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Biochemical assessment of phosphate homeostasis

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ABSTRACT

Phosphate homeostasis is a requirement for normal life. Phosphate is involved in the synthesis of membrane lipids, DNA, RNA, and energy-rich molecules (ATP and GTP), and the regulation of protein activity by phosphorylation/dephosphorylation. Moreover, phosphate is a component of apatite crystals, which provide stability to the bone, and is essential for normal growth. Phosphate balance in the body is the difference between net phosphate absorption through the intestine and phosphate excretion through the kidney. Numerous disorders, both genetic and acquired, may alter phosphate homeostasis. In affected individuals, it is crucial to identify the underlying mechanism(s) to provide adequate treatment; however, phosphate homeostasis assessment remains challenging. Besides the measurement of key hormones involved in the control of phosphate homeostasis (parathyroid hormone, vitamin D and metabolites, fibroblast growth factor 23), assessing the magnitude of phosphate reabsorption by the kidney is a crucial step. It makes it possible to distinguish between a primary disorder of renal phosphate reabsorption, associated with an intrinsic defect or endocrine disturbance, and a nutritional cause of phosphate deficiency. This strategy is described, and the potential consequences for therapeutic decisions are discussed.

1. Abbreviations

Pi inorganic phosphate;

NPT2A, NPT2B, and NPT2C Na⁺-dependent phosphate cotransporters 2A, 2B and 2C;

1,25(OH)₂ vitamin D 1,25-dihydroxyvitamin D;

PTH parathyroid hormone;

FGF23 fibroblast growth factor 23;

CaSR calcium/polycation-sensing receptor;

ADHR autosomal dominant hypophosphatemic rickets;

XLH X-linked hypophosphatemic rickets;

TRP tubular fractional reabsorption of phosphate;

TmPi tubular maximum reabsorption of phosphate;

GFR glomerular filtration rate

2. Introduction

Maintenance of phosphate homeostasis is a requirement for normal life. Phosphate is involved in the synthesis of membrane lipids, DNA, RNA, and energy-rich molecules (ATP or GTP), and in the regulation of protein activity by phosphorylation/dephosphorylation. Further, it is a component of apatite crystals, which provide stability to bone. Overall, adequate phosphate balance in the body results from the net sum of phosphate input and output flows.

Indeed, phosphate homeostasis in the body involves the regulation of numerous molecular and cellular mechanisms controlled by the endocrine system and may be disturbed in many pathological conditions. In individuals with a phosphate homeostasis disorder, it is crucial to identify the underlying mechanism(s) to provide appropriate treatment, but this assessment remains challenging.

3. Phosphate status in the body

Phosphate is the most abundant anion in the human body: on average, a normal adult contains approximately 23 mol (700 g or 8.5-10 g/kg body weight) of phosphate. It is mostly present in bone, 85% of bone mineral being apatite, and in soft tissues, as a component of ATP, phospholipids, and phosphoproteins. Extracellular phosphate only accounts for 1% of total body phosphate, two-thirds of it being organic and one-third inorganic: the latter fraction, inorganic phosphate (Pi), is routinely measured in plasma. Pi circulates as HPO_4^{2-} and H_2PO_4^- , in a 4:1 ratio at normal plasma pH.

Overall, net phosphate balance in the body usually is zero in young adults but positive during growth. Phosphate is absorbed from food in the small intestine. Dietary requirements are approximately 0.5 mmol/kg/day in young adults but are

much greater in growing children. Intestinal phosphate absorption occurs via both active transcellular and passive paracellular pathways [1]. Phosphate absorption is a linear and non-saturable function of phosphate intake. It mainly occurs through the dedicated Na⁺-dependent phosphate type 2B cotransporter (NPT2B) encoded by the *SLC34A2* gene, expressed at the apical membrane of enterocytes. In normal adults, under steady-state conditions, the amount of phosphate excreted in urine per day equals the net intestinal phosphate absorption in this time period, whereas in growing children and teenagers, less phosphate is found in urine than is absorbed due to skeletal and cellular uptake of phosphate. Intestinal absorption of phosphate is enhanced by the active metabolite of vitamin D, 1,25-dihydroxy vitamin D (1,25(OH)₂ vitamin D), which upregulates NPT2B expression. Unbound inorganic phosphate is almost totally absorbed, but cations such as calcium, magnesium, and aluminum bind to phosphate in the intestinal lumen and decrease its absorption.

Therefore, as we usually live in a natural environment of phosphate excess, the kidney, which dominates the phosphate output regulation, plays a significant role in extracellular phosphate homeostasis. Renal handling of phosphate is a filtration-reabsorption process. Highly active tubular Pi reabsorption prevents the body from excessive urinary phosphate loss [1]. Phosphate is weakly bound to proteins, and 80-90% of plasma phosphate is ultra-filterable. The primary site of phosphate reabsorption in rodents and humans is the proximal tubule. Phosphate is transported across the apical membrane of the proximal tubule cell by specific transporters, mainly type 2A and 2C Na⁺-dependent phosphate cotransporters (NPT2A, encoded by *SLC34A1*, and NPT2C, encoded by *SLC34A3*, respectively). The pathway for phosphate to leave the proximal tubular cell remains unclear.

Two hormones are potent inhibitors of proximal phosphate absorption: parathyroid hormone (PTH) and fibroblast growth factor 23 (FGF23). PTH is released by parathyroid chief cells upon inactivation of the calcium/polycation-sensing receptor (CaSR). CaSR expression and activity are down-regulated by a decrease in extracellular free calcium concentration, which results in increased PTH secretion. Hence, PTH's primary role is to control the plasma level of free calcium, its role in adapting the phosphate level being much more accessory. FGF23 is mainly secreted by osteocytes and lining cells. Both PTH and FGF23 act on the renal proximal tubular cells and decrease phosphate reabsorption by inducing endocytosis of the apical sodium-phosphate cotransporters. This effect accounts for the decrease in plasma phosphate concentration that can be seen in patients with hyperparathyroidism (either primary or secondary), as well as in patients with elevated FGF23 concentrations in autosomal dominant hypophosphatemic rickets (ADHR) [2], X-linked hypophosphatemic rickets (XLH) [3], tumor-induced osteomalacia [3,4] and McCune-Albright syndrome with fibrous dysplasia [5].

While PTH and FGF23 have similar effects on proximal phosphate transport, they have opposite effects on the renal synthesis of 1,25(OH)₂ vitamin D: PTH activates, and FGF23 inhibits the activity of the 1-alpha-hydroxylase (CYP27B1), which represents the limiting step in the synthesis of 1,25(OH)₂ vitamin D. In addition, FGF23 activates the CYP24A1 enzyme which inactivates 1,25(OH)₂ vitamin D. Notably, FGF23 has a weak inherent ability to bind to its receptor, fibroblast growth factor receptor 1, in the proximal tubule and requires a cofactor, klotho, for optimal function in the kidney [6]. Growth hormone, insulin-like growth factor 1, and thyroid hormones increase phosphate reabsorption by the proximal tubule, contributing to the adaptation of phosphate levels during growth [7,8]. In particular, insulin-like growth

factor 1 increases the expression of NPT2A at the apical membrane of tubular cells [9].

Phosphate is essential for the apoptosis of hypertrophic chondrocytes through, as demonstrated recently, activation of the ERK1/2 pathway [10,11].

4. Extracellular phosphate measurement

Phosphate concentration is characterized by a high physiological variation, depending on age, sex, and physiological state. Extracellular phosphate concentration also fluctuates with a circadian rhythm. In adults, the concentration rises after 11 am, reaching 0.3 mmol/L higher in the afternoon than the morning; and a second rise begins around 8 pm with a peak at 2-3 am followed by a decrease, the minimum value being reached between 8 and 10 am. Reference values have been determined with samples collected from fasting volunteers in the morning, a time when extracellular phosphate concentration is the lowest [12-15]. The pattern of changes is similar in children, but the mean values are higher than in adults [16-18]. Extracellular phosphate concentration can be measured in serum or heparinized plasma samples; EDTA- or citrate-containing samples should be avoided as both compounds can interfere with the formation of the phosphomolybdate complex. Extracellular phosphate concentration is 0.06 to 0.10 mmol/L lower in plasma than in serum (<http://www.acb.org.uk/whatwedo/science/AMALC.aspx>). Many compounds can interfere with extracellular phosphate measurement, resulting in either pseudohypo- or pseudohyperphosphatemia. Mannitol at high concentrations can bind to molybdate, which results in pseudohypophosphatemia [19-21], while pseudohyperphosphatemia may be observed in the presence of some monoclonal

immunoglobulins [22-26], high concentrations of bilirubin or lipids [27-29], or contamination by tissue plasminogen activator [30,31].

As phosphate, like potassium, is mostly found in intracellular fluid, acute shifts of phosphate into or out of cells can significantly affect extracellular fluid phosphate concentration. An increase in glycolysis, such as that elicited by acute respiratory alkalosis or glucose or fructose intake, can decrease extracellular phosphate concentration by 0.1 mmol/L in healthy individuals [32]. Ideally, extracellular fluid phosphate concentration must be measured in the morning, after an overnight fast, under normal breathing conditions.

5. Assessment of renal tubular handling of phosphate

5.1. Is the renal tubular phosphate (re)absorption altered?

Healthy individuals submitted to an essentially phosphate-free diet show a rapid change in urinary phosphate excretion that decreases to a value close to zero [33]. The change occurs within the first two days. This indicates that the normal renal tubule is able to reclaim almost all phosphate that has been filtered under conditions exposing an individual to the phosphate depletion. Therefore, a low urinary phosphate concentration is expected in patients with hypophosphatemia of extrarenal origin. On the other hand, urinary phosphate concentration is determined not only by the renal tubule's ability to reabsorb phosphate but also by urine flow. Stated differently, urinary phosphate concentration may be low in patients with a renal leak of phosphate and polyuria; and it may be high in patients with extrarenal depletion of phosphate and highly concentrated urine due to water deprivation. A more robust assessment of phosphate's renal tubular handling requires the calculation of the tubular fractional reabsorption of phosphate (TRP), the fraction of

the filtered load that is reabsorbed by the renal tubule. To do so, urine should be collected after fasting to avoid fluctuations due to intestinal phosphate absorption and insulin release [34]. Phosphate and creatinine concentrations are measured in a urine sample, and a blood sample collected simultaneously. TRP is calculated as follows:

$$1 - \frac{UPi \times PCr}{PPi \times UCr}$$

UPi is the urinary concentration of Pi, PCr is the extracellular concentration of creatinine, PPi is the extracellular concentration of Pi and UCr is the urinary concentration of creatinine, all expressed in the same units (mmol/l). Inappropriately high TRP in hyperphosphatemia or inappropriately low TRP in the context of hypophosphatemia suggests a renal proximal tubular abnormality as the underlying cause.

The renal phosphate threshold concentration, computed as the ratio of maximum tubular reabsorption of phosphate (TmPi) to glomerular filtration rate (GFR), may also be used to assess tubular handling of phosphate, and - unlike TRP - this value is independent of GFR. It is derived from a nomogram developed by Walton and Bijvoet [35] (Fig. 1) to correct for the nonlinear relationship between TRP and TmPi/GFR when TRP is higher than 0.86 [36]. This value is the theoretical extracellular phosphate concentration for which all nephrons would reach saturation if they were all identical; however, they are not, and this accounts for the splay in the curve of the relationship between TRP and TmPi/GFR. One assumption when using TmPi/GFR is that creatinine clearance is a proxy for GFR. As extracellular phosphate concentration is usually higher in newborns, infants, and young children, TmPi/GFR might not be accurate in this population. Some authors suggested using another variable to assess the renal handling of phosphate [37]. In older children, a

urine spot technique can be used because the 24-hour collection is difficult and often inaccurate. The urine spot test must be performed in the morning, taking into account the circadian rhythm of plasma phosphate concentration. Normative values of TRP and TmPi/GFR have been provided according to age, these varying slightly throughout infancy and puberty [18]. Hypophosphatemia with a low TRP or TmPi/GFR indicates a defect in renal tubular handling of phosphate.

5.2. Is the alteration in renal tubular phosphate (re)absorption isolated?

When the disorder of extracellular phosphate concentration stems from the renal tubule, the next step is to determine whether the condition is isolated or part of a more complex tubular dysfunction. The proximal tubule, by far the primary site of phosphate reabsorption, also reabsorbs the bulk of filtered bicarbonate, amino acids, and proteins and reclaims all filtered glucose. Further, it is the central place where activated vitamin D, 1,25(OH)₂ vitamin D, is formed and secreted. Isolated disorders of phosphate reabsorption are usually associated with a change in 1,25(OH)₂ vitamin D concentration that is appropriate given the phosphate levels because the activity of the 1-alpha hydroxylase in the proximal tubule is affected by the level of extracellular phosphate: hypophosphatemia increases 1-alpha hydroxylase activity, whereas hyperphosphatemia does the opposite.

Isolated defects in extracellular phosphate concentration (with an appropriate 1,25(OH)₂ vitamin D concentration) commonly result from defects affecting membrane phosphate transporters themselves or disorders in parathyroid hormone secretion. In contrast, abnormalities affecting all secondary active transports in the proximal tubule and 1,25(OH)₂ vitamin D synthesis are present in patients with de Toni-Debré-Fanconi syndrome. Finally, isolated abnormalities of phosphate transport

and an inappropriate (low) $1,25(\text{OH})_2$ vitamin D concentration are typical of disorders affecting the metabolism of FGF23. It may, therefore, be helpful to use a simple algorithm to distinguish between these situations (Fig. 2). Nutritional rickets due to primary vitamin D deficiency, with decreased intestinal phosphate absorption, will result in various degrees of hypophosphatemia, depending on the level of secondary hyperparathyroidism. Figure 3 provides an example of situations in which an assessment of phosphate reabsorption is essential to diagnose the etiology in cases of hypophosphatemia.

6. Hypophosphatemia in primary hyperparathyroidism is not the result of renal phosphate wasting

Establishing phosphate reabsorption status by measuring TRP and/or TmPi/GFR is indeed a crucial step to progress towards identifying the etiology of disorders associated with hypophosphatemia or hyperphosphatemia. Knowledge of the level of phosphate-regulating hormones helps considerably in reaching a diagnosis.

Appropriate (increased) TRP (and TmPi/GFR) levels characterize extrarenal phosphate leak situations where renal conservation of phosphate is a requirement for homeostasis. This is found in advanced nutritional diseases, namely, anorexia nervosa, with dietary phosphate (and frequently vitamin D) deficiency. In such cases, phosphate supplementation is indicated to restore phosphate levels to normal. The same approach is logical in severe vitamin D deficiency cases when phosphate reserves are low, potentially compromising vital cellular functions. These situations are often worsened by secondary hyperparathyroidism.

However, the situation is different in primary hyperparathyroidism, where hypophosphatemia is associated with low TmPi/GFR due to the excess of PTH.

According to Parfitt, the hypophosphatemia of primary hyperparathyroidism is not the result of renal phosphate wasting [38] (Fig. 4), which means that, in steady-state, all the phosphate entering the body is eliminated in the urine, with the risk of favoring nephrocalcinosis or calcium stones in the event of excessive supplementation. The same holds true in X-linked hypophosphatemic rickets where the primary abnormality is the excess of FGF23 leading to chronically low TmPi/GFR, together with a low level of active vitamin D. In this case, phosphate must be administered with caution, with frequent administration of low doses, not to normalize phosphate levels (which could be likened to "trying to fill a bathtub with the plug out"), but rather to generate repeated pulses of phosphate to transfer it to bone [39]. Otherwise, the excess of phosphate will chronically stimulate the parathyroid gland cells and lead to secondary or tertiary hyperparathyroidism, nephrocalcinosis, and renal stones. However, in the future, more specific treatments aiming to decrease the level of active FGF23 (such as anti-FGF23 antibodies) are likely to be able to obviate such side effects [40,41].

7. Phosphate and FGF23

FGF23 has emerged in the last two decades as the major hormone regulating phosphate homeostasis in the body [6]. FGF23 produced in bone circulates as active (native) and inactive (cleaved) forms. It has a wide range of effects apart from phosphate homeostasis, which is beyond the scope of this review. It can be speculated that active FGF23 prevents excessive calcification of soft tissues by maintaining an adequate plasma phosphate level through renal tubular phosphate reabsorption control. Indeed, while the body must frequently deal with calcium insufficiency, phosphate is naturally abundant in a normal diet. Its excess must be

eliminated in the urine; the amount of filtered phosphate that is not reabsorbed in the proximal tubule and subsequently lost in urine is determined in part by FGF23.

On the other hand, various distinct factors play a role, as illustrated by the phosphate retention during the progression of chronic kidney disease despite an increasing level of FGF23 [42]. Defects in FGF23 cleavage in cases of loss of function of N-acetylgalactosaminyltransferase 3, with a highly cleavable form of FGF23 [43-45], or autoimmune expression of FGF23 antibodies [44] result in hyperphosphatemia and tumoral calcinosis. Conversely, several genetic and non-genetic causes of hypophosphatemia are related to FGF23 excess, due to non-cleavable FGF23 (mutation of its cleavage site in ADHR) or excessive FGF23 production, in XLH, tumor-induced osteomalacia or fibrous dysplasia. Thus, when TmPi/GFR and 1,25(OH)₂ vitamin D levels are inappropriate to plasma phosphate levels, the measurement of total and cleaved forms of FGF23 may help identify specific underlying causes.

8. Conclusion

In the context of chronic hypo- or hyperphosphatemia, a set of parameters is needed to determine whether the abnormality stems from the renal proximal tubule or has a non-renal origin. The first step is to obtain an accurate measurement of phosphate levels and analyze whether the renal phosphate reabsorption level is appropriate or not and whether the disorder is isolated. Analysis of essential determinants of phosphate homeostasis (PTH, vitamin D) is mandatory to identify the origin of the disorder. The measurement of FGF23 or 1,25(OH)₂ vitamin D should be considered a later part of the etiological workup. In children, adequate normative values must be evaluated to avoid inconsistent conclusions and inappropriate treatments.

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Contributors

Both authors wrote the manuscript and approved the final version.

Disclosure of interest

Pascal Houillier has served as a speaker and taken part in advisory board meetings for Shire, Kyowa Kirin and Takeda. Jean-Pierre Salles reports no conflict of interest.

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FIGURE LEGENDS

Fig. 1. Nomogram for derivation of renal threshold phosphate concentration.

From Walton and Bijvoet (ref 35). The renal threshold phosphate concentration (Tm_{Pi}/GFR) can usually be derived from the tubular fractional reabsorption of phosphate (TRP) and phosphate levels, assuming that renal creatinine clearance is a proxy for GFR (Alon and Hellerstein ref. 37). For children, age-adjusted normative values must be used.

Fig. 2. Simplified algorithm to identify various causes of hypophosphatemia.

Assessment of tubular fractional reabsorption of phosphate (TRP) and renal threshold phosphate concentration (Tm_{Pi}/GFR) is the essential step in order to ascertain whether renal phosphate reabsorption is appropriate (in cases of

extrarenal phosphate deficiency) or inappropriate in cases of abnormal renal phosphate handling. Hormones involved in the control of plasma phosphate concentration (vitamin D and metabolites, parathyroid hormone (PTH) and fibroblast growth factor 23 (FGF23)) must be taken into account. Abnormal phosphate handling has numerous etiologies, both genetic (abnormalities in Na⁺-dependent phosphate cotransporters, ADHR, autosomal dominant hypophosphatemic rickets; XLH, X-linked hypophosphatemic rickets, complex tubulopathy ...) and acquired (TIO, tumor-induced osteomalacia, fibrous dysplasia, secondary tubulopathy).

Fig. 3. Example of distinctive situations of hypophosphatemia.

A sister (on the left) and brother (on the right) who presented at our hospital with a history of orthopedic surgery. The two children, from a family of eight children from the Comoro Islands, share the same mother and father. The girl had developed progressive bilateral femoral bowing, justifying hemiepiphysiodesis (a). The boy had a long history of bilateral femoral bowing surgery, initially hemiepiphysiodesis, and finally osteotomy with external fixation for reaxation (b and c). Both presented with hypophosphatemia at onset (d) and vitamin D deficiency. Tubular fractional reabsorption of phosphate (TRP) and renal phosphate threshold (TmPi/GFR) were inappropriately low in the girl but not in the boy. The follow-up demonstrated persistent hypophosphatemia in the girl and normalization in the boy after vitamin D supplementation. The final diagnosis was sequelae of severe nutritional rickets in the boy and hypophosphatemic rickets (treated by phosphate and 1-hydroxylated vitamin D) in the girl. All their siblings had various levels of vitamin D deficiency partly attributable to colored skin and excess weight. Notably, FGF23 level was found to be high in the girl and low in the boy. Pi is expressed as mmol/l, parathyroid

hormone levels (PTH) as pg/ml, 25 OH vitamin D as ng/ml, and FGF23 (ELISA Immunotopics) as UI/ml (normal values: 30-60).

Fig. 4. There is no phosphate leak in primary hyperparathyroidism under steady-state conditions.

From Parfitt (ref. 38). It is a misconception that renal phosphate wasting persists under steady-state conditions in hyperparathyroidism (or FGF23 excess). When PTH increases, the renal threshold phosphate concentration (T_{mPi}/GFR) decreases, causing a renal phosphate loss; then, the renal threshold phosphate concentration remains lower than normal but, under steady-state conditions, the net intestinal absorption equals the urinary excretion of phosphate. Excessive elimination of phosphate in urine would occur with inadequate attempts to normalize plasma phosphate levels with inappropriate phosphate intake, which would lead to detrimental excess of urinary phosphate excretion.

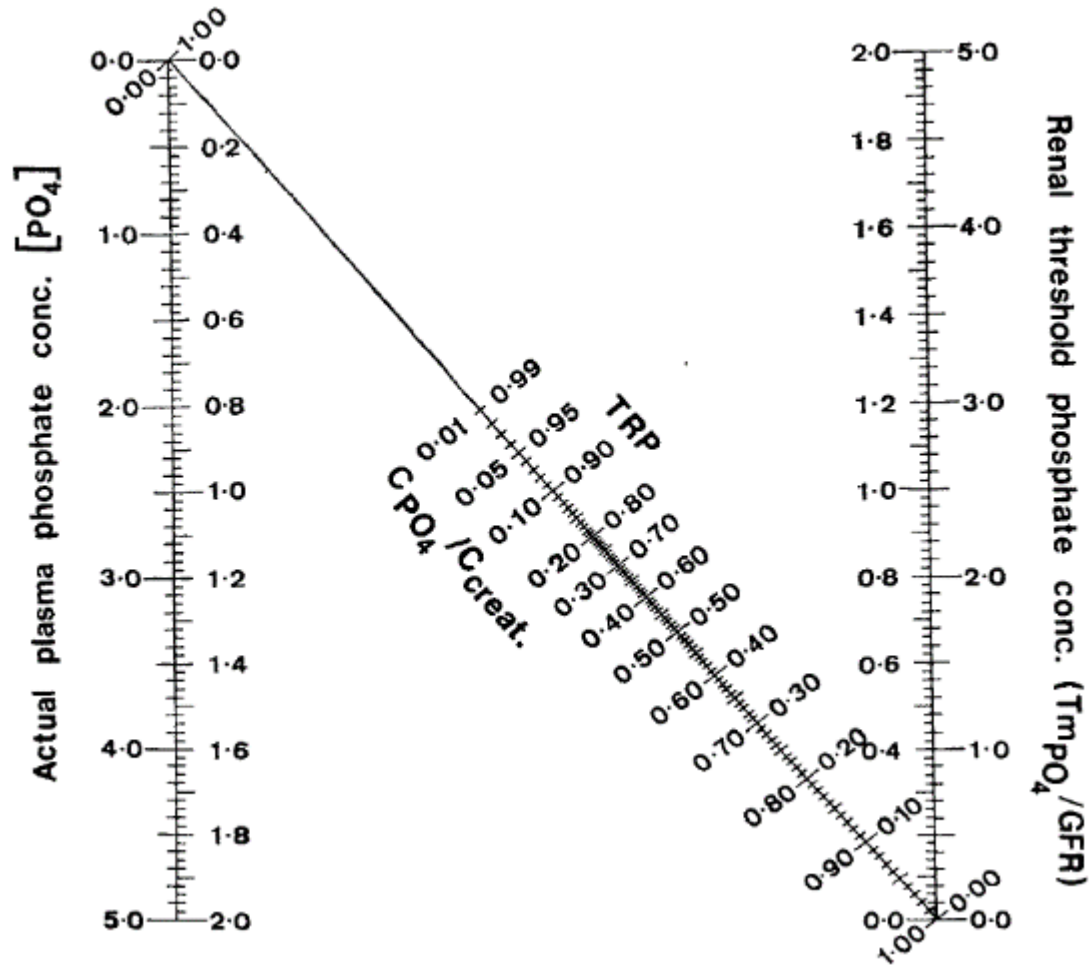


Figure 1

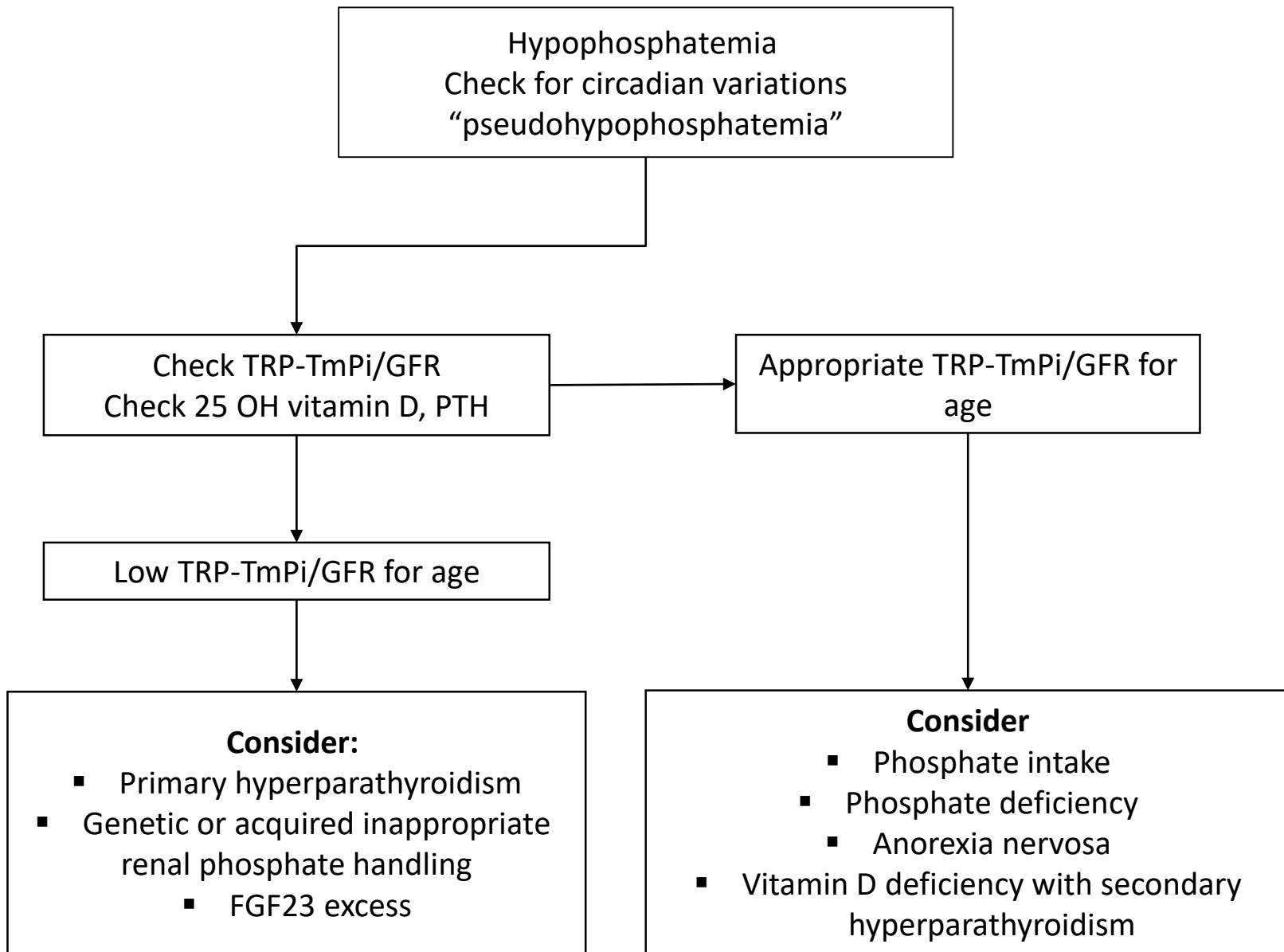
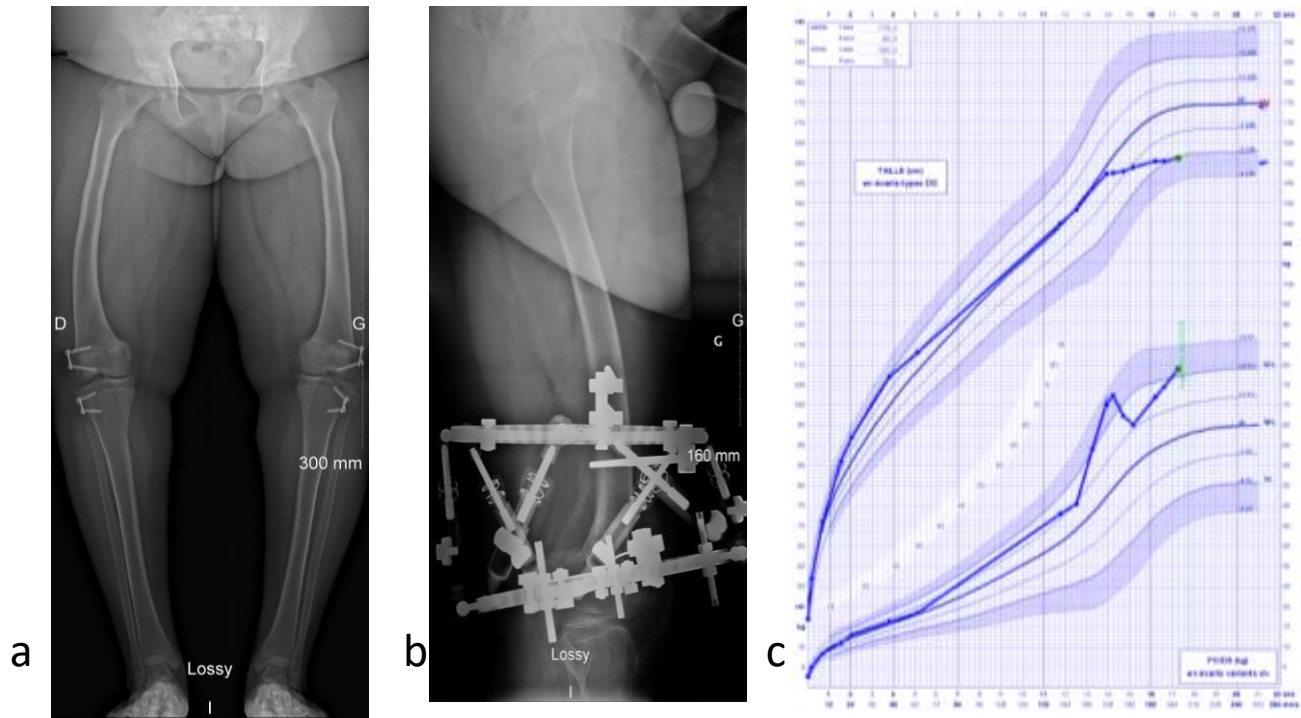


Figure 2



d

Girl	9 years	10 years	13 years
PLASMA			
Phosphate	0.66	0.75	0.78
Alkaline phosphatase	792	753	408
	high	high	
25-OH vitamin D	10	27	18
	low		
PTH	30	20	19
FGF23	78	59	67
	high	high	high
URINE			
Calcium/creatinine	0.51	1.03	1.01
TRP	0.82	0.88	0.87
	low	low	low
TRP/GFR	0.64	0.78	0.72
	low	low	low

Boy	15 years	17 years
PLASMA		
Phosphate	0.79	1.15
Alkaline phosphatase	552	114
	high	
25-OH vitamin D	7	19
	low	
PTH	18	15
FGF23	22	25
	low	low
URINE		
Calcium/creatinine	0.80	0.23
TRP	0.91	0.94
		appropriate
TRP/GFR	0.95	0.9
	appropriate	appropriate

Figure 3

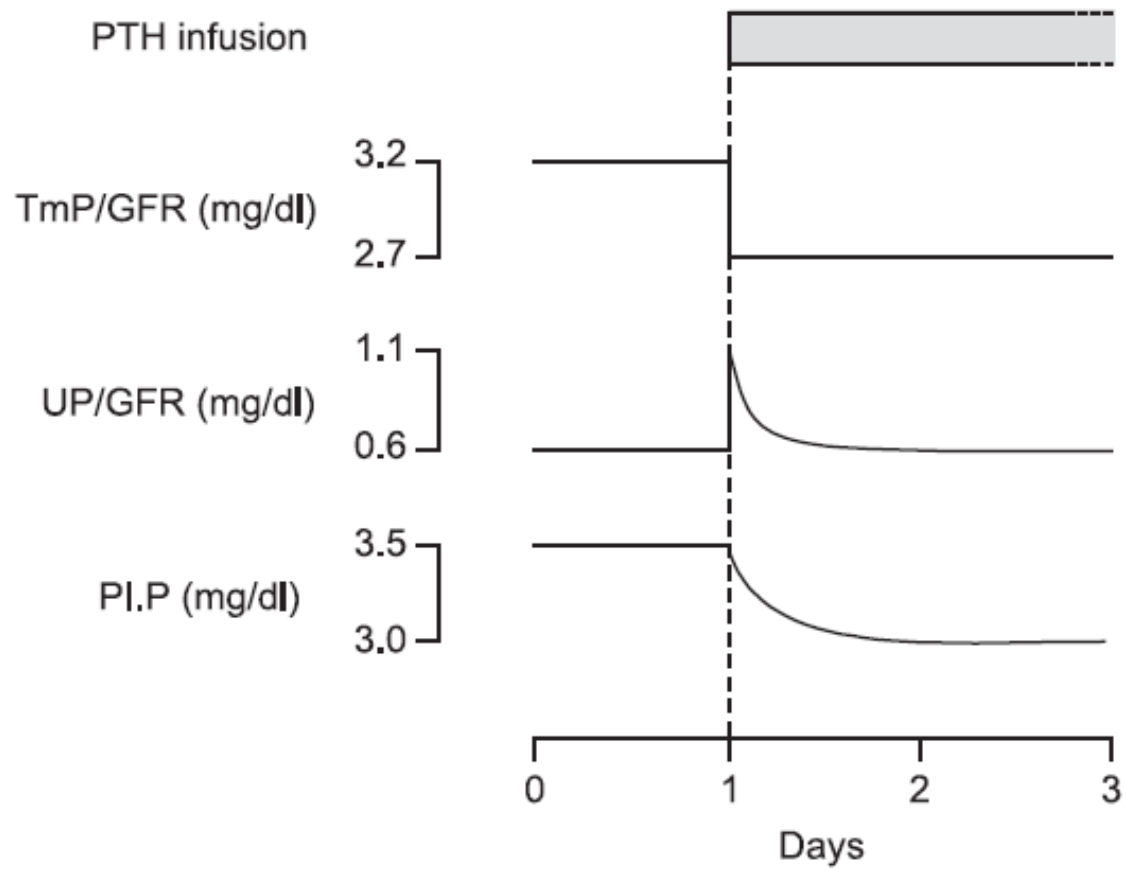


Figure 4