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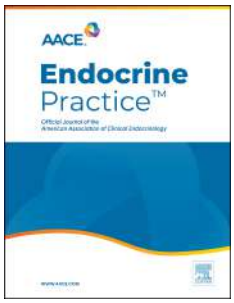

Phosphatonins: From Discovery to Therapeutics

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Phosphatonins: From Discovery to Therapeutics

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Structured Abstract

Phosphate is crucial for cell signaling, energy metabolism, nucleotide synthesis, and bone mineralization. The gut-bone-parathyroid-kidney axis is influenced by parathyroid hormone (PTH), 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), and phosphatonins, and facilitates maintenance of phosphate homeostasis. Phosphatonins including fibroblast growth factor 23 (FGF23), secreted frizzled-related protein 4 (sFRP4), matrix extracellular phosphoglycoprotein (MEPE), and fibroblast growth factor 7 (FGF7) play a pathogenic role in several hypophosphatemic disorders. Excess FGF23 inhibits sodium-dependent phosphate cotransporters (NaPi-2a and NaPi-2c), resulting in hyperphosphaturia and hypophosphatemia. Additionally, FGF23 suppresses $1,25(OH)_2D$ synthesis in the proximal renal tubule, and thus it indirectly inhibits intestinal phosphate absorption. Disorders of FGF23-related hypophosphatemia include X-linked hypophosphatemia (XLH), autosomal dominant hypophosphatemic rickets (ADHR), autosomal recessive hypophosphatemic rickets (ARHR), fibrous dysplasia/McCune-Albright syndrome, and tumor-induced osteomalacia (TIO). Complications of conventional therapy with oral phosphate and vitamin D analogs comprise gastrointestinal distress, hypercalcemia, nephrocalcinosis, and secondary/tertiary hyperparathyroidism. In both children and adults with XLH and TIO, the anti-FGF23 antibody burosumab exhibits a favorable safety profile and is associated with healing of rickets in affected children and improvement of osteomalacia in both children and adults. This review summarizes current knowledge regarding the phosphate homeostasis, phosphatonin pathophysiology, and clinical implications of FGF23-related hypophosphatemic disorders, with specific focus on burosumab treatment.

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Introduction

Phosphate plays crucial roles in cell signaling, energy metabolism, membrane function, and nucleotide synthesis.[1-3] Additionally, phosphate is essential for biomineralization[4] and metabolic pathways including glycolysis, ammoniagenesis, and phosphorylation. Moreover, phosphate regulates oxygen-carrying capacity via the generation of 2,3-diphosphoglycerate in erythrocytes. Approximately 85% of phosphate in the body is present in bone and teeth, 14% exists in soft tissues, and 1% is present in extracellular fluids. In plasma, approximately 85% of phosphate is present as free forms (HPO_4^{2-} and $H_2PO_4^-$), 10% is bound to proteins, and 5% is complexed with cations.[2] Serum phosphate concentrations are higher in children than in adults; the reference range is 4-7 mg/dL in children compared with 2.5-4.5 mg/dL in adults. Serum phosphate concentrations exhibit a biphasic circadian rhythm. Values are lowest in the morning, peak first in the late afternoon and peak again in the late evening. Since transcellular shifting of phosphate occurs under certain circumstances, serum phosphate concentrations may not reflect true phosphate stores. Serum phosphate concentrations are dependent on dietary intake and are regulated by parathyroid hormone (PTH), fibroblast growth factor 23 (FGF23), and 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) through feedback mechanisms (Figure 1).[2]

Intestinal Phosphate Absorption

Dietary phosphate intake averages 1,000-1,500 mg per day. Approximately 60-80% of dietary phosphate is absorbed mostly through the proximal small intestine. Intestinal phosphate absorption occurs via two pathways: a passive paracellular and an active transcellular pathway (Figure 2).[5,6] Passive transport mechanisms depend on electrochemical phosphate gradients across the intestinal epithelial cell layer with paracellular movement mediated by tight junction

proteins. The active transcellular transport of phosphate is mediated by the sodium-phosphate cotransporter 2b (NaPi-2b) on the apical membrane of enterocytes.[7] In addition, PiT-1 and, to a lesser extent, PiT-2 transporters also contribute to transcellular intestinal phosphate absorption.[8] The deletion of NaPi-2b gene results in compensatory increases in the renal tubular phosphate reabsorption which help to normalize serum phosphate concentrations.[9] NaPi-2b expression is upregulated by the administration of $1,25(OH)_2D$ and dietary phosphate restriction.[6] PTH indirectly induces changes in NaPi-2b expression through its stimulatory effect on renal $1,25(OH)_2D$ synthesis, which enhances NaPi-2b expression.[10]

Renal Phosphate Transport

The kidney is central to the regulation of phosphate homeostasis. Approximately 90-95% of circulating phosphate is filtered through the glomerulus, 80% of which is reabsorbed in the proximal tubule with less than 10% being reabsorbed in the distal nephron.[2] The renal tubular reabsorption of phosphate is mainly mediated by sodium-phosphate cotransporters (NaPi-2a, SLC34A1; NaPi-2c, SLC34A3; and PiT-2, SLC20A2) in the apical membrane of the proximal tubule (Figure 3).[2] The abundance of NaPi-2a and NaPi-2c is increased by low-phosphate diet and decreased by PTH.[11] PTH suppresses proximal tubular reabsorption of phosphate by internalizing NaPi-2a and NaPi-2c in the apical membrane through interaction with the scaffold protein Na^+/H^+ exchanger regulatory factor 1 (NHERF1) and by increasing serine phosphorylation of NHERF1.[12] Additionally, dietary phosphate restriction induces apical membrane expression of PiT-2 in the proximal tubule.[13] Xenotropic and polytropic retrovirus receptor 1 (XPR1) could function as a basolateral phosphate transporter in the proximal tubule.[14] Mice with conditional inactivation of XPR1 gene in the renal tubule exhibit hypophosphatemic rickets and Fanconi syndrome with hyperphosphaturia.[14] Additional factors that increase renal

tubular phosphate reabsorption include volume contraction, metabolic alkalosis, $1,25(OH)_2D$, growth hormone, insulin, insulin-like growth factor 1, and thyroid hormone.[2] Conversely, additional factors that decrease renal tubular phosphate reabsorption include phosphate loading, volume expansion, metabolic acidosis, carbonic anhydrase inhibitors, calcitonin, atrial natriuretic peptide, dopamine, estrogen, and glucocorticoids.[15-17]

Phosphatonins

In 1994, the existence of phosphaturic factors ("phosphatonins") that induce renal phosphate wasting in patients with tumor-induced osteomalacia (TIO) was described.[18,19] Conditioned medium from a tumor associated with TIO was found to secrete substances that inhibited sodium-dependent phosphate transport in opossum kidney epithelial cells. This heat-labile substance(s) inhibited phosphate transport via a cAMP-independent mechanism, and its action was not blocked by a PTH antagonist.[18] The phosphatonins have emerged as pivotal regulators of phosphate homeostasis which act by inducing a state of negative phosphate balance via direct inhibition of phosphate reabsorption in the proximal tubule and indirectly via the suppression of $1,25(OH)_2D$ production in the kidney. The currently known phosphatonins include fibroblast growth factor 23 (FGF23), matrix extracellular phosphoglycoprotein (MEPE), secreted frizzled-related protein 4 (sFRP4), and fibroblast growth factor 7 (FGF7).

Fibroblast Growth Factor 23

Fibroblast growth factor 23 (FGF23) is predominantly expressed in bone (osteocytes and osteoblasts). FGF23 is initially produced as a 251-amino acid prohormone which comprises three domains with the first 24 amino acids being the signal sequence, the middle portion of 155 amino acids forming the core FGF homology domain, and the last 72 amino acids forming the C-

terminal domain of FGF23 (Figure 4).[20] After intracellular cleavage of the signal sequence, intact FGF23 (iFGF23) is O-glycosylated at threonine 178 by polypeptide N-acetylgalactosaminyltransferase 3 (GALNT3), which prevents proteolysis and facilitates the secretion of iFGF23 as a 227-amino acid glycoprotein with a molecular mass of 32 kDa and a plasma half-life of 45-60 minutes.[21] Alternatively, iFGF23 can be cleaved intracellularly by subtilisin-like proprotein convertases at the protease recognition site (R176XXR179), yielding biologically inactive N- and C-terminal FGF23 fragments that are cosecreted with iFGF23.[22] The balance between GALNT3-mediated O-linked glycosylation and proprotein convertase cleavage determines the amount of iFGF23 and FGF23 fragment in the circulation.[23]

The effects of FGF23 on phosphate homeostasis are mainly mediated by activation of the FGF receptor 1 (FGFR1) and require an alpha-klotho coreceptor that is highly expressed in the kidney and parathyroid gland.[24] The C-terminal domain of iFGF23 mediates binding of FGF23 to the FGFR-klotho complex.[25] C-terminal FGF23 fragments impair cellular signaling by competing with iFGF23 for binding to the FGFR-klotho complex.[25] FGF23 production in bone is stimulated by a variety of factors including high dietary or serum phosphate levels, PTH, $1,25(OH)_2D$, calcium, iron deficiency, erythropoietin, metabolic acidosis, and inflammatory cytokines.[26]

FGF23 reduces the expression of NaPi-2a and NaPi-2c transporters in the proximal tubule and increases phosphaturia.[2] Evidence shows that hypoparathyroid patients had higher serum phosphate and iFGF23 levels than healthy controls, but the fractional excretion of phosphate was comparable between the two groups.[27] This suggests that the existence of sufficient PTH might be necessary for the phosphaturic effect of FGF23. Further, serum calcium levels may also be involved in the modulation of FGF23 effects on renal tubular phosphate transport in patients with X-linked hypophosphatemia (XLH).[28] In the kidney, klotho is expressed mainly in the distal

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tubule with lower abundance in the proximal tubule.[29] The circulating form of soluble klotho can also act as a coreceptor for FGF23 signaling. Moreover, klotho can act as a phosphaturic factor by diminishing the abundance of NaPi-2a in the proximal tubule.[30] FGF23 reduces serum $1,25(OH)_2D$ level by suppressing renal 1-alpha-hydroxylase (CYP27B1) expression and stimulating the 24-hydroxylase (CYP24A1) that converts 25(OH)D and $1,25(OH)_2D$ into inactive metabolites.[1] Moreover, FGF23 directly suppresses PTH production from parathyroid glands through activation of both the klotho-dependent mitogen-activated protein kinase (MAPK) pathway and the klotho-independent calcineurin-mediated signaling pathway.[31] FGF23 excess causes hypophosphatemia and reduced $1,25(OH)_2D$ levels, whereas FGF23 deficiency causes hyperphosphatemia and elevated $1,25(OH)_2D$ levels.[22]

Matrix Extracellular Phosphoglycoprotein

Matrix extracellular phosphoglycoprotein (MEPE) is also among the most abundantly overexpressed genes found in TIO.[32,33] The intraperitoneal injection of MEPE causes hyperphosphaturia and hypophosphatemia in mice.[34,35] Additionally, MEPE inhibits phosphate uptake in human proximal tubular epithelial cells in vitro. Moreover, MEPE inhibits bone mineralization in vitro and MEPE null mice have increased bone mineralization.[35] These suggest that MEPE may play a role in the pathogenesis of XLH in which there is renal phosphate wasting and evidence for a mineralization defect that is independent of serum phosphate concentrations. Serum MEPE levels are elevated in patients with XLH.[34] PHEX prevents proteolysis of MEPE and release of an acidic serine aspartate-rich MEPE-associated motif (ASARM) peptide, an inhibitor of bone mineralization (minhibin).[36] PHEX and MEPE form a nonproteolytic protein interaction via the MEPE C-terminal ASARM motif. Inactivating mutations of PHEX increases the production of MEPE-derived ASARM peptide and may cause hypomineralization in XLH. A

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subsequent study challenges this conclusion by demonstrating that the selective knockout of PHEX in osteoblasts of normal mice results in a disorder with bone abnormalities and decreased renal phosphate transport, similar to that in the Hyp-mouse, a murine model for XLH in humans. However, in this knockout animal model the serum levels of MEPE are normal, while these variables are elevated in Hyp-mice. These data indicate that MEPE may not participate in the pathophysiology of renal phosphate transport defect and possibly the bone mineralization abnormality in XLH.[37]

Secreted Frizzled-Related Protein 4

Secreted frizzled-related protein 4 (sFRP4) is among the most consistently overexpressed genes found associated with TIO.[32] We have demonstrated that sFRP4 inhibited sodium-dependent phosphate transport by reducing NaPi-2a abundance in the brush border membrane of the proximal tubule and on the surface of opossum kidney epithelial cells.[38] Additionally, the intravenous infusion of sFRP4 increased phosphaturia, decreased serum phosphate, but did not alter renal 1-alpha-hydroxylase mRNA concentrations in rats. However, the expected increase in serum $1,25(OH)_2D$ did not occur. Thus, sFRP4 may inhibit the compensatory upregulation of 1-alpha-hydroxylase activity and $1,25(OH)_2D$ synthesis. The phosphaturic effects of sFRP4 were also demonstrated in thyroparathyroidectomized rats, suggesting the PTH-independent activity of sFRP4.[39] Genetic deletion of the sFRP4 gene in mice does not significantly impact serum or urine phosphate levels. Further, sFRP4 is unable to compensate for the absence of FGF23 or klotho since double knockouts have similar phenotypes as mice with deletion of FGF23 or klotho alone.[40] Furthermore, we observed that serum sFRP4 levels did not change with creatinine clearance or hyperphosphatemia in patients with CKD, and no correlation was found between post-kidney transplant serum sFRP4 levels and hypophosphatemia.[41] We created mice with a

global Phex knockout (Cre-PhexDeltaflox/y mice) and conditional osteocalcin (OC)-promoted Phex inactivation in osteoblasts and osteocytes (OC-Cre-PhexDeltaflox/y). Serum phosphate levels in Cre-PhexDeltaflox/y, OC-Cre-PhexDeltaflox/y, and hyp-mice were lower than those in normal mice. Kidney cell membrane phosphate transport in Cre-PhexDeltaflox/y, OC-Cre-PhexDeltaflox/y, and hyp-mice was likewise reduced compared with that in normal mice. Abnormal renal phosphate transport in Cre-PhexDeltaflox/y and OC-Cre-PhexDeltaflox/y mice was associated with increased bone production and serum FGF23 levels and decreased renal NaPi-2a protein, as was the case in hyp-mice. Additionally, Cre-PhexDeltaflox/y, OC-Cre-PhexDeltaflox/y, and hyp-mice manifested comparable osteomalacia. Serum FGF23, MEPE, and sFRP4 levels were increased in *hyp*- and *Cre-Phex*$^{\Delta flox/y}$ mice. However, serum FGF23, but not MEPE or sFRP4, was increased in *OC-Cre-Phex*$^{\Delta}$*flox/y* mice.[37] Altogether, although sFRP4 acutely alters renal phosphate handling and possibly 1,25$(OH)_2$D physiology, most data demonstrate that it plays a minor role in the regulation of phosphate homeostasis.

Fibroblast Growth Factor 7

Previous research has shown that FGF7 is overexpressed in tumors associated with TIO.[42] FGF7 inhibits sodium-dependent phosphate transport in opossum kidney epithelial cells and enhances phosphaturia in rats.[43] Moreover, circulating FGF7 levels are elevated in some patients with TIO.[44] A recent study showed that low serum FGF7 levels were observed in pediatric patients with hypophosphatasia and hyperphosphatemia.[43]

FGF23-Related Hypophosphatemic Disorders

X-Linked Hypophosphatemia

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X-linked hypophosphatemia (XLH) is the most common inherited form of rickets with an estimated prevalence of 1 case per 20,000 live births.[45,46] XLH is an X-linked dominant disorder characterized by rickets and osteomalacia in children and osteomalacia in adults. The clinical manifestations of XLH are variable, ranging from isolated hypophosphatemia to severe bone disease. XLH generally manifests in the first one to two years of age when rickets and lower-extremity bowing become apparent with the onset of weight bearing.[46,47] Rickets also lead to short stature and growth retardation. The complications that occur more commonly in adults with XLH include osteomalacia with pseudofractures, chronic bone and joint pain, dental abscesses, enthesopathy (calcification of the tendons and ligaments in close proximity to bone), osteoarthritis, muscle weakness, and sensorineural hearing loss.[1,46,47] Laboratory findings show hypophosphatemia, hyperphosphaturia, normocalcemia, low or inappropriately normal serum $1,25(OH)_2D$, and elevated serum alkaline phosphatase and FGF23 levels. FGF23-mediated $1,25(OH)_2D$ deficiency and oral phosphate therapy may lead to elevated PTH levels.[47]

PHEX (phosphate-regulating gene with homologies to endopeptidases on the X chromosome), a member of the M13 family of membrane-bound metalloproteases, has been identified as the gene inactivated in in XLH.[1] PHEX is expressed predominantly in osteoblasts and osteocytes in bone, and odontoblasts in teeth.[46] PHEX is not responsible for direct proteolytic cleavage of FGF23. Instead, PHEX may activate the subtilisin-like proprotein convertase activity by promoting the transcription of its 7B2 chaperone protein.[48] Therefore, inactivating mutations of PHEX lead to reduced levels of 7B2 chaperone protein, diminished activity of subtilisin-like proprotein convertase, reduced FGF23 degradation, and increased serum levels of FGF23.[48] However, the mechanism by which PHEX mutation causes downregulation of 7B2 chaperone protein expression remains unknown. In addition to FGF23-mediated renal phosphate wasting, the

pathophysiology of XLH may involve the abnormal metabolism of PHEX substrates called small integrin-binding ligand N-linked glycoproteins (SIBLING), which include osteopontin, bone sialoprotein, dentin matrix protein 1, dentin sialophosphoprotein, and MEPE.[49] Therefore, PHEX deficiency in XLH patients causes the accumulation of SIBLING proteins and their fragments, which contribute to local inhibition of mineralization. Hypophosphatemia inhibits mineralization and leads to rickets by impairing caspase-mediated apoptosis of hypertrophic chondrocytes.[50] Furthermore, hypophosphatemia-independent effects of excessive FGF23 in bone including local inhibition of $1,25(OH)_2D$ production and downregulation of tissue-nonspecific alkaline phosphatase also play a role in mineralization defect in XLH.[51] $1,25(OH)_2D$ stimulates osteoblast differentiation and induces the generation of mature matrix vesicles, thereby facilitating mineralization process.[52]

Autosomal Dominant Hypophosphatemic Rickets

Autosomal dominant hypophosphatemic rickets (ADHR) is a rare hereditary renal phosphate wasting disorder with phenotypes similar to XLH. The prevalence of ADHR is less than 1 cases per 100,000 live births. Approximately one-half of patients with ADHR present clinically evident disease, including rickets, at one to three years of age. In some affected children, hypophosphatemia persists into adulthood or remits after puberty. The remaining patients have delayed disease onset, ranging from 14 to 45 years. Late-onset ADHR may present with bone pain and hypophosphatemia in adulthood, but no lower-extremity deformities.[46,49,53] ADHR is caused by the mutation disrupting the R176XXR179 protease recognition site in FGF23, and the resultant mutant FGF23 is elevated due to reduced FGF23 cleavage.[46] Serum FGF23 levels usually fluctuate and reflect the disease activity of ADHR, partly depending on iron status.[54,55] Iron deficiency upregulates FGF23 expression in bone, and in normal individuals, intracellular

proteolysis of iFGF23 is efficient, thereby normalizing serum iFGF23 levels. However, inefficient degradation of mutant iFGF23 fails to compensate for increased FGF23 production during iron deficiency, thereby triggering ADHR symptoms. Thus, women with ADHR are at greater risk of developing hypophosphatemia during menstruation or pregnancy and correcting iron deficiency may improve symptoms.[56] Notably, certain intravenous iron preparations, especially ferric carboxymaltose (FCM), can increase serum FGF23 levels presumably by preventing cleavage of FGF23, thereby potentially exacerbating ADHR symptoms.[57] Several studies have reported that FCM resulted in high incidence of hypophosphatemia (serum phosphate <2.0 mg/dL), with values ranging from 64.0% to 75.0%, among patients with iron-deficiency anemia and normal kidney function.[58,59] Hypophosphatemia developed within 1 week of FCM administration, peaked in prevalence by week 2, and persisted through the end of the 5-week study. Reductions in serum phosphate were associated with contemporaneous increases in serum iFGF23, PTH, and $FEPO_4$, and decreases in $1,25(OH)_2D$.[58,59]

Autosomal Recessive Hypophosphatemic Rickets

Autosomal recessive hypophosphatemic rickets (ARHR) is caused by inactivating mutations in the gene encoding dentin matrix protein 1 (DMP1), ectonucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1), or the family with sequence similarity 20, member C (FAM20C).[46] DMP1 belongs to the large SIBLING family of extracellular matrix proteins and is mainly coexpressed with FGF23 in bone. ENPP1 is the transmembrane enzyme critical for the generation of inorganic pyrophosphate which is the mineralization inhibitor.[60] FAM20C directly phosphorylates FGF23 on serine 180, which inhibits GALNT3-mediated O-linked glycosylation, thus increasing intracellular FGF23 cleavage and reducing iFGF23 secretion. The clinical manifestations of ARHR are similar to those of XLH and ADHR. Serum

FGF23 levels are elevated or high normal. Moreover, ENPP1 mutations are associated with generalized arterial calcification in infancy.[61] The mechanism by which inactivating mutations in DMP1 or ENPP1 increase FGF23 levels remains unclear.

Fibrous Dysplasia/McCune-Albright Syndrome

Fibrous dysplasia (FD) is a lesion in which portions of the bone are replaced by fibrous connective tissue and poorly formed trabecular bone. It is caused by somatic activating missense mutation of the guanine nucleotide stimulatory protein (GNAS1) gene that affects bone, skin, and endocrine system function.[62,63] The bones commonly affected by FD are proximal femur, tibia, ribs, and skull. FD may occur in single or multiple bones (monostotic and polyostotic fibrous dysplasia, respectively). McCune-Albright syndrome is characterized by polyostotic FD, café-au-lait macules and a variety of endocrine disorders, including precocious puberty, growth hormone hypersecretion, and hyperthyroidism.[64] Hypophosphatemia due to hyperphosphaturia is observed in approximately 50% of patients with FD/McCune-Albright syndrome and is associated with rickets or osteomalacia. Serum FGF23 levels are elevated due to a mass of FGF23-producing cells in fibrous bone lesions.[65]

Tumor-Induced Osteomalacia (Oncogenic Osteomalacia)

Tumor-induced osteomalacia (TIO) is a rare paraneoplastic syndrome induced by tumoral oversecretion of phosphaturic factors. The tumors frequently associated with TIO are phosphaturic mesenchymal tumors of mixed connective tissue type.[66,67] Additionally, TIO has been reported in patients with other malignancies, including colon, ovaries, prostate, and lymphoma.[68] These tumors typically secrete FGF23 and other phosphaturic proteins, including sFRP4, MEPE, and FGF7.[1,33] Elevated serum FGF23 levels were observed in 19 of 22 patients

with TIO (sensitivity, 86%).[69] TIO is more common in adults than children.[70] Clinical manifestations include muscle weakness, bone pain, fracture, osteomalacia, impaired bone microarchitecture,[71-73] normocalcemia, hypophosphatemia, hyperphosphaturia, elevated alkaline phosphatase, low or inappropriately normal $1,25(OH)_2D$ levels, and normal or elevated PTH. Diagnosis of TIO depends on localization of the underlying tumor, which is usually small and in obscure location (e.g., lower extremities and head and neck regions).[74] Several techniques have been used to identify tumors responsible for TIO, including whole-body magnetic resonance imaging or sestamibi scan, somatostatin receptor scintigraphy, and somatostatin receptor positron emission tomography/computed tomography.[75] Systemic venous sampling for FGF23 is useful to determine whether an identified mass is secreting FGF23.[76] Complete tumor resection leads to an improvement of biochemical abnormalities and bone diseases.

Evaluation of Hypophosphatemia

The etiology of hypophosphatemia is often evident from the clinical history. If the diagnosis is uncertain, then evaluation of the fractional excretion of filtered phosphate ($FEPO_4$) or tubular maximum reabsorption of phosphate per glomerular filtration rate (TmP/GFR) should be helpful. The $FEPO_4$ (phosphate/creatinine clearance ratio) is calculated using the formula: $FEPO_4$ = [urine phosphate (mg/dL) x serum creatinine (mg/dL)] / [serum phosphate (mg/dL) x urine creatinine (mg/dL)]. The fractional tubular reabsorption of phosphate (TRP) is calculated using the formula: TRP = 1 – $FEPO_4$. The nomogram is entered with serum phosphate level and the TRP value, and the intersection of a straight line joining these values with the TmP/GFR scale is read (Figure 5).[77] The normal range for TmP/GFR in adults is 2.5-4.2 mg/dL; it is higher in children. A $FEPO_4$ of less than 20%, or a high TmP/GFR, indicates appropriate low urine phosphate excretion, suggesting that hypophosphatemia is caused by inadequate intake,

transcellular phosphate shift (e.g., respiratory alkalosis, refeeding syndrome), or decreased intestinal phosphate absorption (e.g., chronic diarrhea, malabsorption). Conversely, a $FEPO_4$ of greater than 20%, or a low TmP/GFR indicates renal phosphate wasting, suggesting that hypophosphatemia is caused by primary or secondary hyperparathyroidism, vitamin D resistance, FGF23-dependent hypophosphatemia, or renal tubular defects (Table 1). Euglycemic glycosuria, amino aciduria, tubular proteinuria, hyperuricosuria, and proximal renal tubular acidosis indicate a proximal tubular disorder, such as Fanconi syndrome. The measurement of serum PTH, 25(OH)D, $1,25(OH)_2D$, and serum FGF23 levels are necessary to determine the causes of hypophosphatemia secondary to renal phosphate losses. Hypophosphatemia due to hyperparathyroidism is generally less severe than FGF23-mediated hypophosphatemia because the serum levels of $1,25(OH)_2D$ are higher. High serum FGF23, low serum $1,25(OH)_2D$, and adequate serum 25(OH)D should raise suspicion of FGF23-mediated hypophosphatemia, including XLH, ADHR, ARHR, FD, and TIO. The underlying mechanisms responsible for the increase in FGF23 are shown in Table 2. Although vitamin D deficiency and FGF23-mediated hypophosphatemic disorders sometimes coexist, serum FGF23 levels are elevated in the latter condition.

Measurement of Circulating FGF23

Currently, FGF23 immunoassays are mainly for research use. FGF23 immunoassays can either detect the intact form (iFGF23 immunoassay) or both the intact form and C-terminal fragments (cFGF23 immunoassay) that result from intracellular cleavage (Figure 6). Besides different reported units [iFGF23 in picograms per milliliter (pg/mL) and cFGF23 in relative units per milliliter (RU/mL)], absolute values between the assays vary widely due to different calibration, and no harmonization has been conducted.[78,79] Patients with XLH or TIO usually have serum

iFGF23 level (Kainos assay) above 30 pg/mL.[80] FGF23 is more stable in EDTA plasma than in serum at room temperature. Thus, FGF23 values obtained in EDTA plasma are higher than those obtained in serum. Whether plasma or serum should be used for FGF23 measurement is assay-specific. Previous studies have suggested that iFGF23 immunoassays are more sensitive than cFGF23 immunoassays for the measurement of circulating FGF23 concentrations in patients with TIO and patients with XLH. Additionally, iFGF23 concentrations are not affected by iron deficiency which may lead to false positive results when using cFGF23 immunoassays. Ideally, FGF23 should be measured 1 to 2 weeks after discontinuation of phosphate and $1,25(OH)_2D$ since both agents may increase FGF23 levels.[47] Burosumab therapy may cause analytical interference with certain FGF23 immunoassays.[81]

Management of FGF23-Related Hypophosphatemic Disorders

Conventional treatment of XLH and TIO comprises oral phosphate and vitamin D analogs. Adverse effects of conventional treatment include gastrointestinal discomfort, hypercalciuria, nephrocalcinosis, and secondary/tertiary hyperparathyroidism due to chronic stimulation of parathyroid glands by oral phosphate. Moreover, conventional treatment increases FGF23 levels, potentially aggravating hypophosphatemia and $1,25(OH)_2D$ deficiency. Thus, a reasonable approach is to use burosumab which is a human monoclonal antibody targeted to inhibit excess FGF23 bioactivity. Burosumab is administered subcutaneously, every 2 weeks (for children) or every 4 weeks (for adults) and has demonstrated efficacy with an acceptable safety profile in both pediatric and adult XLH cohorts. Oral phosphate and vitamin D analogs should be discontinued one week before initiating burosumab. Burosumab is contraindicated in patients with creatinine clearance below 30 mL/min. The common side effect of burosumab is a transient injection-site reaction.

The superiority of burosumab over conventional treatment in children with XLH has been demonstrated in terms of serum phosphate normalization, improvements in mineralization defects and lower-extremity deformities, improved physical ability, and reduced pain and the severity of rickets.[82-86] In a double-blind, placebo-controlled, phase 3 study in 134 adults with XLH, burosumab treatment for 24 weeks was associated with improvements in phosphate and vitamin D metabolism, enhanced fracture healing, and reduced stiffness.[87] After 24 weeks, all patients received open-label burosumab until week 48.[88] Burosumab treatment from weeks 24-48 showed that serum phosphate remained normal in 83.8% of subjects who received burosumab throughout and were normalized in 89.4% who received burosumab after placebo. By week 48, 63.1% of baseline fractures/pseudofractures healed completely with burosumab, compared with 35.2% with burosumab after placebo. In both groups, burosumab was associated with clinically significant and sustained improvement from baseline to week 48 regarding stiffness, pain, physical function, and 6-min walk distance. Statistically significant improvements from baseline in patient-reported outcomes and ambulatory function persisted up to week 96 with burosumab treatment, with predefined meaningful changes observed in pain, stiffness, and fatigue.[89] Rates of adverse events were similar for burosumab and placebo. Furthermore, recent research has shown that adults with XLH who received burosumab had a significantly improvement in histomorphometric features of osteomalacia.[90]

Burosumab can also improve biochemical and bone abnormalities in patients with TIO. In an open-label, single-arm, phase 2 study, burosumab treatment of 14 adult patients with TIO up to 144 weeks resulted in improvements in serum phosphate levels and bone histomorphometric measures of osteomalacia, enhanced fracture/pseudofracture healing, reductions in musculoskeletal pain, and improvement in functional mobility.[91] Burosumab exhibited an

acceptable safety profile. Main clinical studies of burosumab in adults with XLH or TIO are summarized in Table 3. Based on these results, burosumab has been approved for the treatment of XLH and TIO in both pediatric and adult patients in several countries including USA. Nevertheless, the current data does not indicate that burosumab treatment results in a complete normalization of osteomalacic disorders. This might be due to delayed treatment initiation or effects of non-FGF23 phosphatonins (FGF7, MEPE, and sFRP4) which are not blocked by burosumab. The use of burosumab in TIO should be limited to patients with unresectable tumors. Long-term efficacy and safety of burosumab on bone histomorphometry, kidney function, and vascular calcification deserve further studies.

Conclusion

Phosphatonins play an important role in phosphate homeostasis and bone mineralization. FGF23 excess is central to the pathogenesis of most hypophosphatemic rickets and TIO. In adults and children with XLH and with TIO, the anti-FGF23 antibody burosumab significantly improves phosphate homeostasis, skeletal abnormalities, and quality of life and exhibits an acceptable safety profile. Whether blocking antibodies to other phosphatonins confer a benefit on certain patient subgroups with XLH or TIO requires further research.

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Figure and Table Captions

Figure 1. Regulation of phosphate homeostasis by the FGF23-PTH-vitamin D axis. FGF23 and PTH reduce phosphate reabsorption in the proximal tubule. PTH also stimulates the renal production of $1,25(OH)_2D$ which subsequently increases phosphate absorption in the intestine and inhibits PTH secretion. In contrast, FGF23 and phosphate suppress the renal production of $1,25(OH)_2D$. In the parathyroid gland, phosphate stimulates secretion of PTH, whereas this secretion is inhibited by FGF23. FGF23 secretion in the bone is stimulated by PTH, $1,25(OH)_2D$, and phosphate. Dashed red arrows indicate inhibition, and solid green arrows indicate stimulation.

Figure 2. Intestinal phosphate absorption occurs in the small intestine via two distinct pathways: the transcellular and paracellular route. The transcellular pathway is characterized by active intestinal phosphate absorption that occurs primarily through NaPi-2b transporters in the apical membrane of enterocytes. Paracellular phosphate absorption occurs passively along concentration gradients through tight junction proteins between adjacent enterocytes. The paracellular pathway is responsible for the majority of intestinal phosphate absorption in humans. Basolateral phosphate efflux possibly occurs by facilitated diffusion through XPR1 phosphate transporter. $1,25(OH)_2D$-mediated VDR activation enhances transcellular phosphate absorption via upregulation of NaPi-2b transporters. VDR, vitamin D receptor; XPR1, xenotropic and polytropic retrovirus receptor 1.

Figure 3. The cellular mechanism of phosphate reabsorption in the proximal tubule. Most of the filtered phosphate is reabsorbed via NaPi-2a and NaPi-2c transporters in the apical membrane and depends on the basolateral Na^+/K^+-ATPase activity to maintain the Na^+ gradient that drives

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the secondary active transport process. PiT-2 transporters also contribute to the apical phosphate influx. PTH and FGF23 induce phosphaturia by reducing expression of NaPi-2a and NaPi-2c transporters. 1,25$(OH)_2$D-mediated VDR activation increases transcellular phosphate absorption. FGF23 suppresses mitochondrial 1α-hydroxylase, but induces 24-hydroxylase expression. PTH has the opposite effects. VDR, vitamin D receptor; XPR1, xenotropic and polytropic retrovirus receptor 1.

Figure 4. FGF23 production and cleavage in osteocytes and osteoblasts. FGF23 is initially produced as a 251-amino acid pre-pro-protein. After intracellular cleavage of the signal peptide, the intact FGF23 (iFGF23) is O-glycosylated by polypeptide N-acetylgalactosaminyltransferase 3 (GALNT3), which prevents proteolysis and facilitates the secretion of iFGF23 as a 227-amino acid glycoprotein. Alternatively, iFGF23 can be cleaved intracellularly by proprotein convertases, yielding biologically inactive N- and C-terminal FGF23 fragments that are cosecreted with iFGF23.

Figure 5. Nomogram for derivation of tubular maximum reabsorption of phosphate per glomerular filtration rate (TmP/GFR).[77] C_{Cr}, creatinine clearance; C_{PO4}, phosphate clearance; TRP, fractional tubular reabsorption of phosphate.

Figure 6. The measurement of FGF23 using immunoassays. A. The intact FGF23 immunoassay uses two monoclonal antibodies that recognize epitopes within the N-terminal and C-terminal domains of FGF23, which flank the proteolytic cleavage site, for the detection of only full-length FGF23. B. For the C-terminal FGF23 immunoassay, detecting antibodies bind to epitopes within the C-terminal domain only and this assay detects both full-length FGF23 and C-terminal FGF23 fragments.

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673 **Table 1.** Clinical conditions associated with hypophosphatemia and other useful laboratory values.

674 **Table 2**. Genetic and acquired disorders of renal phosphate handling and the associated mutations.

675 **Table 3.** Main clinical studies of burosumab in adults with XLH or TIO.

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Table 1. Clinical conditions associated with hypophosphatemia and other useful laboratory values.

Clinical Conditions	S Ca	S PTH	S 25D	S 1,25D	S FGF23	U Ca	TmP/GFR	U aa
VDDR 1	↓	↑	↔	↓	↔	↓	V	V
VDDR 2	↓	↑	↔	↑	↔	↓	V	V
XLH	↔ or ↓	↔ or ↑	↔	↓ or ↔	↑	↔	↓	V
ADHR	↔ or ↓	↔ or ↑	↔	↓ or ↔	↑	↔	↓	V
TIO	↔ or ↓	↔ or ↑	↔	↓ or ↔	↑	↔	↓	V
Nutr Pi deficiency	↔ or ↓	↓	↔	↑	↔	↑	↑	↔
Nutr D Deficiency	↓	↑	↓	↓ or ↔	↔	↓	V	V
PHPT	↑	↑	↔	↑	↔	↑	↓	V
Post-transplant HPT	↑	↑	↔	↑ or ↔	↑ or ↔	V	V	V
Fanconi syndrome	↔ or ↓	↔	↔	↓ or ↔	?	V	↓	↑
Dent's disease	↔ or ↓	↔	↔	↓ or ↔	?	V	↓	↑

↔, no change; ↑, increase; ↓, decrease

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Abbreviations: S = serum; U = urine; TMP/GFR = tubular maximum reabsorption of phosphate/ glomerular filtration rate; aa = amino acid; V = variable; PTH = parathyroid hormone; 25D = 25-hydroxyvitamin D; 1,25D = 1,25-dihydroxyvitamin D; Ca = calcium; Pi = phosphate; VDDR = vitamin D-dependent rickets; XLH = X-linked hypophosphatemia; ADHR = autosomal dominant hypophosphatemic rickets; TIO = tumor-induced osteomalacia; Nutr = nutritional; PHPT = primary hyperparathyroidism.

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Table 2. Genetic and acquired disorders of renal phosphate handling and the associated mutations.

Syndrome	Gene	Mutation type	Mechanism	Serum phosphate	Serum $1,25(OH)_2D$	Serum PTH	Serum intact FGF23
HHRH	NaPi-2c	Loss of function	Decreased NaPi-2c expression in the proximal tubule	↓	↑	↔ or ↓	↔
XLH	PHEX	Loss of function	Increased FGF23 production in bone	↓	↔* or ↓	↔ or ↑	↑
ADHR	FGF23	Gain of function	Decreased FGF23 degradation	↓	↔* or ↓	↔ or ↑	↑
ARHR	DMP1, ENPP1, or FAM20C	Loss of function	Increased FGF23 production in bone	↓	↔* or ↓	↔ or ↑	↑
FD/MAS	GNAS1	Gain of function	Increased FGF23	↓	↔* or ↓	↔ or ↑	↑

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Table 2. Genetic and acquired disorders of renal phosphate handling and the associated mutations.

Syndrome	Gene	Mutation type	Mechanism	Serum phosphate	Serum $1,25(OH)_2D$	Serum PTH	Serum intact FGF23
			production in lesions				
TIO	FN1–FGFR1 or FN1–FGF1	Gain of function	Increased FGF23 production in tumors	↓	↔* or ↓	↔ or ↑	↑
FTC	GALNT3	Loss of function	Increased FGF23 degradation	↑	↔ or ↑	↔	↓

*****Inappropriately normal; ↔, no change; ↑, increase; ↓, decrease

Abbreviations: ADHR, autosomal dominant hypophosphatemic rickets; ARHR, autosomal recessive hypophosphatemic rickets; DMP, dentin matrix protein; ENPP, ectonucleotide pyrophosphatase/phosphodiesterase; FAM20C, family with sequence similarity 20, member C; FD, fibrous dysplasia; FGF, fibroblast growth factor; FGFR, FGF receptor; FN, fibronectin; FTC, familial tumoral calcinosis; GALNT, polypeptide N-acetylgalactosaminyltransferase; GNAS, guanine nucleotide-binding protein G (s) subunit alpha; HHRH, hereditary hypophosphatemic rickets with hypercalciuria; MAS, McCune-Albright syndrome; PHEX, phosphate-regulating gene with homologies to endopeptidases on the X chromosome; PTH, parathyroid hormone; TIO, tumor-induced osteomalacia; XLH, X-linked hypophosphatemia.

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Table 3. Main clinical studies of burosumab in adults with XLH or TIO.

References	Cohort	Phase	Study Design	Burosumab Dose	Outcomes
Insogna et al.[87]	134 adults with XLH	3	Double-blind, placebo-controlled study (Week 24 primary analysis)	1.0 mg/kg s.c. given every 4 weeks	Therapy increased serum phosphate, enhanced fracture/pseudofracture healing, and improved some BPI-SF scores, BFI worst fatigue, and WOMAC stiffness scores.
Portale et al.[88]	Cohort from Insogna et al.[87]	3	Extension (Insogna study) – open-label period until week 48	1.0 mg/kg s.c. given every 4 weeks	- Therapy maintained serum phosphate, enhanced fracture/pseudofracture healing, and improved all BPI-SF and WOMAC scores. - Therapy also improved ambulatory function as measured with 6MWT.
Briot et al.[89]	Cohort from Insogna et al.[87]	3	Extension (Insogna study) – open-label period until week 96	1.0 mg/kg s.c. given every 4 weeks	- Therapy improved all BFI, BPI-SF, and WOMAC scores. - Ambulatory function was consistently improved with therapy.
Insogna et al.[90]	11 adults with XLH and completed bone biopsies at baseline and week 48	3	Open-label, single-arm study	1.0 mg/kg s.c. given every 4 weeks	Therapy increased serum phosphate, reduced pain, and improved osteomalacia as measured by bone histomorphometry.
Beur et al.[91]	14 adults with TIO	2	Open-label study	0.3-2.0 mg/kg s.c. given every 4 weeks	- Therapy increased serum phosphate, enhanced fracture/pseudofracture healing, and improved physical functioning with acceptable safety profile. - Therapy also improved osteomalacia as measured by bone histomorphometry.

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Abbreviations: 6MWT, 6-minute walk test; BFI, Brief Fatigue Inventory; BPI-SF, Brief Pain Inventory - Short Form; TIO, tumor-induced osteomalacia; WOMAC, Western Ontario and McMaster Osteoarthritis Index; XLH, X-linked hypophosphatemia.

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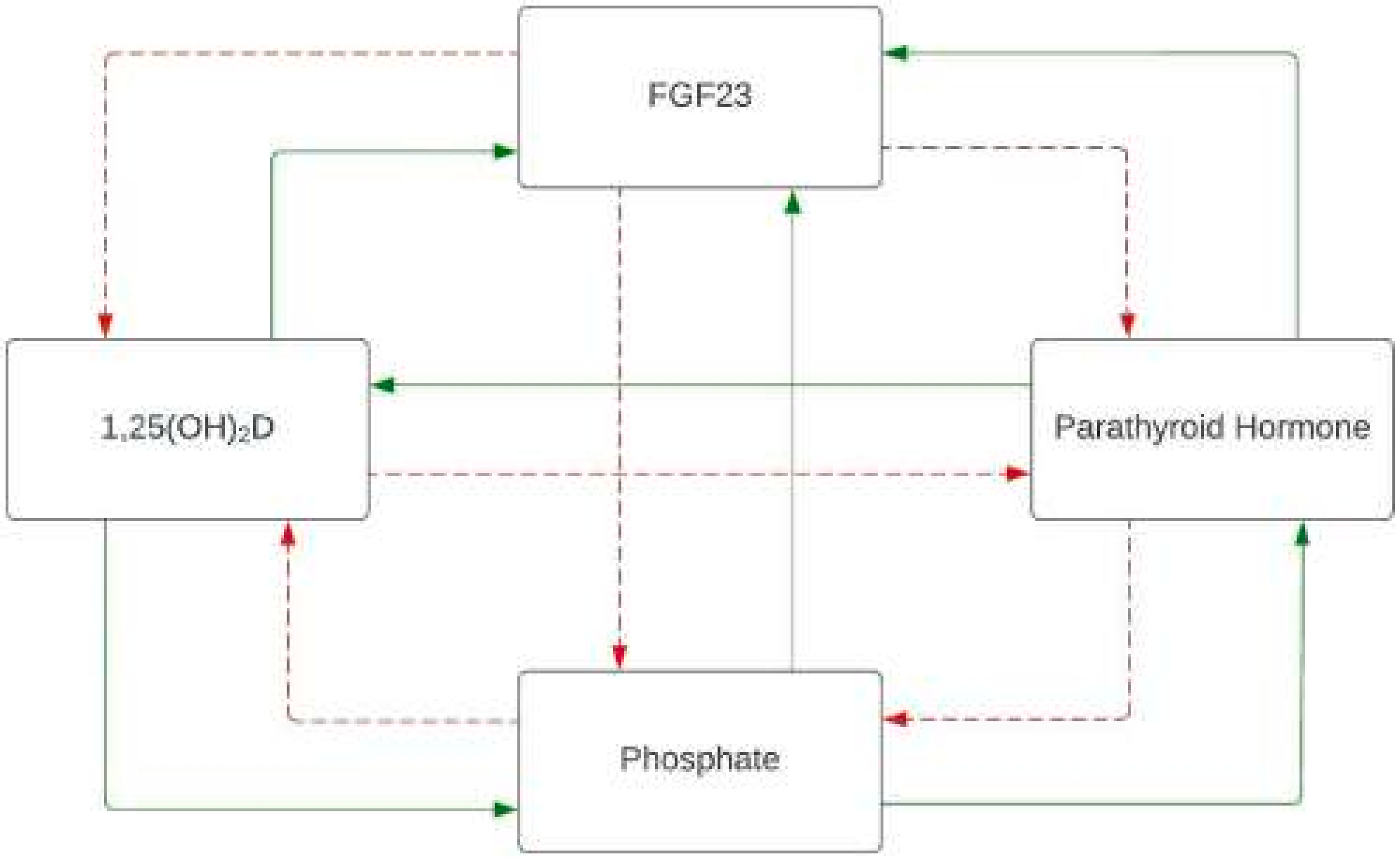
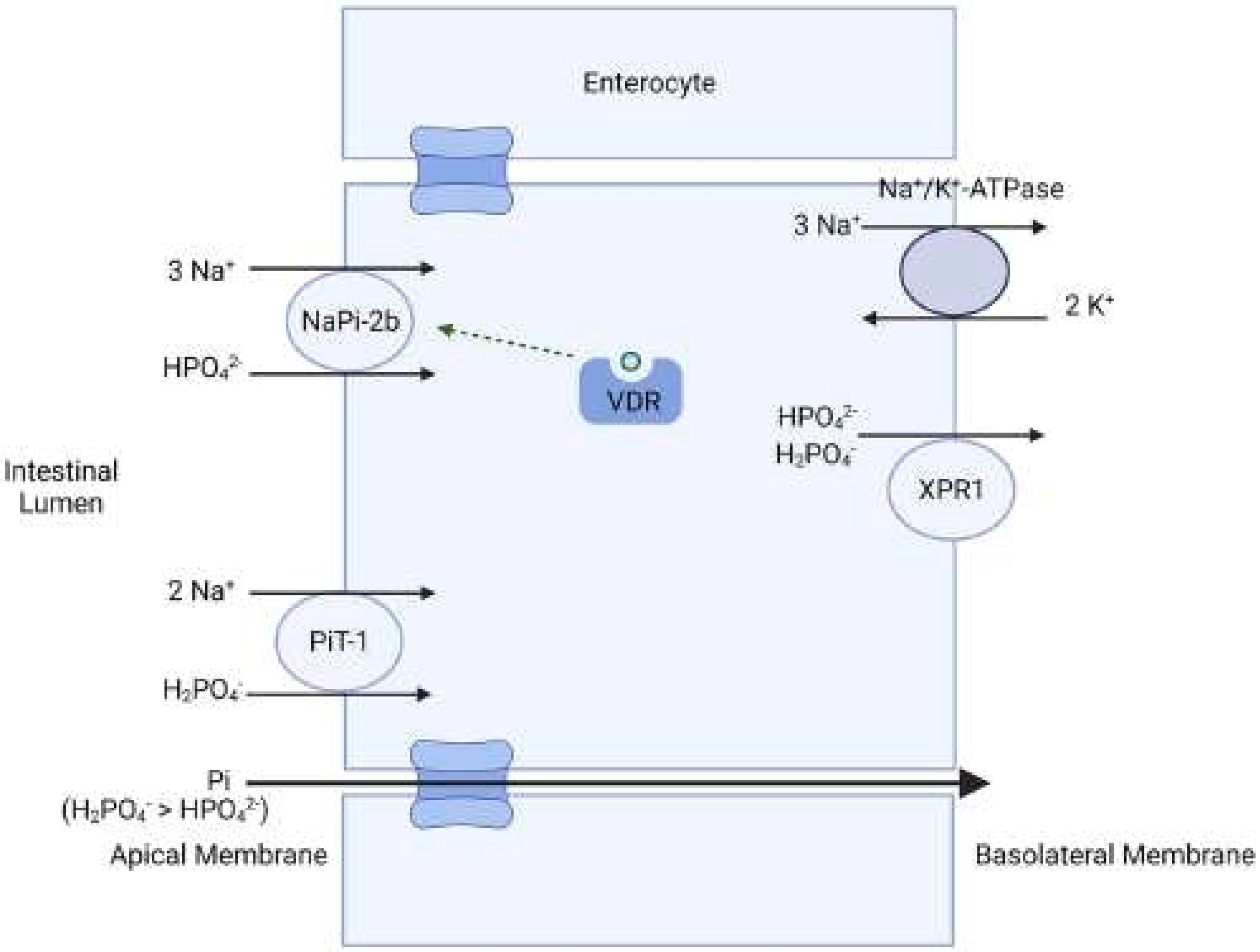
Highlights

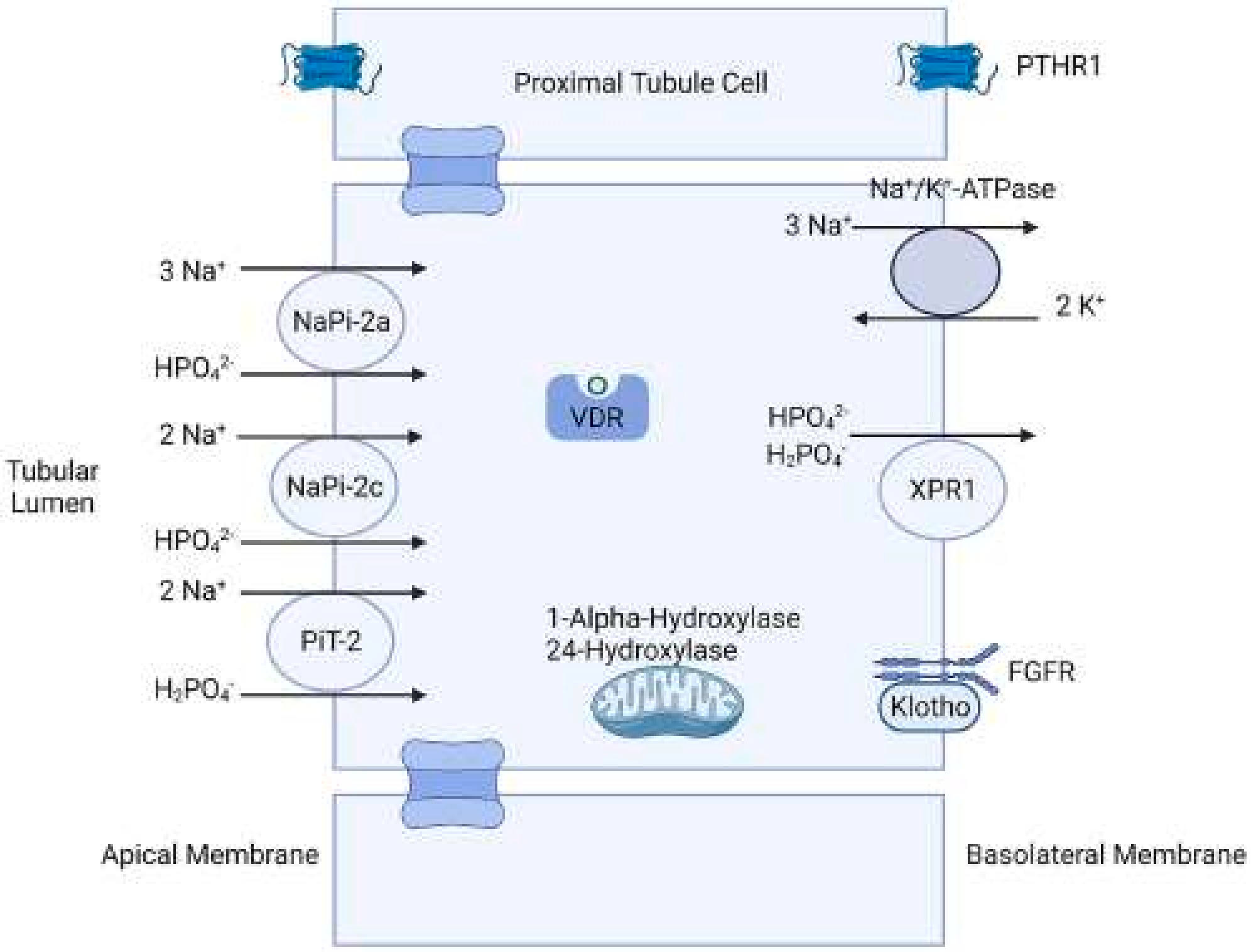
- Inorganic phosphorus is crucial for a variety of biological processes such as cell division, cellular signaling, enzyme function and skeletal integrity.
- Serum phosphate concentrations are regulated by several hormone including parathyroid hormone, 1,25-dihydroxyvitamin D and phosphatonins (especially, fibroblast growth factor 23 or FGF23).
- The absorption of phosphate in the intestine and its reabsorption in the kidney are mediated by sodium-phosphate cotransporters in the apical membrane of epithelial cells that are regulated by PTH, 1,25-dihydroxyvitamin D and FGF23.

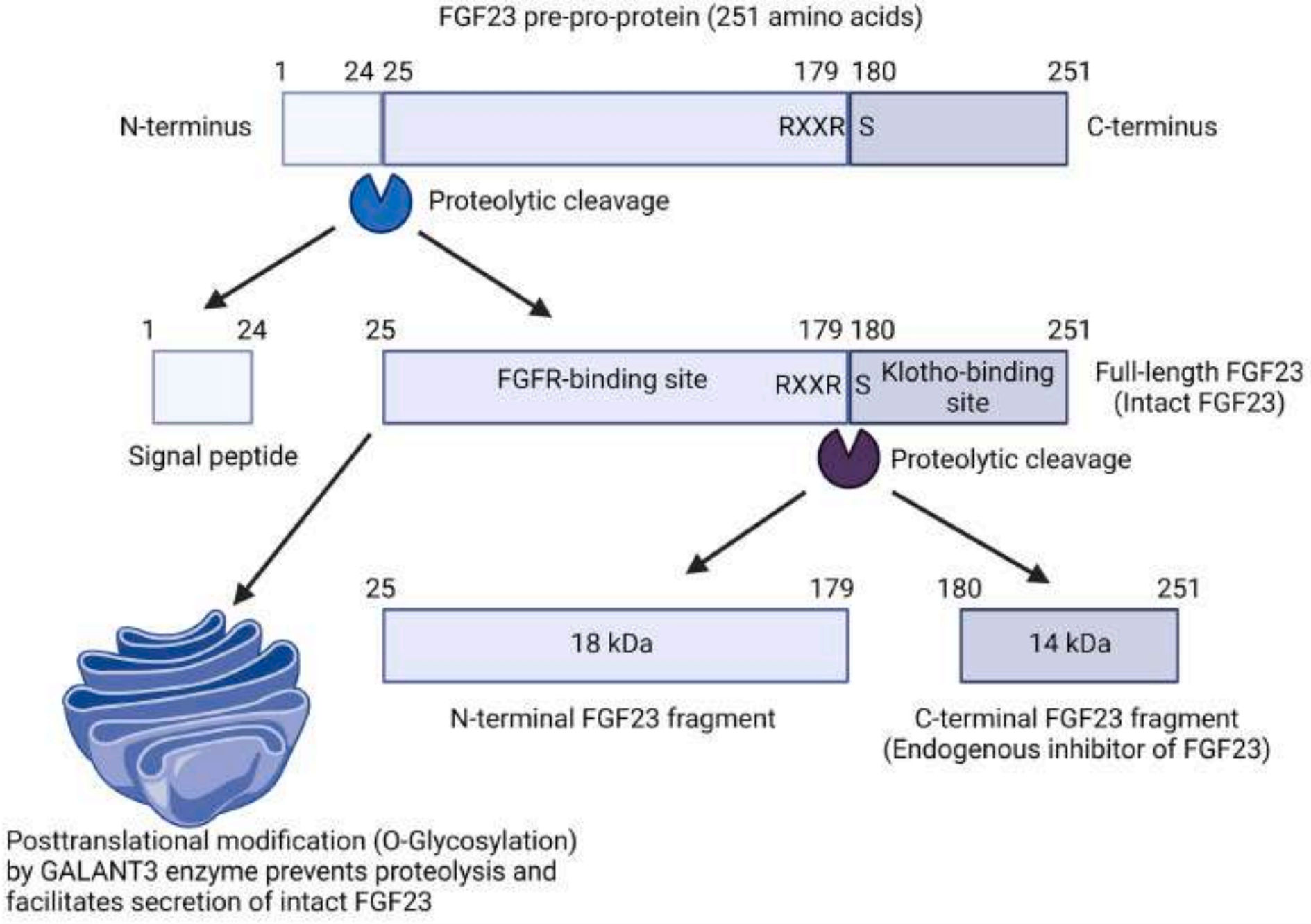
- **Clinical Relevance**: Several hypophosphatemic disorders are mediated by abnormal concentrations of phosphate regulating hormones, especially FGF23. The etiology of these disorders can be readily diagnosed by a thorough clinical evaluation and appropriate laboratory tests. Hypophosphatemic disorders can be treated with oral phosphate, 1,25-dihydroxyvitamin D_3 therapy or FGF23 antibodies.

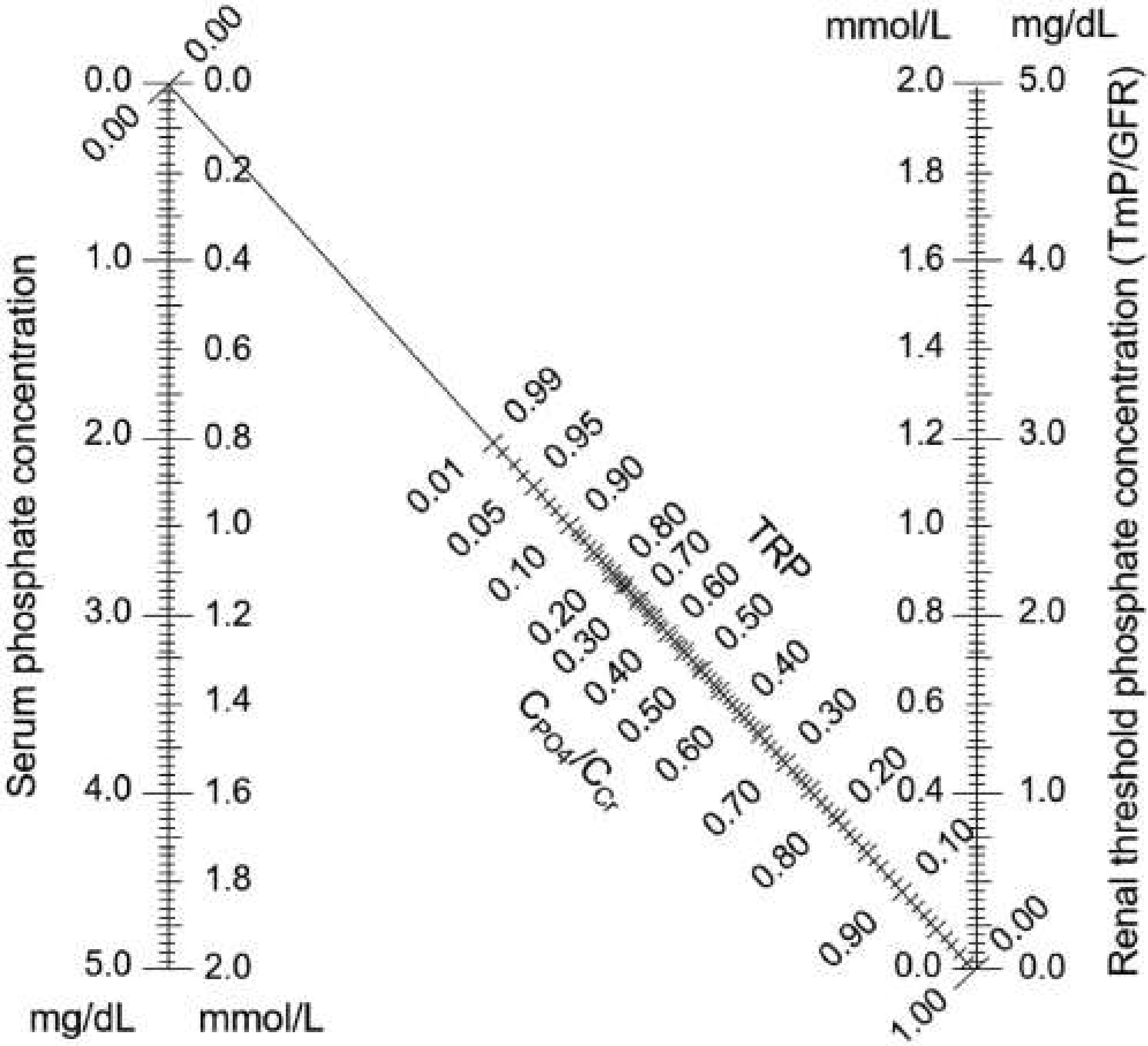
Journal Pre-proof

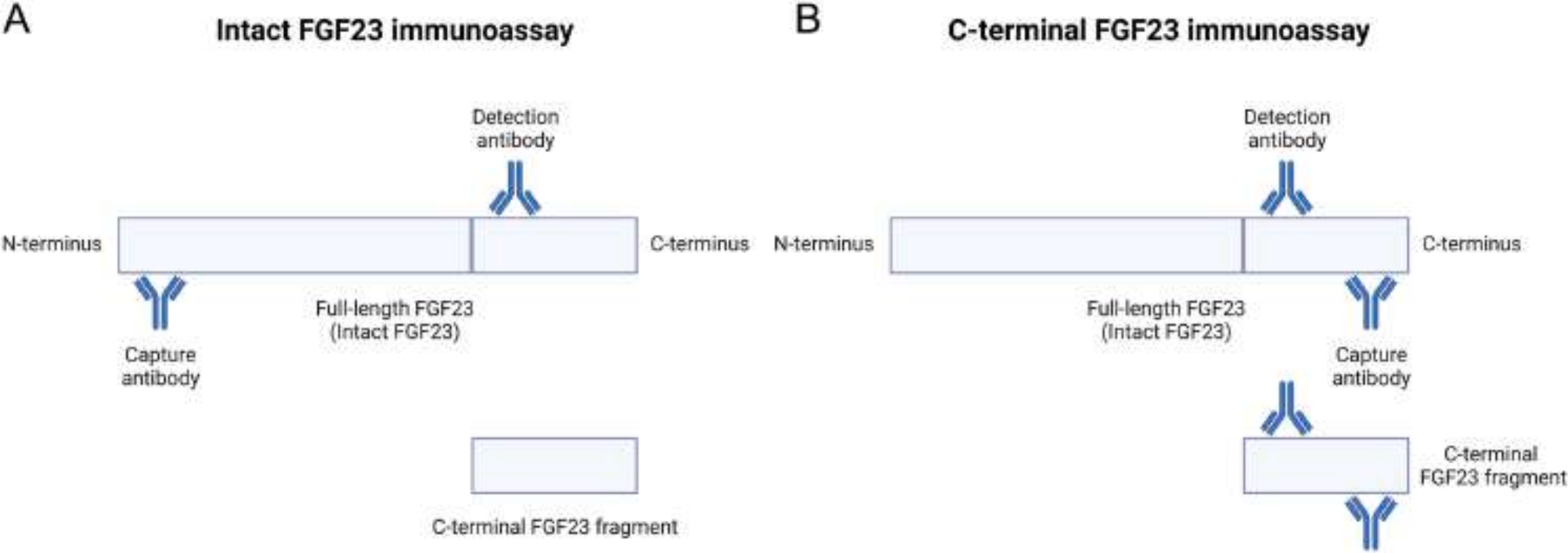

FGF23
1,25(OH)2D
Parathyroid Hormone
Phosphate


Enterocyte
Na+/K+-ATPase
3 Na+
2 K+
3 Na+
NaPi-2b
HPO4 2-
VDR
HPO4 2-
H2PO4-
XPR1
Intestinal Lumen
2 Na+
PiT-1
H2PO4-
Pi
(H2PO4- > HPO4 2-)
Apical Membrane
Basolateral Membrane


Proximal Tubule Cell
PTHR1
Na+/K+-ATPase
3 Na+
2 K+
3 Na+
NaPi-2a
HPO4 2-
VDR
2 Na+
NaPi-2c
HPO4 2-
H2PO4-
XPR1
Tubular Lumen
HPO4 2-
2 Na+
PiT-2
H2PO4-
1-Alpha-Hydroxylase
24-Hydroxylase
FGFR
Klotho
Apical Membrane
Basolateral Membrane


FGF23 pre-pro-protein (251 amino acids)
1
24 25
179 180
251
N-terminus
RXXR
S
C-terminus
Proteolytic cleavage
1
24
25
179 180
251
FGFR-binding site
RXXR
S
Klotho-binding site
Full-length FGF23 (Intact FGF23)
Signal peptide
Proteolytic cleavage
25
179
180
251
18 kDa
14 kDa
N-terminal FGF23 fragment
C-terminal FGF23 fragment (Endogenous inhibitor of FGF23)
Posttranslational modification (O-Glycosylation) by GALANT3 enzyme prevents proteolysis and facilitates secretion of intact FGF23


Serum phosphate concentration
mg/dL
mmol/L
0.0
1.0
2.0
3.0
4.0
5.0
0.00
0.2
0.4
0.6
0.8
1.2
1.4
1.6
1.8
2.0
C_{PO4}/C_{Cr}
0.01
0.05
0.10
0.20
0.30
0.40
0.50
0.60
0.70
0.80
0.90
0.95
0.99
TRP
mmol/L
mg/dL
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
5.0
4.0
3.0
2.0
1.0
0.0
0.00
0.10
0.20
0.30
0.40
0.50
0.60
0.70
0.80
0.90
1.00
Renal threshold phosphate concentration (TmP/GFR)


A
Intact FGF23 immunoassay
Detection antibody
N-terminus
C-terminus
Capture antibody
Full-length FGF23 (Intact FGF23)
C-terminal FGF23 fragment
B
C-terminal FGF23 immunoassay
Detection antibody
N-terminus
C-terminus
Full-length FGF23 (Intact FGF23)
Capture antibody
C-terminal FGF23 fragment