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Can features of phosphate toxicity appear in normophosphatemia?

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Abstract

Phosphate is an indispensable nutrient for the formation of nucleic acids and the cell membrane. Adequate phosphate balance is a prerequisite for basic cellular functions ranging from energy metabolism to cell signaling. More than 85% of body phosphate is present in the bones and teeth. The remaining phosphate is distributed in various soft tissues, including skeletal muscle. A tiny amount, around 1% of total body phosphate, is distributed both in the extracellular fluids and within the cells. Impaired phosphate balance can affect the functionality of almost all human systems, including muscular, skeletal, and vascular systems, leading to an increase in morbidity and mortality of the involved patients. Currently, measuring serum phosphate level is the gold standard to estimate the overall phosphate status of the body. Despite the biological and clinical significance of maintaining delicate phosphate balance, serum levels do not always reflect the amount of phosphate uptake and its distribution. This article briefly discusses the potential that some of the early consequences of phosphate toxicity might not be evident from serum phosphate levels.

Keywords

Klotho; FGF23; Vitamin D; Calcium

Introduction

Phosphorus is regularly consumed through food and, once absorbed, phosphorus binds with oxygen and is distributed in the various parts of the body as phosphate. A number of important cellular and systemic organ functions, including energy metabolism, maintenance of mineral ion equilibrium, and adequate cell signaling require physiological phosphate balance (serum levels around 2.5–4.5 mg/dl in humans). Of relevance, infants have higher serum phosphate levels than adults [1]. Most of the body phosphate is present in the bone as hydroxyapatite, and only 1% of the total phosphate is available in the extracellular compartment (Fig. 1) [2–5]. Serum measurements of extracellular phosphate therefore reveal only a tiny fraction of total body phosphate and might not always reflect the amount of phosphate uptake and its distribution. Of relevance, in a healthy individual, roughly two-

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thirds of consumed phosphate is excreted in urine and one-third is excreted through the stool to maintain homeostatic balance.

The physiological phosphate balance is mostly accomplished by cross-organ talk among kidney, intestine, bone, and parathyroid gland [3, 6–10]. Phosphate absorption, following food ingestion, takes place mostly in the small intestine [11]. Intestinal phosphate absorption is a complex process, partly assisted by sodium-dependent phosphate transporter 2b (NaPi-2b), present in the luminal side of the intestinal cells. The levels of 1,25-dihydroxyvitamin D and dietary phosphate could influence the intestinal transport activity of NaPi-2b [12]. For example, 1,25-dihydroxyvitamin D can induce intestinal NaPi-2b protein to increase phosphate absorption in the gut. In a similar line of observation, following acute phosphate administration, the absorption ability of phosphate was reduced in mice with genetically suppressed *NaPi-2b* activity when compared with control mice [13]. It must be noted that the human relevance of *NaPi-2b* knockout mice is not yet clear, as loss-of-function mutations of human *NaPi-2b* gene do not significantly alter phosphate balance in the affected individual [14].

Phosphate is freely filtered through the glomeruli; more than 80% of the filtered phosphate is reabsorbed in the proximal tubular epithelial cells and a tiny fraction is reabsorbed in the distal tubule. Comparable to intestinal phosphate absorption, renal phosphate reabsorption in the proximal part of the tubules is also partly accomplished by sodium-dependent phosphate uptake through NaPi-2a and NaPi-2c transporters; parathyroid hormone (PTH) has a major influence on the activity of NaPi-2a and NaPi-2c that is present in the luminal side of the proximal tubular epithelial cells [15, 16]. PTH can inhibit NaPi-dependent phosphate reabsorption and thereby increase urinary phosphate excretion [15, 16]. The physiological phosphate balance is uniquely maintained by the ability of the kidney to adjust phosphate handling, as phosphate restriction could increase reabsorption, whereas phosphate intake could reduce the reabsorption process; such adaptation to a low or high phosphate-containing diet is partly accomplished by the insertion or retrieval of renal NaPi transporters from the brush border membrane. Recent studies have shown that fibroblast growth factor 23 (FGF23) and klotho can also suppress renal NaPi activity [17–20].

Our understanding of the critical role of the kidney in the regulation of systemic phosphate metabolism is significantly enhanced by the identification of the FGF23–klotho system. In the accompanying section, we briefly discuss the role for the FGF23–klotho system in phosphate metabolism that is relevant to this article. For further details on the molecular and clinical aspects of FGF23–klotho functions, readers are recommended to see several recently published review articles [3, 7, 21–30].

FGF23–klotho system

Bone-derived FGF23 is a protein of around 30 kDa; the NH_2-terminal contains the FGF receptor (FGFR)-binding domain, and the COOH-terminal contains the klotho-binding site [31]. In vitro studies have shown the binding ability of FGF23 to FGFR1c, FGFR3c, and FGFR4 [17, 32–35], although in vivo studies could not detect noteworthy responses of FGF23 through FGFR3 and FGFR4 [36]. In contrast to other autocrine and paracrine members of FGFs, the interaction between endocrine FGF23 with its receptors and subsequent activation of signaling network requires klotho as a cofactor; the FGF23–klotho–FGFR complex can activate downstream signaling phosphoproteins, including FGF receptor substrate-2a (FRS2a) and extracellular signal-regulated kinase 1/2 (Erk1/2) [37, 38].

Klotho is a 130-kDa membrane protein [28] that can be cleaved from the plasma membrane by disintegrin and metalloproteinases (ADAM-10 and ADAM-17) [39]. The expression of klotho is mostly detected in the kidney (distal convoluted tubules), in the brain (choroid

plexus epithelium), and in the parathyroid gland [40]. Although FGF23 is a circulatory protein, the restricted expression of klotho provides the tissue specificity for FGF23 functions. The essential role of klotho in FGF23-mediated phosphate regulation is convincingly shown in various experimental models. For example, wild-type mice, challenged with bioactive FGF23 protein, had significantly reduced serum phosphate levels, whereas such bioactive protein lost its phosphate-lowering ability in mice without klotho activity (either *klotho* knockout mice or *Fgf23/klotho* double-knockout mice) [41]. Similarly, hypophosphatemic *phex* mutant mice became hyperphosphatemic when klotho function was eliminated, even though the *phex/klotho* double-mutant mice have higher serum levels of FGF23 than *phex* mutant mice [42, 43]. A similar line of observation was also noted in humans; for instance, loss-of-function mutation in the human *klotho* gene resulted in severe hyperphosphatemia, although the affected patient with tumoral calcinosis had high serum levels of FGF23 [44].

The ability to reduce serum phosphate levels by the activated FGF23–klotho system is observed in both animal and human studies [41, 43–52]. For example, in autosomal dominant hypophosphatemic rickets (ADHR) patients, increased activity of FGF23 caused by gain-of-function mutations of the human *FGF23* gene is associated with hypophosphatemia as a result of excessive urinary phosphate loss [46]. Such a phosphate-wasting phenotype is also observed in transgenic mice with forced expression of human *FGF23* [53], and genetically eliminating the functions of *Fgf23* from mice leads to hyperphosphatemia [54]. More importantly, restoring the functions of human FGF23, either by genetic manipulation, or by therapeutic inoculation in the *Fgf23* knockout mice, can reverse hyperphosphatemia to hypophosphatemia, clearly showing the in vivo phosphate-regulating abilities of FGF23 [55].

The interaction of bone-derived FGF23 and mostly kidney-derived klotho can increase urinary phosphate excretion by reducing the reabsorption ability of NaPi-2a and NaPi-2c co-transporters and by inhibiting synthesis of the active vitamin D metabolite, 1,25-dihydroxyvitamin D [17–19]. It is important to mention that whether the suppression of renal NaPi co-transporter activity by the FGF23–klotho pathway is a direct effect [20] or is mediated through other FGF23 target molecules is not yet clear and requires additional experimental clarification.

Because physiological phosphate homeostasis is maintained by delicate cross-organ interactions among the kidney, intestine, bone, and parathyroid gland (Fig. 2) [3, 6–10], pathology involving any of these organs can lead to altered phosphate balance in the form of either hypophosphatemia or hyperphosphatemia. Particularly, the renal transport system is essential for the physiological regulation of organic and inorganic ion balance, and most of the chronic renal diseases without therapeutic intervention usually progress to irreversible renal fibrosis that affects survival [56–59]. The cause and consequences of such obvious dysregulation of phosphate is detailed in numerous publications [60–64], and instead of reproducing such information, we have listed a few disorders related to hypo-and hyperphosphatemic conditions as Table 1.

Human phosphate toxicity

Phosphate toxicity resulting from excessive accumulation of phosphate can provoke a wide range of local and systemic manifestations in the body. A higher occurrence of vascular calcification in patients with chronic kidney disease (CKD) is one of the commonly encountered consequences of phosphate toxicity [65–68]. More importantly, phosphate toxicity and low serum vitamin D levels have been implicated as independent risk factors for patients with CKD. In the 1960s, administration of inorganic phosphate, by intravenous or

oral routes, was used as a therapy to treat hypercalcemia and provided direct evidence of the consequences of human phosphate toxicity. For instance, inorganic phosphate treatment, either orally or intravenously, induced various features of phosphate toxicity, not only in organ damage, but also in their survival; disodium or dipotassium salt (Na_2HPO_4 or K_2HPO_4) was administrated orally (the cumulative dose of phosphate varied from 1.2 to 21.6 g; average, 9.6 g) or administered intravenously (the cumulative dose of phosphate varied from 2.3 to 6.2 g; average, 3.33 g). Despite similar serum phosphate levels in pre- and postphosphate treatment, two patients developed diarrhea during oral phosphate administration with a cumulative dose of 8.8 and 6.5 g, respectively. Pulmonary edema was encountered during phosphate infusion in a patient receiving a cumulative dose of 3.1 g phosphate that raised the serum phosphate level to 5.6 g/dl. In addition, one of the intravenously phosphate-treated patients, who received a cumulative dose of 3.1 g phosphate, died of an acute myocardial infarction. Finally, autopsies were performed on six cases of phosphate-treated patients, and five of those showed features of ectopic calcification, detected mostly in the lungs, heart, and kidney [69].

Phosphate solution was also widely used as an enema, especially for the treatment of constipation and preoperative bowel preparation in children. Phosphate toxicity induced by such phosphate enemas is reported to cause tetany and respiratory and intestinal disorders [70]. For example, a 28-month-old boy who received phosphate enemas for preoperative bowel preparation developed hyperthermia, tachycardia, tachypnea, cyanosis, and tetany immediately after the administration of the phosphate-containing enema; the patient also developed respiratory failure and severe metabolic acidosis and was treated with artificial ventilation [70]. Some of these abnormal symptoms following a phosphate-containing enema can be attributed to abnormal mineral ion and electrolyte balance induced by phosphate toxicity. In a similar line of observation, when hypertonic phosphate solution was rectally administered to a 4-year-old chronically constipated girl with normal renal function, she developed phosphate toxicity (23 mg/dl) with breathing difficulties and a depressed level of consciousness [71]. In accord with the pediatric group of patients, 14 elderly patients who received a phosphate-containing enema showed significantly elevated serum phosphate levels within an hour, followed by a marked reduction of serum calcium levels by 12 h [72].

Recently, complications associated with sodium–phosphate (Na–P) treatment were compiled [73]. The 109 patients treated with Na–P compounds showed numerous complications, including electrolyte disturbances, renal failure, and colonic ulceration [73]. Furthermore, 171 cases of renal failure were reported to the U.S. Food and Drug Administration (FDA) following use of an Na–P compound [73]. In a separate retrospective study using the U.S. Food and Drug Administration Adverse Event Reporting System, analyzing 2,097,223 files from 2004 to 2009 (first 9 months), 178 patients treated with Na–P tablet preparations were reported for higher occurrence of adverse drug reactions in the kidneys [74], again suggesting the potential harmful effects of uncontrolled phosphate ingestion. Dose-dependent time-course animal studies have provided further insights in the pathomechanisms of phosphate toxicity.

Experimental phosphate toxicity

The damage inflected by phosphate toxicity can range from biochemical alterations to tumor growth [75] to mammalian aging, with the resultant effect being compromised survival [45]. Genetically engineered *klotho* knockout mice develop phosphate toxicity by 3 weeks of age that leads to body weight loss, kyphosis, infertility, generalized organ atrophy, and significantly reduced lifespan [43, 45, 48, 76–78]. Some of these changes that were noted in *klotho* knockout mice bear similarities to human aging. Extensive molecular and

biochemical analysis, performed on *klotho* knockout mice, suggests that increased renal activity of NaPi-2a leads to increase serum retention of phosphate; importantly, genetically reducing phosphate toxicity in *klotho* knockout mice, by generating *NaPi2a/klotho* double-knockout mice, could suppress aging phenotypes [45]. Notably, the *klotho* knockout mice regained fertility and extended their survival when serum phosphate levels were genetically reduced, as noted in the *NaPi2a/klotho* double-knockout mice [45]. However, when *NaPi2a/klotho* double-knockout mice were fed with a high-phosphate diet, these mice lost their reproductive abilities and other aging features reappeared, clearly suggesting that diet-induced phosphate toxicity can promote the aging process. Again, when phosphate toxicity was induced in *NaPi2a/klotho* double-knockout mice by providing a high-phosphate diet (1.2%), features resembling premature aging, including generalized tissue atrophy and cardiovascular calcification, reappeared, and all the mice died by 15 weeks of age. Of particular significance, none of the *NaPi2a/klotho* double-knockout mice fed with a normal phosphate diet (0.6%) died until 20 weeks of the observational period [45]. These in vivo genetic and dietary manipulation studies clearly demonstrated that the mammalian aging process could be adversely influenced by phosphate toxicity to compromise survival. In a separate animal study, when acute phosphate toxicity (7- to 20-fold increase over control by 4 h) was induced by using a commercially available phosphate-containing enema (30–50 ml/kg), 100% mortality was noted in the experimental animals [79]. Moreover, genetically inducing phosphate toxicity could significantly impair survival of *leptin*-deficient obese mice [80]. Furthermore, experimental studies have shown that excessive dietary phosphate intake could increase the growth and size of lung tumors [75].

Although the pathological consequences of phosphate toxicity on organ damage and survival are obvious, the mechanism by which phosphate toxicity accelerates tissue injuries is not clear. Phosphate toxicity can exert cytotoxic effects on various organs to compromise their functionality. For instance, an increased rate of apoptosis, induced by phosphate toxicity, could be suppressed by reducing phosphate burden [45]. In fact, patients exposed to a phosphate-containing enema showed features of necrotic changes of the abdominal tissues, including loss of internal and external sphincters as a consequence of extensive tissue necrosis [81]. In a similar line of observation, when hypertonic phosphate solution was intradermally injected to rabbits, a pronounced erythema and indurations were noted by 24 h, which eventually progressed to central necrosis and full-thickness tissue loss by 5–7 days [81], implicating local erosive effects of phosphate toxicity induced by cytotoxic effects. Moreover, dietary phosphate could stimulate the AKT-mediated signaling network and can provoke an increase in lung tumorigenesis [75]. Similarly, studies have found that extracellular phosphate can induce MAPK signaling networks, and particularly can activate Erk1/2 phospho-protein to exert yet to be defined cellular functions [82]. Detailed understanding of phosphate-mediated cell signaling will help to explain the molecular mechanisms of phosphate toxicity.

Can features of phosphate toxicity appear in normophosphatemia?

Evidence from human studies

Recent studies have linked serum phosphate levels with cardiovascular disorders, including calcification. In fact, studies have found increased risk of cardiovascular diseases even when serum phosphate levels were within the upper limit of normal range [83]. In the Cholesterol and Recurrent Events (CARE) study, a relationship was established between serum phosphate levels and the rate of cardiovascular disorders [83]; in this study, the baseline serum phosphate levels were measured in 4,127 fasting participants before the incidence of myocardial infarction and followed up for a median period of 59.7 months. Participants with serum phosphate >3.5 mg/dl had a higher adjusted hazard ratio for death compared with those with serum phosphate <3.5 mg/dl. In a similar line of observation, higher serum levels

of phosphate were found to be associated with increased risk of cardiac dysfunction, although the serum phosphate levels of most of the individuals were within the upper limit of normal range [83].

The Framingham Offspring Study was the first community-based examination of the association between normal-range phosphate levels and cardiovascular disorders in the general population. In this cohort, 3,368 individuals (mean age, 44 ± 10 years) without any cardiovascular or renal disorders were followed up for about 16 years [84]. Higher incidence of cardiovascular diseases was found to be associated with serum phosphate levels; individuals with 3.5 mg/dl serum phosphate levels had 1.55 times higher hazard ratios than those with levels below 2.8 mg/dl [84]. Again, it is important to note that a serum level of 3.5 mg/dl is within the accepted normal range.

In a similar line of study, an association between serum phosphate levels and the Coronary Artery Risk Development in Young Adults (CARDIA) was evaluated for 3,015 individuals (mean age, 25.2 years), with mean serum phosphate and calcium levels of 3.6 (1.3–5.7) and 9.5 (7.1–13.2) mg/dl, respectively [85]. The presence of coronary artery calcium, assessed by computed tomography 15 years later, was found to be significantly associated with serum phosphate levels. There was a clear difference between the groups with serum phosphate values of first quartile (<3.3 mg/dl) versus the fourth quartile (>3.9 mg/dl) [85]. Similarly, in a community-dwelling follow-up study of 12.6 years, higher serum levels of phosphate were shown to be associated with death among 15,732 adult participants with a mean phosphate level of 3.4 mg/dl (1–9.1) [86]. These human studies with a significant number of participants clearly suggest the risk of cardiovascular events and death even within the upper limit of normal serum phosphate levels in the general population. One important question that is not yet clearly understood is why serum phosphate levels differ in the general population, and recent genome-wide association studies have shed some light on this aspect.

Using 16,264 participants of four large cohorts with normal phosphate metabolism, a genome-wide association study found polymorphisms in seven loci with minor allele frequencies of 0.08–0.49 associated with serum phosphate levels [87]. Three loci were identified near genes encoding the NaPi-2a co-transporter, the calcium-sensing receptor (CASR), and FGF23, and the investigators concluded that these genetic variants might determine the serum phosphate levels in the general population [87]. However, additional follow-up studies are needed to validate such observations. It is important to note that in accord with the human studies, experimental studies have also shown various phosphate-induced physical, biochemical and morphological changes, despite the lack of significant changes in the serum levels.

Evidence from experimental studies

The consequences of phosphate toxicity are easier to identify when serum phosphate levels are abnormally high; however, whether certain features of phosphate toxicity might appear even in normophosphatemic conditions are not yet clear. At present, determining serum phosphate level is the gold standard to estimate the overall phosphate status of the body. In this section, we provide experimental evidence suggesting that some of the early consequences of phosphate toxicity might not be evident from serum levels of phosphate because this level does not always reflect the amount of phosphate uptake and its distribution.

The high phosphate-fed experimental animal model helped us to understand whether features of phosphate toxicity might appear even in a normophosphatemic microenvironment. In an experimental study using 8-week-old Wistar male rats fed with various amounts of phosphate (0.3%, 0.6%, 0.9%, 1.2%, or 1.5%) with a constant amount of

calcium (0.6%) for 4 weeks, significantly increased urinary and fecal excretion of phosphate followed the high-phosphate diet consumption [88]. Interestingly, despite the dietary phosphate load for 4 weeks, there was no statistically significant increase in serum phosphate or calcium levels in animals fed 1.5% phosphate compared with 0.3% phosphate-fed control animals. Even though no marked changes in serum phosphate levels were noted between high and normal phosphate-fed animals, the high (1.5%) phosphate-fed rats had lesser body weight gain (58 ± 6 g/4 weeks) as compared with the control (0.3%) phosphate-fed rats (95 ± 5 g/4 weeks), clearly suggesting an adverse impact of the high-phosphate diet without marked changes in serum phosphate levels [88].

In a separate study, 1-month-old male rats ($n = 30$) were fed with a control diet (Ca:P = 1:1) or experimental diets of either Ca:P = 1:2 or Ca:P = 1:3 for 8 weeks. In accordance with the earlier study, a high-phosphate diet reduced growth; the rat that received Ca:P = 1:2 was 11% lighter whereas the rat that received Ca:P = 1:3 was 29% lighter than control rats fed with the Ca:P = 1:1 diet. Moreover, rats that received Ca:P = 1:3 had significantly shorter femurs, compared with rats fed Ca:P = 1:1 and Ca:P = 1:2 ($P = 0.001$ and 0.019, respectively) [89]. Both groups of rates with high phosphate consumption had significantly ($P < 0.001$) lower bone mineral content (BMC) and areal bone mineral density (BMD) than control rats [89]. It is important to note that such extensive changes in the body weight and skeletal anomalies were present in spite of normal serum calcium and phosphate levels in the various groups [89], reinforcing the idea that serum phosphate levels might not always reflect phosphate burden.

It is important to note that high serum phosphate can induce compensatory hyperparathyroidism in both human and animal models [90, 91]. Experimental studies have shown that feeding animals with a high-phosphate diet can induce hyperparathyroidism, even when the serum phosphate level stays within the normal range during the first several months [92]. In an study conducted on rabbits fed with a high-phosphate diet (Ca:P = 1:7) for 1–6 months, compared with a control group that received a normal phosphate diet (Ca:P = 1:0.7), a gradual increase of serum PTH levels was noted in animals that consumed the high-phosphate diet, although serum phosphate levels in high phosphate diet-fed animals remained unchanged over the course of the first 3 months [92]. Summarizing the aforementioned experimental evidence, it is clear that the features of phosphate toxicity can appear in a normophosphatemic microenvironment.

Conclusion

Although measuring serum phosphate level is the gold standard to estimate the overall phosphate status of the body, the amount of intracellular phosphate or phosphate storage is not taken into consideration in such traditional methods of phosphate measurement. In this brief review article, we have provided both human and experimental evidence that clearly suggests that certain features of phosphate toxicity might appear even in normophosphatemic conditions, thereby exposing the limitation of serum phosphate measurements to detect early events of phosphate toxicity. We believe that tissue injuries inflicted by chronic phosphate toxicity caused by extremely high serum retention of phosphate are mostly irreversible, whereas the effects of phosphate toxicity that appear in normophosphatemic microenvironments are more likely to be reversible, or even preventable. Based on the results of human and experimental studies, it is obvious that reducing phosphate burden and maintaining phosphate balance by adequate uptake are crucial for a healthy life because phosphate toxicity can inflict irreversible organ damage that compromises the quality of life.

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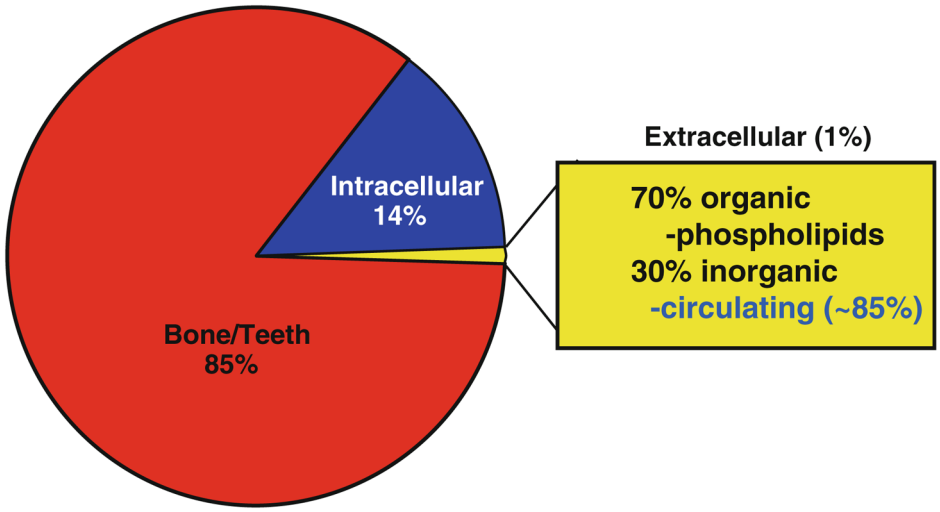

Fig. 1.
Phosphate distribution in various compartments of the body. Please note that circulating serum phosphate measurement estimates only a tiny pool of total body phosphate

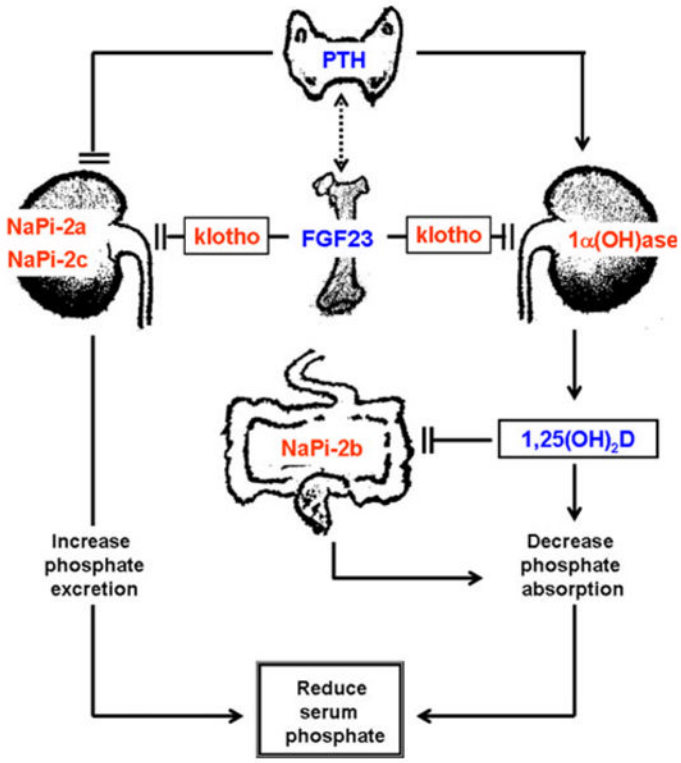

Fig. 2.

Simplified diagram showing multi-organ interactions in regulation of phosphate homeostasis. Fibroblast growth receptor (FGF)23 produced in the bone cells can suppress renal NaPi-2a and NaPi-2c co-transporter activities to increase the urinary excretion of phosphate. Similarly, FGF23 can also suppress renal expression of 1α(OH)ase to reduce production of 1,25-dihydroxyvitamin D [1,25$(OH)_2$D], which can suppress intestinal NaPi-2b activities to reduce phosphate absorption, resulting in decreased serum phosphate levels [3]. Of relevance, parathyroid hormone (PTH) can induce the expression of the 1α(OH)ase, and thereby can increase the production of 1,25$(OH)_2$D, which in turn can inhibit PTH and 1α(OH)ase expression. Such transcriptional repression feedback maintains vitamin D homeostasis. The figure is adopted with modification from our earlier publication [29]

Table 1

Partial list of pathological events or diseases that can induce hypophosphatemia and hyperphosphatemia [3, 27, 93]

Hypophosphatemia
Respiratory alkalosis
Alcoholism
Bone marrow transplant
Chronic diarrhea
Chronic liver disease
Diabetic ketoacidosis
Diuretics
Extensive burns
FGF23-induced hypophosphatemic rickets
FGF23-mediated tumor-induced osteomalacia
Fibrous dysplasia
Hormones (insulin, glucagon, cortisol)
Hyperparathyroidisms
Intestinal malabsorption
MacCune–Albright syndrome
Renal transplant
Renal tubular defects (Fanconi syndrome)
Salicylate poisoning
Severe dietary deficiency
Severe sepsis
Vitamin D deficiency
Hyperphosphatemia
Acromegaly
Bisphosphonate therapy
Hemolysis
Hypoparathyroidism
Intravenous/oral phosphate therapy
Magnesium deficiency
Metabolic acidosis
Phosphate enema
Renal failure
Respiratory acidosis
Rhabdomyolysis
Tumor calcinosis
Tumor lysis syndrome
Vitamin D intoxication