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Redefining the pathogenesis of CKD-MBD: the critical role of FGF-23

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FGF-23 IN THE DEVELOPMENT OF SECONDARY HYPERPARATHYROIDISM

Through signals between the kidney, parathyroid gland, and bone, alterations in kidney function lead to changes in serum biochemical values that accompany progressive skeletal disease. Abnormalities in mineral and bone metabolism occur early in the course of chronic kidney disease (CKD) and progress as renal function declines[(1)]. Traditionally, these abnormalities have been ascribed to an early decline in $1,25(OH)_2$vitamin D (calcitriol) levels, leading to increases in serum PTH and subsequent alterations in calcium and phosphorus metabolism[(1-3)]. However, recent studies have revealed that circulating values of fibroblast growth factor 23 (FGF-23), a key regulator of phosphorus and vitamin D metabolism, rise as renal function declines and may play a key initiating role in the development of abnormal mineral metabolism in patients with CKD[(4)].

The importance of FGF-23 in the regulation of mineral metabolism was first identified in human genetic and acquired rachitic diseases such as autosomal dominant hypophosphatemic rickets (ADHR), tumor induced osteomalacia (TIO) and X-linked hypophosphatemic rickets (XLH)[(5-8)]. In these conditions, increased levels of the protein are accompanied by impaired tubular phosphate reabsorption, hypophosphatemia, low (or inappropriately normal) levels of $1,25\ (OH)_2$vitamin D, and impaired skeletal mineralization (rickets or osteomalacia)[(9-13)]. Subsequently, animal studies confirmed that excess FGF-23, as occurs when FGF-23 is infused into rats with normal renal function, results in renal phosphate wasting through inhibition of renal phosphate cotransporters, NaPi2a and NaPi2c and direct suppression of 1α-hydroxylase activity[(11)]. Moreover, a complete lack of either functional FGF-23 or its co-receptor, Klotho, results in phosphate retention, hyperphosphatemia, and increased circulating calcitriol levels[(12–18)]. More recent studies have also implicated a role for FGF-23 in the regulation of PTH secretion–FGF-23 suppressing PTH secretion both *in vitro* and *in vivo*[(19,20)]. In turn, FGF-23 expression is itself regulated by vitamin D, phosphate, and, potentially, PTH. In both animals and humans, the administration of $1,25(OH)_2$vitamin D increases circulating FGF-23 levels[(21)], apparently due to a direct action of vitamin D on FGF-23 via a vitamin D response element located upstream of the FGF-23 promotor[(22)]. Sustained increases in dietary phosphorus are also associated with increasing FGF-23 levels and declining $1,25(OH)_2$vitamin D levels[(23,24)], while dietary phosphorus restriction reverses these trends[(23,24)]. Parathyroid hormone (PTH) levels may also stimulate FGF-23 expression[(25)]; findings in primary hyperparathyroidism[(25)], McCune-Albright syndrome[(26)], and Jansen's disease[(27)], suggest that osteocytic stimulation by PTH directly increases skeletal FGF-23 release. The mechanism by which phosphate and PTH mediate changes in FGF-23 expression remain unknown and may be either direct effects on FGF-23 gene expression itself or mediated through other potential regulators of FGF-23.

Circulating levels of FGF-23 are elevated as early as stage 2 CKD, before any changes in calcium, phosphorus, calcitriol, or PTH are apparent[(4)]. Values rise progressively throughout the course of CKD and are markedly elevated in patients treated with maintenance dialysis[(28,29)]. This rise

in FGF-23 likely occurs due to a combination of increased phosphate load from declining GFR and decreased renal FGF-23 excretion. As a consequence of rising FGF-23 values, the fractional renal excretion of phosphate increases[4], thus maintaining circulating phosphate concentrations in the normal range despite declining renal mass. Unfortunately, rising FGF-23 values also suppress renal 1α-hydroxylase activity[11]; indeed, $1,25(OH)_2 D_3$ levels decline as CKD progresses and its values are inversely related to levels of circulating FGF-23[4]. These reduced circulating $1,25(OH)_2D_3$ levels contribute to secondary hyperparathyroidism and parathyroid gland hyperplasia in a number of ways: through decreased intestinal calcium absorption, decreased binding to the VDR, reduced CaSR expression, and decreased VDR expression[30,31]. In advanced CKD, declining calcitriol levels allow PTH levels to rise, thereby causing a release of calcium and phosphorus from bone. When compensatory mechanisms fail, severely impaired GFR results in phosphate retention which itself directly suppresses 1α-hydroxylase activity[32]. At this stage, hypocalcemia (from decreased intestinal calcium absorption mediated by declining calcitriol levels), hyperphosphatemia, and low circulating 1,25 dihydroxyvitamin D_3 values all combine to stimulate PTH secretion, thus contributing to the development of secondary hyperparathyroidism[1].

FGF-23 IN THE PATHOGENESIS OF RENAL OSTEODYSTROPHY

Since calcium and phosphorus, in the form of hydroxyapatite, are the building blocks of bone, it is not surprising that disordered mineral metabolism is tightly linked to bone disease in the context of CKD. Aside from basic calcium and phosphorus homeostasis, other hormones play critical roles in the structure and function of bone; alterations in these hormones, as occur in CKD, thus contribute to abnormal bone turnover, mineralization, volume, linear growth and strength which, together, define Renal Osteodystrophy. Recent evidence suggests that FGF-23, aside from its effects on mineral metabolism, may be one such hormone that plays a critical role in the development of Renal Osteodystrophy.

Although the effects of FGF-23 on mineral metabolism obscure the potential direct effects of the protein on bone biology, a growing compendium of data from animals, as well as genetic and acquired human diseases of FGF-23 deficiency and excess, have yielded many insights into the role that FGF-23, and factors that regulate FGF-23, play in bone biology. While FGF-23 is expressed in a variety of tissues, the majority of circulating FGF-23 is derived from osteocytes (in high levels) and osteoblasts (in lower levels)[33] in mineralized bone. Studies of FGF-23 in human genetic and acquired diseases, as well as in animal models, have demonstrated that both under- and over-expression[5,12,13] of the protein results in impairments in bone biology. Although the defective skeletal mineralization observed in patients with FGF-23 excess is likely a consequence of low phosphorus and vitamin D values, studies of FGF-23 deficiency in animal models and in cell culture suggest that FGF-23, and the proteins that regulate FGF-23, also have a direct effect on bone[34]. In these models, FGF-23 appears to directly regulate osteoblast differentiation[34], while a complete lack of the FGF-23 protein impairs skeletal mineralization, despite adequate (even excessive) circulating levels of phosphorus and vitamin D[12,13]. In addition, recent studies suggest that alterations in skeletal FGF-23 expression also coincide with impairments in skeletal metabolism in the CKD population. Indeed, FGF-23 is upregulated early in the course of CKD and is associated with skeletal mineralization indices in these individuals[29].

Particularly during embryonic skeletal development, FGF-23 appears to directly inhibit osteoblast maturation and matrix mineralization[34]. Consistent with an effect of FGF-23 on osteoblast proliferation, FGF-23 expression is much lower in embryonic skeleton than it is in adult animals[35] and, indeed, dysruption of the Wnt signaling pathway –a pathway responsible for osteoblast proliferation and bone matrix mineralization– has been noted in mice with excess skeletal FGF-23 expression[36]. In mature animals, a complete lack of FGF-23 also results in focal alterations in skeletal mineralization, despite adequate (even excessive) serum phosphate, calcium, and vitamin D levels[12,13], suggesting a direct role of the protein on maintaining skeletal mineralization at later stages of development.

The effect of FGF-23 on skeletal mineralization may also be mediated by alterations in other skeletal proteins known to regulate FGF-23. The genetic condition of X-linked hypophosphatemia (XLH) (a condition with a phenotype very similar to that of ADHR) and its mouse homologue, the *Hyp* mouse, are associated with increased FGF-23 levels as a result of defects in phosphate regulating endopeptidase homologue (PHEX). Although the exact actions of PHEX *in vivo* are not completely defined, inactivation of PHEX leads to increased FGF-23 expression by an indirect mechanism. Whether from a direct effect of increased skeletal FGF-23 expression or due to some other factor modulated by loss of PHEX activity, bone from Hyp mice displays an intrinsic mineralization defect that is not corrected by normalization

of circulating calcium and phosphate concentrations; indeed, selective ablation of PHEX in osteoblasts and osteocytes is sufficient to generate a phenotype of osteomalacia in mice[37], while transplantation of *Hyp* mouse bone into wild type mice does not reverse the phenotype of the explanted bone[38].

Skeletal mineralization in various forms of hypophosphatemic rickets may also be regulated through interactions with members of the short integrin binding-ligand, N-linked Glycoprotein (SIBLING) family, particularly dentin matrix protein 1 (DMP1). DMP1, or rather the 2 active (N- and C-terminal) fragments of DMP1 generated by its proteolytic cleavage[39], promotes mineral formation[40]. In both humans and in animals, DMP1 dysfunction results in increased skeletal and circulating FGF-23 values as well as a diffuse skeletal mineralization defect[33,41] and disrupted osteocyte structure[33]. Furthermore, the DMP1/FGF-23 double knockout is phenotypically similar to the FGF-23 knockout[42], suggesting that DMP1 regulates FGF-23 and is located upstream of the FGF-23 molecule.

As in patients with primary excesses in FGF-23[5], defective skeletal mineralization is also common in patients with all stages of chronic kidney disease, in whom increased circulating levels of FGF-23 occur in the presence of normal or elevated serum phosphorus values[12,13]. However, the association between FGF-23 and bone in this population differs greatly from that in the general population. A cross-sectional analysis of 49 pediatric dialysis patients with secondary hyperparathyroidism suggested that high circulating levels of FGF-23 in pediatric dialysis patients are associated with improved indices of skeletal mineralization[29]. Although these results appear to contrast with findings in patients with normal kidney function, they are similar to the mineralization defects found in rodents with a complete lack of FGF-23, despite adequate circulating mineral content[12,13]. Confirming this association, a study of FGF-23 and DMP1 expression in bone tissue of 32 pediatric and young adult patients with CKD demonstrated that both FGF-23 and DMP1 expression were upregulated in trabecular bone in early (stage 2) CKD. In patients with all stages of CKD, the amount of bone FGF-23 correlated directly with bone DMP1 expression and the expression of each was inversely related to osteoid accumulation. Although the simultaneous increase in both DMP1 and FGF-23 expression appears contrary to previous data suggesting that DMP1 acts to suppress FGF-23 expression, other data has suggested that overexpression of DMP1 does not suppress FGF-23 expression[43]. Moreover, DMP1 promotor activity increases in response to increasing phosphate concentrations[44]. Thus, it is possible that the simultaneous increase in bone DMP1 and FGF-23 expression reflects the increasing phosphate burden associated with progressive renal failure. Alternatively, increased DMP1 expression may reflect an alteration in protein function in the context of CKD.

FGF-23 IN THE DEVELOPMENT OF CARDIOVASCULAR DISEASE

Visceral, tumoral or periarticular, and vascular calcifications may develop in patients with CKD. Indeed, the mortality rate in adults and children with CKD is markedly higher than that of the general population, and cardiovascular disease is the leading cause of death in both children and adults treated with maintenance dialysis[45,46]. In contrast to the calcifications of atherosclerotic plaques that develop with age in the vascular intima of individuals with normal kidney function, vascular calcification in the uremic milieu develops primarily in the tunica media. Hyperphosphatemia is associated with soft-tissue and vascular calcifications[47], and, at the molecular level, phosphate plays a major role in the genesis of these lesions[48]. Recently, FGF-23 has also been associated with increased mortality. In a prospective, nested, case-control study of 400 adult patients new to dialysis, the greatest increases in baseline serum phosphate levels were modestly associated with an increased mortality rate during the first year of dialysis. However, concomitant levels of FGF-23 were independently associated with future risk of death in a dose-dependent fashion. Furthermore, increased FGF-23 was associated with increased risk of mortality in every quartile of serum phosphorus value except the highest and this included phosphate levels in the "normal" range for dialysis patients[49]. This association between FGF-23 and mortality was independent of serum phosphate levels, prior phosphate binder use, and follow up treatment with active vitamin D, all of which have themselves been associated with improved survival in other studies[50-53]. This association between FGF-23 and mortality in the dialysis population is likely mediated through cardiovascular disease; indeed, increased FGF-23 levels have been associated with left ventricular hypertrophy in patients with all stages of CKD[54] and with vascular calcification in the dialysis population[55]. Thus, although further studies are needed, these findings suggest that FGF-23 may have physiologic importance, independent of its traditional role in mineral metabolism, in affecting survival. Alternatively, FGF-23 may be a superior biomarker of net phosphorus exposure than even serum phosphate itself.

SUMMARY

FGF-23 plays a central role in mineral, bone, and vascular metabolism. This role was initially delineated by the study of genetic and acquired conditions of hypophosphatemic rickets but the greatest clinical impact of the discovery of FGF-23 may be in the management of CKD patients. FGF-23 and its regulators are made in osteocytes in bone, and in patients with CKD, FGF-23 levels rise as renal function declines, likely due to decreasing capacity of the damaged kidney to excrete dietary phosphorus loads. Rising FGF-23 levels contribute to the development of secondary hyperparathyroidism and may also be linked to alterations in skeletal mineralization and to cardiovascular disease in the CKD population. Thus, through expression of various proteins crucial to mineral metabolism, osteocytes appear to be endocrine cells with a key role in the regulation of skeletal mineralization and vascular calcification.

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