Reciprocal Control of 1,25-Dihydroxyvitamin D and FGF23 Formation Involving the FGF23/Klotho System

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Fibroblast growth factor 23 (FGF23) is a circulating hormone that is synthesized by osteocytes and osteoblasts. This glycosylated peptide controls phosphate balance by modulating urinary phosphate excretion and indirectly intestinal phosphate absorption by reducing expression of the renal and intestinal sodium phosphate transporters. In a feedback loop, 1,25-dihydroxyvitamin D and phosphate intake control FGF23 production. FGF23 is inactivated by cleavage by a still unidentified enzyme. FGF23 cleavage occurs within cells and probably in the circulation. Klotho, a protein expressed at the cell surface of few organs, forms complexes with FGF receptors, which increases their affinity for FGF23. Klotho is also released into the plasma and urine by an enzymatic cleavage. FGF23 plays a central role in vitamin D metabolism: It inhibits calcitriol synthesis in the kidney and stimulates the catabolism of active vitamin D sterols. In turn, calcitriol stimulates FGF23 and Klotho expression. In chronic kidney diseases, FGF23 concentration increases as GFR declines, whereas Klotho tissue expression decreases. The modifications of FGF23 and Klotho expression are probably involved in the genesis of hyperparathyroidism and the resistance to vitamin D receptor (VDR) activation in chronic kidney disease. Low vitamin D, high FGF23 concentrations, and defects in VDR activation are associated with similar risks, which evoke the possibility that potential FGF23 toxicity might be partly mediated by FGF23-induced decrease in calcitriol or 25-hydroxyvitamin D. Conversely, VDR activators could be used to modulate Klotho or FGF23 expression.


Activation of vitamin D receptors (VDR) by 1,25-dihydroxyvitamin D (calcitriol) or pharmacologic VDR agonists is important for the control of phosphate and calcium homeostasis and bone remodeling but could also have beneficial effects by reducing the risk for development of cardiovascular morbidity and mortality, diabetes, autoimmune diseases, and cancer. Microarray assay studies suggested that more than 150 human genes are directly regulated by calcitriol through vitamin D–responsive elements (VDREs) in their promoters (1). Circulating calcitriol is mainly synthesized by the renal proximal tubule. Calcitriol is also produced as an autocrine factor in many tissues. Until recently, parathyroid hormone (PTH) was the main identified peptide known to control calcitriol synthesis in the kidney under physiologic conditions. Ten years ago, genetics analysis of patients with autosomal dominant hypophosphatemic rickets and analysis of tumor-induced osteomalacia resulted in the discovery of fibroblast growth factor 23 (FGF23) (2,3). The sequence of this peptide made it the 23rd member of the FGF family. Rapidly, data accumulated on FGF23 and showed that FGF23 is a hormone that controls serum phosphate concentration and calcitriol metabolism. Klotho, which had been identified a few years earlier, was then recognized as a co-factor that is mandatory for FGF23 action. The couple FGF23-Klotho in the presence of FGF receptors seems to be involved in the pathophysiology of various disorders and associated with adverse or beneficial clinical outcomes. It is interesting that the same outcomes are associated with treatments by active forms of vitamin D derivatives or with the circulating level of 25-hydroxyvitamin D [25(OH)D]. This suggests that the manipulation of the FGF23-Klotho axis through VDR activation or the control of vitamin D metabolism via FGF23-Klotho could be used as therapeutic targets.

Vitamin D Metabolism

Vitamin D synthesis in humans begins in the epidermis, where vitamin D₃ is produced from a UVB-mediated conversion of 7-dehydrocholesterol (Figure 1). Vitamin D₃ is then converted to 25(OH)D₃ in the liver, most likely by the high-affinity cytochrome enzyme CYP2R1. Homozygous mutation of CYP2R1 gene is associated with low 25(OH)D₃ circulating concentrations (4). This step is not regulated by hormones. 25(OH)D₃ is released into the plasma and circulates bound mainly to vitamin D–binding protein (DBP). The affinity of DBP for 25(OH)D₃ is very high, and DBP is present...
in large molar excess in the plasma. Consequently, almost all 25(OH)D3 is bound to this carrier. The plasma concentration of 25(OH)D3 is considered to be a good indicator of vitamin D stores. 25(OH)D3 functions as a prohormone that cannot activate VDR. The 25(OH)D3-DBP complex is captured by renal proximal tubular cells through binding to megalin and cubilin (5). Then 25(OH)D3 is converted to calcitriol by the cytochrome enzyme CYP27B1, also called 25(OH)D-1α-hydroxylase. In the kidney, FGF23 increases the expression of the 24-hydroxylase an enzyme that inactivates calcitriol.

Figure 1. Vitamin D metabolism. Vitamin D3 is produced in the skin from a UVB-mediated conversion of 7-dehydrocholesterol. In the liver, vitamin D is converted to 25(OH)D3, which is released in blood. In the kidney, 25(OH)D3 is converted to 1,25-dihydroxyvitamin D [1,25(OH)2D]. Two hormones regulate this step: PTH and FGF23, a hormone that is synthesized by osteocytes and osteoblasts. In the kidney, PTH stimulates whereas FGF23 represses 25(OH)D-1α-hydroxylation. In the kidney, FGF23 increases the expression of the 24-hydroxylase an enzyme that inactivates calcitriol.

FGF23, Klotho, and Phosphate Homeostasis

FGF23 is a 251 amino acid–glycosylated peptide that was initially identified in patients with autosomal dominant hypophosphatemia or oncogenic osteomalacia. This peptide is present in the plasma of healthy individuals. In blood, FGF23 is present as an intact peptide, which is its active form. In disorders that alter FGF23 glycosylation, intact FGF23 is cleaved within cells, which results in the release of fragments (7–9). Intact circulating FGF23 concentration is low, whereas plasma FGF23 fragment concentration is increased; hence, patients develop hyperphosphatemia and tumoral calcinosis. The enzyme that cleaves FGF23 between amino acids 176 and 179 in vivo is unknown. The main source of FGF23 production in vivo is the osteocyte and osteoblast (10–12). Various bone disorders are associated with FGF23 mRNA overexpression in bone cells (12,13). FGF23 mRNA expression is not restricted to bone, however. It is also present, albeit at lower levels, in the liver and in the kidney (10). One of the principal roles of FGF23 is the control of phosphate balance. Infusion of recombinant FGF23 or overexpression of FGF23 gene in animals constantly results in severe hypophosphatemia as a result of a marked increase in urinary phosphate excretion (3,14–16). This loss of phosphate is due to a decrease in the expression of the main renal phosphate transporters NPT2a and NPT2c (17,18). FGF23 also inhibits the expression of the intestinal phosphate transporter NPT2b (19). This effect is mediated by the reduction of calcitriol levels induced by FGF23, as it is not observed in mice with disrupted VDR gene (20).

FGF23 circulating concentration is modulated by phosphate intake and serum phosphate concentration, which creates a feedback loop. High dietary phosphate intake and hyperphosphatemia stimulate FGF23 production, whereas low phosphate intake reduces FGF23 concentration (21–23). By contrast with other FGF family members, FGF23 specifically modifies phosphate metabolism, suggesting that FGF23 activates a particular FGF receptor. The accidental disruption in mice of a gene encoding a protein that was named Klotho led to a phenotype very similar to that observed in FGF23 knockout mice except for an increase in circulating FGF23 (24,25). Experiments performed in cultured cells showed that FGF23 binds to FGF receptors with low affinity but that expression of Klotho markedly increases the affinity. Co-immunoprecipitation studies showed that in the presence of FGF23, Klotho binds to FGF receptors. Then Klotho seemed mandatory for the activation of the FGF receptor–signaling pathway, which involves extracellular signal–regulated kinase 1/2 and FGF receptor substrate phosphorylation, by FGF23 (24,26). Collectively, these data suggest that Klotho is a co-receptor for FGF23. Furthermore, the phenotype of mice with a double disruption of FGF23 and Klotho gene is similar to that of FGF23 or Klotho knockout mice, suggesting that all of the effects of FGF23 are mediated by Klotho and that the principal function of Klotho is to permit FGF23 action.
Klotho is a 1012–amino acid–long protein. It is expressed at the cell surface and is bound to the plasma membrane by a short one-span transmembrane domain. Its intracellular tail is less than 15 amino acids long. Klotho expression is restricted to a small number of tissues. It is mainly expressed in the kidney, the parathyroid gland, the brain, and the skeletal muscle (27–29). In the kidney, Klotho is mainly expressed in the distal tubule, whereas FGF23 exerts its action on the proximal tubule. The mechanism by which FGF23 modifies proximal tubule functions is unknown. Klotho is also present in plasma and urine. The major soluble form of Klotho originates from the shedding of the full-length protein. A smaller circulating form comes from RNA splicing at exon 3 in Klotho gene. It has been reported that ADAM 10 and 17 can cleave and release Klotho from the cell surface; however, it is unknown whether this is a physiologic role of these enzymes (30). The physiologic role of the circulating forms of Klotho remains to be established.

Mutual Control of Vitamin D Metabolism and FGF23-Klotho

Inappropriate expression of FGF23 or Klotho is constantly associated with abnormal serum calcitriol concentrations. Overexpression of FGF23 leads to a rapid and marked decrease in circulating calcitriol despite severe hypophosphatemia. Conversely, ineffectiveness of FGF23 as a result of FGF23 or Klotho gene disruption or mutations induces an increase in serum calcitriol despite hyperphosphatemia and hypercalcemia. Studies performed in animal models showed that FGF23 is a potent inhibitor of the mRNA expression of 25(OH)D-1α-hydroxylase in the renal proximal tubule. Simultaneously, FGF23 stimulates the expression of CYP24A1 in the kidney (3,16,19,31). By contrast, in the parathyroid glands, FGF23 augments 25(OH)D-1α-hydroxylase expression (29,32). The subsequent increase in local calcitriol production contributes to the observed inhibitory effect of FGF23 on PTH production and secretion, which prevents a dramatic increase in PTH concentration.

Calcitriol in turn controls FGF23 production, creating a feedback loop. A single injection of calcitriol induces an increase in FGF23 mRNA expression in bone cells. The plasma concentration of FGF23 augments within a few hours and returns to previous values 24 hours after the injection. Analysis of the FGF23 gene promoter revealed the presence of a VDRE (33). Specific disruption of VDR in bone cells decreases FGF23 production (34), suggesting that the VDRE in the FGF23 promoter plays a physiologic role. These findings explain how targeted VDR gene disruption in bone induces an increase in circulating calcitriol concentration.

Calcitriol stimulates mRNA Klotho expression in the kidney (35), with timing similar to that observed for FGF23. Calcitriol might directly control Klotho expression because a putative VDRE has been identified in the promoter of the human Klotho gene (1).

Calcitriol versus Phosphate Toxicity in Disorders with FGF23-Klotho Ineffectiveness

Loss of function of the FGF23-Klotho couple in mice and in humans is associated with calcifications of soft tissues, features of accelerated aging, and increased mortality. These detrimental effects could be due to hyperphosphatemia and hypercalcemia, the toxicity of high calcitriol concentration, deleterious effects of high FGF23 concentration, or the absence of Klotho. The similarity of the phenotypes of FGF23 and Klotho knockout mice makes the two last hypotheses unlikely. The disruption of the 25(OH)D-1α-hydroxylase gene in FGF23−/− mice normalizes the phenotype that is in favor of the toxicity of high calcitriol levels (36). This manipulation, however, also normalizes serum calcium and phosphate concentrations. Selective normalization of serum phosphate and calcium concentration by a low-phosphate diet fully reversed the phenotype of FGF23−/− mice, whereas normalization of calcitriol concentration with the persistence of hyperphosphatemia improved the phenotype but did not normalize it (37). These data suggest a preponderant effect of hyperphosphatemia over that of high calcitriol levels in the genesis of the phenotype. In line with these findings, correction of hyperphosphatemia in patients with tumoral calcinosis as a result of FGF23 ineffectiveness is able to cure soft tissue calcifications (9).

Altered FGF23-Klotho Expression and Vitamin D Deficiency as Risk Factors

FGF23 concentration increases whereas GFR decreases in chronic kidney disease (CKD). This increase occurs early in the course of renal function impairment because it begins when GFR goes below 70 ml/min (Figure 2). In ESRD, FGF23 concentration is >1000 times above the upper limit of the normal range (38,39). FGF23 accumulates as intact active peptide, as suggested by a biological assay (38) and by the inverse correlation between circulating FGF23 and calcitriol levels in dialysis patients (40). Two mechanisms could account for the augmentation of FGF23. First, the decrease in GFR tends to retain phosphate. This stimulates FGF23 production that in turn lowers renal tubular reabsorption of phosphate and thereby prevents serum phosphate from increasing. FGF23 elevation also...

Figure 2. Correlation between measured GFR and plasma concentration of FGF. GFR was measured by inulin or iothexol clearance in 312 patients. Plasma FGF23 concentration was assessed by the Immulitec c-terminal assays. Similar results were obtained with Kainos intact FGF23 assay. Below GFR values of 75 ml/min, FGF23 is inversely correlated with GFR.
Disclosures
None.

References


42. Koh N, Fujimori T, Nishiguchi S, Tamori A, Shiomi S, FGF23/Klotho Vitamin D


