Advances in understanding of phosphate homeostasis
and related disorders

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Abstract. Inorganic phosphate (Pi) in the mammalian body is balanced by its influx and efflux through the intestines, kidneys, bones, and soft tissues, at which several sodium/Pi co-transporters mediate its active transport. Pi homeostasis is achieved through the complex counter-regulatory feedback balance between fibroblast growth factor 23 (FGF23), 1,25-dihydroxyvitamin D (1,25(OH)2D), and parathyroid hormone. FGF23, which is mainly produced by osteocytes in bone, plays a central role in Pi homeostasis and exerts its effects by binding to the FGF receptor (FGFR) and αKlotho in distant target organs. In the kidneys, the main target, FGF23 promotes the excretion of Pi and suppresses the production of 1,25(OH)2D. Deficient and excess FGF23 result in hyperphosphatemia and hypophosphatemia, respectively. FGF23-related hypophosphatemic rickets/osteomalacia include tumor-induced osteomalacia and various genetic diseases, such as X-linked hypophosphatemic rickets. Coverage by the national health insurance system in Japan for the measurement of FGF23 and the approval of burosumab, an FGF23-neutralizing antibody, have had a significant impact on the diagnosis and treatment of FGF23-related hypophosphatemic rickets/osteomalacia. Some of the molecules responsible for genetic hypophosphatemic rickets/osteomalacia are highly expressed in osteocytes and function as local regulators of FGF23 production. A number of systemic factors also regulate FGF23 levels. Although the mechanisms responsible for Pi sensing in mammals have not yet been elucidated in detail, recent studies have suggested the involvement of FGFR1. The further clarification of the mechanisms by which osteocytes detect Pi levels and regulate FGF23 production will lead to the development of better strategies to treat hyperphosphatemic and hypophosphatemic conditions.

Key words: Phosphate, Fibroblast growth factor 23, Bone, Osteocyte, Mineralization

Introduction

Phosphorus mediates the majority of biological processes in living organisms, including cell membrane integrity, the maintenance and inheritance of genetic information, energy metabolism, signal transduction, and skeletal mineralization in vertebrates [1]. The findings of a cadaver study published in 1945 showed that an adult male body weighing 70.55 kg contained 544 g of phosphorus, approximately 90% of which was present in the skeleton. Most of the remaining phosphorus was distributed in soft tissues, with less than 1% in extracellular fluid [2]. Phosphorus is present in serum as free ions of inorganic phosphate (Pi), such as HPO42− and H2PO4−, the ratio of which at physiological pH is approximately 4:1 [3]. Phosphate that is clinically measured is Pi, and hypophosphatemia and hyperphosphatemia indicate low and high levels of Pi, respectively. In contrast to serum calcium levels, which are maintained within a narrow range, serum Pi levels are influenced by various factors, including the time of day, age, dietary intake, and serum pH [3, 4]. Serum Pi is maintained at higher levels in children than in adults, which may reflect the needs of the growing body [4]. Phosphorus exists as organic phosphate in addition to Pi, which includes phosphate esters, phospholipids, nucleotides, and high-energy phosphate compounds, such as adenosine 5′-triphosphate [1].

The majority of phosphorus in the body is stored in the skeleton as hydroxyapatite (calcium-Pi crystals); therefore, a prolonged deficiency leads to impaired skeletal mineralization, namely, rickets in children and osteomalacia in adults [5]. At the beginning of this century, fibroblast growth factor 23 (FGF23) was found to be responsible for some types of hypophosphatemic rickets/osteomalacia [4, 6]. Our understanding of the mechanisms underlying Pi homeostasis has since increased, leading to advances in the diagnosis and treatment of
hyperphosphatemic and hypophosphatemic diseases.

This review describes current concepts for the molecular mechanisms underlying Pi homeostasis and related disorders.

**Pi Balance and Sodium/Pi (Na\(^+\)/Pi) Co-transporters**

In mammals, the extracellular pool of Pi is maintained in a dynamic equilibrium by influx and efflux through the intestines, kidneys, bone, and soft tissues. A positive Pi balance occurs when intake and accrual exceed loss, as observed in growing children, whereas a negative Pi balance arises when loss is greater than intake and accrual.

Phosphorus in food is absorbed by the small intestines, which is mediated by two types of transport processes: non-saturable paracellular Pi absorption by passive diffusion and saturable active transcellular Pi absorption [1]. Pi absorbed by the intestines is excreted by the kidneys; however, the majority of Pi filtered by glomeruli is reabsorbed by the proximal tubules and returned into the circulation. The renal reabsorption of Pi is mainly mediated by an active transcellular transport process, which begins with the entry of Pi into proximal tubular cells on the apical brush border membrane (BBM) [1]. Hyperphosphatemia is common in patients with renal failure in whom the excretion of Pi is impaired. On the other hand, hypophosphatemia may be caused by the insufficient intestinal absorption of Pi, renal Pi wasting, the impaired mobilization of Pi from bone, such as hungry bone syndrome, and the transient shift of Pi into cells [7].

The active transport of Pi is mediated by Na\(^+\)/Pi co-transporters. Mammalian Na\(^+\)/Pi co-transporters are classified into 3 families: types I, II, and III, based on similarities in their amino acid sequences [8]. However, type I co-transporters also transport iodine, bromide, chloride, and organic anions, such as urate [8, 9], and are now considered to play a minor role in Pi homeostasis. Type II Na\(^+\)/Pi co-transporters belong to the solute carrier family 34 (SLC34) family and include type Ila, Iib, and Iic co-transporters, which are encoded by SLC34A1, SLC34A2, and SLC34A3, respectively, in humans [8].

The active transport of Pi in the intestines is mediated by type Ila Na\(^+\)/Pi co-transporters (NaPi-IIa) localized to the apical membrane of small intestinal epithelial cells [10, 11]. The expression of NaPi-IIb was previously shown to be increased by a low intake of phosphorus and 1,25-dihydroxyvitamin D (1,25(OH)\(_2\)D), the active metabolite of vitamin D [12-14]. NaPi-Iib is expressed in various organs in addition to the intestines, and inactivating mutations in NaPi-Iib cause pulmonary alveolar microlithiasis and testicular microlithiasis, which are characterized by the deposition of calcium/Pi microliths in the lung and testis, respectively [15].

Type Ila and Iic co-transporters (NaPi-Ila and NaPi-Iic, respectively) are predominantly expressed in the renal proximal tubules and are responsible for the reabsorption of Pi. The findings of analyses of knockout models suggested that NaPi-Ila was more important than NaPi-Iic for renal Pi reabsorption in mice [16, 17]. However, inactivating variants in the human SLC34A3 gene encoding NaPi-Iic cause hereditary hypophosphatemic rickets with hypercalciuria (HHRH) characterized by hypophosphatemia due to renal Pi wasting [18, 19], indicating the critical role of NaPi-Iic in Pi homeostasis in humans. In patients with HHRH, hypophosphatemia elevates serum levels of 1,25(OH)\(_2\)D, which, in turn, causes secondary hypercalciuria by accelerating the intestinal absorption of calcium. On the other hand, inactivating variants in SLC34A1 have been reported in patients with nephrolithiasis and osteoporosis associated with hypophosphatemia, Fanconi renitubular syndrome 2, and idiopathic infantile hypercalcemia type 2 [20-22]. The activity of Pi reabsorption in the proximal tubules is assessed by the protein levels of NaPi-Ila and NaPi-Iic on the BBM, which are regulated by several hormones, including parathyroid hormone (PTH) and FGF23 as well as dietary phosphorus intake [23-25].

Type III Na\(^+\)/Pi co-transporters are classified as SLC20 family members and include PiT-1 and PiT-2, which are encoded by the SLC20A1 and SLC20A2 genes, respectively, in humans [8]. PiT-1 and PiT-2 are expressed at different levels in a broad range of tissues [1]. Although the mRNA expression of both SLC20A1 and SLC20A2 was detectable in rat kidneys, protein localization on BBM was only reported for PiT-2 [26]. Inactivating SLC20A2 variants in humans cause familial idiopathic basal ganglia calcification (IBGC), an autosomal dominant neurodegenerative disorder characterized by symmetric calcification in the basal ganglia and other brain regions [27]. The deletion of SLC20A2 in mice resulted in brain calcification, reproducing the manifestation of human IBGC, and this was associated with a higher concentration of Pi in the cerebrospinal fluid (CSF) than in wild-type mice despite similar serum Pi levels [28]. Human diseases caused by variants of the SLC20A1 gene have not yet been identified, and its deletion in mice was previously reported to result in embryonic lethality. Table 1 summarizes the properties of type II and III Na\(^+\)/Pi co-transporters.

Although xenotropic and polytropic retrovirus receptor 1 (XPR1) functions as a Pi exporter, it was initially identified as a receptor for xenotropic-murine leukemia viruses [29]. XPR1 is actively expressed in neural stem cells and brain regions, and inactivating variants of this gene are responsible for a subgroup of IBGC [30].
Defects in PiT-2 and those in XPR1 both cause IBGC; however, the mechanisms underlying brain calcification may differ. Calcium/Pi deposits in PiT-2 deficiency may form extracellularly in association with elevated Pi levels in CSF, whereas those in XPR1 deficiency appear to form intracellularly due to the lower efflux of Pi. Furthermore, mice with the conditional deletion of Xpr1 in the renal tubules showed similar phenotypes to Fanconi syndrome with impaired renal Pi reabsorption [31]. However, the precise roles of XPR1 in Pi homeostasis currently remain unclear.

### Endocrine Regulators of the Phosphate Balance

Endocrine factors, such as PTH, 1,25(OH)₂D, and FGF23, mediate interorgan communication to maintain Pi homeostasis. PTH increases renal Pi excretion by decreasing the amounts of NaPi-IIa and NaPi-IIc on the BBM of proximal renal tubules mainly by facilitating their internalization [23, 24, 32], and hyperparathyroidism causes renal Pi wasting and hypophosphatemia. However, the main function of PTH is to maintain the homeostasis of serum calcium, and its synthesis and secretion are regulated by extracellular calcium [33].

1,25(OH)₂D increases intestinal Pi absorption by inducing the expression of NaPi-Iib [12]. Since 1,25(OH)₂D also stimulates intestinal calcium absorption, disorders associated with the impaired effects of vitamin D metabolites, such as vitamin D deficiency and vitamin D-dependency type 1 and type 2, may result in hypophosphatemia and/or hypocalcemia. Vitamin D-dependency type 1A is caused by inactivating variants in the CYP27B1 gene encoding 25-hydroxyvitamin D 1α-hydroxylase [34], whereas type 2A is caused by those in the vitamin D receptor (VDR) gene [35].

FGF23 plays a central role in Pi homeostasis and is mainly produced by osteoblasts and their descendants, osteocytes in bone [36]. It consists of 251 amino acids, including a 24-amino acid signal peptide [25]. The N-terminal domain of FGF23 binds to FGFR, while its C-terminal domain binds to αKlotho [37]. FGF23 is inactivated by proteolytic cleavage between Arg₁⁷⁹ and Ser₁₈₀ within a recognition motif for the subtilisin-like proprotein convertase, R₁⁷⁶XXR¹⁷⁹/S¹₈₀ [38]. UDP-N-acetyl-α-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 3 (GalNAc-T3), which has been suggested to prevent the proteolytic cleavage of FGF23 at Thr₁⁷⁸, which has been suggested to prevent the proteolytic cleavage of FGF23 between Arg₁⁷⁹ and Ser₁₈₀ [39]. There are two main types of immunoassays for measuring FGF23 levels. Intact assays use antibodies that recognize epitopes on either side of the cleavage site and measure only intact bioactive FGF23 proteins [40]. On the other hand, C-terminal assays utilize antibodies that bind to epitopes in the C-terminal region and capture both intact FGF23 and its C-terminal fragments produced by cleavage [41].

In contrast to canonical FGF, which function as local factors, FGF23 has low binding affinity for heparin/heparan sulfate, which confers its endocrine effects in distant organs [37]. FGF23 at physiological concentrations requires αKlotho, which is a single-pass transmembrane protein with a large extracellular region, as a cofactor to exert signals through FGFR receptors (FGFR). The binding of FGF23 to a FGFR/αKlotho complex activates FGFR and intracellular signaling cascades, including the mitogen-activated protein kinase (MAPK) pathway [42, 43]. Organs and tissues expressing both FGFR and αKlotho may be physiological targets for FGF23, which include the kidneys, parathyroid glands, placenta, and choroid plexus [44]. Elevated levels of FGF23 under pathological conditions may activate FGFR independently of αKlotho in a broad range of tissues.

In the kidneys, FGF23 increases the excretion of Pi by decreasing the expression of NaPi-Ila and NaPi-Ilc...
co-transporters in the proximal tubules [25]. Furthermore, FGF23 inhibits the production of 1,25(OH)\(_2\)D by reducing the renal expression of Cyp27b1 and increasing that of Cyp24a1 encoding 25-hydroxyvitamin D-24-hydroxylase. The decrease in 1,25(OH)\(_2\)D levels suppresses the absorption of Pi in the intestines [4]. Therefore, the FGF23-mediated bone kidney axis plays a central role in Pi homeostasis.

Although the renal effects of FGF23 are mainly exerted in the proximal tubules, the expression of αKlotho is markedly higher in the distal tubules than in the proximal tubules [45]. It currently remains unclear whether αKlotho in the proximal tubules or that in the distal tubules contributes to the effects of FGF23 in the proximal tubules. Olauson et al. reported that a mutant mouse with the conditional deletion of αKlotho in the distal tubules exhibited hyperphosphatemia and elevated FGF23 levels, which suggested that αKlotho in the distal tubules received the initial signal of FGF23 and transduced it into the proximal tubules [46]. On the other hand, Takeshita et al. demonstrated that the conditional deletion of αKlotho in the proximal tubules led to the loss of the effects of FGF23 on Pi reabsorption and vitamin D metabolism [47]. Andrukhova et al. also showed that FGF23 directly acted on the proximal tubules to activate the extracellular signal-regulated kinase (ERK)1/2-SGK1 signaling pathway and increase Pi excretion [48]. αKlotho undergoes physiological cleavage by a disintegrin and metalloproteinase 10 (ADAM10) and ADAM17 to generate soluble circulating forms of the protein [49], which may mediate the FGF23 signal in the absence of the transmembrane form of αKlotho. However, based on the tissue-specific activities of FGF23, soluble forms of αKlotho appear to require supraphysiological concentrations of FGF23 to mediate the signal.

The parathyroid glands also express FGFR and αKlotho, and a previous study using rats reported that the administration of FGF23 suppressed the production and secretion of PTH through the MAPK pathway [50]. However, in mice with the parathyroid-specific conditional deletion of αKlotho, the acute administration of FGF23 still suppressed the secretion of PTH through an NFAT pathway [51]. Therefore, FGF23 may regulate the secretion of PTH in both αKlotho-dependent and -independent manners. A previous study using a mouse model of chronic kidney disease (CKD) with high levels of FGF23 suggested that persistent FGF23 signaling in the parathyroid glands stimulated cell proliferation and PTH secretion [52]. Therefore, FGF23 appears to exert context-dependent effects on the parathyroid glands.

We previously demonstrated that FGF23 also directly affected the placenta. FGFR1 and αKlotho co-localized at the feto-maternal interface of the placenta: at syncytiotrophoblasts and mononuclear trophoblasts in mice and at syncytiotrophoblasts in humans [53, 54]. Using pregnant female Hyp mice, a mouse model for human X-linked hypophosphatemic rickets (XLH), we showed that elevated levels of FGF23 in the maternal blood of Hyp pregnancy induced the placental expression of Cyp24a1, which affected fetal vitamin D metabolism [54]. In contrast to the altered expression of Cyp24a1, the placental expression of Na\(^+\)/Pi co-transporters was not affected by elevated levels of FGF23 in the maternal blood of Hyp pregnancy [54]. Although maternal FGF23 may regulate fetal vitamin D metabolism, analyses of FGF23-null fetuses suggested that FGF23 was unlikely to be involved in prenatal Pi homeostasis [55]. Fig. 1 summarizes the effects of FGF23 on the kidneys, parathyroid glands, and placenta.

**FGF23-related Hyperphosphatemic and Hypophosphatemic Disorders**

Since Pi is indispensable for skeletal mineralization, a prolonged deficiency may cause rickets and osteomalacia. In addition to skeletal manifestations, hypophosphatemia may be associated with a number of symptoms, such as myopathy, weakness, myocardial dysfunction, respiratory failure, and neurological manifestations [7]. On the other hand, phosphorus overload also causes severe cellular and tissue damage, which includes the ectopic calcification of soft tissue and vascular cells, inflammation, tissue/organ dysfunction, increased cell death, premature aging, impaired fertility, and increased tumorigenesis. Oxidative stress and dysregulated signal transduction as well as pathological calcification have been suggested to contribute to the harmful effects of phosphorus overload [1, 56]. However, this review focuses on disorders related to deficient and excess FGF23.

Inactivating variants in the FGF23 gene cause hyperphosphatemic familial tumoral calcinosis (HFTC), an autosomal recessive disease characterized by hyperphosphatemia, normal or elevated levels of serum 1,25(OH)\(_2\)D, and ectopic calcification [57, 58]. Most cases of HFTC are caused by inactivating variants in the GALT3 gene [59], with low levels of intact FGF23 and high levels of C-terminal FGF23 fragments being detected in patients [60]. A case of HFTC caused by a homozygous variant in the KLOTHO gene encoding αKlotho was reported in 2007. The identified variant, p.His193Arg was located in the FGF23-binding domain of the αKlotho protein. In this patient, the level of intact FGF23 was very high, suggesting resistance to FGF23 [61].

Excess FGF23 has been implicated in various types of hypophosphatemic rickets/osteomalacia, including
Endocrine disorders as well as tumor-induced osteomalacia (TIO) [62, 63]. These diseases are characterized by urinary Pi wasting, hypophosphatemia, inappropriately low or normal levels of serum 1,25(OH)₂D, and impaired skeletal mineralization, and are collectively referred to as FGF23-related hypophosphatemic rickets/osteomalacia [62, 63].

Among FGF23-related hypophosphatemic rickets/osteomalacia, autosomal dominant hypophosphatemic rickets (ADHR) is caused by missense variants in the \( \text{FGF23} \) gene itself, at the amino acid Arg₁₇₆ or Arg₁₇₉ [6]. Since these arginines are located within the recognition motif for cleavage by subtilisin-like proprotein convertase, variants at these residues are expected to make the protein resistant to inactivation by cleavage [64, 65]. However, serum levels of intact FGF23 are not always high in individuals carrying these variants, and ADHR may manifest as early or delayed onset with variable expressivity [66]. The delayed onset of ADHR is often detected in females after puberty, who are prone to iron deficiency [67]. A previous study reported that iron deficiency elevated intact FGF23 levels in ADHR patients, but not in healthy subjects. In healthy subjects, low serum levels of iron increased the C-terminal fragments of FGF23, but not intact FGF23, suggesting that the cleavage of FGF23 and its production were accelerated [67]. A translational mouse study demonstrated that a low-iron diet elevated serum levels of both intact FGF23 and C-terminal fragments of FGF23 and caused hypophosphatemia in \( \text{Fgf23} \) knock-in mice carrying the p.R176Q ADHR variant, whereas wild-type mice fed the low-iron diet had normal serum levels of intact FGF23 and normophosphatemia with elevated levels of the C-terminal fragments of FGF23 [68], supporting the contribution of iron deficiency to the manifestation of symptoms in ADHR.

XLH is the most common form of hereditary hypophosphatemic rickets [69]. It is caused by inactivating variants in the \( \text{PHEX} \) gene, located at Xp22.1 and shows X-linked dominant inheritance [70]. \( \text{PHEX} \) is expressed in osteoblast/osteocyte lineage cells, with higher expression levels in osteocytes, which is similar to the expression pattern of \( \text{Fgf23} \) [36]. Although the possible product of \( \text{PHEX} \) is a cell surface-bound, zinc-dependent protease [71], its physiological substrates have not yet been identified. Patients with XLH have elevated serum levels of intact FGF23, which causes urinary Pi wasting, hypophosphatemia, and inappropriately low levels of 1,25(OH)₂D [69]. The impaired activation of vitamin D leads to a poor therapeutic response to native vitamin D at dosages that cure vitamin D-deficient
rickets; therefore, XLH was initially called vitamin D-resistant rickets [72]. The symptoms and complications of XLH include rickets, bone deformities, and a short stature in children and osteomalacia, enethopathies (calcification of the tendons, ligaments, and joint capsules), osteoarthritis, pseudo-fractures, and hyperparathyroidism in adults [69]. Patients with XLH are also prone to dental abscesses. The hypophosphatemic Hyp mouse harboring a spontaneous 3'-large deletion in the Phex gene is widely used as a model for XLH and shows the increased production of FGF23 by osteoblasts and osteocytes [36, 73]. Although these findings suggest that PHEX functions as a negative local regulator of FGF23, the mechanisms underlying the overproduction of FGF23 in XLH remain unclear. Since FGF23 is not a substrate for PHEX [74], its regulation by PHEX may involve other molecule(s).

In patients with XLH, various types of variants have been identified in the PHEX gene, including missense variants, nonsense variants, frameshift variants, splicing variants, large deletions, and large duplications. More than 1,000 PHEX variants have been identified to date and are summarized in: https://www.rarediseasesgenes.com/. However, the genotype-phenotype relationship in XLH patients remains unclear [75]. We recently examined the genotype-phenotype relationship in 39 Japanese patients with XLH using 3-dimensional structure modeling. Serum levels of intact FGF23 were significantly higher in patients with PHEX variants causing defects in zinc-binding sites or cavities in the putative products, which suggested that these regions are crucial for the functions of PHEX proteins [76].

Autosomal recessive hypophosphatemic rickets type 1 (ARHR1) and type 2 (ARHR2) are caused by biallelic inactivating variants in the dentin matrix protein 1 (DMP1) and ectonucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1) genes, respectively, and show hypophosphatemia associated with elevated levels of FGF23 [77-80]. DMP1 encodes an extracellular matrix belonging to the small integrin-binding ligand, N-linked glycoproteins (SIBLINGs) family, and is highly expressed in osteocytes, similar to dentin. ENPP1 encodes an enzyme that produces pyrophosphate, an inhibitor of mineralization. Biallelic inactivating variants in the ENPP1 gene are also responsible for hypermineralization disorders, such as generalized arterial calcification of infancy [81].

Family with sequence similarity 20, member C (FAM20C), which is also called dentin matrix protein 4 (DMP4), encodes a kinase that phosphorylates caseins and various secreted proteins, including FGF23 and members of the SIBLINGs family of proteins, such as DMP1, osteopontin, and matrix extracellular phosphoglycoprotein [82]. The FAM20C gene is highly expressed in odontoblasts, and biallelic inactivating variants in this gene cause Raine syndrome (RNS), a neonatal osteosclerotic bone dysplasia with a poor prognosis [83]. Surviving patients with mild RNS may manifest elevated levels of serum FGF23 and hypophosphatemia as well as dental anomalies [84, 85].

Some skeletal dysplasias may also be associated with FGF23-related hypophosphatemia. Osteoglophonic dysplasia is an autosomal dominant disease caused by activating variants in the FGFR1 gene and is characterized by rhizomelic dwarfism, non-ossifying bone lesions, craniosynostosis, and distorted faces with frontal bossing and a depressed nasal bridge. Some patients with osteoglophonic dysplasia manifest FGF23-related hypophosphatemia [86]. Hypophosphatemia with elevated FGF23 levels may also be associated with Jansen-type metaphyseal chondrodysplasia, an autosomal dominant disease caused by activating variants in the PTH1R gene encoding the receptors for PTH and PTH-related protein [87].

FGF23-related hypophosphatemia may also occur in other conditions, such as TIO, McCune-Albright syndrome caused by somatic variants in GNAS1, linear nevus sebaceous syndrome caused by somatic variants in KRAS/HRAS, and the intravenous administration of iron preparations, such as saccharated ferric oxide, ferric carboxymaltose, and iron polymaltose [62, 63, 88]. TIO is a paraneoplastic syndrome caused by the overproduction of FGF23 by phosphaturic mesenchymal tumors (PMT) and is treated surgically by removing the responsible tumor. Previous studies identified fibronectin 1 (FN1)-FGFR1 and FN1-FGF1 fusion genes in subgroups of PMT [89, 90]. In addition, positive immunoreactivity for FGFR1 was detected in 82% of PMT patients, suggesting the involvement of FGFR1 signaling in the pathogenesis of PMT [90].

The measurement of serum levels of intact FGF23 is useful for differentiating FGF23-related hypophosphatemia from other hypophosphatemic diseases, including vitamin D deficiency, and renal tubular dysfunction, such as Fanconi syndrome. A previous study proposed a serum level of intact FGF23 of 30 pg/mL or higher in the presence of chronic hypophosphatemia for the diagnosis of FGF23-related hypophosphatemia [91]. In 2019, the measurement of serum levels of intact FGF23 by a chemiluminescent enzyme immunoassay to diagnose FGF23-related hypophosphatemia was covered by the national health insurance system in Japan [92]. Genetic testing is also useful for confirming the diagnosis of hereditary hypophosphatemic rickets/osteomalacia, such as XLH; however, it is not yet covered by the national health insurance system in Japan.

Oral Pi salts and active vitamin D metabolites are
administered as conventional medical treatments for FGF23-related hypophosphatemia represented by XLH to correct their deficiencies [69]. This is different from HHRH caused by variants in the SLC34A3 gene encoding NaPi-IIc, where the administration of Pi alone may cure hypophosphatemia and rickets/osteomalacia [93]. In pediatric patients with XLH, conventional treatment initiated in early infancy improved growth, biochemical, and radiological outcomes, but did not completely normalize skeletal development [94]. In adult patients, conventional treatment may attenuate bone pain and osteomalacia and improve oral health. However, it does not prevent or ameliorate enthesopathies or hearing loss. Furthermore, conventional treatment with Pi supplementation and active vitamin D may lead to adverse effects, including hypercalciumia, nephrocalcinosis, and secondary/tertiary hyperparathyroidism [69].

In 2018, burosumab, a humanized monoclonal neutralizing antibody to FGF23, was approved for the treatment of XLH by the European Medicines Agency and the United States Food and Drug Administration based on the promising findings of clinical trials. In Japan, burosumab was approved in 2019 for the treatment of all patients with FGF23-related hypophosphemic rickets/osteomalacia by the Pharmaceuticals and Medical Devices Agency. In a phase 3 clinical trial on pediatric patients with XLH, greater improvements were reported in the severity of rickets and growth and biochemical parameters in patients treated with burosumab than in those who continued conventional therapy [95]. The beneficial effects of burosumab have also been demonstrated in adult patients with XLH and those with TIO [96-98]. The long-term safety and efficacy of burosumab as well as its effects on complications, such as enthesopathies, warrant further study.

**Local Regulators of FGF23**

As described above, the main source of FGF23 is osteocytes in bone. Osteocytes are defined as dendritic cells embedded in the bone matrix and are differentiated from osteoblasts. In adult bone, osteocytes account for more than 90% of all bone cells and have the longest life span [99, 100]. Studies conducted in the last few decades revealed that osteocytes play profound roles in bone homeostasis by sensing mechanical strain and controlling bone formation and resorption. They produce sclerostin, a secreted inhibitor of Wnt/β-catenin signaling. Mechanical strain and PTH have been shown to suppress the production of sclerostin in osteocytes, which increases osteoblastic bone formation through the enhancement of Wnt/β-catenin signaling [101, 102]. Furthermore, osteocytes produce larger amounts of receptor activator of NF-κB ligand and have a greater capacity to support osteoclastogenesis than osteoblasts and bone marrow stromal cells [103]. In addition to these roles in bone homeostasis, osteocytes are essential for Pi metabolism. As osteoblasts mature into osteocytes, they acquire the high expression of multiple genes involved in Pi metabolism, including Fgfr23, Phex, Dmp1, and Fam20c, which indicates the key role of osteocytes in Pi homeostasis [36]. Enpp1 was also reported to be expressed by osteoblasts and osteocytes [104]. Since inactivating variants in PHEX, DMP1, FAM20C, and ENPP1 cause FGF23-related hypophosphemic rickets/osteomalacia, these molecules appear to function as local negative regulators of FGF23 production.

The mechanisms underlying the overproduction of FGF23 in most types of FGF23-related hypophosphatemia have not yet been elucidated in detail. However, previous findings suggested the involvement of enhanced FGF/FGFR signaling in the overproduction of FGF23 in XLH and ARHR1 [36, 105, 106]. In our previous study using Phex-deficient Hyp mice, we demonstrated that the osteocytic expression of the genes encoding canonical FGF ligands (Fgfl and Fgfl2), FGF receptors (Fgfr1–3), and their downstream target gene, early growth response 1, was higher in Hyp mice than in their wild-type littermates [36]. Furthermore, another group reported that the Dmp1-Cre-mediated conditional deletion of Fgfr1 from osteocytes and mature osteoblasts in Hyp mice partially restored elevated FGF23 levels and hypophosphatemia [106]. In the Dmp1 knockout mouse, a model for human ARHR1, a treatment with SU5402, an inhibitor of FGFR signaling, prevented increases in FGF23 expression levels in a culture of bone marrow stromal cells [105]. These findings suggest that the overproduction of FGF23 associated with Phex and Dmp1 deficiencies was due to the enhanced activation of FGFR signaling.

The regulation of FGF23 production by FGFR signaling has also been implicated based on findings showing FGF23-related hypophosphatemia in patients with osteoglophonic dysplasia caused by activating variants in FGF1R1 [86]. In addition, transgenic mice overexpressing high-molecular-weight (HMW) isoforms of FGF2 in osteoblast lineage cells showed elevated levels of serum FGF23 and hypophosphatemic rickets [107]. HMW-FGF2 isoforms increased FGF23 promoter activity through the intracellular FGF1R1/CBP/CREB pathway in osteoblasts, whereas low-molecular-weight isoforms of FGF2 transactivated the FGF23 promoter through the membranous FGFR-mediated PLCγ/calcineurin/NFAT and MAPK pathways [108]. Therefore, FGF/FGFR signaling in osteoblast lineage cells appears to locally regulate FGF23 production. As described above, enhanced FGFR signaling was suggested in a subgroup of PMT...
Systemic Regulators of FGF23

In addition to the local regulators described above, various systemic factors also regulate FGF23 levels and include 1,25(OH)2D, PTH, Pi, iron, hypoxia, inflammation, insulin, estrogen, and the circadian rhythm [68, 114-121]. Therefore, the regulation of FGF23 is complex and multifactorial and has not yet been elucidated in detail. This review describes some of the systemic regulators. There are already several excellent reviews on the regulators of FGF23 [114, 119].

Among systemic regulators, 1,25(OH)2D has been shown to increase the expression of FGF23 in osteoblast lineage cells by stimulating the transcription of the gene via VDR [115, 122]. Since FGF23 decreases 1,25(OH)2D by suppressing CYP27B1 and inducing CYP24A1, FGF23 is considered to function as a counter-regulatory hormone for 1,25(OH)2D [115]. Patients with vitamin D deficiency have low levels of serum FGF23, which supports the importance of 1,25(OH)2D in the regulation of FGF23 [91].

PTH has also been suggested to stimulate FGF23 production. As described earlier, Jansen-type metaphyseal chondrodysplasia caused by activating variants in PTHHR may be associated with elevated serum levels of FGF23 [87]. In addition, in a mouse model of hyperparathyroidism, a positive correlation was reported between serum levels of FGF23 and PTH [123]. Other studies also suggested the stimulation of FGF23 expression by PTH [124, 125]. A distal enhancer that mediated the PTH-induced expression of the mouse Fgf23 gene was identified and was also shown to mediate the induction of the gene by inflammation and early onset kidney disease [116].

Pi functions as a systemic stimulator of FGF23 production. Previous studies on humans and mice indicated that dietary phosphorus loading increased the serum levels of FGF23 [117, 118]. However, Pi exerts context-dependent effects on FGF23 levels. Acute elevations in serum Pi levels following the intravenous infusion of potassium Pi solution failed to increase serum levels of FGF23 in healthy human subjects [126]. In a cell study, the expression of Fgf23 in the rat osteoblastic cell line ROS17/2.8 was markedly up-regulated by a 24-hour treatment with a high level of 1,25(OH)2D, but not Pi [115]. Using primary osteoblasts and osteocytes isolated from mouse bones, we also demonstrated that a 24-hour treatment with a high level of Pi did not alter the expression of Fgf23 [36]. Increases in the production of FGF23 by phosphorus loading may take time and involve other molecule(s) and/or osteoblast maturation. Elevated levels of Pi were shown to up-regulate the expression of Galnt3 in osteoblast lineage cells in a FGFR1-dependent manner.
manner, leading to the increased production of FGF23 [127].

Iron deficiency enhances the production and cleavage of FGF23 as a physiological response [128]. As described earlier in this review, iron deficiency increases serum FGF23 levels in ADHR patients, with the production, but not cleavage, of FGF23 being accelerated due to the resistance of mutant FGF23 to cleavage [67, 68]. Furthermore, iron deficiency has been shown to induce the cellular accumulation of hypoxia-inducible factor 1α (HIF1α), which transactivates the Fgf23 promoter [68]. Iron deficiency also increased the levels of the hematopoietic hormone erythropoietin (EPO) through the regulation of HIF, and transgenic mice overexpressing EPO showed significantly elevated serum levels of both intact FGF23 and C-terminal fragments of FGF23, suggesting the direct effects of EPO on FGF23 [129].

Inflammatory activity may be associated with increased levels of FGF23 in inflammatory diseases, such as rheumatoid arthritis and inflammatory bowel disease [114]. Inflammation may promote the release of FGF23 from bone by accelerating bone resorption. Furthermore, various pro-inflammatory cytokines, including tumor necrosis factor α, IL-1β, and IL-6, have been shown to up-regulate the expression of Fgf23 [119]. Although iron deficiency and the up-regulation of HIF1α may contribute to inflammation-associated increases in the expression of FGF23 [130], additional mechanisms, such as the involvement of NF-κB and signal transducer and activator of transcription 3, have been proposed [131, 132]. A recent study indicated that lipocalin 2 mediated the induction of FGF23 by inflammation in CKD [133].

Insulin signaling has been suggested to decrease the expression of FGF23 through the activation of AKT. In women given an oral glucose load, increases in plasma insulin levels negatively correlated with plasma FGF23 levels [120]. Furthermore, in the cultured osteoblastic cell line UMR106, insulin and insulin-like growth factor 1 (IGF-1) reduced the production of FGF23 by inhibiting forkhead box protein 1 (FOXO1) through the activation of the phosphoinositide 3-kinase/AKT pathway [120]. On the other hand, we recently reported that the production of FGF23 was reduced in mice with the osteocyte-specific deletion of phosphatase and tensin homolog deleted from chromosome 10 (PTEN), which decreased the renal excretion of Pi and increased serum Pi levels [134]. Furthermore, the knockdown of PTEN expression in UMR106 cells activated AKT/mechanistic target of rapamycin complex 1 (mTORC1), which reduced the expression of Fgf23 [134]. These findings suggest that the insulin- and IGF1-induced activation of AKT inhibited the production of FGF23 through the FOXO1 and mTORC1 pathways.

Estrogen is also suggested to influence serum FGF23 levels. A clinical cross-sectional study with 987 participants aged 67 ± 11 years reported that women who were not taking estrogen had higher levels of serum FGF23 than those in men or women on estrogen therapy [135]. On the other hand, in vitro studies using MPC2 cells, which differentiate into osteocyte-like cells, demonstrated that a treatment with 17β-estradiol up-regulated the expression of FGF23 in a dose-dependent manner [136].

The circadian clock network is an evolutionarily conserved system for the adaptation of organisms to environmental factors, including the availability of nutrients [137, 138]. In addition to the central clock located in the suprachiasmatic nucleus, peripheral tissues have their own clocks and synchronize with the central clock through neuronal and hormonal signals. The peripheral circadian clock is also entrained by various factors, such as restricted feeding. Serum levels of phosphate have been shown to exhibit a circadian profile in both mice and humans [139, 140], indicating the regulation of Pi metabolism by the circadian clock system. Sympathetic tone also displays a circadian profile and is activated by food intake, and we previously suggested that food intake-associated sympathetic activation was involved in the regulation of Pi metabolism [121]. The skeletal expression of Fg23 showed a circadian rhythm with higher expression during the dark phase in animals fed standard chow ad libitum. The increased expression of Fg23 during the dark phase was associated with elevated serum levels of FGF23 and urinary Pi excretion. When feeding was restricted to the light phase, the peak expression of skeletal Fg23 and urinary Pi excretion shifted from the dark phase to the light phase. Sympathetic activation by the administration of the β-adrenergic agonist isoproterenol up-regulated the skeletal expression of Fg23 in a circadian-dependent manner; however, this was not observed in mice deficient for the core clock gene Bmal1. These findings suggest that skeletal Fg23 expression is regulated by circadian rhythms and food intake, at least partly through alterations in sympathetic activity [121]. The complex regulation of FGF23 by various local and systemic factors is shown in Fig. 2.

How are Pi Levels Sensed?

Organisms need to sense and adapt to Pi availability in their environment in order to maintain Pi homeostasis. The molecular mechanisms responsible for Pi sensing have been defined in unicellular organisms, such as bacteria and yeasts. They use a system called the pho/PHO regulon, which is activated by Pi depletion, for Pi
sensing and adaptation [1]. Some types of Pi transporters, such as Pst in bacteria and Pho transporters in yeasts, function as Pi sensors, and other molecules, including kinases, are also involved [1]. In contrast, the mechanisms underlying Pi sensing in mammals remain largely unknown. However, we and others previously reported that an elevated level of extracellular Pi directly exerted its effects on various cell types, including bone cells, to activate signaling pathways, such as the FGFR and Raf/MEK (MAPK/ERK kinase)/ERK pathways, and regulate gene expression and cell behavior [1, 141-144]. The responsiveness of mammalian cells to alterations in extracellular Pi levels suggests that phosphate availability is sensed at the cellular level. The treatment of the human embryonic kidney cell line, HEK293 with a high level of Pi induced the phosphorylation of FRS2α (FRS2α) and ERK1/2, which was diminished by silencing the expression of FGFR1 [141]. Similar findings were obtained for osteoblastic MC3T3-E1 cells: a treatment with a high level of Pi induced the phosphorylation of FRS2α and expression of Dmp1, and these effects were abolished by a co-treatment with SU5402, an inhibitor of FGFR [143]. Furthermore, phosphorus loading was shown to up-regulate the skeletal expression of Galnt3 by activating FGFR, which increased the production of FGF23 in mice [127]. These findings suggest the involvement of FGFR in Pi sensing in mammals. Taken together with enhanced FGFR signaling in the osteoblasts/osteocytes of Phex- or Dmp1-deficient mice [36, 105, 106], impaired Pi sensing in these cells may contribute to the overproduction of FGF23 in XLH and ARHR1.

**Conclusion**

Mammalian Pi homeostasis is maintained by fluxes in the intestines, kidneys, bone, and soft tissues, in which type II and III Na+/Pi co-transporters play critical roles. FGF23 is an endocrine factor that functions as a key molecule in Pi homeostasis and regulates Pi and vitamin D metabolism along with 1,25(OH)2D and PTH. Excess or deficient FGF23 causes disorders associated with abnormal Pi and vitamin D metabolism. The measurement of serum levels of FGF23 is useful for diagnosing FGF23-related hypophosphatemic rickets/osteomalacia and is covered by the national health insurance system in Japan. The recent approval of burosumab, a humanized antibody against FGF23, has changed the treatment of FGF23-related hypophosphatemic rickets/osteomalacia; however, its long-term efficacy and effects on complications, such as entheseopathies, remain unclear. Further studies are needed to clarify the molecular mechanisms underlying Pi sensing and the regulation of FGF23.
Acknowledgments

This manuscript was prepared with the support of a grant from the Japan Society for the Promotion of Science (JSPS KAKENHI Grant Numbers 21K07835 to TM).

Disclosure

The author has no conflicts of interest to disclose.

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