online). These data suggest that functional iron deficiency alone can stimulate FGF23 production and preferentially increase circulating concentrations of cFGF23 fragments that are detected as elevated cFGF23 levels.

Chronic inflammation increases circulating levels of biologically active FGF23

Twelve days after the single BA injection, iron levels remained significantly decreased compared with controls (Figure 3a), and serum ferritin remained significantly increased (Figure 3b). At day 12, cFGF23 levels remained markedly increased compared with control, and iFGF23 levels were modestly increased (Figure 3c and d), although the result was of borderline statistical significance ($P=0.051$). Femoral