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Phosphate and FGF-23

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Fibroblast growth factor (FGF)-23 is probably the most important regulator of serum phosphate and calcitriol ($1,25(OH)_2D_3$) levels. It is secreted by osteocytes and osteoblasts in response to oral phosphate loading or increased serum $1,25(OH)_2D_3$ levels. In human chronic kidney disease (CKD), plasma FGF-23 appears to be a sensitive biomarker of abnormal renal phosphate handling, as FGF-23 levels increase during early stages of kidney malfunction. In humans and animals with CKD, elevated FGF-23 levels increase fractional phosphate excretion, reduce serum phosphate levels, and reduce 1α-hydroxylase activity, which reduces $1,25(OH)_2D_3$ formation thereby increasing parathyroid hormone (PTH) secretion. FGF-23 thus has a key adaptive role in maintaining normophosphatemia. Plasma FGF-23 continues to increase as CKD progresses, increasing by orders of magnitude in end-stage renal disease. At the same time, responsiveness to FGF-23 declines as the number of intact nephrons declines, which is associated with reduced expression of Klotho, the co-receptor required for FGF-23 signaling. In late CKD, FGF-23 cannot reduce serum phosphate levels, and abnormally high plasma FGF-23 concentrations appear to exert unwarranted off-target effects, including left ventricular hypertrophy, faster CKD progression, and premature mortality. Lowering serum phosphate levels through the use of oral phosphate binders and/or long-acting PTH agents may reduce FGF-23 levels in early CKD stages, thereby limiting off-target effects, which may improve patient outcomes.

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Phosphate is essential for many cellular functions. It is a constituent of DNA, membrane lipids, high-energy phosphates, and second messengers, that is, inositol trisphosphate, cyclic adenosine monophosphate (cAMP), and cyclic guanosine monophosphate, and protein phosphorylation is an essential process, which helps regulate enzyme and receptor activities. Because phosphate is scarce in nature, vertebrate physiology has evolved to conserve phosphate through the efficient use of three sodium-dependent phosphate co-transporters (NaPi-IIa, NaPi-IIb, and NaPi-IIc, also referred to as NPT2a, NPT2b, and NPT2c) that are highly conserved throughout vertebrate taxa, from fish[1] to humans.[2] Additional molecules contributing to the regulation of phosphate homeostasis include Pit2 and the 1α-hydroxylase, which allows the formation of calcitriol ($1,25(OH)_2D_3$). Expression of these proteins in the proximal renal tubules is regulated by serum phosphate concentration and different hormonal systems. For example, low serum phosphate levels induce NPT2b expression in the intestinal tract, thus enhancing absorption of this mineral from the diet. In addition, low serum phosphate levels induce NPT2a and NPT2c expression in the proximal tubules of the kidney, thus maximizing the reabsorption of phosphate and minimizing urinary losses of this mineral. The efficiency of this latter process was strikingly demonstrated in a 1976 study, in which healthy human volunteers on a low-phosphate diet (90 mg/day) reduced their phosphate excretion considerably, thereby avoiding profound hypophosphatemia.[2] In contrast to the efficient adaptation to hypophosphatemia, renal excretion of excess phosphate is a more difficult problem for human physiology to solve.

The principal hormones that regulate renal phosphate handling are parathyroid hormone (PTH), which is produced by the parathyroid gland, and fibroblast growth factor (FGF)-23, which is produced by osteocytes and osteoblasts in bone. In healthy individuals, increasing serum phosphate concentration induces secretion of PTH and FGF-23. These two phosphaturic hormones reduce expression of NPT2a and NPT2c in the proximal renal tubules, thereby diminishing phosphate reabsorption and increasing urinary phosphate excretion.[3,4] PTH also increases the 1α-hydroxylase in the kidney, thereby increasing $1,25(OH)_2D_3$ production, thus enhancing intestinal calcium and phosphate absorption. Some evidence suggests that PTH induces expression and secretion of FGF-23; FGF-23 in turn decreases $1,25(OH)_2D_3$ production, which is an inhibitor of PTH production.

In chronic kidney disease (CKD), increased FGF-23 production enhances the excretion of phosphate per nephron, thereby restoring normophosphatemia. However, it also reduces $1,25(OH)_2D_3$ levels,[5] contributing to an increase in PTH secretion, which appears to occur after FGF-23 levels increase. This process disrupts the bone–kidney–parathyroid endocrine axis and eventually fails to prevent the development of hyperphosphatemia as CKD progresses. The resulting changes, constituting CKD-related mineral and bone disorder (CKD-MBD), are a reflection of the trade-offs postulated by Bricker's[6] intact nephron hypothesis: 'If solute intake does not diminish as the number of excretory units diminishes, the adaptive increase in excretion rate per nephron may be accomplished only at the expense of one or more abnormalities of the uremic state.' This review will explore preclinical and clinical studies of the role of FGF-23 in phosphate metabolism in CKD and the contribution of FGF-23 to CKD-MBD.

RENAL REGULATION OF PHOSPHATE AND CALCIUM HOMEOSTASIS

Role of PTH: lessons from familial pseudohypoparathyroidism

NPT2a and NPT2c facilitate the efficient reabsorption of phosphate in the proximal tubule; expression of both transporters is regulated by PTH and FGF-23. The acute PTH-dependent regulation of NPT2a and NPT2c expression is mediated predominantly through the cAMP and protein kinase A signaling pathway.[4] The PTH-induced increase in urinary phosphate is coupled to cAMP excretion from proximal tubules. Cyclic AMP production requires $G_s\alpha$, which appears to be derived in this portion of the kidney predominantly, if not exclusively, from the maternal allele, thereby potentially limiting the maximal amount of $G_s\alpha$ protein that can be generated in this tissue. As a consequence of this parent-specific expression of $G_s\alpha$ in the proximal renal tubules, maternally inherited, heterozygous *GNAS* mutations cause PTH resistance, that is, pseudohyperparathyroidism.[7] Inactivating *GNAS* mutations in those exons that encode $G_s\alpha$ cause pseudohypoparathyroidism type Ia, whereas microdeletions within or upstream of *GNAS* cause pseudohypoparathyroidism type Ib and are associated with *GNAS* methylation changes. The mechanism by which cAMP is excreted into the urine remains unknown, but is expected to involve a specific transporter that is expressed in the proximal tubule. In the distal tubule, PTH diminishes calcium excretion through cAMP/protein kinase A- and possibly also through inositol trisphosphate/protein kinase C-dependent actions on the function of calbindin-D and TRPV5. In contrast to the findings in proximal tubular cells, $G_s\alpha$ is expressed in the distal tubular cells from both parental alleles, and maternally inherited *GNAS* mutations consequently leave the PTH-induced regulation of calcium excretion intact.

Role of FGF-23: lessons from CKD

Similar to the actions of PTH, FGF-23 controls NPT2a and NPT2c expression. FGF-23 is secreted by bone cells in response to $1,25(OH)_2D_3$, which increases the mRNA levels encoding this phosphaturic hormone.[8] FGF-23 mediates its action in the kidney through an FGF receptor (FGFR)/Klotho complex to downregulate NPT2a and NPT2c expression in the proximal tubules. However, it is uncertain whether FGF-23 signals initially through receptors in the distal convoluted tubule cells in which ERK phosphorylation occurs in response to FGF-23.[9] These cells are adjacent to NPT2a-expressing proximal tubular cells and it has therefore been speculated that a paracrine signal from the distal to the proximal tubules is required for decreasing NPT2a expression. Even small changes in plasma FGF-23 levels are associated with significant changes in urinary phosphate excretion, as shown by the small increase in FGF-23 levels after unilateral nephrectomy in healthy kidney donors, which is associated with increased urinary phosphate excretion.[10]

Besides its effect on tubular phosphate handling, FGF-23 reduces PTH secretion, and it inhibits 1α-hydroxylase leading to a decrease in $1,25(OH)_2D_3$ production, which contributes to the development of hypocalcemia and leads to an increase in PTH production.[11] The $1,25(OH)_2D_3$ itself increases FGF-23 production.

FGF-23 AND PHOSPHATE IN CKD

FGF-23 appears to be an important biomarker for an abnormal regulation of phosphate homeostasis in CKD and is likely to be involved in CKD-MBD pathophysiology. As CKD progresses, plasma FGF-23 levels increase. This increase occurs earlier and to a greater extent than observed for serum phosphate; in late CKD, plasma FGF-23 levels can be elevated by several orders of magnitude.[5,12] Findings in experimental renal disease suggest that the FGF-23 increase also precedes the increase in PTH levels.[11] In fact, bone biopsies of patients with CKD have shown increased expression of FGF-23 already by CKD stage 2, along with a marked increase in DMP1 protein (a negative regulator of FGF-23), which may be improperly processed and could thus be inactive.[13] Furthermore, in a prospective study of patients with mild-to-moderate CKD,[14] higher plasma FGF-23 levels were shown to predict a more rapid progression toward end-stage renal disease (ESRD); similarly elevated FGF-23 levels predict CKD progression in patients with diabetic nephropathy.[15] In addition, elevated FGF-23 levels are independently associated with left ventricular hypertrophy in patients with CKD,[16] an important finding because CKD-MBD, and particularly hyperphosphatemia, worsen the cardiovascular prognosis in CKD. Aortic calcification, a major reason for cardiovascular morbidity, in hemodialysis recipients is independently predicted by plasma FGF-23.[17]

Phosphate is a major regulator of FGF-23 expression.[18] Dietary phosphate loading increases FGF-23 expression, whereas phosphate depletion with binders decreases the circulating levels of this hormone.[19] In mild CKD, FGF-23 seems to function as a protective factor, as it triggers adaptive changes that maintain normophosphatemia. For example, in animal models, FGF-23 protects against hyperphosphatemia

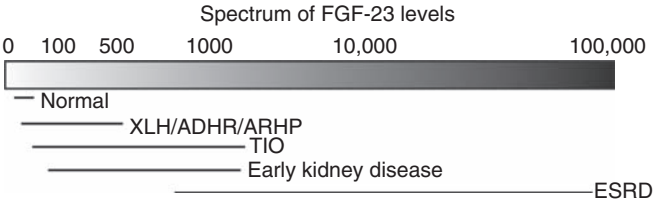
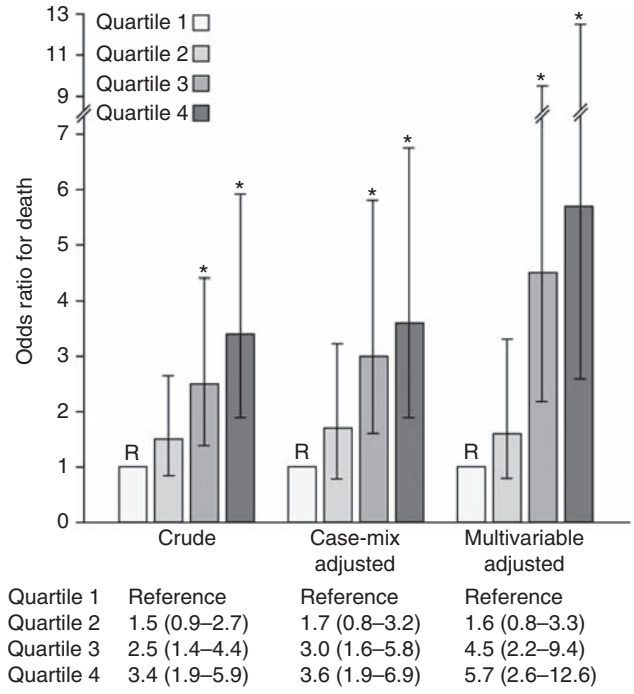


Figure 1 | Spectrum of serum fibroblast growth factor (FGF)-23 levels in early chronic kidney disease and end-stage renal disease (ESRD) as compared with the normal condition and with different disorders affecting FGF-23. ADHR, autosomal dominant hypophosphatemic rickets; ARHP, autosomal recessive hypophosphatemia; TIO, tumor-induced osteomalacia; XLH, X-linked hypophosphatemia. Adapted with permission from Isakova *et al.*[20]

by increasing urinary phosphate excretion and reducing $1,25(OH)_2D_3$ production; diminished $1,25(OH)_2D_3$ levels lead to hypocalcemia and thus to an increase in PTH levels, which further enhances renal phosphate excretion.[11] The importance of FGF-23 in mediating these effects is shown by the effects of anti-FGF-23 antibodies in rats with experimental CKD.[11] Animals with early CKD that were treated with anti-FGF-23 antibodies showed an increase in $1,25(OH)_2D_3$ levels, a normalization of serum calcium levels, and a decrease in PTH levels. Furthermore, fractional phosphate excretion decreased because of inactivation of FGF-23 resulting in hyperphosphatemia.

As CKD progresses, elevated FGF-23 levels are no longer able to enhance urinary phosphate excretion, thus leading to the development of hyperphosphatemia. This may be partly related to declining Klotho expression and a reduction in functional nephrons. When patients require treatment by dialysis, the levels of immunoreactive FGF-23 can be markedly elevated (Figure 1).[20] However, unlike the appearance of large amounts of C-terminal PTH fragments, most of which are biologically inactive, practically all of the circulating FGF-23 present in patients with ESRD is intact and biologically active. This conclusion is based on observations with a reporter-cell assay (human embryonic kidney cells expressing Klotho and a luciferase reporter expressed under the control of the EGR-1 promoter) designed to quantify biologically active FGF-23, which demonstrated excellent correlation with immunoreactive measurement of FGF-23. The immunometric assays used for these measurements quantify the intact FGF-23 molecule alone (Kainos assay) or the intact protein along with a C-terminal fragment (Immutopics assay).[21,22] Consistent with the excellent correlation between bioactive and immunoreactive FGF23, Western blot analyses of plasma FGF-23 from dialysis patients showed that most of the circulating FGF-23 is intact.[21] Thus, with CKD progression, the excess of biologically active FGF-23, which is thought to occur in response to hyperphosphatemia, ceases to be protective and may lead to pathological off-target effects that potentially contribute to the increase in mortality as FGF-23 increases in patients with ESRD[23,24] (Figure 2).

Interventions that lower serum phosphate, such as oral phosphate binders to prevent intestinal absorption or



	Crude	Case-mix adjusted	Multivariable adjusted
Quartile 1	Reference	Reference	Reference
Quartile 2	1.5 (0.9–2.7)	1.7 (0.8–3.2)	1.6 (0.8–3.3)
Quartile 3	2.5 (1.4–4.4)	3.0 (1.6–5.8)	4.5 (2.2–9.4)
Quartile 4	3.4 (1.9–5.9)	3.6 (1.9–6.9)	5.7 (2.6–12.6)

Figure 2 | Mortality in patients with end-stage renal disease (ESRD) receiving hemodialysis in relationship to quartiles of serum fibroblast growth factor (FGF)-23 concentration at initiation of dialysis. R, reference. Reproduced with permission from Gutiérrez *et al.*[23] Copyright The Massachusetts Medical Society.

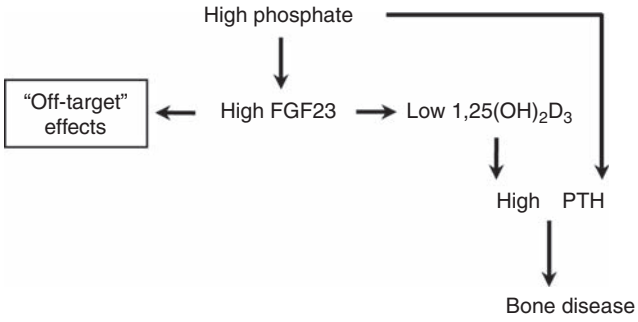


Figure 3 | 'Trade-off hypothesis' revisited. Involvement of fibroblast growth factor (FGF)-23 in end-stage renal disease (ESRD) pathology, reflecting potentially adverse effects of increased secretion of this phosphaturic hormone, which helps normalize phosphate homeostasis but contributes to the development of secondary hyperparathyroidism. PTH, parathyroid hormone.

possibly long-acting PTH analogs that reduce NPT2a or NPT2c expression, may help prevent CKD-related FGF-23 increases (Figure 3). For example, two recently reported, long-acting PTH analogs (M-PTH(1-28) and Trp1-M-PTH(1-28)) were found to efficiently reduce NPT2a expression in proximal tubules, leading to sustained hypophosphatemia in wild-type mice.[25] Targeting the proximal tubules with long-acting PTH agents in early CKD may add to the effects of phosphate binders in normalizing serum phosphate levels and may help avoid an increase in FGF-23, unless PTH has a role in the synthesis and/or secretion of

FGF-23. Proactive interventions to avoid the development of transient hyperphosphatemia in CKD may thus prevent an increase in FGF-23 and the hormone's putative off-target effects in CKD-MBD. In support of this conclusion, a recent *post hoc* analysis of a randomized clinical trial has shown that treatment with phosphate binders can markedly decrease FGF-23 levels in patients undergoing hemodialysis.[26]

CONCLUSIONS

FGF-23 is a bone-generated major regulator of phosphate homeostasis, which appears to be an important biomarker of phosphate homeostasis in patients with CKD. Plasma FGF-23 concentrations begin to increase early in CKD, with increases by orders of magnitude by the time patients reach ESRD. Increasing FGF-23 levels are independently associated with left ventricular hypertrophy, CKD progression, and mortality, possibly through off-target actions. Lowering serum phosphate levels through the use of phosphate binders may lower FGF-23 levels. Further research is needed to show whether lowering FGF-23 levels improves outcomes in patients with CKD.

DISCLOSURE

HJ has served as a paid consultant to Genzyme and Roche, and is a named inventor on the patent outlining the development of immunometric assays for the detection of FGF-23.

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