New Concepts in Pathogenesis of Renal Hypophosphatemic Syndromes

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During the past decades, our knowledge of renal phosphate handling has advanced dramatically. This advance is primarily due to the discovery of sodium-phosphate transport channels and their regulation in health and disease. The discovery of phosphatonins, initially in patients with tumor-induced osteomalacia, has not only allowed us to develop a better understanding of several rare diseases including vitamin D-resistant rickets, but also it has expanded our knowledge of the dynamic interaction between the bone and the kidney critical to bone mineralization. In this review, the author focuses on these new developments and their importance to our understanding of phosphate homeostasis in health and disease.

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INTRODUCTION

Renal hypophosphatemic syndromes are a group of disorders characterized by phosphaturia due to inhibition of proximal tubular phosphate reabsorption. These disorders may present as isolated phosphaturia or as full-blown Fanconi syndrome. They could be due to a primary proximal tubular defect or presence of circulating factors with the ability to inhibit phosphate reabsorption by the proximal tubule. Finally, these syndromes could be hereditary or acquired in nature (Table). During the past decade significant progress has been made in our understanding of phosphate homeostasis in health and disease. This progress is partly due to advances in our understanding of rare acquired or hereditary diseases presenting with renal phosphate wasting. This review will focus primarily on these developments and their relevance to both normal phosphate homeostasis as well as renal hypophosphatemic disorders.

BACKGROUND

Phosphorous, presenting primarily as inorganic phosphate, is the most abundant anion in human body. It plays a major role in many biologic systems including cell membrane function, energy metabolism, cell signaling, and oxygen transport. The total body phosphorous pool is 99% intracellular, of which 85% is bound in the skeleton and the remainder in the soft tissues. The extracellular pool of phosphorous of approximately 600 mg exists primarily as $H_2PO_4^-$ and HPO_4^{2-} with a ratio of 4 divalent to 1 monovalent molecule. The concentration of plasma inorganic phosphate, reported as the concentration of elemental phosphorous, is between 2.5 mg/dL to 4.5 mg/dL (range, 0.8 mmol/L to 1.4 mmol/L). Given the fact that the vast majority of phosphorous is intracellular, serum phosphorous is not a good indicator of the total body phosphate. In addition, the ratio of intracellular to extracellular phosphate is affected by multiple factors including acid-base status, glucose metabolism, and several hormones, especially insulin and catecholeamines.[1,2]

Phosphate intake, expressed as the amount of elemental phosphorous intake, is about 20 mg/kg/day or 1400 mg in a 70 kg man. Approximately, 80% of phosphorous intake (1100 mg) is absorbed

Classification of Renal Hypophosphatemic Syndromes*

Cause	Disease
Primary proximal tubular disorder	
Hereditary	Mutation in phosphate transporters (Na-Pi-IIc transporter mutation [HHRH]) Fanconi syndrome due to inborn error of metabolism (Wilson disease and cystinosis)
Acquired	Drugs (tenefovir and isofosfamide) Toxins (lead and mercury) Tumor products (light-chain nephropathy and lysozymuria)
Presence of circulating factor/hormone	
Hereditary	X-linked hypophosphatemic rickets Autosomal dominant hypophosphatemic rickets Autosomal recessive hypophosphatemic rickets
Acquired	Primary hyperparathyroidism Tumor-induced osteomalacia Posthepatic resection hypophosphatemia

*Na-Pi indicates sodium-phosphate and HHRH, hereditary hypophosphatemic rickets with hypercalciuria.

in the upper intestine, primarily in the proximal jejunum. This is counterbalanced by secretion of 200 mg of absorbed phosphorous in bile and saliva. The net absorption of phosphorous is therefore 900 mg or 64% of the total intake. In adults, but not in children, bone resorption and formation are equal and this dynamic process therefore has no impact on the overall phosphate homeostasis. In such individuals, renal excretion of phosphorous is equal to the net phosphorous absorption.

Intestinal phosphate absorption is both active (through sodium-phosphate transporters) and passive (by diffusion). Sodium-phosphate transporters, part of a large family of sodium-phosphate type II (Na-Pi-II) transporters, are located in the luminal membrane of both the renal proximal tubule and the upper intestine.[3] Intestinal phosphate absorption is regulated by the shuffling of Na-Pi-IIb transporters between the intracytoplamic vesicles and luminal membrane. At low phosphate intake, most of the intestinal phosphate absorption occurs through active pathway, while at high phosphate intake, absorption mostly occurs passively.[4] Phosphate absorption is regulated by 1,25-vitamin D through the modulation of the number of sodium-phosphate transporters in the luminal membrane of enterocytes, primarily in the proximal jejunum.[4] As the activation of vitamin D is regulated by both parathyroid hormone (PTH) and serum phosphorous, phosphate absorption is therefore regulated through an interaction between serum phosphorous, PTH, and 1,25-vitamin D.[5]

RENAL REGULATION OF PHOSPHATE

Phosphate is relatively freely filtered and then actively reabsorbed, resulting in a fractional excretion of about 10% to 15%. Phosphate reabsorption occurs primarily in the proximal tubule through Na-Pi-IIa, and to a lesser extent, Na-Pi-IIc transporters. By the end of the accessible proximal tubule, less than 30% of the filtered phosphate remains in the tubular fluid. The remainder of the filtered phosphate is reabsorbed in the distal portion of the proximal tubule with a small amount reabsorbed in the distal nephron. Phosphate is not secreted by any segment of the nephron.

Major regulators of renal phosphate handling are PTH, dietary phosphate, and to a lesser extent, vitamin D. Parathyroid hormone, by binding to its receptor on the apical and basolateral membrane of the proximal tubules, decreases the abundance of Na-Pi-IIa and Na-Pi-IIc in the apical brush border membrane. Acutely, this is due to the movement of Na-Pi transporter from the brush border membrane to the intracellular pool. Long-term regulation is through the intracellular degradation of Na-Pi transporters. The basolateral membrane exit pathway for phosphate, however, remains unknown. Parathyroid hormone action is through activation of phospholipase C and protein kinase C, as well as protein kinase A pathways.[6,7] Dietary phosphate, independent of the PTH level, also rapidly modulates phosphate reabsorption in the proximal tubules through similar mechanisms.[6,8] The effect of vitamin D depends on its duration of administration as well as the state of the total body phosphate. Short-

term administration is phosphaturic in phosphate replete and antiphosphaturic in phosphate-depleted individuals.[9,10] Chronic administration of vitamin D increases gastrointestinal absorption of phosphate that secondarily increases urinary phosphate excretion.[11] Many other hormones also affect phosphate handling by the kidney. Growth hormone, insulin, and thyroid hormone increase, while calcitonin, glucocorticoids, and atrial natriuretic factor decrease phosphate reabsorption (reviewed in reference 6). These hormones and factors primarily exert their effect through regulation of Na-Pi-IIa transporter. Estrogen replacement is known to cause hypophosphatemia. This effect was very recently shown to be due to downregulation of Na-Pi-IIa transporter in the proximal tubules.[12]

PHOSPHATONINS

In 1994, Cai and colleagues documented the presence of a circulating inhibitor of phosphate transport in the serum of a patient with tumor-induced osteomalacia (TIO). This patient presented with hypophosphatemia, phosphaturia, low 1,25-vitamin D, and rickets, which resolved with the removal of the tumor.[13] This circulating inhibitor was later shown to be fibroblast growth factor 23 (FGF-23).[14] The elevated serum level of this peptide is normalized with the removal of the tumor.[15] Fibroblast growth factor 23, a peptide with 251 amino acids, is synthesized by osteocytes. It is a member of a large family of FGFs that shows affinity for FGF receptors.[16] This peptide inhibits renal and intestinal absorption of phosphate directly by inhibition of Na-Pi-II transporters. It also inhibits activation of vitamin D to 1,25-vitamin D through inhibition of 1-α-hydroxylase enzyme directly (Figure 1).[17,18]

Patients with TIO have similar biochemical findings as 2 genetic syndromes, autosomal dominant hypophosphatemic rickets (ADHR) and X-linked hypophosphatemic rickets (XHR). In patients with ADHR, a mutated form of FGF-23 with prolonged half-life induces severe phosphaturia, hypophosphatemia, and rickets.[19-21] Patients with XHR have a mutation in a gene called *phosphate-regulating gene with homology to endopeptidases on X-chromosome* (*PHEX*).[22] This gene is expressed primarily in the osteoblast lineage cells in the bone and the teeth.[23] Mutation

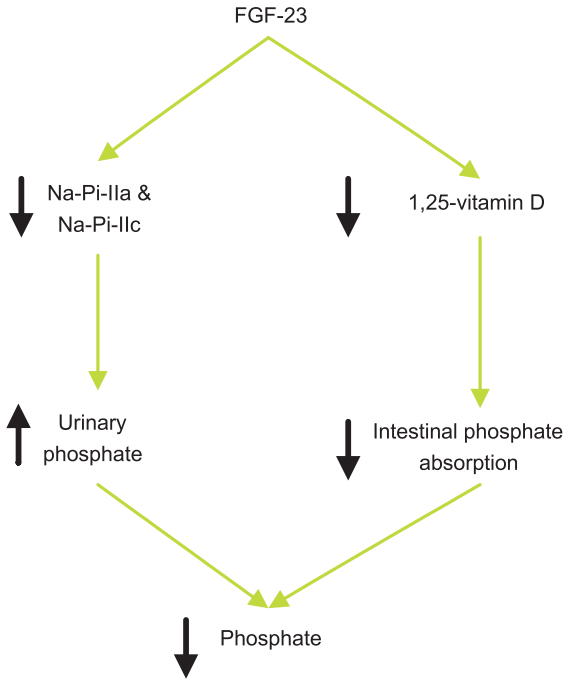

Figure 1. Fibroblast growth factor 23 (FGF-23) and regulation of serum phosphorous. Na-Pi IIa indicates sodium-phosphate type II a transporter.

in this gene is associated with an increase in serum concentration of FGF-23 in patients with XRH.[18] Although the mechanism by which *PHEX* regulates FGF-23 level remains controversial, the elevated level of FGF-23 in these patients is supportive of a mechanistic role for this factor in the development of hypophosphatemic rickets.[18] It is postulated that this enzyme normally breaks down FGF-23 and other phosphatonins, and therefore, an inactivating mutation could result in elevated levels of FGF-23. In a newly described hereditary disease, autosomal recessive hypophosphatemic rickets, mutation in another bone matrix protein, dentin matrix protein 1, is reported. These patients also have elevated levels of FGF-23 resulting in phosphaturia and rickets.[24] The mechanism by which this mutation leads to elevation in FGF-23 is unknown.

The physiological role of FGF-23 in normal individuals is still controversial. Dietary manipulation of phosphate intake results in either no change or only modest alterations in serum concentration of FGF-23.[25,26] An important regulator of FGF-23 is 1,25-vitamin D. In mice, administration of 1,25-vitamin D results in rapid upregulation of FGF-23 transcription in the

osteocytes, followed by an increase in its serum concentration. Osteoblast cells maintained in constant calcium and phosphate environment, increase their synthesis of FGF-23 in response to the administration of 1,25-vitamin D.[27] This effect creates a negative feedback loop which is important in the regulation of serum phosphorous. 1,25-vitamin D increases serum phosphorous by stimulating gastrointestinal absorption of phosphate, while at the same time, increasing concentration of FGF-23 results in inhibition of phosphate reabsorption by the kidney.[25,26] Hence, 1,25-vitamin D plays a dual and opposing role in phosphate homeostasis (Figure 2).

In patients with chronic kidney failure, serum levels of 1,25-vitamin D and FGF-23 are inversely correlated.[28] Fibroblast growth factor 23 in knocked out mice develop hyperphospahtemia and decreases renal excretion of phosphate.[16] These findings together support the concept that FGF-23 has an important physiologic role in phosphate homeostasis.

The mechanism of action of this peptide in the kidney is through interaction with FGF receptor on the basolateral membrane of the proximal tubule. This interaction requires presence of a unique protein encoded by the *klotho* gene.[29] This interesting gene, known as anti-aging gene in mice, encodes 2 transcripts; a membrane-bound and a secreted protein.[30] Klotho protein acts as a cofactor for FGF-23 by binding to the FGF receptor in the basolateral membrane of the proximal tubule. This binding increases affinity of the receptor for FGF-23.[29] Mice deficient in klotho develop multiple defects including hyperphosphatemia due to a decrease in renal excretion of phosphate.[31] Recently, a patient has been reported with hypophosphatemic rickets and hyperparathyroidism due to a mutation resulting in elevated level of alpha-klotho protein.[32]

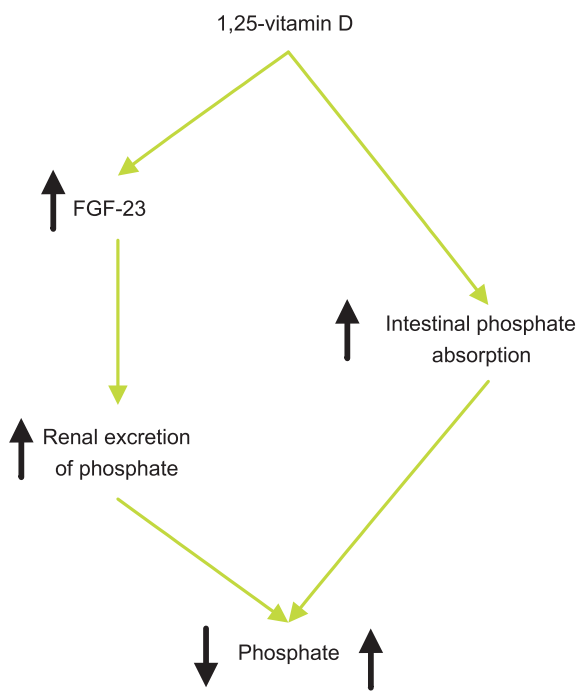

Figure 2. Dual action of vitamin D on serum phosphate. FGF-23 indicates fibroblast growth factor 23.

Several other phosphatonins, including matrix extracellular phosphoglycoprotein, frizzle-related protein 4, and FGF-7 are also secreted in TIO. These peptides are all phosphaturic through inhibition of Na-Pi transporters. The frizzle-related protein 4 inhibits the expected rise in 1,25-vitamin D level. In contrast, matrix extracellular phosphoglycoprotein infusion is associated with an increase in the level of this metabolite.[17,18] While these three peptides do contribute to hypophosphatemia seen in TIO as well as XHR, their role in phosphate homeostasis in the human is still speculative.

HYPOPHOSPHATEMIA DUE TO MUTATION IN SODIUM-PHOSPHATE TRANSPORTER

Hereditary hypophosphatemic rickets with hypercalciuria, first reported in Bedouin tribes, is a rare but interesting genetic disease that presents with renal hypophosphatemic rickets coupled with hypercalciuria. These patients, in contrast to those with XHR or ADHR, have appropriate increase in 1,25-vitamin D and decrease in PTH level. Recent work has shown a mutation in a candidate gene (*SLC34C3*) that encodes Na-Pi-IIc transporter. Although this transporter, which is located on the luminal membrane of renal proximal tubule, was thought to be responsible for a relatively small fraction of phosphate transport (about 15%), patients with homozygous or compound heterozygous mutation present with renal phosphate wasting. This finding therefore raises the possibility that Na-Pi-IIc transporter has a more important role in phosphate transport in human than previously thought. The resultant hypophospatemia activates 1,25-α-hydroxylase enzyme resulting in increased level of 1,25-vitamin D. This would increase calcium absorption from the gastrointestinal tract inhibiting

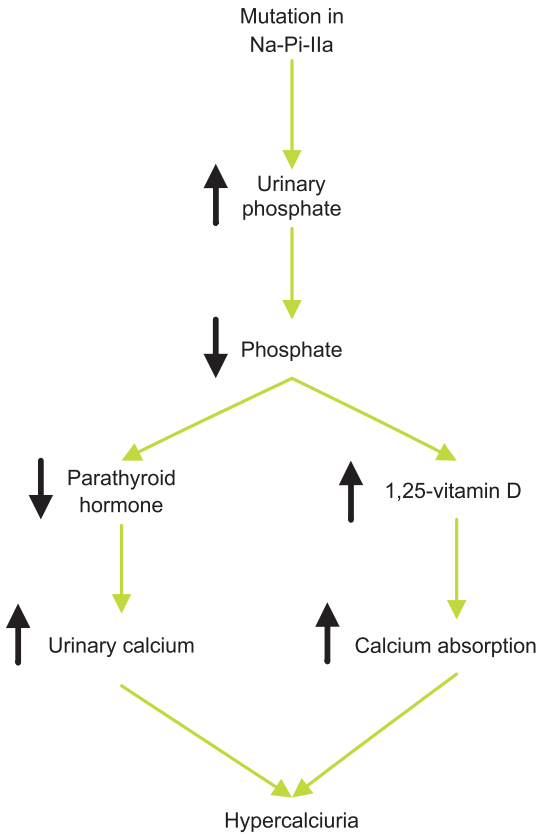

Figure 3. Pathogenesis of hereditary hypophosphatemic rickets with hypercalciuria. Na-Pi IIa indicates sodium-phosphate type II a transporter.

PTH secretion and resulting in hypercalciuria (Figure 3).[33,34]

SUMMARY

Our understanding of phosphate homeostasis has expanded rapidly by the discovery of a new class of phosphate-regulating hormones, the phosphatonins. These hormones, secreted by osteobalst-linage cells, allow a dynamic dialogue between the bone and the kidney, through which they play a critical role in skeletal development. Such a discovery, while adding a newer layer to the complexity of calcium-phosphate homeostasis, has provided a molecular explanation for two enigmatic genetic diseases, ADHR and XHR. Further research is needed to better define the role of these hormones in phosphate homeostasis in health and disease, especially in patients with chronic kidney disease.

CONFLICT OF INTEREST

None declared.

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