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Invited Review

Inorganic phosphate homeostasis and the role of dietary phosphorus

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Abstract

Inorganic phosphate (Pi) is required for cellular function and skeletal mineralization. Serum Pi level is maintained within a narrow range through a complex interplay between intestinal absorption, exchange with intracellular and bone storage pools, and renal tubular reabsorption. The crucial regulated step in Pi homeostasis is the transport of Pi across the renal proximal tubule. Type II sodium-dependent phosphate (Na/Pi) cotransporter (NPT2) is the major molecule in the renal proximal tubule and is regulated by Pi, parathyroid hormone and by 1,25-dihydroxyvitamin D. Recent studies of inherited and acquired hypophosphatemia [X-linked hypophosphatemic rickets/osteomalacia (XLH), autosomal dominant hypophosphatemic rickets/osteomalacia (ADHR) and tumor-induced rickets/osteomalacia (TIO)], which exhibit similar biochemical and clinical features, have led to the identification of novel genes, PHEX and FGF23, that play a role in the regulation of Pi homeostasis. The PHEX gene, which is mutated in XLH, encodes an endopeptidase, predominantly expressed in bone and teeth, but not in kidney. FGF-23 may be a substrate of this endopeptidase and may therefore accumulate in patients with XLH. In the case of ADHR mutations in the furin cleavage site, which prevent the processing of FGF-23 into fragments, lead to the accumulation of a "stable" circulating form of the peptide which also inhibits renal Pi reabsorption. In the case of TIO, ectopic overproduction of FGF-23 overwhelms its processing and degradation by PHEX, leading to the accumulation of FGF-23 in the circulation and inhibition of renal Pi reabsorption. Mice homozygous for severely hypomorphic alleles of the Klotho gene exhibit a syndrome resembling human aging, including atherosclerosis, osteoporosis, emphysema, and infertility. The KLOTHO locus is associated with human survival, defined as postnatal life expectancy, and longevity, defined as life expectancy after 75. In considering the relationship of klotho expression to the dietary Pi level, the klotho protein seemed to be negatively controlled by dietary Pi.

Keywords: phosphate • sodium dependent phosphate cotransporter • PHEX • FGF23 • Klotho • aging

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Introduction

Inorganic phosphate (Pi) plays a critical role in skeletal development, mineral metabolism, and diverse cellular functions involving intermediary metabolism and energy-transfer mechanisms. It is a vital component of bone mineralization, phospholipids in membranes, nucleotides that provide energy and serve as components of DNA and RNA, and phosphorylated intermediates in cellular signaling. Pi is abundant in the diet, and intestinal absorption of Pi is efficient and minirnally regulated. The kidney is a major regulator of Pi homeostasis and can increase or decrease its Pi reabsorptive capacity to accommodate Pi need. The bulk of filtered Pi is reabsorbed in the proximal tubule where sodium-dependent Pi (Na/Pi) transport systems in the brush-border membrane mediate the rate-limiting step in the overall Pi reabsorptive process [1]. Three classes of Na/Pi cotransporters have been identified in mammalian kidney and considerable progress has been made in our understanding of their function and regulation.

Na/Pi cotransporters

cDNAs encoding three classes of Na/Pi cotransporters have been identified (Table1). The gene encoding the type I Na/Pi cotransporter, NPT1, has been mapped to human chromosome 6p22 [2] and is expressed predominantly in the proximal tubular brush-border membrane [3]. Recent studies suggest that Npt1 gene expression is transcriptionally regulated [4] and that Npt 1 may function as a modulator of intrinsic cellular Pi transport rather than as a Na/Pi cotransporter *per se* [5]. The type III Na/Pi cotransporters, GLVR-1 (PIT-1) and RAM-1 (PIT-2), which map to human chromosomes 2 and 8, respectively, are cell surface retroviral receptors [6]. They are ubiquitously expressed and, in the kidney, appear to be localized to the basolateral membrane where they serve a housekeeping function. The type I and III Na/Pi cotransporters have no sequence similarity.

NPT2 is responsible for most of Pi reabsorption in the kidney and can be strictly regulated by Pi regulating hormone such as PTH and vitamin D [1]. Recently, three isoforms of NPT2 have been identified. NPT2a (type Ila) is originally cloned and mainly express in the kidney. NPT2b (type llb) specifically express in the small intestine and can be regulated by vitamin D [7, 8]. NPT2c (type Ilc) also express in the kidney but is not regulated by PTH [9]. Recently, NPT2c is identified as growth-related Pi transporter expressing in the kidney [10]. Those transporters strictly localize in the apical brush-border membrane of the renal proximal tubular cells or intestinal epithelial cells.

Hypophosphatemic rickets/osteomalacia

There are several kinds of diseases that cause hypophosphatemic rickets/osteomalacia (Table 2). Of these, X-linked hypophosphatemic rickets / osteomalacia (XLH), autosomal dominant hypophosphatemic rickets/ osteomalacia (ADHR) and tumor-induced rickets/osteomalacia (TIO) show very similar biochemical features. These diseases are characterized by hypophosphatemia due to reduced TmP/GFR. In addition, hypophosphatemia usually stimulates renal 25-hydroxyvitamin D-1α-hydroxylase (1α-hydroxylase) and increases serum 1,25-dihydroxyvitamin D [1,25$(OH)_2$D] level. In contrast, patients with XLH, ADHR or TIO show inappropriately normal or low serum 1,25$(OH)_2$D Ievel. Therefore, regulatory mechanisms for both tubular reabsorption of Pi and vitamin D metabolism are deranged in these hypophosphatemic diseases. The elucidation of the pathogenesis of inherited and acquired hypophosphatemic syndromes has rapidly advanced our understanding of not only the syndromes themselves but the general mechanism of Pi homeostasis.

X-linked hypophosphatemia (XLH)

XLH, the most prevalent of the renal Pi wasting disorders, is a dominant disorder of Pi homeostasis. It is characterized by growth retardation, rachitic and osteomalacic bone disease, hypophosphatemia, and renal defects in the reab-

Table 1. Sodium dependent phosphate cotransporters.

	Type I (NPT1)	Type II			Type III (NPT3)
		Type IIa (NPT2a)	Type IIb(NPT2b)	Type IIc (NPT2c)	
Molecule	NaPi-I, Npt-I, NPT-I	NaPi-2, Npt-3, NaPi-7	NaPi-IIb NaPi-5		Glvr-I (PiT-I), Ram-I (PiT-2)
Gene locus	Chromosome 6 (human NPT-I)	Chromosome 5 (human NaPi-3)	Chromosome 4	Chromosome 2 (mouse)	Chromosome 2 (human NPT-I) Chromosome 8 (human PiT-2)
Structure					
Amino acid	465	635	690	601	679,656
Transmembrane	7-9 times	8 times	8 times	8 times	10 times
Function					
Substrate	Pi, organic anions	Pi	Pi	Pi	Pi
Affinity for Pi	5-10 nM	0.1-0.2 mM	0.05mM	0.1-0.2mM	0.025mM
Affinity for Na	50-60mM	50-70mM	33mM	50mM	40-50mM
pH dependency	None	+	+(high in acidic pH)	+ (high in neutral and alkaline pH)	+
Regulating factors	Insulin, glucose, glucagon	Dietary and serum Pi value, PTH, GH $1.25(OH)_2D_3$, others	Pi concentrarion	Pi concentrarion	Pi concentration IGF-1
Expressed tissues	Kidney, liver	Kidney, lung	Small intestine, lung	kidney	Kidney, brain, lung, heart, liver, muscle, osteoblast

Pi: phosphate; PTH: parathyroid hormone; GH: growth hormone; $1,25(OH)_2D_3$: 1,25-dihydroxyvitamin D_3; IGF-1: insulin - like growth factor-1.

sorption of filtered Pi and the metabolism of vitamin D [11–13]. Aberrant regulation of the 1α-hydroxylase accounts for the paradoxical occurrence of inappropriately normal or reduced calcitriol levels in the face of hypophosphatemia. The pathogenesis of the renal Pi wasting is dissociable from the vitamin D synthetic defect. Npt2 null mice have renal phosphate wasting, but exhibit an appropriate increase in calcitriol levels and synthesis.

When a normal mouse and a Hyp mouse were connected by parabiosis experiment, serum Pi of the normal mouse decreased [14]. In addition, even when kidneys from normal mice were trans-

Disease	Cause	FGF 23 level	1.25(OH)2D level
XLH	PHEX gene mutation	↑	↘
ADHR	FGF 23 gene mutation	↑	↘
NPT2a deficiency	NPT2a gene mutation	↓	↑
CFDH	FGFR I gene mutation	↗	↘
TIO	FGF 23 overproduction	↑	↘

Table 2. Causes of genetic and acquired hypophosphatemia.

XLH : X-linked hypophosphatemia ; ADHR: Autosomal dominant hypophosphatemic rickets; CFDH: Craniofacial dysplasia with hypophosphatemia; TIO: Tumor-Iinduced rickets / osteomalacia; NPT2 : Type II sodium dependent phosphate transporter; PHEX: Phosphate regulating gene with homologies to endopeptidase on X chromosome; FGF23 : Fibroblast growth factor-23 ; FGFR1: FGF receptor 1.

planted into Hyp mice, hypophosphatemia of Hyp mice did not improve [15]. In contrast, phosphaturia was not observed when kidneys from Hyp mice were transplanted into normal mice. When normal kidney was transplanted into a XLH patient with chronic renal failure, hypophosphatemia recurred [16]. These results indicate that there is no intrinsic defect of Pi reabsorption in kidney of Hyp mice or XLH patients. The international collaborative group identified the responsible gene for XLH by positional cloning in 1995 [17], and named it PHEX (phosphate regulating gene with homologies to endopeptidases on the X chromosome).

Autosomal dominant hypophosphatemic rickets (ADHR)

ADHR is a inherited Pi wasting disorder with features similar to XLH [13, 18]. These include short stature, bone deformities, renal Pi wasting, hypophosphatemia and inappropriate serum levels of $1,25(OH)_2D$ for the degree of hypophosphatemia. ADHR is less common than XLH and exhibits male lineage transmission, variable age of onset, and incomplete penetrance.

Linkage studies of one large ADHR kindred demonstrated linkage of the ADHR trait to chromosome 12pI3 [19]. Subsequently, specific mutations in fibroblast growth factor-23 (FGF-23) were identified in several kindreds with ADHR [20]. Composed of a 10-kb genomic sequence; three exons and encoded by a 2.3-kb cDNA. FGF-23 is expressed at low levels in brain, thymus, small intestine, heart, liver, lymph node, thyroid / parathyroid and bone marrow [21–22]. The gene encodes a 251-amino-acid peptide that is secreted and processed at a pro-protein convertase (furin) consensus site Arg-His-Thr-Arg (RHTH) to N- and C-terminal peptides [22]. Three different missense mutations (R176Q, R179W and R179W) in FGF23 have been identified in ADHR patients, and all involve the furin processing site [23].

Metabolic changes	Clinical findings
Increased PTH level	Osteomalacia
Increased FGF23 level	Osteoporosis
Reduced $1,25(OH)_2D$ level	Ectopic calcification
Reduced Klotho protein	Aging

Table 3. Possible effects of high phosphorus intake.

Consistent with these findings is the demonstration that recombinant FGF-23 peptides harboring these mutations are not processed to their N- and C-terminal peptide products [23, 24].

TIO /oncogenic osteomalacia

TIO is typically caused by a variety of benign primitive mesenchymal tumors that secrete factors collectively termed "phosphatonin" [25] that can inhibit proximal renal tubular Pi reabsorption and impair synthesis of calcitriol. The resulting hypophosphatemia impairs skeletal mineralization and causes rickets or osteomalacia. Tumor extracts and conditioned media from cultured tumors can inhibit phosphate transport by renal cell lines *in vitro* [26-30], and can induce phosphaturia and hypophosphatemia when administered *in vivo* to mice [31].

NPT2a deficiency

Prie and coworkers found that two patients had missense mutations in conserved residues of NPT2a [32]. The only child of one of the two patients also had hypophosphatemia and low bone mass and had inherited the mutant gene. Both patients had a high level of urinary calcium excretion. One had a clearly elevated level of $1,25(OH)_2D$; the other had a level at the high end of the normal range. Thus, it shows that mutations in NPT2a can contribute to nephrolithiasis and low bone mass in humans.

Craniofacial dysplasia with hypophosphatemia (CFDH)

CFDH kindred is distinguished by short limb dwarfism and brachydactyly, as well as craniofacial deformities, including craniosynostosis, prominent superorbital ridge, and depressed nasal bridge. A missense mutation in FGF receptor 1 in patients with CFDH resulted both in renal Pi wasting and in impaired vitamin D regulation [33]. Therefore, it is suggested that FGF-23 signals through FGF receptor 1 to regulate kidney Pi reabsorption and $1,25(OH)_2D$ production.

Novel factors regulating Pi homeostasis

PHEX

The PHEX gene encodes a 749 amino acid protein that is a member of the M13 family of membrane-bound metalloproteases [17]. By analogy to other members of this family, such as neutral endopeptidase 24. 11. (NEP) that have been shown to inactivate hormones [34] and endothelin-converting enzyme (ECE) that process hormones to a mature form [35]. Based on the actions of NEP and ECE- I and ECE-2, it has been postulated that PHEX plays a role in the activation or inactivation of peptide factors involved in the regulation of skeletal mineralization renal Pi transport and vitamin D metabolism [12].

The expression of PHEX is reported in bone, teeth, brain, muscle, testis, ovary and parathyroid gland, but not in kidney [36–40]. This means that abnormal PHEX protein itself cannot directly invoke derangements of Pi and vitamin D metabolism in kidney. Therefore, it has been hypothesized that there is a humoral phosphaturic factor that is inactivated by PHEX protein in healthy individuals. Abnormal PHEX proteins seen in XLH patients seem to be unable to degrade the factor and evoke hypophosphatemia by excess phosphaturic action of this humoral factor.

It was demonstrated that depletion of PHEX mRNA and protein in human osteoblasts, achieved by transfection with a PHEX antisense vector, Ied to impaired calcium incorporation and mineralized nodule formation when compared to osteoblasts transfected with the corresponding PHEX sense vector [41]. Furthermore, retroviral-mediated overexpression of PHEX in osteoblasts cloned from Hyp mice led to a partial rescue of the mineralization defect [42]. The results of both studies suggest that PHEX is an important determinant of osteoblast mineralization.

FGF23

The conditioned medium of COS cells expressing either wild-type FGF-23 or mutant FGF-23 found

in ADHR patients inhibited Pi transport of OK cells [24]. In addition, this inhibition of Pi transport was blocked by adding heparin [24]. FGF-23 was also cloned from TIO tumors and shown to be phosphaturic when administered to mice *in vivo* [22] and to inhibit Na/Pi cotransport *in vitro* [24]. Moreover, in another study, several TIO tumors were shown to express high levels of FGF-23 mRNA and protein [43]. Taken together, these data support the hypothesis that FGF-23 is one of the tumor factors responsible for renal Pi wasting in TIO.

FGF-23 may be a substrate of this endopeptidase and may therefore accumulate in patients with XLH. FGF-23 causes hypophosphatemia when injected into mice, and mice with ablation of the FGF-23 gene have hyperphosphatemia and high levels of 1, 25 (OH) 2D [44]. Thus, FGF-23 has a role in both normal Pi homeostasis and several diseases of Pi metabolism. Furthermore, injection of FGF-23 in mice decreases NPT2a levels and suppresses expression of 1α-hydroxylase [45]. The latter action of FGF-23 presumably explains why levels of $1,25(OH)_2D$ are not increased in the diseases associated with increased activity of FGF-23 (XLH, ADHR and TIO), despite hypophosphatemia.

FGF-23 has been proposed to have either paracrine and or endocrine roles. Decreased FGF-23 secretion could represent an endocrine response to dietary Pi restriction and, thus circulating FGF-23 might provide a closed feedback loop between a putative Pi sensor and renal Pi reabsorption [46]. FGF-23 may function physiologically as a locally-acting factor, but may also function pathophysiologically when secreted in excess into the circulation where it can cause marked renal loss of Pi. Current evidence supports the notion that under normal physiologic states, the concentration of FGF-23 in serum is regulated *via* PHEX-dependent proteolysis. However, conditions that lead to excess circulating FGF-23 concentrations or activity are associated with a marked depression in proximal renal tubular reabsorption of Pi and hypophosphatemia. It is clear that FGF-23 plays a central role in the pathophysiology of these disorders. Furthermore, it is suggested that FGF-23 signals through FGF receptor 1 to regulate kidney Pi reabsorption and $1,25(OH)_2D$ production from the study of CFDH [32] (Fig. 1).

Functional role of Pi

Role of Pi in aging

Mice homozygous for severely hypomorphic alleles of the Klotho gene (klotho mice) exhibit a syndrome resembling human aging, including atherosclerosis, osteoporosis, emphysema, and infertility [47]. These include ectopic calcification in the arterial wall and stomach, atrophy of the thymus, disruption of Purkinje cells, gonad atrophy (sterility), thinning of the skin, ataxia and abnormality of the pituitary gland, as well as hypoglycemia and hyperphosphatemia [47]. The data obtained to date support the idea that klotho plays an important role in aging and senescence-related diseases. In klotho mutant mice, the coding region of the klotho gene is preserved but its expression is markedly decreased by the insertion of an exogenously introduced nonfunctional gene within its promoter region [47].

Analysis of cDNA revealed that the klotho gene also expresses a secreted form, lacking KL2, transmembrane, and intracellular domains, due to alternative RNA splicing. Thus, klotho may also act as a humoral factor [47, 48]. Previous experiments have shown that endothelium-dependent vasodilation of the aorta and arterioles was impaired in heterozygous klotho mice, but could be restored by parabiosis with wild-type mice [49]. In addition, nitric oxide metabolites (NO_2 and NO_3) in urine are significantly lower in heterozygous klotho mice [49]. These results suggest that klotho protein may protect the cardiovascular system through endothelium-derived NO production [49]. Remarkably, *in vivo* klotho gene delivery can ameliorate vascular endothelial dysfunction, increase nitric oxide production, reduce elevated blood pressure, and prevent medial hypertrophy and perivascular fibrosis in a rat model with multiple atherogenic risk factors including hypertension, diabetes, obesity, and hyperlipidemia [50].

The average life span of the klotho homozygotes was 9.6 ± 1.1 wk and their average body weight did not exceed 10 g when they were fed the nonpurified diet, CE-2. However, when the klotho homozygous males were fed low phosphorus diet (0.4 g/100 g) starting at wk 3 of age, they continued to gain body weight until reaching 20 g

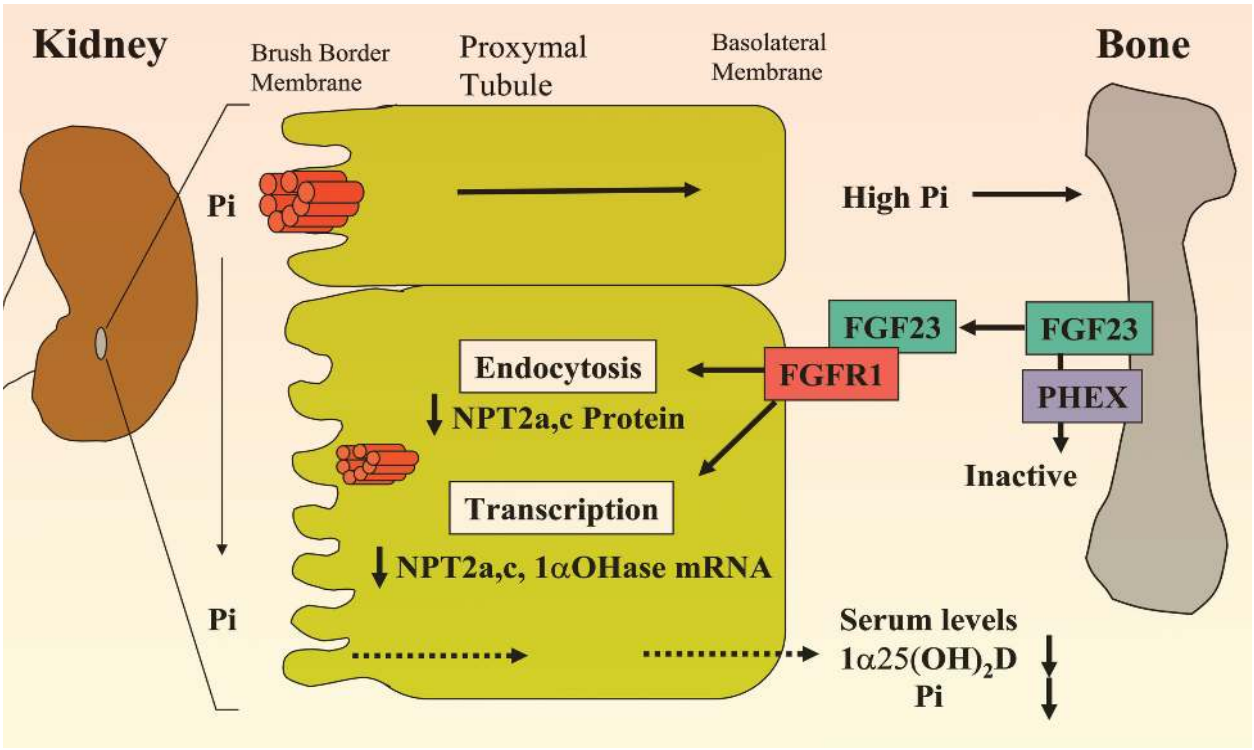

Fig. 1 Phosphate homeostasis regulated by FGF23.

or more at wk 20 of age [51]. Four males examined survived to the end of the experiment at wk 29 of age. In considering the relationship of klotho expression to the dietary phosphorus level, the klotho protein seemed to be negatively controlled by dietary phosphorus.

Human KLOTHO shows 86% amino acid identity with the mouse protein, and is encoded by a gene that spans over 50 kb on chromosome 13q12 [48]. To date, no premature-aging syndromes have been linked to this region. A population-based association study employing two newly characterized flanking microsatellite markers was investigated to determine whether the KLOTHO gene is involved in human aging [52]. The evidence suggests that the KLOTHO locus is associated with human survival, defined as postnatal life expectancy, and longevity, defined as life expectancy after 75.

Role of Pi in quality of life (QOL)

In a survey in the United States, phosphorus intake has increased, but the amount of phosphorus as food additives was not completely estimated [53]. In Japan, the amount of phosphorus from food estimated by the Food Balance Sheet increased gradually from 1,243 mg/d in 1960 to 1,332 mg/d in 1975 and to 1,421 mg/d in 1995 [54]. Phosphorus containing food increased by approximately 17% for the decade until 1993 [55], and the use of phosphorus as food additives may continue to increase. In the Framingham Offspring Study, women, but not men, consuming more than three servings of cola (all types) /day has significantly lower bone mineral density (BMD) at each of the three hip sites, relative to those consuming less than one serving/day: 2.3% lower at the trochanter ($p=0.05$), 3.3% lower at the femoral neck ($p<0.001$), and 5.1% lower at Ward's area ($p<0.0005$). For the spine those with the highest cola intake had BMD 1.2% lower than those consuming less than one servind/day, but this was not significant. The phosphorus content of regular cola is 44-62 mg, and of diet cola 27-39 mg, *per* oz serving, while most other carbonated beverage contain no phosphorus. These suggest that cola, but not other carbonated soft drink consumption, contributes to lower BMD in adult women [56].

Thus, high phosphorus and moderately low calcium intake produced hormonal changes of mild secondary hyperparathyroidism in human, and with prolonged intake lower calcitriol concentrations, the body's main homeostatic mechanism for adaptation to low dietary calcium.

Conclusions

The homeostasis of phosphorus as well as calcium is considered to be modulated by vitamin D and phosphorus restriction is reported to improve calcium utilization [57]. It is thought that excess amounts of phosphorus intake for long periods are a strong factor in bone impairment and aging (Table 3). The proper ratio of calcium to phosphorus in the diet consumed is also important for mineral mobilization and bone mineralization. The restriction of phosphorus intake seems to be important under low calcium intake to keep QOL high. Therefore, further studies on the amount and the source of phosphorus intake is important, particularly in regard to the amount of phosphorus from processed foods, imported foods, and phosphorus-containing food additives.

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