Although phosphate is important in skeletal mineralization, energy metabolism, and multiple enzymatic processes, little has been understood about the regulation of phosphate in health and disease until recently. Genetic and acquired disorders of phosphate homeostasis have begun to reveal important mechanisms for the regulation of phosphate metabolism. Candidate phosphate-regulating hormones (“phosphatonin”) have been discovered, and their actions and interactions continue to be elucidated in an exciting area of ongoing clinical and basic research. Autosomal dominant hypophosphatemic rickets (ADHR), X-linked hypophosphatemic rickets (XLH), tumor-induced osteomalacia (TIO), and fibrous dysplasia (FD) have similar phenotypes of hypophosphatemia, urinary phosphate wasting as measured by a low tubular maximum reabsorption of phosphate per deciliter of glomerular filtrate (TmP/GFR), osteomalacia, and rickets. The phenotypic similarities suggest a common metabolic pathophysiology. In addition, recent evidence concerning tumoral calcinosis, which results in hyperphosphatemia, inappropriately normal calcitriol concentrations, and soft tissue calcifications and can be considered the phenotypic “converse” of the phosphate wasting disorders, indicates that this disorder may result from perturbations of some of the same metabolic pathways. Fibroblast growth factor 23 (FGF23) is a recently discovered, novel, secreted protein hormone involved in the pathogenesis of these disorders. This review focuses on the relationship of FGF23 to various disorders of phosphate homeostasis and its potential regulation and function in normal physiology.

**Hypophosphatemic Disorders**

**ADHR**

ADHR, initially described by Bianchine et al. (1), is characterized by renal phosphate wasting, hypophosphatemia, low or inappropriately normal calcitriol concentrations, and osteomalacia. Econs and McEnery (2) described a large ADHR kindred. Affected patients presented either in childhood with hypophosphatemia and rickets, which resulted in lower extremity deformities, or after puberty with hypophosphatemic osteomalacia, manifesting in weakness, fatigue, bone pain, and fractures. Patients with adult onset of symptoms clinically resemble TIO, but the disease is not a paraneoplastic process. To date, delayed onset of clinically evident disease has been observed only in female individuals. ADHR also exhibits incomplete penetrance, and, in some cases, the clinical and biochemical phenotype spontaneously resolved in patients who were previously documented to have hypophosphatemic rickets (2).

The ADHR consortium used the positional cloning approach to identify the gene responsible for ADHR, FGF23 (3). ADHR results from mutations in either arginine 176 or arginine 179 in FGF23. These arginines make up an RXXR cleavage site, and mutations in either of these arginines protect the protein from degradation, potentially increasing the circulating concentration of FGF23 and, thereby, leading to the disease phenotype (4–6). FGF23 is a 251–amino acid protein in the FGF family with a novel C-terminal end and an N-terminal signal peptide. FGF23 is expressed primarily in bone (7–10) but is also expressed in heart and liver (3,10). FGF23 is present in the circulation of normal individuals (11).

Wild-type and mutant FGF23 decrease serum phosphate and increase the fractional excretion of phosphorous in vivo when injected into mice, whereas the cleavage products do not lower serum phosphorous (5). FGF23 infusion results in hypophosphatemia and low 1,25-dihydroxyvitamin D levels in mice within hours (12). Messenger RNA for 1-α-hydroxylase is also decreased in rodent kidneys within hours of FGF23 infusion (12). Further evidence for FGF23 as the cause of phosphaturia came from studies that used overexpression models of FGF23. Chinese hamster ovary (CHO) cells that are stably transfected with wild-type or mutant FGF23, which then are implanted into nude mice, result in phosphaturia, low serum phosphate and calcitriol concentrations, and rachitic changes (4,5). These results were confirmed by transgenic mouse models of wild-type FGF23 and R176Q FGF23 expression under various promoters (13–15). In addition, both FGF23-transgenic mice and mice bearing FGF23-transfected CHO cells exhibited decreased renal brush border sodium phosphate co-transporter 2a (Npt2a), growth retardation, and parathyroid hyperplasia (4,13–15). Thus, overexpression of either wild-type or mutant FGF23 mimics the phenotypes found in ADHR and XLH.

At the renal proximal tubule brush border, FGF23 infusion rapidly decreases expression of Npt2a (12). Parathyroid hormone (PTH) also directly decreases Npt2a at the renal proximal tubule brush border via a cAMP/protein kinase A–mediated pathway (16,17). Yamashita et al. (18) demonstrated that FGF23 requires the presence of heparin molecules to inhibit phosphate...
uptake in OK cells, that FGF23 activated members of the Ras/mitogen-activated protein kinase pathway, and that the activity of FGF23 could be blocked by tyrosine kinase inhibitors. In addition, infusion of FGF23 does decrease serum phosphorous in thyroparathyroidectomized rats (12,19). Thus, the action of FGF23 on renal phosphate reabsorption is independent of PTH and may be complementary.

That the phenotype is variable in ADHR and may resolve spontaneously suggest that some patients are able to compensate, at least temporarily, for the abnormal phosphate and vitamin D metabolism induced by the mutant FGF23. The mechanism for this compensation has not yet been established but may involve an ability to block the effect of FGF23 at its receptor or its postreceptor signaling, to inhibit the production of FGF23, or to inactivate FGF23 via an alternative pathway.

TIO

TIO is an acquired paraneoplastic disorder characterized by hypophosphatemia, low or inappropriately normal calcitriol concentrations, and osteomalacia. Patients with TIO frequently present with fatigue, bone pain, fractures, and proximal muscle weakness. Children develop rickets and lower extremity deformities similar to those in ADHR and XLH. Many of these tumors are small and can be very difficult to locate. Multiple imaging techniques, including computed tomography, magnetic resonance imaging, octreotide scans, and positron emission tomography—computed tomography may be required in an attempt to localize the tumor. Frequently, a tumor is not found despite an extensive search. However, when a tumor is found, removal cures the phosphate, vitamin D, and bone abnormalities. The vast majority of these tumors are benign. However, even benign tumors that cause TIO may recur years after the original tumor is removed (20).

Tumors that cause TIO overexpress FGF23 (11,21,22). These tumors have also been found to express matrix extracellular phosphoglycoprotein (MEPE) (23,24), frizzled related protein-4 (24), and recently FGF7 (25). Because hypophosphatemia is a characteristic of this disorder, all of these substances are candidate phosphatonin. Although a variety of tumors have been implicated, Folpe et al. (26) analyzed pathologic samples of patients with TIO and found that most cases could be classified as phosphaturic mesenchymal tumor of a mixed connective tissue type. His group was able to detect FGF23 by immunohistochemistry in 81% of pathology specimens tested in their study (26).

Sandwich two-site ELISA have been developed for measuring FGF23. A C-terminal assay utilizes two antibodies to epitopes on the C-terminal side of the RXXR cleavage site and theoretically measures both intact FGF23 and some breakdown products of FGF23 (11). Intact assays have also been developed using antibodies to epitopes on either side of the RXXR cleavage site (27). These antibodies are expected to measure FGF23 differently, especially in conditions that might result in an excess of circulating degradation products of FGF23, such as chronic renal disease. FGF23 intact and C-terminal assay results are expected to be similarly elevated in conditions that overproduce FGF23. As has been the case for assays of a variety of hormones, the characteristics of assays for FGF23 should continue to improve with further generations of development.

Circulating FGF23 is elevated in many patients with TIO, and FGF23 concentrations decline after tumor removal (11,27–29). The use of selective venous sampling for FGF23 has been reported to be useful in localizing a tumor that caused TIO in a single case (30); however, further studies are necessary before the practice can be recommended clinically. Failure of FGF23 concentrations to decline after surgery are indicative of the presence of residual disease (31).

FD and Osteoglophonic Dysplasia

FD lesions, particularly in the setting of McCune-Albright syndrome, frequently result in renal phosphate wasting, hypophosphatemia, and low or inappropriately normal calcitriol concentrations. The lesions result from somatic activating mutations of GNAS1 that cause constitutive activation of various hormone receptors using G-protein signaling (32). Approximately half of patients with McCune-Albright syndrome develop hypophosphatemia and localized mineralization defects (33). Rimunici et al. (8) measured FGF23 concentrations with a C-terminal assay in patients with FD and found elevated serum FGF23 concentrations that correlated negatively with the serum phosphate concentration. The serum FGF23 concentration correlated with disease burden of FD lesions and the presence of phosphate wasting. In bone biopsy specimens from patients with FD, both normal osteogenic cells and osteogenic cells with GNAS1 mutations expressed FGF23 mRNA. This disorder is analogous to TIO in that overexpression of wild-type FGF23 results in renal phosphate wasting. However, significant disease burden of FD lesions (as estimated by bone scintigraphy [34]) is required to produce elevations of circulating FGF23 (8), compared with the relatively small size of most tumors that are responsible for TIO.

White et al. (35) recently described patients with activating mutations in FGFR1 resulting in osteoglophonic dysplasia (OD). OD is characterized by multiple craniofacial abnormalities, craniosynostosis, rhizomelic dwarfism, brachydactyly, and insufficiency fractures. Biochemically, some patients manifest renal phosphate wasting and inappropriately low calcitriol concentrations. The inheritance pattern is autosomal dominant. Mutational analysis revealed three novel missense mutations in FGFR1 (N330I, Y372C, and C379R) (35). Of note, patients with OD and renal phosphate wasting have nonossifying bone lesions, which are similar to FD, and FGF23 concentrations were elevated in one patient with OD and phosphate wasting, whereas they were normal in a patient with OD and no phosphate wasting (35).

XLH

XLH is the most common hereditary form of rickets with a prevalence of approximately 1:20,000. It is an X-linked dominant disorder. Patients frequently present with short stature, lower extremity deformity from rickets, bone pain, joint pain and stiffness as a result of enthesopathy, and dental abscesses. Disease severity is extremely variable, even among affected individuals from the same family. Biochemically, the disorder...
is characterized by hypophosphatemia as a result of renal phosphate wasting and inappropriately low or normal calcitriol concentrations. Radiographic evidence for rickets is common in children, although not universally present (36), and osteomalacia is seen on bone biopsy.

Several murine models of XLH exist, including the Hyp, Gy, and Ska 1 mouse (37–39). Early parabiosis experiments indicated that the cause of phosphaturia in Hyp was a circulating factor (40), and cross-transplant experiments with Hyp and normal mice confirmed that the location of the defect in the Hyp mouse was not in the kidney (41).

A positional cloning approach was used to identify the PHEX gene (phosphate-regulating gene with homologies to endopeptidases on the X chromosome), which encodes a member of the M13 family of endopeptidases (42). More than 170 different mutations have been described to cause XLH, including missense and nonsense mutations, deletions, frame shifts, and splice site mutations (http://www.phexdb.mcgill.ca). PHEX expression is greatest in bone (osteoblasts and osteocytes) and teeth but is also expressed to a limited extent in lung, brain, ovary, and testes (43,44). PHEX is a membrane-bound enzyme, and the exact substrate or substrates of PHEX have yet to be identified. It is not immediately obvious how an inactivating mutation of an enzyme leads to a dominant pattern of inheritance. However, because a circulating factor was responsible for urinary phosphate wasting in XLH, normal PHEX may serve either to degrade or to inhibit production of that factor. FGF23 concentrations are elevated 10-fold in Hyp mice compared with normal littermates (45) and are also elevated, but to a lesser degree, in patients with XLH (11,27).

An attractive initial hypothesis was that FGF23 was a PHEX substrate. However, in vitro experiments revealed that cleavage of FGF23 by endogenous enzymes in cell culture medium was independent of the presence of PHEX (9) and that FGF23 is a substrate for subtilisin-like proprotein convertases (46). Liu et al. (9) showed by real-time PCR that FGF23 gene expression is increased in Hyp mouse bone and Hyp osteoblast cultures, suggesting that PHEX normally inhibits FGF23 expression. An inactivating PHEX mutation results in abnormal regulation of FGF23 and possibly other molecules, resulting in the phenotype of XLH. In addition, PHEX mRNA is increased in the growth plates of transgenic mice that express FGF23 (R176Q), further suggesting a feedback mechanism for control of FGF23 concentrations (13).

FGF23 knockout mice exhibit the opposite phenotype to FGF23 excess, with elevated serum phosphate, elevated 1α-hydroxylase and 1,25-dihydroxyvitamin D, and abnormal bony nodules, along with early death from renal failure (7,47). In addition, injection of neutralizing antibodies to FGF23 into normal mice resulted in similar elevations in serum phosphate and increased renal expression of Npt2a and 1α-hydroxylase (48). Both models suggest an important role for FGF23 in normal phosphate handling and skeletal development.

Because FGF23 mRNA expression in bone is increased in Hyp mice (9) and circulating FGF23 concentrations are elevated in the Hyp mouse and in patients with XLH (11,27), Sitara et al. (7) bred FGF23 knockout mice with Hyp mice to see the effect of removing FGF23 from the Hyp mouse. The skeletal phenotype was indistinguishable from that of the FGF23 knockout mouse. In addition, these mice had elevated serum phosphorous concentrations, demonstrating that the Hyp mouse phenotype is dependent on FGF23. Aono et al. (45) injected neutralizing antibodies to FGF23 into Hyp mice and found dose-dependent increases in serum phosphate and 1,25-dihydroxyvitamin D. Treatment with repeated doses of antibodies to FGF23 over 4 wk improved rachitic bone lesions and bone growth (45). Data from these studies support the hypothesis that abnormal regulation of FGF23 is responsible for the phenotype seen in XLH and that the ability to neutralize FGF23 may be a useful therapeutic modality in hypophosphatemic disorders.

MEPE (also known as osteoblast/osteocyte factor 45) has also been cloned from tumors that are responsible for TIO (23,24) and may play a role in XLH. Circulating levels of MEPE-ASARM peptides (a C-terminal fragment of MEPE) are elevated in patients with XLH and Hyp mice (49), but intact MEPE levels were not elevated in patients with TIO and XLH (50). Intact MEPE did correlate with the phosphate concentration and bone mineral density in normal humans (51). However, the MEPE null mouse model shows increased bone mass without a phosphate or vitamin D phenotype (52). In addition, Liu et al. (53) recently published studies in which MEPE null mice were bred with Hyp mice, and the absence of MEPE failed to correct the skeletal and phosphate defects of the Hyp mouse.

### Tumoral Calcinosis

Hereditary tumoral calcinosis results in elevated calcitriol concentrations and painful, debilitating deposition of calcium phosphate in soft tissue and joints. The first causative mutation discovered was an inactivating mutation in UDP-N-acetyl-α-D-galactosamine/polypeptide N-acetylgalactosaminyl transferase 3 (GALNT3), an enzyme responsible for initiating O-linked glycosylation of proteins (54). GALNT3 is expressed in bone as well as a variety of other tissues. Although previous data suggested that both autosomal recessive and dominant forms of the disease occurred, recent data indicate that the inheritance pattern is recessive, but heterozygotes may have a mild biochemical phenotype (54,55). The family that was described previously to have autosomal dominant inheritance later proved to be carrying compound heterozygous recessive mutations in GALNT3 (55,56). Of note, FGF23 concentrations, as measured by a C-terminal ELISA assay that measures both intact and C-terminal fragments of FGF23, were markedly elevated in patients with inactivating mutations in GALNT3 (54). However, intact FGF23 concentrations have not been reported.

In addition to mutations in GALNT3 causing tumoral calcinosis, recent reports demonstrated that inactivating mutations in FGF23 also result in tumoral calcinosis. Mutation in serine 71 (S71G), which also result in tumoral calcinosis. Mutation in serine 71 (S71G), which is conserved across multiple species, causes tumoral calcinosis (57,58). This mutation is predicted to affect FGF23 structure, and these patients have high FGF23 concentrations as measured by a C-terminal assay (57,58) but low or low normal FGF23 concentrations using an assay that measures only intact FGF23 (58). Benet-Pages et al. (57) demonstrated in cell culture studies that only C-terminal fragments
are secreted from HEK293 cells transfected with the S71G mutant FGF23. These data indicate that the disease results from an inability to secrete intact, active FGF23 protein. In addition to the reported S71G mutation, an S129F mutation in FGF23 has been reported to cause tumoral calcinosis (59). Preliminary data indicate that serine 129 may be O-glycosylated, potentially establishing a pathophysiologic link between the pathogenesis of tumoral calcinosis caused by GALNT3 mutations and that caused by FGF23 mutations.

In summary, FGF23 plays a key role in the pathogenesis of a variety of disorders of phosphate homeostasis. Figure 1 summarizes the authors’ current views.

**Role of FGF23 in Normal Phosphate and Vitamin D Homeostasis**

In light of the fact that FGF23 seems to play an important role in the pathogenesis of a variety of disorders of phosphate homeostasis, it is logical to ask whether FGF23 has a role in the
day-to-day maintenance of serum phosphate concentrations. If FGF23 has a role in normal phosphate homeostasis, then levels should respond to states of hyperphosphatemia and hypophosphatemia induced by other mechanisms (e.g., chronic kidney disease, hypoparathyroidism, dietary phosphate modification).

**Dietary Phosphate**

To assess the response to dietary phosphate, Ito et al. (60) fed normal mice low-, medium-, and high-phosphate diets for 7 d and measured circulating FGF23 concentrations using an intact FGF23 assay. Plasma phosphate levels reflected the dietary phosphate content, and FGF23 concentrations were significantly increased in mice that were fed the high-phosphate diet and significantly decreased in mice that were fed the low-phosphate diet (60). They expressed FGF23 promoter constructs in transiently transfected K562 cells in media with various concentrations of phosphorous and showed an increase in promoter activity with the high-phosphate medium. There was no promoter response to low- or medium-phosphate concentrations. This study demonstrates physiologic regulation of FGF23 by phosphate concentrations in a normal state, similar to that shown by other investigators in models of renal insufficiency (61). The effect of dietary phosphate is magnified in renal insufficiency. Saito et al. (61) performed partial nephrectomies on rats and placed the rats on low-, medium-, and high-phosphate diets. Serum phosphorous correlated with the dietary phosphorous and FGF23 concentrations showed increasing elevations with the low-, medium-, and high-phosphate diets, compared with sham-operated rats.

Conflicting results have been reported in human studies of the effect of dietary phosphorous or calcitriol on serum FGF23 concentrations in healthy individuals. FGF23 did not show circadian variation in six healthy men who were fed a typical diet in a study by Larsson et al. (62). They then restricted dietary phosphate in these individuals for 2 d, followed by 3 d of phosphate supplementation, and did not find a difference in FGF23 concentrations.

In a larger study, Ferrari et al. (63) measured FGF23 concentrations with a C-terminal assay in 29 healthy men who were treated sequentially with 5 d of dietary phosphate restriction with phosphate binders, followed by an equilibration period, then 5 d of a high-phosphate diet. The dietary modifications were accompanied by appropriate alterations in 24-h urine phosphate excretion, TmP/GFR, and, to a lesser degree, serum phosphate. Mean serum FGF23 concentrations decreased 29.1 ± 6.5% during phosphate restriction and increased 31.1 ± 9.5% during phosphate supplementation. Changes in FGF23 concentrations correlated negatively with changes in calcitriol concentrations and changes in TmP/GFR.

Similar results were found by Burnett et al. (64), who randomly assigned 60 healthy men and women to dietary phosphate restriction and phosphate binders or to phosphate supplementation for 4 d. With the use of a C-terminal FGF23 assay, concentrations increased 23% with oral phosphate loading and initially decreased 15% with phosphate restriction, but the difference from baseline was not maintained over the full 4 d of restriction. These studies demonstrate a role of FGF23 in the normal homeostatic regulation of serum phosphate in the setting of dietary phosphate variation.

**Vitamin D**

The first evidence of a role for FGF23 in vitamin D homeostasis comes from disorders of excess FGF23, which are characterized by inappropriately low or normal 1,25-dihydroxyvitamin D concentrations. This is confirmed in rodent models of these disorders. Hyp mice have abnormal 1-α-hydroxylase responses to PTH infusion and to low-phosphate diet (65–67). Yuan et al. (67) demonstrated a normal increase in 1-α-hydroxylase mRNA in Hyp mice after PTH infusion, similar to that seen in normal mice after PTH infusion. In their study, this was not accompanied by an increase in renal 1-α-hydroxylase enzyme, suggesting a posttranscriptional or posttranslational modification of the 1-α-hydroxylase response to PTH and low-phosphate diet. However, other authors have noted decreases in levels of both 1-α-hydroxylase mRNA and enzyme activity in response to these stimuli in Hyp mice (65,68). In addition, differential regulation of production of 1-α-hydroxylase mRNA versus protein seems inefficient and is inconsistent with the finding of decreased 1-α-hydroxylase mRNA reported with FGF23 administration to normal mice (12). Infusion of FGF23 reduces calcitriol in hours, before the observed decrease in serum phosphate, via decreased 1-α-hydroxylase expression (12).

It is interesting that there is discrepancy among transgenic models of FGF23 overexpression. Using mice transgenic for human FGF23 overexpression, Larsson et al. (14) found a nonsignificant decrease in 1-α-hydroxylase mRNA in male mice but a significant increase in female mice at 2 mo of age. Despite hypophosphatemia in the FGF23 transgenic mouse, which should be a stimulus for calcitriol production, 1,25-dihydroxyvitamin D concentrations were significantly lower in male transgenic mice (and nonsignificantly lower in female transgenic mice) than in wild-type mice at 18 d of age but at 2 mo of age were not significantly different between male and female transgenic mice and wild-type mice that were fed the same diet. In a FGF23(R176Q) transgenic model, Bai et al. (13) found that 1-α-hydroxylase mRNA was decreased at 1 mo of age but mRNA increased at 2 mo of age without any increase in immunoreactivity for 1-α-hydroxylase. 1,25-Dihydroxvitamin D levels were decreased at 1 and 2 mo of age in these mice. It seems that short-term exposure to FGF23 excess acutely lowers transcription of 1-α-hydroxylase mRNA, whereas longer exposure may increase transcription, possibly mediated by increased PTH, but may also alter translation or posttranscriptional modification. The degree and duration of FGF23 elevation or the presence of mutant FGF23 may explain the different effects reported by these groups.

FGF23 has corresponding effects on the inactivation of calcitriol. Mouse models of FGF23 overexpression have increased renal tubular expression of 24-hydroxylase, which is responsible for inactivating 1,25-dihydroxyvitamin D (4,13,14). The combined effect of decreased production and increased metabolism of calcitriol led to the inappropriately low levels seen in phosphate-wasting disorders.

Similar effects on calcitriol production have been docu-
mented in patients who have XLH and were treated with infusion of PTH (69). However, administration of calcitonin, another known stimulus to calcitriol production, results in normal stimulation of Hyp mouse 1α-hydroxylase (67,70) and normal stimulation of serum calcitriol in patients with XLH (71). This discrepancy suggests that the defect underlying the abnormal calcitriol production in XLH, as well as in ADHR and TIO, is incomplete and may be overcome with the appropriate stimulation of a PTH-independent pathway.

Removal of FGF23 has the opposite effect of FGF23 excess on vitamin D metabolism. Neutralizing antibodies to FGF23 increased 1α-hydroxylase expression and 1,25-dihydroxyvitamin D in normal mice (48) and in Hyp mice (45), and FGF23 null mice have elevated 1,25-dihydroxyvitamin D (7,47). In addition, calcitriol downregulates normal PHEX mRNA (72). Because inactivating mutations of PHEX cause elevations in FGF23, normal PHEX may decrease FGF23 concentrations, resulting in a potential feedback loop of 1,25-dihydroxyvitamin D, PHEX, and FGF23. However, there is evidence that 1,25-dihydroxyvitamin D directly affects the expression of FGF23.

FGF23, being an endocrine factor, is regulated by feedback from downstream metabolites. Ito et al. (60) isolated the FGF23 promoter and undertook studies to identify FGF23 regulatory factors in the leukemic K562 cell line. Despite the lack of the typical upstream vitamin D response element, there was a dose-dependent increase in FGF23 promoter activity with increasing 1,25-dihydroxyvitamin D concentrations. In confirmation of these findings, cotransfection with the human vitamin D receptor (VDR) further increased FGF23 promoter activity. Of note, there was also a 1.7-fold increase in promoter activity under high-phosphate cell culture conditions, and this effect was synergistic in the presence of 1,25-dihydroxyvitamin D (60). VDR null mice, which have hypocalcemia, hypophosphatemia, and hyperparathyroidism, have decreased or undetectable FGF23 concentrations compared with normal mice, indicating that serum phosphate or vitamin D concentrations may directly regulate FGF23 (60). It is interesting that administration of calcitriol to thyroparathyroidectomized rats results in a dose-dependent increase in FGF23 levels; however, this response is not seen in VDR null mice, indicating that vitamin D works through genomic actions to directly increase FGF23 production (61). Recent investigations have complemented these initial studies and established independent pathways for FGF23 regulation. It was reported that dietary increases in inorganic phosphate correspond with increasing concentrations of serum FGF23 (60). To dissect the mechanisms of vitamin D and dietary phosphate regulation of FGF23 production, Yu et al. (73) used the VDR null mice. As described above, on standard diets, these mice are hypophosphatemic. When given diets that consist of high phosphate, calcium and lactate to raise serum phosphate concentrations, circulating FGF23 levels dramatically increase, indicating that phosphate can regulate FGF23 independently from the genomic actions of vitamin D.

Thus, FGF23 is upregulated by 1,25-dihydroxyvitamin D, whereas 1,25-dihydroxyvitamin D is downregulated by FGF23, providing a mutual feedback loop with implications for phosphate and bone homeostasis. As described in the previous section, phosphate balance also regulates FGF23 concentrations. Given that current therapy for XLH and ADHR involves the use of high doses of calcitriol and phosphate, it is reasonable to expect that this therapy may directly increase the levels of FGF23. It is unclear what the long-term consequences of this increase may be. However, treatments that directly address the underlying problem with FGF23 may be preferable to those that are currently available.

**PTH**

Hyperparathyroidism in patients with primary renal phosphate-wasting disorders is generally thought to be due to complications of therapy. However, patients who have XLH and have never been treated may have elevated PTH concentrations (74,75). Patients who are treated with phosphate and calcitriol will occasionally require parathyroidectomy (76), and hyperparathyroidism may play a role in hypertension in these patients (77). Animal models of overexpression of FGF23 also demonstrate diffuse parathyroid hyperplasia and secondary hyperparathyroidism (13,14). Certainly, hyperparathyroidism can contribute to renal phosphate wasting. Traditionally, the mechanism by which treatment caused hyperparathyroidism was thought to be transient hypocalemia induced by the phosphate treatment, with calcitriol blunting this effect. However, calcitriol therapy could potentially increase FGF23 expression. Because FGF23 decreases intrinsic production of calcitriol, feedback inhibition of parathyroid cells could be diminished, particularly during the nadir between doses of oral calcitriol. The inappropriately low calcitriol level in phosphate-wasting disorders may lead to stimulation of the parathyroid glands in untreated patients. In patients who are on treatment with calcitriol, however, PTH elevations still occur, indicating that calcitriol treatment alone is unable to completely inhibit the PTH production in these patients. There may also be a direct effect of FGF23 on the parathyroid. Currently, there are insufficient data to warrant any change in therapy, and it should be noted that calcitriol is necessary to heal the osteomalacia in XLH (78).

In any event, current data in humans and in animal models suggest that elevation in PTH may be an inherent feature of disorders of phosphate wasting, as well as a potential complication of treatment. PTH elevation may result from the defect in 1,25-dihydroxyvitamin D production seen in these disorders, from increased FGF23 concentrations, and/or from transient hypocalemia after doses of phosphate. This requires close monitoring by the clinician and adjustment of treatment to attempt to minimize the development of parathyroid dysfunction.

In primary hyperparathyroidism, two competing factors may influence FGF23 concentrations: hypophosphatemia and increased calcitriol. To assess this, Yamashita et al. (79) measured full-length FGF23 concentrations in 98 patients with primary hyperparathyroidism. In these patients, FGF23 concentrations were mildly higher than that in control subjects, but there was no difference between control subjects and patients with hyperparathyroidism and normal creatinine clearance. Postoperative values were measured in 11 patients, and there was no change in full-length FGF23 concentration after parathyroidec-
tomy (79). These results suggest that in the setting of primary hyperparathyroidism, FGF23 is not a major regulator of phosphate or calcitriol, or perhaps the competing effects of hypophosphatemia and elevated calcitriol cancel each other out in patients with normal renal function.

Saito et al. (61) took thyroparathyroidectomized rats and found that serum FGF23 concentrations were elevated, but FGF23 elevations did not normalize the serum phosphorous. However, PTH infusion did normalize the serum calcium, phosphorous, and FGF23 levels in these rats, indicating that FGF23 elevations in response to hyperphosphatemia associated with hypoparathyroidism are compensatory but insufficient for normalizing the phosphate.

Similar data have been shown in humans. Hypoparathyroidism with associated hypophosphatemia results in higher FGF23 concentrations with positive correlation to serum phosphate levels (80). This is consistent with one role of FGF23 being to lower phosphorous by decreasing renal tubular reabsorption. Although the FGF23 concentrations did not correlate with morning 1,25-dihydroxyvitamin D concentrations (which would be a trough level) (80), there still may be some influence of treatment with calcitriol on the circulating FGF23 concentration.

If FGF23 plays a physiologic role as a phosphatonin, then one would expect FGF23 to increase to the level required to maintain normal phosphate levels in hypoparathyroidism. However, FGF23 also decreases the production of 1,25-dihydroxyvitamin D and increases its degradation via 24-hydroxylase stimulation (4,13,14). Further increasing FGF23 would decrease 1,25-dihydroxyvitamin D and could decrease the calcium level. This would not be advantageous in hypoparathyroidism and may reflect the relative acute clinical importance of hypocalcemia compared with mild hyperphosphatemia. It is possible that hypocalcemia may inhibit the response of FGF23 to hyperphosphatemia in the setting of an already low intrinsic 1,25-dihydroxyvitamin D in patients with normal renal function. Calcium, however, does not seem to directly regulate the FGF23 promoter region in cell culture studies (60).

Renal Failure

In light of the phosphate and vitamin D abnormalities present in chronic renal failure, it is not surprising that significant attention has been focused on FGF23 and its relationship to phosphate homeostasis in renal failure. Larsson et al. (62) and Imanishi et al. (81) demonstrated that patients with ESRD have elevated FGF23 concentrations that correlate with serum phosphate concentrations. Both of these studies used an ELISA that recognizes the C-terminal portion of FGF23 and thus may measure both full-length and inactive cleavage products of FGF23. However, elevations in intact FGF23 in renal failure have been reported by multiple authors using full-length FGF23 assays (82–85). Okada et al. (84) evaluated full-length FGF23 concentrations in a cohort of patients with mild chronic renal insufficiency. FGF23 levels correlated with phosphorous, creatinine clearance, intact PTH, and 1,25-dihydroxyvitamin D. Shigematsu et al. (85) measured levels of full-length FGF23 in 62 patients with chronic glomerulonephritis before beginning dialysis. FGF23 levels correlated with serum phosphorous and intact PTH and inversely with creatinine clearance. Elevations of FGF23 in chronic renal disease likely are due to decreased phosphate clearance as the number of functioning nephrons decrease.

Concentrations of FGF23 may also provide prognostic information. In dialysis patients with secondary hyperparathyroidism and PTH >300 pg/ml, FGF23 levels were more predictive of successful treatment with calcitriol to lower PTH than pretreatment PTH levels (82). In another study by Nakanishi et al. (83), 103 dialysis patients with starting PTH values <300 pg/ml were assessed for full-length FGF23 concentrations and were followed for 2 yr. FGF23 concentrations at the beginning of the study were the only significant predictor of refractory hyperparathyroidism despite treatment (P = 0.0112). Even the pretreatment PTH did not predict refractory hyperparathyroidism (83).

As noted before, parathyroid hyperplasia is seen in animal models of FGF23 excess, suggesting that FGF23 may be involved in the pathogenesis of increased PTH concentrations (13,14), and it is possible that this effect contributes to the hyperparathyroidism seen in renal disease. The converse also may be true. FGF23 concentrations declined after parathyroidectomy for hyperparathyroidism as a result of renal disease in one small study (86), suggesting that hyperparathyroidism in renal disease may drive some of the excess FGF23 production, although this is not seen in primary hyperparathyroidism in patients with normal renal function (79). However, Nakanishi et al. (83) did not find FGF23 expression in surgically removed parathyroid glands of patients with refractory hyperparathyroidism as a result of renal disease, indicating that the parathyroid glands are not the source of FGF23 elevation. Thus, although FGF23 increases PTH production, it might also be increased by excess PTH.

FGF23 Receptor

The receptors for the FGF are tyrosine kinase family members with an extracellular ligand-binding domain, a single transmembrane domain, and an intracellular tyrosine kinase region. There are four genes that code for the seven classic FGF receptors (1b, 1c, 2b, 2c, 3b, 3c, and 4) (87). A fifth receptor has been described, but the intracellular domain does not contain a tyrosine kinase domain (88,89). Because FGF23 activates its receptor by activation of a tyrosine kinase, FGF receptor 5 is an unlikely candidate receptor (18). Yamashita et al. (18) found that FGF23 bound with high affinity to FGFR3c in opossum kidney cells. However, the FGFR3 knockout mouse does not have abnormalities of phosphate or vitamin D metabolism (90). Whereas the FGFR3 receptor is yet to be determined, it may be that FGF23 mediates its effects through more than one receptor.

Conclusion

Phosphate regulation is important for normal cellular and skeletal function. Multiple disorders have revealed the phosphate- and vitamin D–regulating properties of FGF23, although the mechanisms are not yet completely understood. XLH, TIO, ADHR, and FD all show evidence of phosphate wasting and
inhibition of production of 1,25-dihydroxyvitamin D as a result of increased FGF23 concentrations. Substantial evidence exists for a role for FGF23 in the pathogenesis of tumoral calcinosis. FGF23 functions in a regulated manner in normal phosphate homeostasis via its actions to decrease phosphate reabsorption in the kidney and to decrease gut phosphate absorption (via its effects on 1,25-dihydroxyvitamin D) and as compensation for hyperphosphatemia in ESRD and hypoparathyroidism. Although FGF23 has been most important in elucidating the pathophysiology of some rare genetic and acquired disorders, there is a broader clinical significance. Measurement of FGF23 seems to have prognostic significance in the treatment of secondary hyperparathyroidism as a result of renal disease and may have a role in the diagnosis and management of clinical hypophosphatemia. If hypophosphatemia is FGF23 mediated, then therapy might be directed at reducing FGF23 concentrations and/or blocking FGF23 action with an antagonist. Hyperphosphatemia is a more common clinical scenario, especially in the setting of renal disease. Recombinant FGF23 might become useful in the treatment of hyperphosphatemia as a result of nonoliguric renal disease or tumor lysis syndrome. Familial tumoral calcinosis may also be treatable by recombinant FGF23. Thus, insight into the role of FGF23 in a variety of disorders has set the stage for development of new treatments for several disorders of phosphate homeostasis.

Acknowledgment

This work was supported by National Institutes of Health Grant R01 AR42228.

References


