Phosphatonins and the Regulation of Phosphate Homeostasis

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Key Words

phosphatonins, fibroblast growth factor-23, secreted frizzled related protein-4, matrix extracellular phosphoglycoprotein, fibroblast growth factor-7, sodium-phosphate cotransporters, parathyroid hormone, 1α,25-dihydroxyvitamin D

Abstract

Inorganic phosphate (P_i) is required for energy metabolism, nucleic acid synthesis, bone mineralization, and cell signaling. The activity of cell-surface sodium-phosphate (Na^+-P_i) cotransporters mediates the uptake of P_i from the extracellular environment. Na^+-P_i cotransporters and organ-specific P_i absorptive processes are regulated by peptide and sterol hormones, such as parathyroid hormone (PTH) and 1α,25-dihydroxyvitamin D (1α,25$(OH)_2D_3$), which interact in a coordinated fashion to regulate P_i homeostasis. Recently, several phosphaturic peptides such as fibroblast growth factor-23 (FGF-23), secreted frizzled related protein-4 (sFRP-4), matrix extracellular phosphoglycoprotein, and fibroblast growth factor-7 have been demonstrated to play a pathogenic role in several hypophosphatemic disorders. By inhibiting Na^+-P_i transporters in renal epithelial cells, these proteins increase renal P_i excretion, resulting in hypophosphatemia. FGF-23 and sFRP-4 inhibit 25-hydroxyvitamin D 1α-hydroxylase activity, reducing 1α,25$(OH)_2D_3$ synthesis and thus intestinal P_i absorption. This review examines the role of these factors in P_i homeostasis in health and disease.

Na^+-P_i cotransporters: sodium-phosphate cotransporters

$1\alpha,25(OH)_2D_3$: 1α,25-dihydroxyvitamin D

PTH: parathyroid hormone

THE IMPORTANCE OF PHOSPHOROUS IN BIOLOGICAL PROCESSES

Phosphorus and inorganic phosphate (P_i) play important roles in a variety of biological processes such as cell signaling, nucleic acid synthesis, energy metabolism, membrane function, and bone mineralization (1–5). P_i is required for optimal cellular growth, and serum P_i concentrations, as well as renal P_i reabsorption, are higher in rapidly growing animals than in adults (6, 7). Owing to the involvement of P_i in diverse biological processes, decrements in serum P_i concentrations and a negative P_i balance can result in serious disease. Acute decreases in serum P_i concentrations can result in myopathy, cardiac dysfunction, abnormal neutrophil function, platelet dysfunction, and red-cell membrane fragility (8, 9). Chronic serum P_i deficiency results in impaired bone mineralization, rickets, and osteomalacia because the rate of bone matrix mineralization depends on the availability of phosphorus and calcium (5). Elevated serum P_i concentrations contribute to the pathogenesis of secondary hyperparathyroidism in patients with chronic renal failure (10–14).

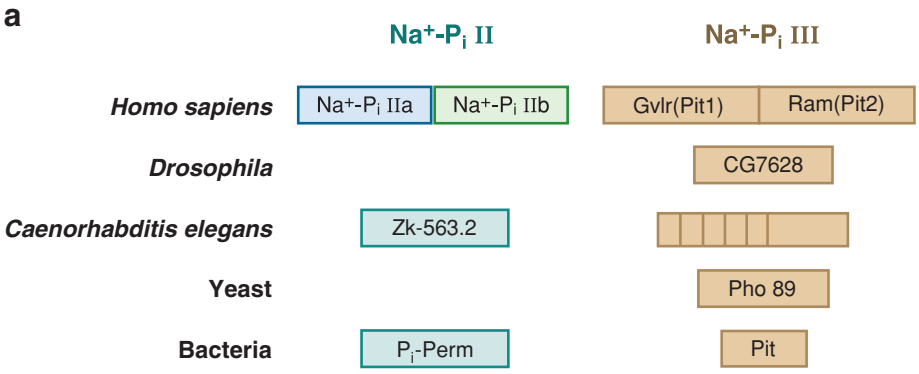
MECHANISMS OF CELLULAR P_i UPTAKE

Because a cell's interior is electronegative relative to the exterior, the movement of P_i into the cell does not occur by simple diffusion (15). Various H^+- or Na^+-coupled P_i cotransporters mediate the transport of P_i across cell membranes (15). The structure and function of these have been extensively reviewed, and the reader is directed to other publications in this regard (15–20). The Na^+-coupled P_i cotransporters that are important in P_i uptake in vertebrates belong to two large families, the Na-P_i type II and the Na-P_i type III families. **Figure 1*a*** shows the different families of Na-P_i transporters, and **Figure 1*b*** summarizes the relationships of Na-P_i type II protein sequences to one another (15). The Na-P_i transporters are highly homologous. Na^+-P_i IIa (Npt2) transporters are the most abundant in the kidney and contribute approximately 85% of proximal tubule Na-P_i reabsorption (19, 20). Researchers recently identified, in rat and human kidney, a Na-P_i type IIc transporter that is maximally upregulated in *Npt2*$^{-/-}$ mice and is thought to account for the residual P_i transport in the *Npt2* knockout mice (21, 22).

PHOSPHATE HOMEOSTASIS IN HUMANS AND MAMMALS

In humans, absorption and reabsorption of P_i occur primarily in the intestine and kidney, respectively (see **Figure 2**). In states of neutral P_i balance, the amount of P_i absorbed in the intestine (approximately 1–1.5 g per 24 h) is equivalent to the amount excreted in the urine. 1α,25-Dihydroxyvitamin D ($1\alpha,25(OH)_2D_3$) increases the efficiency of P_i absorption in the intestine, although there is evidence for a $1\alpha,25(OH)_2D_3$-independent increase in P_i transport during P_i deprivation that occurs in the absence of the $1\alpha,25(OH)_2D_3$ receptor (23–28). In the kidney, P_i is reabsorbed along the proximal convoluted and proximal straight tubules (29, 30). Various factors, most importantly parathyroid hormone (PTH), influence the efficiency of renal P_i reabsorption (29, 31–33).

The vitamin D endocrine system and PTH interact to regulate P_i absorption in the intestine and reabsorption in the kidney, as demonstrated in **Figure 3** (30). Animals fed a low-P_i diet have decreased serum P_i concentrations that are associated with a reciprocal increase in circulating plasma calcium concentrations. The increase in plasma calcium concentrations inhibits PTH release, which in turn reduces the renal excretion of P_i. Additionally, a low-P_i diet and reductions in serum P_i are associated with increased $1\alpha,25(OH)_2D_3$ synthesis as a result of stimulation of 25-hydroxyvitamin D 1α-hydroxylase activity (27, 34). Conversely, when animals are fed a high-P_i diet, serum



b

	Source RNA	Length	Identity with human isoform
Na^+-P_i IIa	Mouse kidney	637	90.9
	Rat kidney	638	91.2
	Human kidney	639	100
	Rabbit kidney	642	89.5
	Sheep kidney	639	89.5
	Opossum kidney cells	653	82.3
	Chicken kidney	240*	73.8
Na^+-P_i IIb	Trout kidney	290*	62.4
	Shark kidney	290*	65.9
	Skate kidney	290*	66.2
	Xenopus intestine	674	63.3
	Chicken intestine	230*	70.4
	Human intestine	690	100
	Mouse intestine	697	78.7
	Bovine cell line	693	67.7
	Carp kidney	636	64.3
	Zebrafish kidney	520*	62.8
	Trout intestine	290*	68.8
	Flounder kidney/intestine	636	63.3
	Zebrafish intestine	632	65.8
	Shark intestine	230*	67.0
	Skate intestine	230*	63.0

*Fragment

Figure 1

(*a*) Different families of P_i translocating proteins. (*b*) Summary of Na^+-P_i-related protein sequences from vertebrates. Both *a* and *b* are from Reference 15 with permission.

calcium concentrations decrease, and PTH release is increased. Recent data suggest that PTH release can occur in the absence of changes in serum P_i or calcium concentrations. Following the instillation of high-P_i diets directly into the intestine, changes in PTH secretion in response to dietary P_i occur rapidly (within 10 min) and independently of changes in serum P_i or calcium (35, 36). A signal emanating from the intestine, similar to that which has been documented for Na^+ (37–39), may affect P_i reabsorption by the kidney (40, 41). An elevation in serum P_i after a high-P_i meal reduces 25-hydroxyvitamin D 1α-hydroxylase activity and results in reduced circulating $1\alpha,25(OH)_2D_3$ and diminished

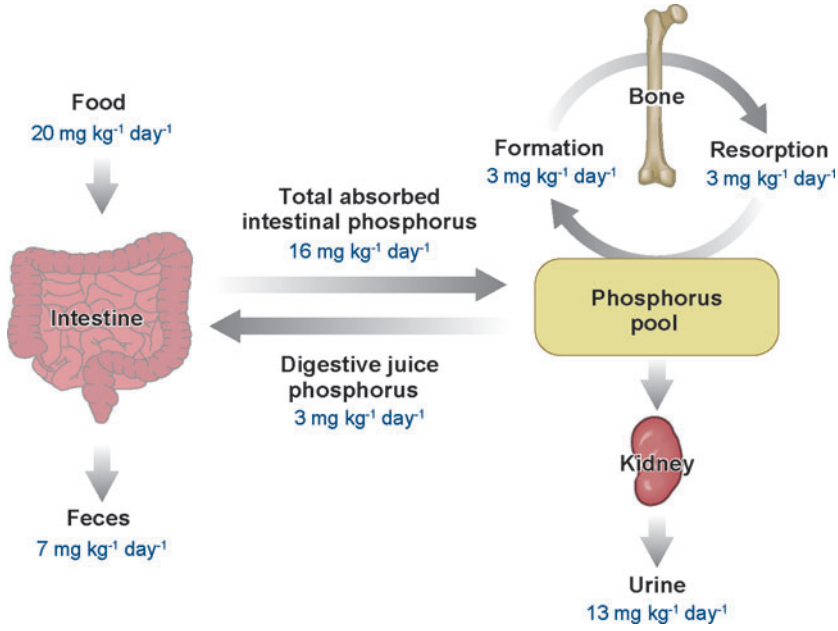


Figure 2

Quantitative aspects of phosphorus homeostasis in humans. Based on Reference 30. The intestine and kidney play major roles in phosphate (P_i) homeostasis by regulating absorption and excretion, respectively. The movement of P_i into bone and soft tissue also determines serum P_i concentrations.

intestinal P_i absorption. **Table 1** lists various factors influencing phosphate absorption in the intestine.

Given the importance of P_i ions in cellular function, various other mechanisms have evolved to maintain P_i balance. Although PTH and the vitamin D endocrine system are involved in the regulation of P_i absorption in the intestine and reabsorption in the kidney, other factors such as arterial CO_2 ($paCO_2$), adrenergic agents, and dopamine can rapidly modulate the phosphaturic effect of PTH to

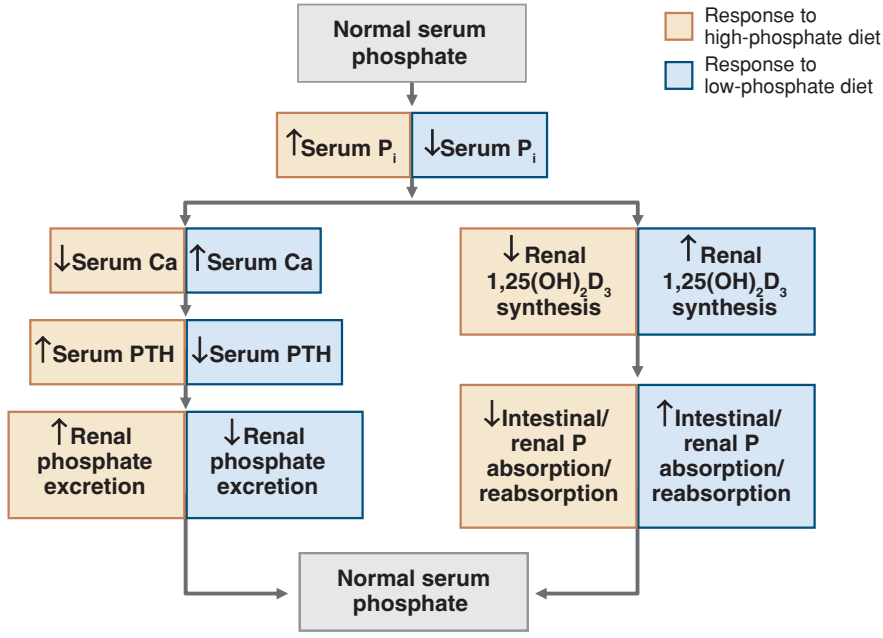


Figure 3

Mechanisms by which phosphate (P_i) homeostasis is maintained. In response to dietary phosphate intake, the vitamin D endocrine system and parathyroid hormone (PTH) interact to regulate phosphate absorption in the intestine and reabsorption in the kidney. From Reference 30 with permission.

alter P_i homeostasis (29). The phosphaturic response to PTH is progressively attenuated by a low-P_i diet. P_i deprivation for two days or fewer results in an attenuated phosphaturic response to PTH that is restored by propanolol infusion, suggesting that stimulation of β adrenoreceptors occurs during short-term P_i deprivation (42). However, P_i deprivation for three days or more results in complete resistance to the phosphaturic response to PTH.

The enhanced renal P_i reabsorption as a result of selective P_i deprivation is rapidly reversed by total fasting through mechanisms that are not understood (43, 44). Rapid changes in renal P_i reabsorption occur independently of changes in serum PTH as well as in the absence of PTH. Cultured proximal tubule cells exposed to low P_i concentrations in the culture medium rapidly increase P_i transport, demonstrating an intrinsic ability of these proximal tubule cells to sense P_i and adapt by increasing P_i transport (45). Serum P_i concentrations are also altered as a result of the movement of P_i from the extracellular fluid space into bone and soft tissues, and the rate at which this occurs is influenced by a number of factors, including blood pH and various peptide hormones. Taken together, these observations suggest the existence of autocrine and paracrine factors that acutely alter the phosphaturic response to PTH and/or directly alter renal P_i reabsorption. **Table 2** summarizes various factors influencing phosphate reabsorption in the kidney.

Table 1 Factors influencing phosphate absorption in the intestine

Factors that increase P_i absorption
Reduced dietary intake of phosphate
Elevated serum 1α,25-dihydroxyvitamin D
Factors that reduce P_i absorption
Reduced serum 1α,25-dihydroxyvitamin D
Elevated concentrations of calcium salts or phosphate binders, e.g., aluminum hydroxide and sevelamer, in intestinal lumen

THE PHOSPHATONINS AND THE REGULATION OF RENAL P_i REABSORPTION

In 1994, our laboratory described the existence of a factor or factors (called the phosphatonins) that induced renal P_i wasting in patients with tumor-induced osteomalacia (TIO) (46, 47). Patients with TIO typically exhibit low serum P_i concentrations, normal or slightly low serum calcium concentrations, normal PTH concentrations, inappropriately low serum $1\alpha,25(OH)_2D_3$ concentrations, renal P_i wasting, and a defect in bone mineralization (47–49). This phenotype is also observed in patients with autosomal-dominant hypophosphatemic rickets (ADHR) and X-linked hypophosphatemic rickets (XLH) (50–53). We demonstrated that conditioned medium from a tumor associated with TIO produced a substance or substances that inhibited Na^+-dependent P_i transport in cultured opossum kidney cells (46). This heat-labile substance(s) was of M_r 10–30,000 and inhibited Na^+-dependent P_i transport by a process independent of cyclic AMP. The activity of the substance was not blocked by a PTH receptor antagonist. This suggested that the factor was

Table 2 Factors influencing phosphate reabsorption in the kidney

Factors that increase P_i reabsorption
Phosphate depletion
Parathyroidectomy
$1\alpha,25(OH)_2D_3$
Volume contraction
Hypocalcemia
Hypocapnia
Factors that decrease P_i reabsorption
Phosphate loading
Parathyroid hormone and cyclic AMP
Volume expansion
Hypercalcemia
Carbonic anhydrase inhibitors
Dopamine
Glucose and alanine
Acid-base disturbances (increased bicarbonate, hypercapnia)
Metabolic inhibitors (e.g., arsenate)
FGF-23
SFRP-4
MEPE
FGF-7

not one already known to influence P_i transport in epithelia.

Several laboratories subsequently showed that factors such as fibroblast growth factor-23 (FGF-23), secreted frizzled related protein-4 (sFRP-4), fibroblast growth factor 7 (FGF-7), and matrix extracellular phosphoglycoprotein (MEPE) are present in these tumors and contribute to the phosphaturia associated with this syndrome (30, 53–61). Identification of these phosphatonin molecules has led to the recognition that these proteins are also involved in other pathophysiological conditions associated with P_i wasting and may contribute to the physiological regulation of renal P_i reabsorption.

Fibroblast Growth Factor-23

Biological properties of FGF-23 and its role in the pathogenesis of ADHR, TIO, and XLH. Patients with ADHR typically exhibit low serum P_i concentrations, normal or slightly low serum calcium concentrations, normal PTH concentrations, low or inappropriately normal serum $1\alpha,25(OH)_2D_3$ concentrations, renal P_i wasting, and a defect in bone mineralization. Using positional cloning methods, the ADHR Consortium (53) identified the mutant gene responsible for the disease in these patients. The mutant gene encodes a novel fibroblast growth factor, FGF-23. The *FGF-23* mutation results in the production of a stable and long-lived form of FGF-23. The wild-type FGF-23 protein contains a furin proconvertase site (176 RHTR 179), and proteolysis results in the formation of the amino-terminal fragment of approximately 16 kDa and a smaller fragment of approximately 12 kDa (62). In ADHR patients, the FGF-23 expressed from the mutant gene lacks a normal furin proconvertase site (176 QHTR 179; 176 RHTW/Q 179) (53, 63).

Shimada et al. (64) showed that FGF-23 was overexpressed in a tumor of a patient with TIO. Recombinant FGF-23 administered intraperitoneally to mice induced hypophosphatemia but did not alter serum calcium concentrations. When Chinese hamster ovary cells transfected with an FGF-23 expression plasmid were implanted in nude mice, these animals became hypophosphatemic, and the urinary fractional excretion of P_i was increased within 10 days. Radiological and histological signs of rickets in the long bones were seen after several weeks. Decreased mRNA for the 25-hydroxyvitamin D 1α-hydroxylase cytochrome P450 was observed in the kidneys of these nude mice. In support of these studies, Bowe et al. (65) demonstrated that recombinant FGF-23 inhibited Na^+-dependent P_i transport in opossum kidney cells. Additionally, these authors showed that FGF-23 was the subject of proteolysis by recombinant PHEX (phosphate-regulating gene with homology to endopeptidases on the X chromosome), the endopeptidase mutated in patients with XLH (52). Intravenous infusion of recombinant FGF-23 into mice caused a rapid, dose-dependent increase in the fractional excretion of P_i with little or no change in Na^+ excretion, suggesting that FGF-23 has direct actions on renal P_i transport (**Figure 4**) (60). Thus, FGF-23 is at least one of the phosphatonins responsible for the pathogenesis of hypophosphatemia, renal P_i wasting, and reduced serum $1\alpha,25(OH)_2D_3$ concentrations in patients with TIO. Cure of the disease phenotype and decreases in FGF-23 serum concentrations after tumor removal in patients with TIO support the role of FGF-23 in the pathogenesis of this condition (66–69).

Researchers have generated transgenic animals overexpressing FGF-23 to elucidate further the ability of FGF-23 to decrease serum P_i concentrations, increase renal P_i excretion, and inhibit 25-hydroxyvitamin D 1α-hydroxylase activity (70, 71). As expected, these mice are hypophosphatemic, exhibit excessive renal P_i excretion, and have rickets and either reduced serum $1\alpha,25(OH)_2D_3$ concentrations or 25-hydroxyvitamin D 1α-hydroxylase activity. Conversely, mice in which the *FGF-23* gene has been ablated demonstrate hyperphosphatemia, reduced renal P_i excretion, and elevated serum

Phosphatonins: phosphaturic substances responsible for the pathogenesis of disorders such as tumor-induced osteomalacia, X-linked hypophosphatemic rickets, and autosomal-dominant hypophosphatemic rickets

Tumor-induced osteomalacia (TIO): a syndrome associated with mesenchymal tumors and characterized by hypophosphatemia, renal phosphate wasting, diminished or inappropriately low serum $1\alpha,25(OH)_2D_3$ concentrations, and rickets or osteomalacia

Autosomal-dominant hypophosphatemic rickets (ADHR): an inherited disorder with phenotype similar to that seen in TIO and XLH

X-linked hypophosphatemic rickets (XLH): an inherited disorder with phenotype similar to that seen in TIO

Secreted frizzled related protein-4 (sFRP-4): a Wnt antagonist

MEPE: matrix extracellular phosphoglycoprotein

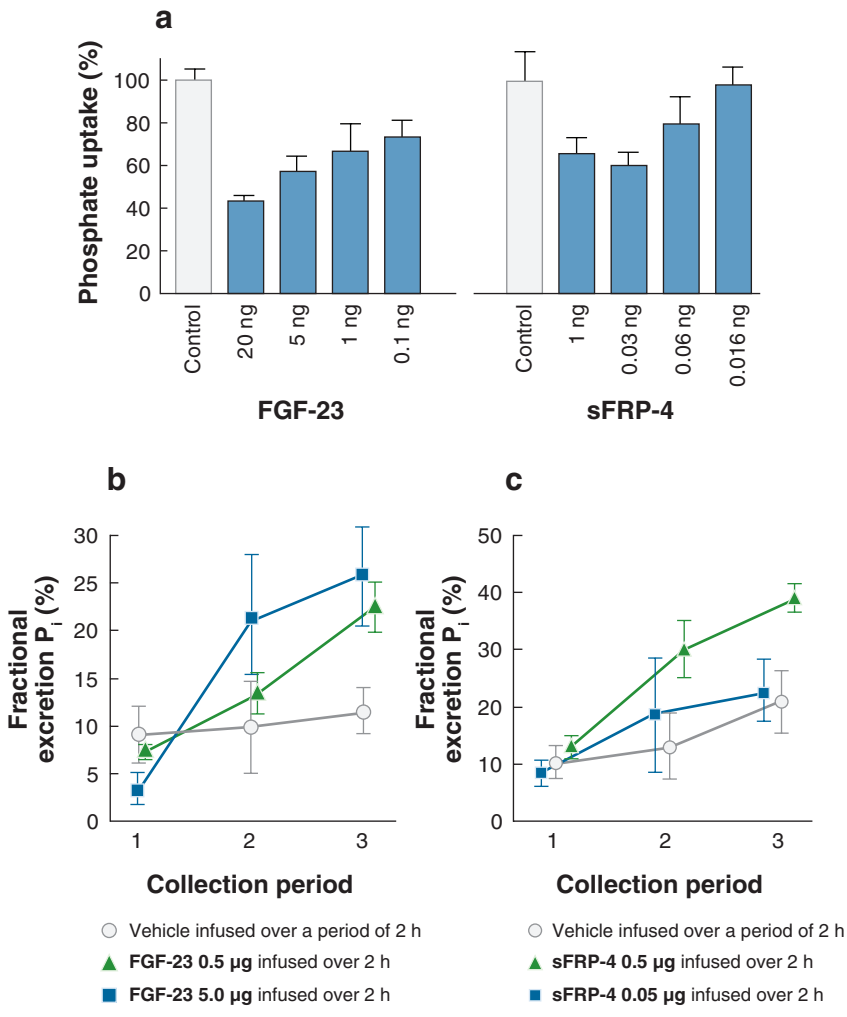


Figure 4

Biological effects of FGF-23 and sFRP-4 in opossum kidney cells in vitro (*a*) and in normal mice in vivo (*b*). (*a*) Opossum kidney cells were maintained in culture, and Na^+-dependent P_i transport was measured following the addition of either FGF-23 (*left panel*) or sFRP-4 (*right panel*). (*b*) The effect of infused FGF-23 on the fractional excretion of P_i in mice. (*c*) The effect of infused sFRP-4 on the fractional excretion of P_i in mice. Figure from Reference 30 with permission.

$1\alpha,25(OH)_2D_3$ concentrations and renal 25-hydroxyvitamin D 1α-hydroxylase mRNA expression (72).

In patients with XLH, mutations of the gene encoding the endopeptidase PHEX are believed to be responsible for the disease phenotype (50–52). Parabiosis and kidney cross-transplantation have clearly shown that there is a circulating hypophosphatemia-inducing factor present in the serum of *Hyp* mice (the mouse homolog of human XLH) (73–75). PHEX may be responsible for the degradation of a phosphatonin (65, 76). FGF-23 is likely the phosphatonin degraded by PHEX. Studies by Bowe et al. (65) with an FGF-23 and recombinant PHEX and the studies of Campos et al. (76) with FGF-23 peptides support a role for PHEX in processing and inactivating FGF-23. Recently, Liu et al. (77) demonstrated that deletion of the *FGF-23* gene in PHEX-deficient mice results in the abolition of the hypophosphatemic phenotype seen in mice deficient in PHEX alone. Furthermore, individuals with XLH and *Hyp* mice have elevated FGF-23 concentrations (67).

Thus, FGF-23—by virtue of its ability to increase renal P_i excretion, inhibit 25-hydroxyvitamin D 1α-hydroxylase activity and $1\alpha,25(OH)_2D_3$ synthesis, and reduce intestinal P_i absorption—likely is involved in

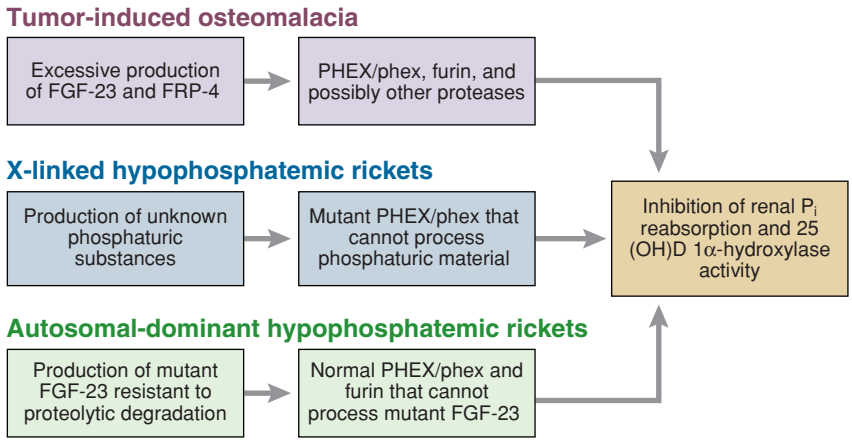


Figure 5

Mechanisms by which hypophosphatemia occurs in tumor-induced osteomalacia (TIO), X-linked hypophosphatemic rickets (XLH), and autosomal-dominant hypophosphatemic rickets (ADHR).

the pathogenesis of at least three hypophosphatemic conditions, namely, ADHR, TIO, and XLH. The mechanisms by which the disease phenotype occurs in each of these conditions are summarized in **Figure 5**.

The study of patients with a rare disorder, tumor calcinosis (TC), has also yielded interesting information concerning the biological properties of FGF-23. Patients with TC have a biochemical phenotype that is the opposite of that seen in patients with TIO, XLH, and ADHR. TC subjects have hyperphosphatemia, reduced renal P_i excretion, and elevated $1\alpha,25(OH)_2D_3$ concentrations. Two different types of mutations account for this syndrome. The first type of mutation is in the gene *GALNT3*, which encodes a glycosyltransferase responsible for initiating mucin-type O-glycosylation (79). Patients with this syndrome have elevated concentrations of FGF-23, as measured by an assay that detects the carboxyl-terminal portion of the molecule. These patients have relatively low normal concentrations of FGF-23, as measured by an assay that detects only the intact protein. The defect in glycosylation may interfere with the processing of FGF-23, and low concentrations of the intact peptide may be responsible for the biochemical phenotype. The second type of mutation seen in patients with tumoral calcinosis occurs within the *FGF-23* gene itself (80–83). Two recessive mutations in FGF-23, serine 71/glycine (S71G) and serine 129/phenylalanine (S129F), have been identified in patients with TC. These patients have elevated FGF-23 concentrations, as measured by an assay that detects carboxyl-terminal fragments and the carboxyl-terminal portion of the protein. However, FGF-23 concentrations measured by the full-length protein assay are apparently low to normal. Defects in the processing of these proteins in the Golgi apparatus may be responsible for the differences in FGF-23 concentrations measured by the two assays.

Fibroblast growth factor-23 (FGF-23) and fibroblast growth factor-7 (FGF-7): growth factors known to cause hypophosphatemia and the inhibition of sodium-phosphate cotransport in renal epithelial cells. FGF-23 also inhibits 25-hydroxyvitamin D 1α-hydroxylase activity, thereby reducing $1\alpha,25(OH)_2D_3$ synthesis

Tumoral calcinosis (TC): a disorder that is the mirror image of TIO, XLH, and ADHR and characterized by hyperphosphatemia, reduced renal phosphate excretion, and elevated or inappropriately high serum $1\alpha,25(OH)_2D_3$ concentrations

Mechanism of action of FGF-23. In vitro binding studies suggest that FGF-23 binds and signals through one of the known FGF receptors. Yamashita et al. (84) demonstrated that FGF-23 binds with high affinity to the FGF receptor 3c, which is mainly expressed in opossum kidney cells. Yu et al. (85) showed that FGF-23 is bound to the c splice isoform of FGFRs 1–4. Yan et al. (86) showed that FGF-23 binds FGFR2 in opossum kidney cells but also presented evidence for a novel FGF-23 receptor on the basolateral surface of the cells. Kurosu et al. (87) recently demonstrated that the binding of FGF-23 to FGFRs 1c, 3c, and 4 is enhanced in the presence of klotho, a membrane protein that shares sequence similarity with the β-glucosidase enzymes. The published data suggest that FGF-23 binds to the c splice isoforms of FGFRs and that the interaction is enhanced by klotho. That other, novel

receptors for FGF-23 may be present in cells that respond to FGF-23 cannot be excluded.

FGF-23 activates the mitogen-activated protein kinase (MAPK) pathway, which is the major intracellular signaling pathway of FGF-23 (84). An inhibitor for tyrosine kinases of the FGF-23 receptor, SU 5402, blocks the activity of FGF-23 (84). Additionally, inhibitors of the MAPK pathway, PD98059 and SB203580, also block the activity of FGF-23. Given that klotho increases the affinity of FGF-23 for FGFRs, it is interesting that klotho significantly enhances the ability of FGF-23 to induce phosphorylation of a FGF receptor substrate and extracellular signal–regulated kinase (ERK) in various types of cells (87). FGF-23 causes a redistribution and internalization of Na^+-P_i IIa cotransporters on the surface of renal epithelial cells (71, 86, 88).

Regulation of FGF-23 by P_i. From a physiological perspective, it would be appropriate for FGF-23 concentrations to be regulated by the intake of dietary phosphorus and by serum P_i concentrations. Studies in humans as well as animal models have examined FGF-23 concentrations following changes in dietary P_i intake. In humans, short-term alterations in dietary P_i intake do not influence FGF-23 concentrations. Larsson et al. (78) fed human subjects normal, high-P_i, or low-P_i diets for 72 h. FGF-23 concentrations did not change substantially, suggesting that dietary P_i does not regulate FGF-23 concentrations. In a subsequent study, Ferrari et al. (89) administered a high- or a low-P_i diet to humans, with concomitant changes in dietary calcium designed to minimize changes in PTH concentrations (89). Modest decreases or increases within normal range in FGF-23 concentrations were observed following the administration of a low- or high-P_i diet, respectively. In neither of these studies were short-term changes in urinary P_i excretion studied to determine whether temporal changes in the renal excretion of P_i directly correlated with temporal changes in FGF-23 concentrations. Thus, in humans, dietary variation in P_i intake apparently has no effect, or at most an extremely modest effect, on P_i excretion in the kidney. A recent study has shown that the administration to humans of a high-P_i meal is associated with an increase in the fractional excretion of P_i within an hour of ingestion of that meal (90). In this study, there were modest changes in PTH concentrations and no changes in FGF-23 concentrations until eight hours after eating the meal, suggesting that changes in P_i excretion by the kidney were unrelated to changes in FGF-23 concentrations. These data suggest that early and rapid changes in renal P_i excretion occur following a high-P_i meal and are independent of FGF-23 concentration.

Perwad et al. (91) have shown that in mice dietary P_i intake influences FGF-23 concentrations. Within five days, a high-P_i diet increased, and a low-P_i diet decreased, serum FGF-23 concentrations in these animals. The changes in serum FGF-23 concentrations in these mice were correlated with changes in serum P_i concentrations. Similar studies from our laboratory suggest that, within 24 h, serum FGF-23 and PTH concentrations increase in response to increased dietary P_i intake and decrease in response to low P_i intake in rodents. However, serum FGF-23 concentrations did not correlate with serum P_i in animals fed a high-P_i diet (92).

Regulation of FGF-23 by $1\alpha,25(OH)_2D_3$. $1\alpha,25(OH)_2D_3$ regulates FGF-23 synthesis (93–96). Increasing doses of $1\alpha,25(OH)_2D_3$ proportionately increase FGF-23 serum concentrations. $1\alpha,25(OH)_2D_3$ administration is also associated with increases in serum P_i concentrations. The elevated concentrations of serum P_i may directly inhibit the synthesis of $1\alpha,25(OH)_2D_3$ in the kidney, but increasing FGF-23 concentrations also may inhibit $1\alpha,25(OH)_2D_3$ synthesis. Thus, there may be a negative feedback loop inhibiting $1\alpha,25(OH)_2D_3$ synthesis that involves both P_i and FGF-23. These relationships are summarized in **Figure 6**.

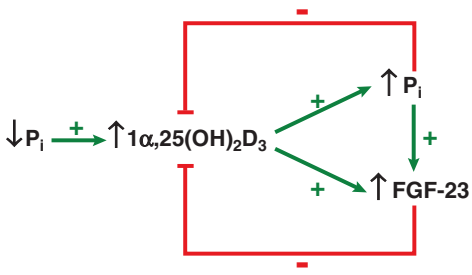


Figure 6

Relationships between concentrations of $1\alpha,25(OH)_2D_3$, serum P_i, and FGF-23.

Secreted Frizzled Related Protein-4

We used serial analysis of gene expression to detect genes that were consistently overexpressed in tumors of patients with TIO (57). sFRP-4 was among the most consistently overexpressed genes found in these tumors. To determine whether sFRP-4 played a role in the pathogenesis of this disorder, we expressed sFRP-4, using recombinant methods, and infused the protein intravenously into rats or mice (30, 56). We also tested the properties of recombinant sFRP-4 by adding it to the medium of opossum kidney cells and examining whether there was an inhibition of Na^+-P_i uptake (56). The intravenous infusion of sFRP-4 into rats increased P_i excretion at both 2 and 8 h; the phosphaturia observed at 8 h was associated with hypophosphatemia. **Figure 4** shows representative results obtained following the intravenous administration of sFRP-4 in mice. There is a dose-dependent increase in the renal fractional excretion of phosphorus in mice given intravenous sFRP-4 at 60 min (clearance 2) and 120 min (clearance 3) after initiation of the sFRP-4 infusion. The effects of sFRP-4 on 25-hydroxyvitamin D 1α-hydroxylase cytochrome P450 messenger RNA concentrations were determined following an 8-h infusion of the protein in rats (56). As noted above, serum P_i concentrations decreased. However, the expected upregulation of 25-hydroxyvitamin D 1α-hydroxylase cytochrome P450 mRNA did not occur, suggesting that sFRP-4 blocked the compensatory upregulation of 25-hydroxyvitamin D 1α-hydroxylase activity and $1\alpha,25(OH)_2D_3$ synthesis.

Mechanism of action of sFRP-4. The secreted frizzled related proteins function as antagonists of the Wnt proteins. Wnt proteins signal in cells by binding to the seven-transmembrane frizzled receptor and to its coreceptor, LRP 5/6. In cells not activated by Wnt, a complex between β-catenin, Axin, APC, and GSK3 causes phosphorylation of β-catenin and its consequent destruction (97–100). Following the binding of Wnt to frizzled receptors and LRP 5/6, phosphorylation of β-catenin is inhibited, and unphosphorylated β-catenin enters the nucleus to activate a variety of genes (97–100). When the secreted frizzled related proteins antagonize Wnt activity, the amount of phosphorylated β-catenin is increased. We demonstrated that the infusion of sFRP-4 into rats was associated with phosphaturia, a concomitant increase in the amount of phospho-β-catenin, and a decrease in the amount of nonphosphorylated β-catenin (56). Thus, sFRP-4 antagonizes Wnt signaling in the kidney. Subsequent studies demonstrated that infusion of sFRP-4 decreased Na^+-P_i cotransporter abundance in the brush border membrane of the proximal tubule and reduced surface expression of the Na^+-P_i IIa cotransporter in the proximal tubules as well as on the surface of opossum kidney cells (**Figures 7** and **8**).

Regulation of sFRP-4 by dietary P_i. sFRP-4 protein concentrations were increased in the homogenates from kidneys of rats that were fed a high-P_i diet for two weeks but not in animals fed a low-P_i diet. This suggests a possible role for sFRP-4 during increases in P_i intake (92).

MEPE

MEPE is also among the most abundantly overexpressed mRNA species found in

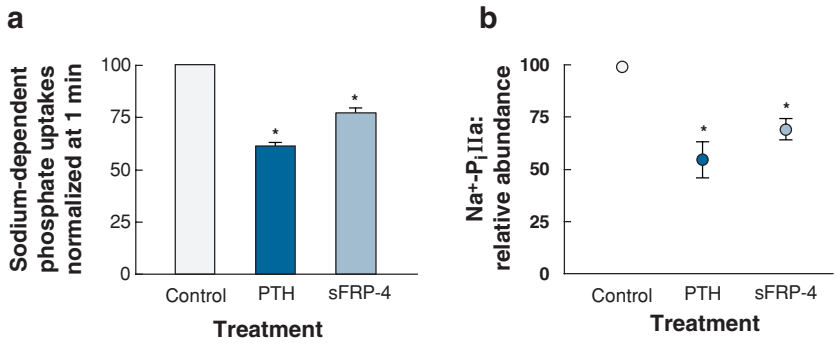


Figure 7

(*a*) Na^+-dependent P_i uptakes into brush border membrane vesicles (BBMV) and Na^+-P_i IIa abundance. Na^+-dependent Pi uptake into renal BBMV prepared from rats infused with vehicle, PTH, or sFRP-4 was measured. Data show uptake rates at 1 min in the PTH- and sFRP-4-treated groups. All data were normalized against the respective control (vehicle-infused) groups. Na^+-dependent P_i uptake following PTH or sFRP-4 treatment is statistically significant, $P < 0.001$, when compared with control. (*b*) Relative abundance of Na^+-P_i IIa protein (Na^+-P_i IIa/actin ratio) in BBMV from the same group of animals used for uptake measurements. Data are normalized and expressed as percentages relative to vehicle-infused rats ± SEM (control group = 100%); $^*P < 0.001$ for PTH compared with control; $^*P < 0.01$ for sFRP-4 versus control as analyzed by ANOVA and Bonferroni's multiple comparison test; $n = 5$–9. From Reference 105 with permission.

tumors associated with renal P_i wasting and osteomalacia (54). Recombinant MEPE expressed in insect cells induces phosphaturia and decreases serum P_i concentrations when administered to mice in vivo (55). Additionally, inhibition of Na^+-dependent P_i uptake was noted in opossum kidney cells incubated in the presence of the recombinant protein. MEPE also inhibits bone mineralization in vitro, and MEPE-null mice have increased bone mineralization (101). Thus, MEPE may be important in the pathogenesis of hypophosphatemia in renal P_i wasting observed in patients with TIO. However, MEPE infusion does not recapitulate the defect in vitamin D metabolism seen in patients with TIO. As noted above, patients with TIO have low serum P_i concentrations and inappropriately reduced or normal concentrations of serum $1\alpha,25(OH)_2D_3$. Infusion of MEPE reduces serum P_i concentrations, and serum $1\alpha,25(OH)_2D_3$ concentrations increase following MEPE infusion, as would be expected in the face of hypophosphatemia (55). Thus, in patients with TIO, it is likely that MEPE contributes to the hypophosphatemia but that other products such as FGF-23 and sFRP-4 inhibit $1\alpha,25(OH)_2D_3$ concentrations by inhibiting the activity of the 25-hydroxyvitamin D 1α-hydroxylase.

MEPE may play a role in the pathogenesis of XLH, in which there is P_i wasting and evidence for a mineralization defect that is independent of low P_i concentrations in the extracellular fluid (50, 51). Recent evidence suggests that MEPE concentration is increased in the bones of mice with the *Hyp* mutation (102). Under normal circumstances, MEPE is proteolyzed to release a peptide containing an ASARM (acidic serine-aspartate-rich motif) sequence. The latter peptide acts as an inhibitor of mineralization. MEPE may be a substrate for PHEX, and PHEX may prevent proteolysis of MEPE and release of the protease-resistant MEPE-ASARM peptide, an inhibitor of mineralization (minhibin) (102). In patients with XLH and in mice with the *Hyp* mutation, PHEX is mutated and therefore cannot bind to either MEPE or the ASARM peptide. This results in the release of MEPE into the circulation, thereby causing hypophosphatemia in renal P_i wasting.

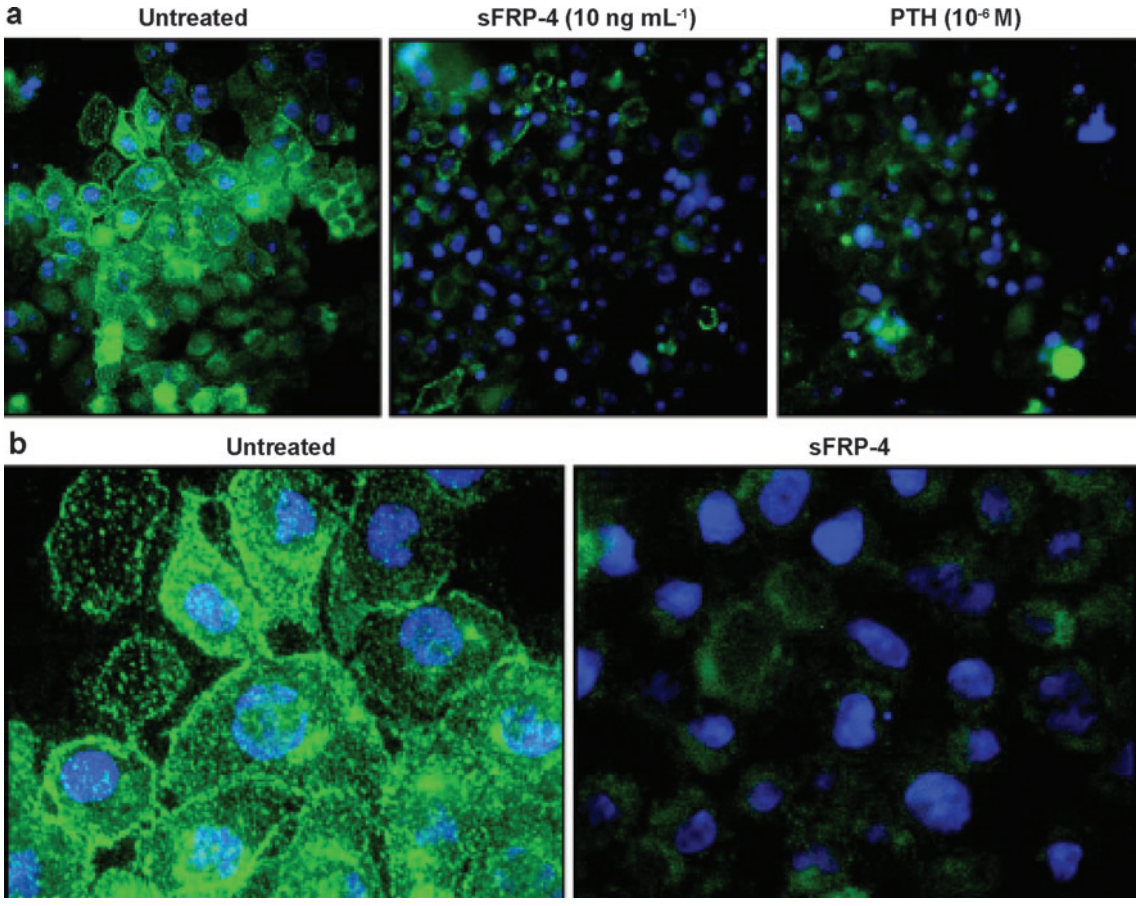


Figure 8

(*a*) Effect of addition of sFRP-4 (10 ng ml^{-1}) or PTH (10^{-6} M) on Na^+-P_i IIa distribution in opossum kidney cells expressing a chimeric Na^+-P_i IIa–V5 transporter (200 times magnification). sFRP-4 or PTH were added to cells in the concentrations indicated for 3 h. Na^+-P_i IIa distribution was examined using an antibody directed against the V5 epitope. (*b*) Effect of sFRP-4 on Na^+-P_i IIa protein distribution in opossum kidney cells expressing a chimeric Na^+-P_i IIa–V5 transporter (400 times magnification). Cells were treated with sFRP-4 (10 ng ml^{-1}) for a period of 3 h. From Reference 105 with permission.

Increased constellations of MEPE-ASARM peptide have been measured in humans with XLH and in *Hyp* mice (103). MEPE concentrations have been measured in normal humans, and concentrations of the protein appear to correlate positively with bone mineral density and serum P_i concentrations (104).

FGF-7

FGF-7, also known as keratinocyte growth factor, is overexpressed in tumors associated with osteomalacia and renal P_i wasting (58). FGF-7 protein inhibited Na^+-dependent P_i transport in opossum kidney cells. Anti-FGF-7 antibodies attenuated the inhibitory effect of tumor supernatants on Na^+-dependent P_i transport. In this study (58), low concentrations of FGF-23 were present in the conditioned medium of tumor cells. FGF-7 is present in normal plasma; however, whether it is elevated in the plasma of subjects with TIO or in response to alterations in dietary P_i intake has not been determined. Nevertheless,

the report does point to the complexity of factors involved in the pathogenesis of TIO.

FUTURE ISSUES

Three key issues need to be addressed; they relate to how mammalian organisms respond to changes in P_i intake. A low-P_i dietary state is somewhat artificial because virtually all foods contain substantial amounts of P_i. Indeed, renal P_i reabsorption responses obtained in the fasting state are considerably different than those obtained in situations in which only P_i is (by artificial means) removed from the diet. The adaptation of greatest consequence is that which is required following the ingestion of a high-P_i diet. The compensatory changes that occur should facilitate the excretion of excessive P_i from the body. It would be extremely important to define these mechanisms. In this regard, it is becoming increasingly clear that short-term rapid responses are sensed by, and mediated through, the intestine and that these mechanisms are key to the ability of the mammalian organism to excrete excessive amounts of P_i via the kidney. We believe that the delineation of this "enteric-renal" P_i regulatory pathway requires further definition and study.

A second area of investigation needed is to define the interactions between the phosphatonins. Do these factors act independently, or do they influence one another?

The third area of investigation that will yield considerable insights into the manner in which P_i is transported across epithelia relates to the structure of the Na^+-P_i cotransporter proteins. Structural studies that delineate the three-dimensional topology of these proteins and the manner in which they move P_i across the lipid bilayer will undoubtedly yield significant information about the transport of this important ion.

SUMMARY POINTS

1. P_i homeostasis is preserved during alterations in P_i intake by a variety of phosphaturic peptides.
2. PTH is a key hormone in the regulation of P_i homeostasis.
3. Phosphatonin molecules, initially identified as a result of the study of patients with rare disorders associated with renal P_i excretion, may contribute to the physiological regulation of renal P_i reabsorption.
4. FGF-23, sFRP- 4, MEPE, and FGF-7 all inhibit renal P_i reabsorption.
5. FGF-23 and sFRP-4 synthesis may be regulated by the intake of dietary P_i.
6. Unidentified P_i regulatory factors likely mediate the rapid changes in P_i reabsorption by the kidney in response to alterations in dietary P_i intake.

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Errata

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