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Novel Bone Endocrine Networks Integrating Mineral and Energy Metabolism

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Abstract

The skeleton is an endocrine organ that regulates energy metabolism through the release of the osteoblast-derived hormone, osteocalcin (Ocn), and phosphate and vitamin D homeostasis through the secretion by osteoblasts and osteocytes of the novel hormone, FGF23. Ocn activates a widely expressed G-protein coupled receptor, GPRC6A, to regulate insulin secretion by pancreatic β -cells, testosterone secretion by testicular Leydig cells, fatty acid metabolism in the liver, and insulin sensitivity of muscle and fat, as well as other functions. FGF23 targets a limited number of tissues, including kidney, parathyroid gland, choroid plexus and pituitary gland that co-express FGF receptors and α -Klotho complexes. Ectodomain shedding and secretion of a soluble form of Klotho also is purported to act as an anti-ageing hormone. Further elucidation of these novel endocrine networks is likely to lead to new appreciation of the cooperation between various organ systems to regulate phosphate, vitamin D, and energy metabolism.

Keywords

Bone; osteoblast; osteocyte; extracellular matrix; mineralization; fibroblastic growth factors; α -Klotho; fibroblastic growth factor receptor; hypophosphatemia; vitamin D; Cyp27b1; Cyp24; PTH; G-protein coupled receptors; GPRC6A; L-arginine; testosterone; osteocalcin; insulin resistance; insulin secretion; metabolic syndrome; hypophosphatemia

Introduction

The adult skeleton is composed of metabolically active bone tissue that determines body size and shape. Bone is composed of bone forming and bone resorbing cells that are held together in an extracellular matrix framework containing calcium and phosphorus. Bone is a support structure and a target for hormones regulating mineral metabolism. New concepts are arising from the finding that bone also releases hormones that regulate several biological processes. A better understanding of these interconnections between bone and other organs may lead to new insights into physiological and pathological processes.

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Conflict of Interest

M Pi declares no conflicts of interest.

Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors.

Bone remodeling and mineralization maintains the structural integrity of the skeleton

The primary function of bone is to maintain its structural integrity through a self-renewal process, called bone remodeling (1). Indeed bone undergoes continuous replacement and renewal, which consists of removal of a quantum of mineralized bone tissue by osteoclasts, leaving a resorptive cavity that is filled by the migration of osteoblast precursors and their differentiation into mature osteoblasts that produce a mineralized extracellular matrix. Osteocytes, cells representing the terminal stage of the osteoblast lineage embedded in the bone matrix, play an important role in regulation of both remodeling and mineralization process. Osteoblasts and osteocytes also sense mechanical forces polycystin/ primary cilia complexes as well as channels and other mechanosensors to maintain bone mass (2).

Bone remodeling imbalances caused by aging, inactivity, alterations in reproductive hormones, excess parathyroid hormone, vitamin D deficiency and other disease processes alters the structural integrity of bone (3). Of the bone remodeling abnormalities osteoporosis is the most significant clinical problem that increases the risk of bone fracture. Decreases in osteoblast-mediated bone formation and/or increases in osteoclast-mediated bone resorption underlie osteoporotic disorders. Impaired mineralization of extracellular matrix that leads to rickets and osteomalacia is another common form of metabolic bone disease. Reduction in both bone formation and resorption leads to low turnover, metabolically inactive bone disease. Less commonly, inhibition of bone resorption leads to abnormally dense or overly mineralized bone.

The skeleton is a target for local factors and hormones regulating mineral homeostasis

The second function of bone is to participate in the physiological regulation of mineral metabolism (4). Bone is a reservoir for calcium and phosphate and other minerals that are in equilibrium with the extracellular fluid. The influx and efflux of calcium and phosphorus from bone is both a passive process regulated by physiochemical forces and an active process under control of hormones such as parathyroid hormone and $1,25(\text{OH})_2\text{D}$, which directly target PTH and vitamin D receptors in osteoblasts to regulate bone remodeling.

Bone remodeling processes are regulated by a variety of local factors and systemic hormones (5). Locally derived paracrine/autocrine factors that are secreted by osteoblasts and/or stored in the extracellular matrix regulate both the renewal and differentiation of precursors from the bone marrow progenitor pool as well as the functions of bone forming and bone resorbing cells. Notable local regulators are Opg/RankL, the SOST/Wnt, and BMPs/SMAD signaling pathways (1). The PTH-Vitamin D axis regulates serum calcium concentrations through the combined actions of increased $1,25(\text{OH})_2\text{D}$ to stimulate gastrointestinal calcium absorption and PTH effects on bone to increase calcium efflux and on the kidney to reduce calcium excretion in the urine. Also, calcitonin, released from C-cells in the thyroid, suppresses bone resorption and calcium efflux and functions to protect against hypercalcemia. Sex hormones are also important regulators of bone remodeling.

Loss of estrogen or androgen function leads to osteopenia through respective increases in bone resorption and decrements in bone formation.

Bone is also a target for other endocrine pathways regulating fat and energy metabolism, including the central nervous system, adipocytes, pancreatic beta-cells, and small intestines. For example, insulin acting through insulin receptors in osteoblasts stimulates osteoclast-mediated bone resorption. In addition, fibroblast growth factor 21 (FGF21), a member of the circulating family of fibroblastic growth factors that is produced by the liver and adipose tissue and functions as a regulator of glucose and lipid metabolism, is a negative regulator of bone mass through apparent direct actions to inhibit osteoblastogenesis and enhance marrow adipogenesis (6). Leptin, produced by adipocytes, regulates bone mass through a CNS relay leading to activation of the sympathetic nervous system. Leptin may also act to stimulate osteoblast proliferation and inhibit osteoclastogenesis through direct actions on osteoblasts. Gut-derived serotonin may inhibit bone formation by directly targeting osteoblasts.

The mineralization process is actively regulated by proteins and physiochemical processes. In the osteoblast microenvironment, tissue-nonspecific alkaline phosphatase (TNAP) and ectonucleoside triphosphate/diphosphohydrolase 5 (ENTPD5) initiate mineralization by converting pyrophosphate or other substrates to free phosphate necessary for nucleation of crystal growth (7). On the other hand, pyrophosphate, a local inhibitor of mineralization, is regulated by the pyrophosphate channel ANK and ectonucleotide pyrophosphatase phosphodiesterase 1 (ENPP1). The local ratio of PPi and Pi regulates mineralization. In addition, small integrin-binding ligand, N-linked glycoproteins (SIBLINGs), including osteopontin (OPN), dentin matrix protein-1 (DMP1), bone sialoprotein (BSP), matrix extracellular phosphoglycoprotein (MEPE), and dentin sialophosphoprotein (DSPP) are implicated in the biomineralization process. For example, dentin matrix protein 1 is a potent inducer of mineralization. The function of at least some members of the SIBLING family requires phosphorylation by FAM20C (family with sequence similarity 20 (Fam20)), a protein kinase expressed in bone that is dedicated to the phosphorylation of extracellular proteins (8).

Bone is an endocrine organ

The third and newly discovered function of bone is its endocrine function. Indeed, osteoblasts/osteocytes secrete two hormones, osteocalcin and FGF23, into the circulation to regulate functions of other organs and create complex endocrine feedback loops regulating mineral and energy metabolism.

Osteocalcin-bone-pancreas-testes axis regulating energy metabolism and sex hormone production

Regulation of energy metabolism has recently expanded to include the involvement of bone in regulating glucose homeostasis, both as a target for insulin and as an endocrine organ that releases undercarboxylated Ocn, a hormone regulating insulin secretion in β -cells(9). Briefly, Ocn, an osteoblast derived factor that is stored in the extracellular matrix and released during the process of osteoclast-mediated bone resorption, participates in the

regulation of systemic glucose metabolism and insulin resistance as well as regulates sex hormone production. A new endocrine loop has been identified in experimental models that consists of insulin activation of insulin receptors (IR) in osteoblasts to increase Ocn secretion and bioactivity by decarboxylating Ocn. In turn, circulating underdecarboxylated Ocn activates the Ocn-sensing receptor GPRC6A in β -cells (9, 10) to regulate insulin secretion and other β -cell functions (11) and GPRC6A in Leydig cells to stimulate testosterone secretion. This endocrine paradigm is supported by the observations that osteoblast-specific deletion of IR results in loss of insulin-mediated release of bioactive Ocn from bone (12); that ablation of osteocalcin (*Ocn*^{-/-}) leads to glucose intolerance in mice, while genetically modified mice with an increase in uncarboxylated Ocn are protected from type 2 diabetes mellitus (T2DM) and obesity (13); and by the finding that administration of recombinant Ocn to mice stimulates β -cell functions, including increases in β -cell mass and insulin secretion (10, 13).

The study of the osteocalcin sensing receptor (14), GPRC6A, confirms the existence of the bone-pancreas-testes endocrine loops. Indeed, the phenotype of the global knockout of *Gprc6a* in mice resembles that of Ocn null mice, consistent with its role in mediating the end-organ effects of Ocn. *Gprc6a* null mice, like Ocn null mice have glucose intolerance and impaired insulin secretion (10, 15, 16) as well as reductions in serum testosterone levels. Global *Gprc6a*^{-/-} and *Ocn*^{-/-} mice also resemble mice with conditional deletion of the insulin receptor in osteoblasts (*IR*^{ob-cko}) (17).

Ocn binds to and activates GPRC6A (14, 17). GPRC6A, a member of the C family of GPCRs that are structurally characterized by a very large “venous fly trap (VFT)” extracellular evolutionary domain that primitive organisms used to sense multiple ligands in nutrient rich primordial milieu (14, 18–20). GPRC6A is capable of sensing amino acids, such as L-arginine, extracellular cations, such as calcium and zinc, osteocalcin (Ocn), anabolic steroids, such as testosterone, and organic compounds, such as calcimimetics, calcilytics, and catechins (a component of green tea) (14, 21–23). GPRC6A is expressed in bone, pancreas, muscle, fat, testes, brain, liver, and prostate, as well as many other tissues.

The Ocn-GPRC6A axis regulates several important functions. First, *Gprc6a* is highly expressed in the mouse pancreatic β -cell line where it mediates the effects of Ocn to activate ERK and insulin secretion (10, 15). Administration of Ocn stimulates ERK activity in the pancreas and increases serum insulin levels in wild-type mice, but these responses are markedly attenuated in *Gprc6a*^{-/-} mice (10). *Gprc6a*^{-/-} mice also have decreased islet size and number, suggesting that GPRC6A also regulates β -cell mass. Selective deletion of *Gprc6a* in β -cells(24) respectively reduces insulin secretion. Ocn stimulation of insulin secretion and insulin regulation of Ocn release from bone creates a positive feedback loop regulating glucose homeostasis.

Second, selective deletion of *Gprc6a* Leydig cells reduces testosterone secretion and Ocn stimulates testosterone secretion. This creates another endocrine network whereby bone release of Ocn regulates sex hormone production. During rapid skeletal growth, increments in testosterone levels initiated by alterations in the hypothalamic-pituitary axis, may be

further augmented by increasing Ocn due to skeletal growth, thereby increasing bone size in males (25).

Third, the Ocn-GPRC6A endocrine network may also include effects on hepatic, fat and muscle metabolism. In this regard, *Gprc6a*^{-/-} mice exhibited hepatic steatosis, and decreased glycogen storage in the liver, increased triglycerides and cholesterol levels. *Gprc6a*^{-/-} mice also have decreased muscle mass, increased visceral fat, glucose intolerance, insulin resistance and impaired insulin secretion (26, 27). Loss of *Gprc6a* was also associated with increased serum leptin and adipocyte hypertrophy, but no change in adiponectin levels. This suggests that GPRC6A may regulate Ocn function in peripheral tissues regulating glucose production and utilization. GPRC6A loss-of-function are consistent with the phenotype of *Ocn*^{-/-} mice, and suggests the actions of rOcn to decrease hepatic steatosis and improve insulin sensitivity when administered to mice fed high fat diets are mediated through the activation of GPRC6A. Male mice exhibited an increase in serum estradiol and decrease in testosterone, and decreased expression in the testes of *Cyp17*, a key enzyme involved with the androgen biosynthesis pathway, and *SULT1E1*, which inactivates estrogens (15, 16). Further studies are needed to evaluate the selective functions of GPRC6A in liver, adipocytes and muscle. However, if this schema proves to be correct, activation of GPRC6A with high affinity compounds that bind to this receptor may counter the three major abnormalities leading to T2D, namely decreased insulin secretion, reduced β -cell mass (decompensation) and increased insulin resistance (28).

Fourth, *Gprc6a* is also expressed in the brain. Hypothalamic-pituitary abnormalities were also observed, including a paradoxical stimulation of Luteinizing hormone (LH) in response to T administration, and an increase in growth hormone and decrease in IGF1 levels (16). In addition, there is also preliminary evidence that *Gprc6a*^{-/-} mice have abnormalities in myelin production and decreased seizure thresholds.

The ability of GPRC6A to bind to additional ligands may lead to novel coordinated effects on energy metabolism and glucose homeostasis. For example, deletion of *Gprc6a* in mice results in the loss of the ability of systemically administered T, Ocn, or L-Arg to stimulate ERK activity and *Egr-1* expression in bone, testis, and pancreas (10, 16, 21). GPRC6A is also activated by insulin secretagogues (e.g., testosterone, calcium, and L-Arg) (14, 16). Islets isolated from *Gprc6a*^{-/-} mice exhibit impaired glucose and L-Arg stimulated insulin secretion *ex vivo*. GPRC6A in the cell surface membrane also has been shown to bind extracellular testosterone and transduces the rapid, “non-genomic” effects of testosterone both in vitro and in vivo in several cell types and tissues (16). Non-classical signaling by androgens has been implicated in a variety of physiological processes, including changes in neuronal activity, the hypothalamic GnRH pituitary LH secretion axis, facilitation of the sperm acrosome reaction, oocyte maturation, insulin secretion as well as insulin sensitivity in adipocytes, obesity and metabolic syndrome, maintenance of bone mass, endothelial dysfunction, and vasodilatation as well as others (29–34). Thus, some of the purported non-genomic effect of T may be mediated through GPRC6A and Ocn stimulation of testosterone may be creating additional positive feed forward loops. Additional studies are needed to understand the interaction between Ocn and testosterone.

GPRC6A may be involved in regulating prostate cell proliferation. Three genome-wide association studies have identified *GPRC6A* as a novel genetic locus highly associated with prostate cancer in the Asian population (35–37). In addition, *GPRC6A* is expressed at higher levels in prostate cancer cells and prostate cancer tissues, siRNA knockdown of GPRC6A attenuates prostate cancer growth in human prostate cancer cell lines (38), *GPRC6A* is coupled to signaling pathways, such as PI3K and cAMP known to be deregulated in prostate cancer (22, 39), and transfer of global *Gprc6a* deficiency onto a TRAMP mouse model of prostate cancer significantly retarded PCa progression and improved survival of compound *Gprc6a*^{-/-}/TRAMP mice (38). If GPRC6A is involved in the pathogenesis of PCa, then agonists would be expected to accentuate PCa severity, whereas antagonists might inhibit prostate cancer progression. GPRC6A is a multiligand receptor. Many of these GPRC6A ligands are known to stimulate human prostate cancer cell proliferation, migration and gene expression. For, example, GPRC6A appears to be the cell surface testosterone receptor. In addition, another ligand Ocn is highly expressed in prostate cancer cells (40, 41) and elevated in the serum of prostate cancer patients, where it is a predictor of bone metastasis (42) and tumor progression (40).

Finally, while the Ocn-GPRC6A network has robust physiological effects in the mouse, the clinical relevance of these endocrine pathways in humans is less certain and, at present, no mutations of Ocn or GPRC6A have been reported to cause a specific hereditary disease in humans. Since metabolic rate is inversely related to body size, the effects of these endocrine pathways may be accentuated in smaller animals(43). Nevertheless, polymorphisms in the Ocn receptor, GPRC6A, are associated with osteopenia in humans (23) and the GPRC6a locus is associated with increased prostate cancer risk in Asian males (35). Genome-wide association studies show that GPRC6A is a genetic locus highly associated with C-reactive protein (CRP) levels (44), a heritable marker of chronic inflammation that is strongly associated with diabetes mellitus (45) and cardiovascular diseases (46, 47). Clinical association studies also support the relevance of the Ocn-GPRC6A network. Circulating Ocn is inversely correlated with body mass index, fasting glucose and insulin, triglycerides, and leptin and positively correlated with adiponectin (48).

FGF23 bone-kidney axis

Osteoblasts and osteocytes also secrete FGF23 (49–51), a hormone that regulates phosphate and vitamin D metabolism (52). FGF23 differs from the classical autocrine /paracrine FGFs by its ability to diffuse into the circulation and activate FGF receptors by binding to a co-factor, α -Klotho (Kl), a transmembrane protein that shares homology with the β -glucuronidase family of proteins. The N-terminal domain of FGF23 binds to FGF receptors (FGFR) and its 71 amino acid C-terminus binds to Kl (53–55). Consequently, the physiological effects of FGF23 are limited to organs that co-express FGFR/Kl complexes (54, 55), which include the kidney, parathyroid gland, pituitary gland, and choroid plexus (55).

The principal biological action of FGF23 in the kidney is to inhibit phosphate reabsorption by decreasing Na-dependent Pi co-transporters. FGF23 also suppresses 1,25(OH)₂D levels by inhibiting Cyp27b1 and by stimulating the catabolism of 1,25(OH)₂D by activating the

24-hydroxylase (Cyp24) (56–59). In the parathyroid gland, it has been proposed that FGF23 suppresses PTH, although most conditions of excess FGF23 are associated with elevated PTH. The role of FGF23 in the central nervous system is not known.

FGF23 participates in several endocrine feedback loops (52). The best characterized is the FGF23-1,25(OH)₂D endocrine loop. FGF23 acts as a counter regulatory factor for 1,25(OH)₂D (60) in that 1,25-(OH)₂D stimulates FGF23 production by bone through VDR-dependent mechanisms and elevated circulating FGF23 suppresses 1,25-(OH)₂D production in the kidney (60, 61). The physiological role of FGF23 may be to prevent vitamin D toxicity. PTH and FGF23 have different effects on 1,25(OH)₂D production (*i.e.*, increased by PTH and decreased by FGF23) and 1,25(OH)₂D has opposite effects on these hormones (*i.e.*, suppresses PTH and stimulates FGF23).

Whether FGF23 also participates in a parathyroid gland-bone endocrine loop is controversial (62–64), even though FGFR/Kl complexes are expressed in this tissue (65). On the one hand, FGF23 directly suppresses PTH mRNA expression *in vitro* and decreases serum PTH *in vivo* (62). However, FGF23 does not prevent the development of HPT in any clinical circumstance and there is a strong association between elevated FGF23 levels and the severity of HPT in CKD and other disorders (55), suggesting FGF23 may promote the development of HPT (55, 63, 64). Moreover, excess soluble secreted Klotho results in elevated serum FGF23 levels. Hereditary hypophosphatemic disorders also have elevated FGF23 concentrations in association with increased PTH levels. Effects of PTH to regulate FGF23 expression in bone are also variable, with some studies showing that activation of PTH-dependent pathways stimulate FGF23 secretion by bone (55, 63, 64, 66–69), whereas in other studies PTH either inhibits (70) or fails to stimulate FGF23 (60, 71). Serum FGF23 levels are dependent upon calcium and vitamin D concentrations, which may account for the variable effects of PTH on FGF23 levels. In VDR^{-/-} mice FGF23 is undetectable in spite of elevations of PTH (72). The ability of PTH to regulate FGF23 is also modified by whether PTH induces a net anabolic or catabolic effect on bone as well as by the level of serum calcium (*i.e.*, hypocalcemia prevent PTH stimulation of FGF23) (68, 71).

FGF23 causes hypophosphatemia, but its regulation is not tightly coupled to serum phosphate as might be expected if FGF23 participated in a serum phosphate regulating feedback loop. Indeed, in spite of a positive correlation between serum phosphate levels and elevations in FGF23 levels in ESRD (73), phosphate restriction and/or loading has minimal and/or delayed effects on FGF23 levels in both animal models and clinical settings, both in the presence of normal and impaired renal function (74–78). At present direct evidence that phosphate regulates FGF23 gene transcription is lacking (60). Phosphate effects on FGF23 might be indirectly mediated by bone mineralization, which could account for the delay in the effect of phosphate on FGF23 expression.

FGF23 production by bone is under control of local factors that regulate the mineralization process. It has been proposed FGF23 secretion changes in response to impaired mineralization and/or bone formation to coordinate bone phosphate flux with the renal handling of phosphate. Several mutations support the notion. Indeed, Phex, Dmp1, ENPP1, FAM20C, and ENTPD5 mutations, which cause hereditary hypophosphatemic rickets, are

regulators of both bone mineralization and FGF23 production (79). The mechanisms whereby alterations in the matrix milieu leads to increased FGF23 are poorly understood, but may involve activation of FGFR1-dependent and/or other signaling pathways leading to increased FGF23 gene transcription (80).

The idea that FGF23 can only act on tissues that co-express FGF receptors and membrane α -Klotho has recently been challenged in two important ways. First, there are data that FGF23 can activate FGFRs in the absence of membrane bound α -Klotho, at least under some conditions. For example, it has been proposed that FGF23 directly targets FGFRs in the myocardium to stimulate LVH (81). Second, soluble, secreted Klotho is released into the circulation from the distal tubule by either ectodomain shedding or secretion of an isoform lacking the membrane domain, and is purported to function as an anti-ageing hormone (82–84). α -Klotho is stimulated by $1,25(\text{OH})_2\text{D}$ and inhibited by FGF23 thereby creating still another endocrine loop, as circulating Klotho can activate FGF23-FGFR signaling, inhibit insulin and insulin-like growth factor receptor pathways (85) and suppress Wnt signaling through binding to various Wnt family members (86).

Regardless, alterations of circulating FGF23 are both physiologically and clinically important. FGF23 has essential biological functions, since ablation of FGF23 is lethal in the early postnatal period due to hyperphosphatemia and excessive $1,25(\text{OH})_2\text{D}$ production. As noted above, elevations of circulating bioactive FGF23 concentrations cause hereditary and acquired hypophosphatemic disorders, whereas reductions in circulating FGF23 concentrations cause familial tumoral calcinosis (80). There is emerging evidence that the increase in FGF23 is an initial positive adaptive response for maintenance of phosphate balance at the expense of suppressing $1,25(\text{OH})_2\text{D}$ production in early CKD, but becomes maladaptive with more advanced CKD. Indeed, epidemiological studies in both ESRD and CKD find that elevated circulating FGF23 levels are a strong independent risk factor for both renal failure progression and cardiovascular mortality, independent of serum phosphate levels (87). Elevated circulating FGF23 concentrations are also associated with progression of renal disease (88) and left ventricular hypertrophy, fat mass and dyslipidemia in elderly patients without CKD (89). This positive correlation between FGF23 and mortality is also found in the general population with coronary artery disease (90). Speculatively, FGF23 adverse effects on the cardiovascular system could be due to direct effects on the myocardium or indirect effects resulting from FGF23 mediated suppression of angiotensin-converting enzyme 2 (ACE2) expression in the kidney or through FGF23 mediated reduction in α -Klotho.

Cross-talk between Ocn/GPRC6A and FGF23 endocrine networks?

Given the importance of cellular uptake of phosphate for energy utilization in peripheral tissues, FGF23 and Ocn may be coordinately regulated. In this regard, leptin, the adipocyte derived hormone that suppresses appetite, limits bone mass accrual and increases energy expenditures (91), directly stimulates FGF23 synthesis in bone cells (92). and indirectly, through activation of the sympathetic nervous system, inhibits Ocn release from bone (93). Theoretically this might allow renal phosphate handling to be coordinated with cellular uptake of phosphate. Insulin, acting on InsR in renal proximal tubule also stimulates

phosphate reabsorption. Insulin effects to promote renal phosphate retention may ensure an adequate supply of phosphate to support energy production at a time when insulin is promoting phosphate and glucose uptake in peripheral tissues. Whether insulin regulates FGF23 secretion from bone is not known. However, insulin and IGF-1 can regulate renal tubular phosphate transport through activation of protein kinase B, serum- and glucocorticoid-regulated kinase (SGK) and glycogen synthase kinase (PKB/SGK/GSK3) pathways that lead to secondary changes in FGF23. In addition, SGK3 null mice have decreased bone density and increased phosphaturia that is associated with decreased expression of FGF23 in bone.

FGF23 may also be linked to energy metabolism and insulin signaling through secreted Klotho (94). FGF23 either directly or indirectly, through suppression of $1,25(\text{OH})_2\text{D}$, inhibits K1 expression in the kidney, whereas sKL is capable of stimulating FGF23 production by bone (95, 96), thereby creating a FGF23-sKL endocrine loop. Insulin is reported to stimulate cleavage and release of the extracellular domain of Klotho by ADAM10 and ADAM17 (97). Finally, FGF23 in combination with sKL is reported to inhibit Ocn message expression by osteoblasts through a direct activation of FGFR1 (98).

Conclusion

The production and release of Ocn and FGF23 from bone defines new endocrine networks and feedback loops between organs that previously were not known to be connected. The physiological significance of many of these interconnections remains uncertain. With the possible exception of measurement of FGF23 to diagnose hypophosphatemic disorders, few specific diagnostic tests or therapeutic interventions have evolved from this new knowledge, although an anti-FGF23 antibody is currently in phase 1–2 clinical trials for the treatment of XLH. With regard to the GPRC6A-dependent endocrine loops, however, development of agonists might be exploited to stimulate insulin secretion, insulin sensitivity and prevent hepatic steatosis, while also stimulating testosterone secretion. Further studies are needed to determine whether the GPRC6A-dependent endocrine networks can be exploited in the diagnosis and treatment of metabolic syndrome and related disorders. With regards to FGF23/Klotho pathways, development of ways to prevent the apparent toxicity of FGF23 in chronic kidney disease through developing ways to block FGF23 effects may ultimately prove to be important.

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