“Phosphatonins” and the regulation of phosphorus homeostasis

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The normal adult body contains 15–20 mol of phosphate, and males have slightly more phosphate than females (39). A majority of phosphate (80–90%) is present in bone, with the remainder distributed between other tissues and extracellular fluid. Phosphate is present as inorganic phosphate and in a variety of organic substances including sugars, phosphoproteins, phospholipids, and nucleic acids. All tissues absorb and secrete phosphate into the extracellular fluid, and phosphate moves between the extracellular fluid pool and bone. It is not surprising that multiple factors and regulatory mechanisms play a role in maintaining serum phosphate concentrations within a physiological range. Currently, our understanding of phosphate regulation is limited to the major pathways associated with intestine, bone, and kidney (Fig. 2).

A normal diet adequate in phosphate normally contains ~20 mg·kg⁻¹·day⁻¹ (~1,500 mg) of phosphorus. Of this, ~16 mg·kg⁻¹·day⁻¹ (~1,100 mg) are absorbed in the proximal intestine, predominantly in the jejunum. Approximately 3 mg·kg⁻¹·day⁻¹ (~200 mg) are secreted into the intestine via pancreatic and intestinal secretions, giving a net phosphorus absorption of ~13 mg·kg⁻¹·day⁻¹ (~900 mg). Approximately 7 mg·kg⁻¹·day⁻¹ of phosphorus appear in the feces. Absorbed phosphorus enters the extracellular fluid pool and moves in and out of bone as needed (~3 mg·kg⁻¹·day⁻¹, ~200 mg). The rate of bone remodeling (both resorption and mineralization) is important in determining the concentration of serum phosphorus in the extracellular fluid pool. Plasma phosphorus is filtered in the glomerulus and enters the tubular fluid. Unlike plasma calcium, which is bound to plasma proteins and only partly filtered, plasma phosphorus is filtered almost completely at the glomerulus and enters the tubular fluid in approximately the same concentrations as are present in

PHOSPHATE HOMEOSTASIS IN MAMMALS

Importance of Phosphorus in Biological Processes

Phosphorus plays an important role in a number of biological processes and is an exceptionally important component of hydroxyapatite, the major component of bone mineral (88). In addition, phosphorus is present in nucleic acids, bioactive signaling proteins, phosphorylated enzymes, and cell membranes (27, 51, 52, 63). A prolonged deficiency of phosphorus and inorganic phosphate results in serious biological problems, including impaired mineralization of bone resulting in osteomalacia or rickets, abnormal erythrocyte, leukocyte, and platelet function; impaired cell membrane integrity that can result in rhabdomyolysis; and impaired cardiac output (61, 62, 66, 67, 101). Therefore, the maintenance of appropriate phosphorus homeostasis is critical for the well-being of the organism.

During skeletal growth and bone remodeling, calcium and phosphate are required for the formation of hydroxyapatite and other mineral-phase components. The rate at which mineralization occurs is dependent, in part, on the availability of phosphorus and calcium. In the absence of these ions, mineralization is impaired, resulting in the formation of poorly mineralized bone that is characteristic of osteomalacia or rickets (68). This defect is clearly demonstrated by reduced tetracycline uptake and minimal separation of sequential tetracycline labels (Fig. 1). The amount of nonmineralized matrix is significantly increased in osteomalacia as a consequence of reduced mineralization (68).

Phosphorus Metabolism in Humans

The normal adult body contains 15–20 mol of phosphate, and males have slightly more phosphate than females (39). A majority of phosphate (80–90%) is present in bone, with the remainder distributed between other tissues and extracellular fluid. Phosphate is present as inorganic phosphate and in a variety of organic substances including sugars, phosphoproteins, phospholipids, and nucleic acids. All tissues absorb and secrete phosphate into the extracellular fluid, and phosphate moves between the extracellular fluid pool and bone. It is not surprising that multiple factors and regulatory mechanisms play a role in maintaining serum phosphate concentrations within a physiological range. Currently, our understanding of phosphate regulation is limited to the major pathways associated with intestine, bone, and kidney (Fig. 2).

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References

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Phosphorus is excreted in the urine in states of phosphate equilibrium or balance and normal renal function, the amount appearing in urine is remarkably similar to that absorbed in the intestine and can serve as a rough approximation of the amount absorbed in the intestine. In states of phosphate deprivation resulting from inadequate intake of phosphorus or low absorption of phosphorus from the intestine, urinary phosphorus concentrations are low and serve as appropriate indicators of altered phosphorus regulation.

Factors Regulating Phosphorus Homeostasis

There are a number of hormones involved in the control of phosphorus metabolism. Parathyroid hormone and 1,25(OH)2D3 are among the best understood. Concentrations of these hormones are regulated by phosphorus in a manner that is conducive to the maintenance of normal phosphate balance. In addition, a number of other substances such as growth hormone and insulin-like growth factor 1, alter phosphorus balance although their circulating concentrations are not directly controlled by ambient phosphorus concentrations. Recent studies have identified several new factors that also play a role in the regulation of phosphorus transport and homeostasis. These include the "phosphotonins" fibroblast growth factor 23 (FGF-23) and secreted frizzled related protein-4 (sFRP-4) that induce a state of negative phosphate balance directly, by inhibiting renal phosphate reabsorption in the proximal tubule, and indirectly, by inhibiting the synthesis of 1,25(OH)2D3 and reducing the intestinal absorption of phosphorus.
absorption of phosphorus. Two recently described factors, fibroblast growth factor 7 (FGF-7) and matrix extracellular phosphoglycoprotein (MEPE), have been shown to inhibit phosphate transport in renal epithelial cells in culture, and in the case of matrix extracellular phosphorus glycoprotein, to induce phosphaturia in mice. FGF-7 and MEPE, however, have not been demonstrated to prevent compensatory increases in serum 1,25(OH)2D3 concentrations seen in hypophosphatemic states or to directly inhibit 25-hydroxyvitamin D 1-hydroxylase activity.

Figure 3 summarizes the factors currently known that control phosphorus homeostasis in mammals. Parathyroid hormone (PTH) inhibits renal phosphate reabsorption, whereas, 1, 25-dihydroxyvitamin D increases renal phosphate reabsorption and intestinal phosphate absorption. It is now proposed that fibroblast growth factor (FGF)-23, secreted frizzled related protein-4 (sFRP-4), matrix extracellular phosphoglycoprotein (MEPE), and FGF-7, together with PTH inhibit renal phosphate reabsorption. FGF-23 and sFRP-4 also inhibit the formation of 1, 25-dihydroxyvitamin D, thereby reducing intestinal phosphate absorption.

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Figure 3 summarizes the factors currently known that control phosphorus homeostasis in mammals. Parathyroid hormone, by virtue of its phosphaturic effect in the kidney (11, 26, 39, 81), decreases overall phosphate retention, whereas 1α,25(OH)2D3 increases phosphate retention by enhancing the efficiency of phosphorus absorption in the intestine and in the kidney (116, 121–125). It should be noted that parathyroid hormone has two opposing effects. As noted above, parathyroid hormone increases urinary phosphate excretion. At the same time, it also increases the synthesis of 1α,25(OH)2D3 by stimulating the activity of the 1α-hydroxylase enzyme in the kidney (17, 30, 41). 1α,25(OH)2D3, in turn, increases the efficiency of phosphorus absorption in the intestine and kidney. In contrast, the phosphatonin increase renal phosphate excretion and inhibit 25-hydroxyvitamin D 1α-hydroxylase activity, thereby further decreasing the retention of phosphorus.

In Figure 4 are shown physiological changes that are known to occur with low or high dietary intakes of phosphate. A decrease in serum phosphate concentrations, such as occurs following the ingestion of a diet low in phosphorus, results in increased ionized calcium concentrations, decreased parathyroid hormone secretion, and a subsequent decreased renal phosphate excretion, such that serum phosphate concentrations remain normal (11, 39, 81). At the same time, by parathyroid hormone-independent mechanisms, there is increased renal 25-hydroxyvitamin D 1α-hydroxylase activity, increased 1α,25(OH)2D3 synthesis, and increased phosphate absorption in the intestine and reabsorption in the kidney (31, 43–45, 121–125). Conversely, with elevated phosphate intake, there are decreased calcium concentrations, increased parathyroid hormone release from the parathyroid gland, and increased renal phosphate excretion. Increased serum phosphate concentrations inhibit renal 25-hydroxyvitamin D 1α-hydroxylase, and decrease 1α,25(OH)2D3 synthesis. Reduced 1α,25(OH)2D3 concentrations decrease intestinal phosphorus absorption as well as renal phosphate reabsorption. All of these factors tend to bring serum phosphate concentrations back into the normal range.

![Figure 3. Model depicting factors that regulate phosphate homeostasis and renal phosphate reabsorption. Parathyroid hormone (PTH) inhibits renal phosphate reabsorption, whereas, 1, 25-dihydroxyvitamin D increases renal phosphate reabsorption and intestinal phosphate absorption. It is now proposed that fibroblast growth factor (FGF)-23, secreted frizzled related protein-4 (sFRP-4), matrix extracellular phosphoglycoprotein (MEPE), and FGF-7, together with PTH inhibit renal phosphate reabsorption. FGF-23 and sFRP-4 also inhibit the formation of 1, 25-dihydroxyvitamin D, thereby reducing intestinal phosphate absorption.](http://ajprenal.physiology.org/)

![Figure 4. Mechanisms by which phosphate homeostasis is maintained. S, serum.](http://ajprenal.physiology.org/)
Factors Influencing Intestinal Phosphate Absorption and Renal Tubular Phosphate Reabsorption

The absorption of phosphate in the intestine is dependent on the amount and availability of phosphorus present in the diet (31, 43, 45, 121, 122). For example, a diet containing an exceptionally low amount of phosphorus results in increased phosphorus absorption in the proximal intestine, predominantly the jejunum. Phosphate binders, such as aluminum hydroxide, calcium salts, lanthanum, and sevelamer (Renagel), reduce the amount of available phosphorus, thereby decreasing phosphate absorption and serum phosphate levels. 1α,25-(OH)2D increases the efficiency of phosphorus absorption in the jejunum, and, conversely, low 1α,25-(OH)2D concentrations reduce the efficiency of phosphorus absorption in the intestine (122, 123).

A variety of factors influence bulk phosphate reabsorption along the proximal convoluted and straight tubule of the kidney (11, 81). In animals with intact parathyroid glands, phosphate reabsorption along the proximal convoluted tubule exceeds fluid reabsorption, such that the phosphate concentration in the proximal tubules is 70% of the plasma level (11, 81). In the absence of parathyroid hormone, phosphate reabsorption increases and the phosphate concentration in the proximal tubule is ∼30% of that in plasma. There is little phosphate reabsorption in the proximal straight tubule in the presence of parathyroid hormone. However, in the absence of parathyroid hormone, phosphate is avidly reabsorbed along the proximal straight tubule, resulting in very low urinary excretion of phosphate (11, 81). Hormonal and nonhormonal factors that influence phosphate reabsorption along the proximal tubule include parathyroid hormone concentrations, sodium reabsorption, serum calcium concentrations, 1α,25(OH)2D, serum bicarbonate concentrations, hypercapnia or hypocapnia, dopamine, and serotonin (11, 81). Table 1 summarizes the influence of some of these factors on the efficiency of phosphorus reabsorption by the proximal tubule.

Sodium-Phosphate Cotransporters Mediate the Uptake of Phosphate in Intestinal and Renal Epithelial Cells

The uptake of phosphate is mediated by sodium-phosphate cotransporters that are located at the apical border of intestinal cells (NaPi-Ilb) and at apical borders of proximal tubule cells (NaPi-IIa and NaPi-IIc) (5, 12–15, 28, 48, 49, 59, 69, 76–80, 82–85, 90–92, 113, 129, 130, 132, 133). The structure and physiology of these cotransport molecules have been extensively reviewed, and the reader is directed to other publications in this regard (12, 14, 15, 76–84, 117). Suffice it to say that the sodium-phosphate cotransporters are highly homologous and are predicted to have similar structures (59, 83).

Parathyroid hormone and 1α,25(OH)2D maintain phosphate homeostasis through their regulation of the sodium-phosphate cotransporters in the kidney and intestine. The numbers of renal sodium-phosphate cotransporters is reduced along the apical borders of proximal tubular cells following the administration of parathyroid hormone 1–34, but not by the administration of parathyroid hormone 3–34, (129, 130). The renal sodium-phosphate cotransporter NaPi-IIa have been shown to be internalized and degraded within the lysosomes (49, 60, 78, 84).

In the intestine, the number of NaPi-IIb cotransporters is increased within the apical membrane of absorptive cells following the administration of a diet low in phosphorus (48). Because phosphorus deprivation is also associated with an increase in the synthesis of 1α,25(OH)2D, it is logical to assume that the increase in the number of transporters is due to the increased serum concentration of 1α,25(OH)2D. The administration of 1α,25(OH)2D is associated with an increase in the numbers of sodium-phosphate cotransporters present within apical membranes of intestinal cells (48). However, recent evidence suggests that in vitamin D receptor mutant mice, phosphate deprivation results in an upregulation in the numbers of transporters in intestinal cells even in the absence of vitamin D activity (103).

“PHOSPHATONIN”

Definition of Phosphatonin

The term phosphatonin was introduced to describe a factor or factors responsible for the inhibition of renal phosphate reabsorption and altered 25-hydroxyvitamin D 1α-hydroxylase regulation observed in patients with tumor-induced osteomalacia (35). Cai et al. (22) described a patient with tumor-induced osteomalacia in whom the biochemical characteristics of hypophosphatemia, renal phosphate wasting, and reduced 1α,25(OH)2D, disappeared following removal of the tumor. Culture of tumor cells demonstrated the presence in tumor cell supernatants of 10- to 30-kDa heat-sensitive factor(s) that inhibited sodium-dependent phosphate transport (but not the transport of other substances such as glucose and amino acids) in opossum kidney cells, proximal tubular-like epithelial cells that have been previously shown to transport phosphate in a sodium-dependent manner. Unlike parathyroid hormone, this substance(s) did not increase intracellular cAMP concentrations. Furthermore, its activity was not blocked by parathyroid hormone receptor antagonists. When tumor cells were implanted in nude mice, the animals became hypophosphatemic within 3–6 mo following implantation of the cells. These observations suggested that a novel substance(s) capable of modulating phosphate transport in the proximal tubule cells was present in tumor cells. The term phosphatonin was coined to describe this circulating phosphaturic factor(s) that functions via cAMP-independent pathways and prevents or attenuates increased 25-hydroxyvitamin D 1α-hydroxylase activity that normally occurs in response to hypophosphatemia.

Table 1. Factors affecting phosphate reabsorption in the proximal nephron (11)

<table>
<thead>
<tr>
<th>Factors That Decrease P, Reabsorption</th>
<th>Factors That Increase P, Reabsorption</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increased phosphate intake</td>
<td>Phosphate depletion</td>
</tr>
<tr>
<td>Parathyroid hormone/cAMP</td>
<td>Parathyroidectomy</td>
</tr>
<tr>
<td>Volume expansion</td>
<td>Volume contraction</td>
</tr>
<tr>
<td>Hypercalcemia</td>
<td>Hypercalcemia</td>
</tr>
<tr>
<td>Carbonic anhydrase inhibitors</td>
<td>1α,25(OH)2D</td>
</tr>
<tr>
<td>Acid-base disturbances</td>
<td>Growth hormone</td>
</tr>
<tr>
<td>Hypercapnia</td>
<td>Hypocapnia</td>
</tr>
<tr>
<td>Dopamine</td>
<td>Serotonin</td>
</tr>
<tr>
<td>FGF-23, sFRP-4, MEPE, FGF-7</td>
<td></td>
</tr>
</tbody>
</table>

FGF-23, fibroblast growth factor 23; sFRP-4, secreted frizzled related protein-4; MEPE, matrix extracellular phosphoglycoprotein.
Biochemical Similarity Among Patients with Tumor-Induced Osteomalacia, X-Linked Hypophosphatemic Rickets, and Autosomal Dominant Hypophosphatemic Rickets

A similar biochemical phenotype exists in patients with X-linked hypophosphatemic rickets (XLH) and the animal model, the Hyp mouse (32, 33, 36, 37, 93, 94). Several investigators have shown the presence of circulating factors in the serum of Hyp mice that inhibit sodium-dependent phosphate transport in the kidney (74, 75, 87). Hence, it was reasonable to assume that a factor similar to that found in patients with tumor-induced osteomalacia was also present in patients with XLH. Positional cloning experiments identified the mutant gene, but, surprisingly, the gene encoded an endopeptidase, PHEX, that resembled a protease responsible for the degradation of atrial natriuretic factor (2, 34, 73). It was postulated that the endopeptidase PHEX was responsible for the degradation of this phosphaturic peptide and that excessive amounts of this peptide circulated in serum when the endopeptidase was unable to degrade the peptide in question.

Further interesting information became available when it was demonstrated that patients with the disease, autosomal dominant hypophosphatemic rickets, had activating mutations within the fibroblast growth factor homolog FGF-23 (1). The mutation in the FGF-23 gene resulted in the modification of a cleavage region of the encoded peptide. Replacement of the arginine residues at either position 176 or 179 eliminated the cleavage site. Subsequent studies showed that the uncleaved mutant form of FGF-23 had a long half-life (7, 107, 134). Its persistence in the circulation resulted in the biochemical phenotype of hypophosphatemia, renal phosphate wasting, and low 1α,25(OH)2-D concentrations.

Identification of Phosphaturic Peptides from Tumors Responsible for Tumor-Induced Osteomalacia

FGF-23 overexpression was also associated with tumor-induced osteomalacia (16, 106). FGF-23 infused or injected into mice was shown to induce hypophosphatemia, renal phosphate wasting, and reduced renal 25-hydroxyvitamin D 1α-hydroxylase activity following the induction of hypophosphatemia. Thus FGF-23 was a substrate for PHEX inasmuch as recombinant PHEX degraded FGF-23 in vitro in rabbit reticulocyte lysate system. This latter observation has not been validated by others using somewhat different systems, although another report has shown that FGF-23-derived peptides are processed by PHEX (9, 23, 46).

In addition to FGF-23, serial analysis of gene expression (SAGE) identified additional genes that were overexpressed in tumors associated with tumor-induced osteomalacia (29). Table 2 summarizes identified genes validated by other methods. Genes encoding secreted factors were expressed using recombinant methods and tested for phosphate-regulating activity in vitro. Similar to FGF-23, sFRP-4 was shown to inhibit sodium-dependent phosphate transport in opossum kidney cells and phosphate reabsorption in vivo in rats. In addition, infusion of sFRP-4 also prevented a compensatory increase in 25-hydroxyvitamin D 1α-hydroxylase activity following the induction of hypophosphatemia. Thus sFRP-4 has the properties of a phosphatonin.

Rowe and co-workers (98, 100) also isolated another molecule, MEPE, from tumors associated with tumor-induced osteomalacia (98, 100). This protein, when infused into mice, caused hypophosphatemia and renal phosphate wasting (100). Furthermore, it inhibits sodium-dependent phosphate transport in opossum kidney cells. Recently, Carpenter and co-workers (24) have also shown that FGF-7 is overexpressed in tumors associated with tumor-induced osteomalacia and may be a phosphaturic factor as it also inhibits phosphate transport in opossum kidney cells (24).

In summary, using a variety of biochemical and molecular biological methods, at least four phosphate-regulating substances have been isolated from phosphate-wasting tumors associated with tumor-induced osteomalacia: FGF-23, sFRP-4, MEPE, and FGF-7. In the following sections, we will review the biological activity of the different phosphaturic peptides described above and discuss information concerning their role in various clinical disorders.

Table 2. Serial analysis of gene expression in tumors associated with osteomalacia

<table>
<thead>
<tr>
<th>Gene</th>
<th>Accession No.</th>
<th>Function</th>
<th>Control</th>
<th>OOM</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEPE</td>
<td>NM_020203</td>
<td>Regulation of bone mineralization</td>
<td>C1 0</td>
<td></td>
</tr>
<tr>
<td>FRP-4</td>
<td>AF026692</td>
<td>Secreted factor</td>
<td>C2 0</td>
<td></td>
</tr>
<tr>
<td>DMP-1</td>
<td>U65378</td>
<td>Bone/teeth ECM protein</td>
<td>Hp1 107</td>
<td></td>
</tr>
<tr>
<td>Candidate 11</td>
<td>Novel</td>
<td>Unknown</td>
<td>Hp2 75</td>
<td></td>
</tr>
<tr>
<td>Integrin-o 10</td>
<td>AF113245</td>
<td>Plasma membrane</td>
<td>Hp3 31</td>
<td></td>
</tr>
<tr>
<td>Neurogranin RC3</td>
<td>U89165</td>
<td>Intracellular</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pit/Ghv-1</td>
<td>L20859</td>
<td>Ubiquitous phosphate transporter</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placental nucleoside transporter</td>
<td>U81375</td>
<td>Nucleoside transporter</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serine protease 11</td>
<td>AF70555</td>
<td>Serine protease of unknown function</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FGF-23</td>
<td>AF263537</td>
<td>Secreted factor mutated in ADHR</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

OOM, oncogenic osteomalacia; Hp, hemangiopericytoma (29).

As noted above, FGF-23 was initially postulated to be the factor responsible for autosomal dominant hypophosphatemic...
rickets (1). A mutation in the FGF-23 gene resulted in expression of an FGF-23 protein that was resistant to proteolysis and with a prolonged half-life and biopotency (7, 107, 134). Recombinant FGF-23 produced hypophosphatemia when administered intraperitoneally to mice (106). Serum calcium concentrations did not change following the administration of the peptide. When Chinese hamster ovary cells were transfected with an FGF-23 expression plasmid and the cells were implanted in nude mice, the animals became hypophosphatemic and the fractional excretion of phosphate was increased within 10 days. Alkaline phosphatase concentrations increased in the serum, consistent with changes in bone mineralization. Radiological evidence of rickets in the long bones and histological evidence of rachitic changes were observed after several weeks. There was a decrease in the amount of mRNA for the 25-hydroxyvitamin D 1α-hydroxylase.

In support of these studies, Bowe et al. (16) demonstrated that recombinant FGF-23 inhibited sodium-dependent phosphate transport in opossum kidney cells (see Fig. 5A). Furthermore, intravenous infusion of recombinant FGF-23 into mice caused a rapid, dose-dependent increase in the fractional excretion of phosphate with little or no change in sodium excretion (101) (Fig. 5B). These studies suggest that FGF-23 has direct actions on renal phosphate transport.

The physiological role of FGF-23 in modulating plasma phosphate concentrations and 25 hydroxyvitamin D 1α-hydroxylase levels was further supported by the generation of FGF-23 null mice (105). These mice had a reduced growth rate and died 10–14 wk after birth. Serum phosphate concentrations were elevated within 10 days after birth, and serum calcium concentrations became moderately elevated 2 wk after birth. Interestingly, these mice developed increased 25 hydroxyvitamin D 1α-hydroxylase messenger RNA levels and associated increases in serum 1α,25(OH)₂D concentrations. A moderate increase in serum calcium concentrations was also observed that could be a consequence of the increased 1α,25(OH)₂D concentrations and increased intestinal calcium transport. Parathyroid hormone concentrations were diminished in the homozygous mutant mice only at 9 wk of age. Long bones displayed abnormal mineralization and a reduced growth plate. TmP/GFR was significantly increased in FGF-23 null mutant animals.

Conversely, transgenic mice overexpressing FGF-23 have reduced serum phosphate concentrations, increased phosphate excretion, and reduced renal sodium-phosphate cotransporter NaPi-IIa (70, 108).

Numerous studies have now demonstrated that FGF-23 is associated with increased phosphate excretion and decreased plasma phosphate concentrations. In addition to changes in phosphate homeostasis, chronic overexpression of FGF-23 has also been linked to disturbances in vitamin D metabolism, calcium homeostasis, and increased parathyroid hormone levels (6). The exact interaction and the relative contribution of FGF-23, parathyroid hormone, and vitamin D on phosphate homeostasis in these models of chronic FGF-23 excess remain an open question.

The mechanism of action of FGF-23 on phosphate transport is currently unknown. Limited in vitro binding studies suggest that FGF-23 may bind to FGFR-3c fusion proteins. Furthermore, tyrosine kinase inhibitors that are known to inhibit signaling through FGFRs block the effect of FGF-23 on sodium-dependent phosphate transport in opossum kidney cells (138). These results raise the possibility that FGF-23 may signal through one of the known FGFRs. Further clarification...
will require demonstration of FGF-23 binding to a receptor under physiologically relevant conditions.

**FGF-23 in Normal and Clinical Disorders**

Recent studies demonstrate that FGF-23 is present in normal plasma and that it is increased in a variety of circumstances associated with altered phosphate reabsorption. Elevations in circulating FGF-23 have been associated with various clinical disorders.

**Oncogenic osteomalacia.** Numerous reports now show that serum FGF-23 is elevated in some, but not all, patients with oncogenic or tumor-induced osteomalacia (16, 55, 56, 72, 86, 120, 139). Removal of the tumor is generally associated with a reduction in serum FGF-23 concentrations, and there is a temporal association between the reduction in serum FGF-23 concentrations, the increase in serum phosphate concentrations, a decrease in renal phosphate wasting, and an increase in serum 1α,25(OH)₂D₃ concentrations (56, 120, 139). In some patients, FGF-23 concentrations are not greatly increased and do not dramatically decrease following tumor removal (56). The increase in serum FGF-23 concentrations is consistent with the overexpression of mRNA and protein for FGF-23 within the tumors themselves. Recently, venous sampling has demonstrated a gradient between FGF-23 concentrations in tumor venous effluent and FGF-23 concentrations in peripheral blood, suggesting that elevations of serum FGF-23 are due to direct secretion from the tumors (120).

**Autosomal dominant hypophosphatemic rickets.** As noted earlier, mutations in the FGF-23 gene within a proconvertase processing site were identified as the cause for autosomal dominant hypophosphatemic rickets (1). These mutations appear to prevent processing of FGF-23 in mice (7, 107, 134).

**X-linked hypophosphatemic rickets.** The evidence for elevated FGF-23 expression in patients with X-linked hypophosphatemic rickets is conflicting. Jonsson and co-workers (56) describe elevated serum FGF-23 concentrations in many subjects, whereas, Weber and colleagues (131) reported only modestly increased or normal concentrations of FGF-23. In the Hyp mouse, preliminary reports suggest that FGF-23 serum concentrations are elevated and neutralization of FGF-23 with an antibody ameliorates hypophosphatemia and rickets (3). These data are consistent with the premise that PHEX processes FGF-23 either directly or indirectly under normal circumstances. Inactivation of PHEX, as occurs in individuals with X-linked hypophosphatemic rickets and in Hyp mice, would reduce FGF-23 degradation and cause renal phosphate wasting and hypophosphatemia. As described earlier, it is unclear whether FGF-23 is a direct substrate for PHEX (9, 16, 23, 46).

**Fibrous dysplasia.** Riminucci and co-workers (96) examined the concentrations of FGF-23 in the plasma of patients with fibrous dysplasia, some of whom have hypophosphatemia. These investigators demonstrated that those patients who had low phosphate concentrations had elevated FGF-23 concentrations in the blood, whereas those who had normal phosphate concentrations did not have elevations in FGF-23 (96). These results support the association of elevated FGF-23 and hypophosphatemia.

**Chronic renal failure and hemodialysis.** Serum FGF-23 concentrations are elevated in patients with chronic renal failure and in patients on hemodialysis (53, 71, 104, 137). It is attractive to hypothesize that the synthesis of FGF-23 is induced by retention of phosphate that occurs in patients at late stages of renal insufficiency. Current data demonstrating direct regulation of FGF-23 concentrations by changes in serum phosphate are conflicting. In a study by Jonsson and colleagues (71), ingestion of a diet that was either high or low in phosphate did not appreciably alter FGF-23 concentrations. A recent study suggested that serum FGF-23 concentrations are modestly increased in serum in normal human subjects receiving a high-phosphate diet; alterations in parathyroid hormone secretion were prevented by the concomitant administration of a large amount of dietary calcium (38). In this latter study, changes in FGF-23 with variations in dietary phosphate were modest, and it remains uncertain whether changes of the magnitude demonstrated in this study are sufficient to alter phosphate excretion.

Many of the initial reports concerning elevated serum FGF-23 levels in patients with chronic renal insufficiency were obtained using an assay that recognizes both COOH-terminal fragments of the FGF-23 as well as the intact molecule. Therefore, it is difficult to assess whether the large increases in serum FGF-23 seen in these patients are due to decreased degradation of full-length FGF-23 or are FGF-23 fragments that may not have biological activity. Recent reports would suggest that the intact FGF-23 is elevated in patients with chronic renal failure (104). In many of the studies reported, a direct relationship among serum FGF-23, parathyroid hormone, phosphate, and creatinine concentrations was observed. To address the issue as to whether parathyroid hormone regulates serum FGF-23 levels, investigators have examined FGF-23 levels in patients with primary hyperparathyroidism without chronic renal failure (127, 136). There is no relationship between parathyroid hormone and FGF-23 concentrations in these patients. Furthermore, parathyroidectomy does not result in a significant alteration in FGF-23 even though parathyroid hormone concentrations decrease dramatically following surgery. Thus, the stimulus driving the elevation of serum FGF-23 in chronic renal failure is not certain. Many of the uncertainties concerning the role of FGF-23 and chronic renal insufficiency would be resolved with an analysis of the different types of peptide present within the serum of such patients and a determination of the bioactivity of FGF-23 fragments. Such studies are currently under way.

**Humoral hypercalcemia of malignancy and hyperparathyroidism.** Patients with humoral hypercalcemia of malignancy or primary hyperparathyroidism have elevated serum calcium concentrations as well as hypophosphatemia. It is possible that increased circulating FGF-23 could contribute to the hypophosphatemia seen in individuals with these disorders. Recently, serum FGF-23 has been shown to be elevated in patients with humoral hypercalcemia of malignancy (127). The concentrations are elevated 5- to 10-fold. Interestingly, the elevations in FGF-23 are not correlated with the concentrations of serum phosphorus. The finding of elevated FGF-23 concentrations in patients with humoral hypercalcemia of malignancy may explain the reduced 1α,25-dihydroxyvitamin D concentrations seen in these patients (118). Patients with primary hyperparathyroidism have slightly elevated FGF-23 concentrations that do not change after parathyroidectomy (127).
Ovarian cancer. Because FGF physiology is known to be altered in patients with ovarian cancer, we measured FGF-23 concentrations in patients with early and late ovarian cancer (126). Interestingly, we found that patients with stage three and four ovarian cancer had elevated FGF-23 concentrations when measured by both COOH-terminal and intact FGF-23 immunoassays. It is interesting to speculate that FGF-23 may be a tumor marker as it is increased in both ovarian cancers and in patients with humoral hypercalcemia of malignancy. In these circumstances, FGF-23 appears not to correlate with phosphate concentrations and might indicate that FGF-23 concentrations need to be significantly elevated to induce hypophosphatemia, or that other factors are involved in the pathogenesis of low phosphate concentrations and renal phosphate wasting.

Tumoral calcinosis. Tumoral calcinosis is an unusual disorder associated with hyperphosphatemia, elevated 1α,25(OH)2D3 concentrations, and reduced excretion of phosphorus in the urine. This disorder has recently been shown to be due to a defect in the activity of an O-linked glycosylating enzyme, GALNT3 (128). Interestingly, serum FGF-23 concentrations are high in these patients. It is uncertain from the published report whether the serum FGF-23 concentrations were measured using an assay that detects intact FGF-23 or FGF-23 fragments as well as the intact molecule. It is possible that there could be defects in the processing of FGF-23 as a result of a failure in glycosylation of the molecule, or as a result of the chronic hypophosphatemia seen in these patients. A recent report suggests that the hyperostosis-hyperphosphatemia syndrome is an allelic disorder similar to tumoral calcinosis (40). No reports of FGF-23 concentrations in this syndrome are available.

BIOLICAL PROPERTIES OF sFRP-4

sFRP-4 was among the most consistently overexpressed genes found associated with oncogenic osteomalacia (Table 2). To assess whether sFRP-4 has phosphatonin activity, it was expressed by recombinant methods in COS or insect cells (10). Increasing concentrations of the recombinant protein were added to opossum kidney cells to determine whether it inhibits sodium-dependent phosphate transport. We observed that sFRP-4 inhibited sodium-dependent phosphate transport in opossum kidney cells in a dose-dependent manner at concentrations in the picograms per milliliter range (Fig. 5A). When infused into rats, sFRP-4 increased renal phosphate excretion at both 2 and 8 h following initiation of the sFRP-4 infusion (10) (Figs. 5C and 6A).

![Fig. 6. A: effect of infusion of sFRP-4 on solute excretion in intact rats. Intact rats were administered sFRP-4 (filled bars; group 2) at a dose of 0.3 μg·kg⁻¹·h⁻¹ or vehicle (open bars; group 1) by intravenous infusion over a period of 2 h. C1, equilibration period before the infusion of sFRP-4 or vehicle; C2, experimental period during which sFRP-4 or vehicle was infused. Fractional excretion of inorganic phosphate (FEPi), sodium (FENa), and calcium (FECa) were measured as described in the text. *P < 0.05. From Ref. 10 with permission. B: effect of the infusion of sFRP-4 on solute excretion in thyroparathyroidectomized rats. Thyroparathyroidectomized rats were administered sFRP-4 (filled bars; group 4) at a dose of 0.3 μg·kg⁻¹·h⁻¹ or vehicle (open bars; group 3) by intravenous infusion over a period of 2 h. FEpi, FENa, and FECa were measured as described in the text. *P < 0.05. From Ref. 10 with permission. C: Western blots of renal homogenates obtained from rats infused with vehicle (control) or sFRP-4 for 8 h. Antibodies against β-catenin (top) or phosphorylated β-catenin (phospho β-catenin; bottom) were used to detect proteins. From Ref. 10 with permission.](http://ajprenal.physiology.org/)}
Minimal changes in sodium excretion were seen, and calcium excretion did not change. Interestingly, the effects of sFRP-4 were also demonstrated in parathyroidectomized rats, thus demonstrating that parathyroid hormone was not essential for the phosphaturic effect of sFRP-4 (Fig. 6B). During an 8-h intravenous infusion of sFRP-4, serum phosphate concentrations decreased and phosphate excretion increased. However, no change in 25-hydroxyvitamin D 1α-hydroxylase mRNA concentrations was noted in the kidney. The infusion of sFRP-4 was associated with a decrease in β-catenin concentrations in renal cells and an increase in phosphorylated β-catenin, thereby demonstrating that sFRP-4 may act as an antagonist against Wnt molecules in the kidney (Fig. 6C). Additionally, sFRP-4 was detected in the plasma of patients with tumor-induced osteomalacia, although elevated levels were not found with the current assay. Thus the data published to date suggest that sFRP-4 is a phosphatonin. Details concerning the mechanism by which FRP-4s inhibits renal phosphate reabsorption and its relationship to FGF-23 will need to be elucidated in the future.

BIological PROPERTIES OF MEPE

MEPE is also among the most abundantly overexpressed mRNA species found in tumors associated with renal phosphate wasting and osteomalacia (29, 98, 100). Recently, MEPE has been expressed in insects cells and administered to mice in vivo (100). The protein causes renal phosphate wasting and a reduction in serum phosphate concentrations in vivo. Additionally, inhibition of sodium-dependent phosphate uptake was noted in opossum kidney cells exposed to the protein. MEPE also appears to inhibit bone mineralization in vitro, and MEPE null mice have increased bone mineralization (42). This suggests that it may play a role in the pathogenesis of X-linked hypophosphatemic rickets, in which there is phosphate wasting and evidence for a mineralization defect that is independent of low phosphate concentrations in the extracellular fluid (135).

Recent evidence suggests that concentrations of this substance is increased in the serum of patients with X-linked hypophosphatemic rickets (18). It has been suggested that MEPE is a substrate for PHEX (47, 99) and that PHEX prevents proteolysis of MEPE and release of a protease-resistant MEPE-ASARM peptide, an inhibitor of mineralization (minihinbin). Phex may be acting to interfere with the actions of other enzymes that degrade extracellular matrix proteins. PHEX and MEPE form a nonproteolytic protein interaction via the MEPE COOH-terminal ASARM motif. The ASARM peptide is believed to inhibit mineralization in vivo. The binding of MEPE and ASARM peptide by PHEx may explain why loss of functional osteoblast-expressed PHEX results in defective mineralization in Hyp. MEPE concentrations have been measured in normal humans, and concentrations of the protein appear to correlate with bone mineral density and serum phosphate concentrations (54).

FGF-7 IS OVEREXPRESSED IN TUMORS ASSOCIATED WITH OSTEOMALACIA AND RENAL PHOSPHATE WASTING

A recent report has shown that FGF-7 is overexpressed in tumors associated with osteomalacia and renal phosphate wasting (24). FGF-7 protein inhibited sodium-dependent phosphate transport in opossum kidney cells. Anti-FGF-7 antibodies attenuated the inhibitory effect of tumor supernatants on sodium-dependent phosphate transport. Only low concentrations of FGF-23 were present in the conditioned medium of tumor cells. At present it is not known whether FGF-7 circulates in plasma, whether it alters 25 hydroxyvitamin D 1α-hydroxylase levels, or whether it is elevated in the plasma of subjects with tumor-induced osteomalacia. Nevertheless, the report does point to the complexity of factors involved in the pathogenesis of tumor-induced osteomalacia.

UNANSWERED QUESTIONS AND FUTURE DIRECTIONS FOR RESEARCH

Several new phosphaturic peptides have now been isolated from tumors associated with renal phosphate wasting and osteomalacia. The most information concerning these various peptides is available for FGF-23. Concentrations of this protein are elevated in most patients with tumor-induced osteomalacia and are reduced following removal of the tumor. Of interest, however, is the observation that FGF-23 concentrations are elevated in patients who do not have oncogenic osteomalacia but have tumors associated with humoral hypercalcemia of malignancy. Another report shows that FGF-23 concentrations are elevated in patients with malignancies not associated with phosphate wasting. Hence, it is possible that FGF-23 concentrations must be significantly elevated before the occurrence of hypophosphatemia or that other factors might also need to be formed in excessive amounts to cause hypophosphatemia in tumor-induced osteomalacia.

Another key, and unresolved question, relates to whether these peptides play a role in the control of phosphate homeostasis under normal circumstances. It is attractive to consider that FGF-23 may play a role in the control of renal phosphate reabsorption under normal circumstances. Studies performed in humans, however, have shown either no or only modest changes in FGF-23 concentrations following the alteration of dietary phosphate. Preliminary data in rodents suggest that serum FGF-23 may be significantly altered by dietary phosphate (89). Thus it is still unclear whether changes in serum FGF-23 may be responsible for alterations in phosphate excretion in response to changes in serum phosphate levels.

Less information is available concerning role of sFRP-4 and MEPE in various clinical disorders. In part, this relates to the fact that robust assays that distinguish modified forms such as proteolytic fragments derived from the intact molecule are not available for these proteins. Furthermore, analysis of FRP-4 and MEPE expression in various clinical conditions is just beginning.

The question as to why there are multiple phosphatonin molecules needs to be resolved in the future. It is possible that one or the other molecule acts downstream of the other. For example, the administration of FGF-23 may be associated with an induction of sFRP-4 in renal cells. FGF-23 may have similar effects on MEPE in bone cells. Suffice it to say that study of rare diseases associated with phosphate wasting or abnormal phosphate retention has provided new insights into the mechanisms by which phosphate homeostasis is regulated.

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REFERENCES


In this invited review, Hernando N, Forgo J, Biber J, and Murer H. discuss the regulation of small intestinal Na-P, type IIb cotransporter by dietary phosphate intake. They explore the role of PTH in regulating phosphate homeostasis and the impact of phosphate on tumor-induced osteomalacia. Additionally, they examine the effects of dietary phosphate on renal phosphate transport in normal and uremic dogs, and the role of fibroblast growth factor 23 in oncogenic osteomalacia. The review also covers the impact of dietary phosphate on the kidney: physiology and pathophysiology, and the interplay between phosphorus, calcium, and hypophosphatemia. The authors conclude with a discussion on the clinical implications of these findings, emphasizing the importance of dietary phosphate in maintaining bone health and preventing metabolic bone disease.


