TRIENNIAL GROWTH SYMPOSIUM: A novel pathway for vitamin D-mediated phosphate homeostasis: Implications for skeleton growth and mineralization1,2

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ABSTRACT: Systemic factors that ultimately affect skeletal growth involve interrelationships among Ca, parathyroid hormone (PTH), and conversion of 25-OH vitamin D₃ to the active hormone, 1α,25-(OH)₂D₃. These interrelationships, with a focus on mechanisms that affect Ca homeostasis, are referred to as the Ca, PTH, and vitamin D axis. Relatively little research has focused on these interrelationships and P homeostasis. In the past decade, discovery of a previously unrecognized hormone involved in a pathway for P homeostasis offers opportunities to improve P efficiency without compromising skeletal growth and animal well-being. The objective of this review was to summarize pivotal research discoveries that led to the current understanding of the roles of fibroblast growth factor 23 (FGF23) in P homeostasis that are independent from the well-described pathways involved with Ca homeostasis. The novel pathways are referred to as the FGF23, P, and vitamin D axis. The peptide, FGF23, directly affects P homeostasis via action on renal target tissues to regulate Na-P transport proteins and renal 25(OH)D₃-1α hydroxylase activity. Identification of bone as the primary site for FGF23 production ascribes an endocrine gland function to bone. Within 9 h after a single injection of recombinant FGF23, mice displayed hypophosphatemia and urinary P wasting. In contrast, FGF23 knockout mice displayed hyperphosphatemia and renal P conservation. These responses were independent of PTH. Applications of the FGF23, P, and vitamin D axis in dietary strategies for animal agriculture need to be explored. Development of dietary inputs to balance both Ca and P homeostasis are needed to improve skeletal growth and nutrient efficiency.

Key words: calcium homeostasis, fibroblast growth factor 23, sodium-phosphate transporter 2a

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INTRODUCTION

The well-described axis for mammalian regulation of Ca homeostasis involves serum Ca, parathyroid hormone (PTH), and the conversion of vitamin D into an active hormone. Within the past decade, research discoveries, primarily involving transgenic mouse models, have challenged the traditional axiom and disclosed additional pathways. These novel pathways involve signals and feedback inhibition among P, vitamin D metabolites, and PTH with a critical regulatory component attributed to fibroblast growth factor 23 (FGF23). The central component that linked P homeostasis and renal function was the identification of bone tissue as the primary site for FGF23 synthesis, in essence ascribing an endocrine gland function to bone.

The objective of this review was to summarize interrelations of dietary P, vitamin D, and the role of FGF23 on P homeostasis. The peptide hormone FGF23 directly affects P homeostasis via action on renal Na-P transport proteins and renal 25(OH)D₃-1α hydroxylase activity. This review will first provide a brief overview of the traditional axis for Ca homeostasis and then identify key discoveries that implicated FGF23 as a central, systemic signal involved in P homeostasis. Implications of the novel FGF23, P, and vitamin D axis that can be applied to solutions of issues in agricultural animal nutrition will be discussed.

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TRADITIONAL AXIS FOR REGULATION OF CALCIUM HOMEOSTASIS

Decades of research have been devoted to develop the intricate interrelationships involved in Ca homeostasis. Relationships among systemic factors are summarized in Figure 1, which displays the glands, organs, and target tissues involved in the Ca, PTH, and vitamin D axis. Briefly, a decrease in serum Ca induces the release of PTH from the parathyroid gland. Circulating PTH acts directly on bone to increase resorption of Ca and P. Increased PTH also upregulates renal 25(OH)D3-1α hydroxylase activity (1α-hydroxylase), which increases synthesis of the active form of vitamin D [1α,25-(OH)2D3]. Increased PTH and 1α,25-(OH)2D3 target bone to induce a net resorption of Ca and P from mineralized tissue into circulation. Increased 1α,25-(OH)2D3 also targets the small intestine to stimulate active absorption of Ca and P via upregulation of proteins involved in Ca transport including calmodulin (CaM), calbindin (CaBP), transient receptor potential channel vanilloid 6 (TRPV6, also referred to as Ca transport protein 1), and Ca ATPase. The kidneys are ultimately required to restore serum Ca concentrations and maintain a Ca-to-P ratio. The increased 1α,25-(OH)2D3 stimulates renal Ca reabsorption via upregulation of Ca transport proteins and downregulation of P transport proteins (NaPi2a and NaPi2c). The net renal response results in a decreased excretion of P. Increased 1α,25-(OH)2D3 also serves as a feedback regulator to decrease 1α-hydroxylase activity. The net response to a decrease of serum Ca is a restoration of serum Ca with no effect on serum P.

The above abridged description of the Ca, PTH, and vitamin D axis for regulation of Ca homeostasis has been described in greater detail in numerous reviews (Jones et al., 1998; Holick, 2004) and in reference texts (Crenshaw, 2001; Seeman, 2008). The decades of contributions led by Hector DeLuca, his students, and his co-workers toward discovering intricate details of vitamin D and Ca homeostasis are evident in these reviews. In a recent publication, DeLuca and coworkers (Kutuzova et al., 2008) provided an excellent summary of vitamin D and Ca homeostasis that briefly described key research discoveries that spanned more than 70 yr. Ironically, results in this article challenged the traditional Ca, PTH, and vitamin D axiom for regulation of Ca homeostasis. The group demonstrated that expression of TRPV6 increased almost 2-fold over that of control rat duodenum within 3 h after intravenous injection of 90 ng of 1α,25-(OH)2D3 per kg of BW. However, Ca absorption in TRPV6-null transgenic mice was still upregulated...
after treatment with 1α,25-(OH)2D3, implying that the Ca channel protein was not uniquely required for active Ca absorption. Results from similar experiments that involved gene knockouts of calbindin and even double knockouts of TRPV6 and calbindin imply that additional mechanisms are yet to be discovered. One such mechanism may involve feedback relationships among FGF23, P, and vitamin D signals. The FGF23 research has focused interest on the endocrine roles of mineralized bone tissue.

ADVANCES IN OUR UNDERSTANDING OF THE FUNCTIONAL ROLE OF BONE

Elementary descriptions of the functional roles for mineralized skeletal tissue (i.e., bone) have focused primarily on the roles that involved structural support for locomotion and protection of vital organs with occasional inferences about the importance of bone as a reservoir for minerals. This perspective of bone was summarized by a young participant at a 4-H livestock clinic who provided a definition of bone as “what you hitch muscle to” (anonymous). In contrast to the elementary perspective, an invited presentation (Loveridge, 1999) was titled “Bone: More Than a Stick.” Loveridge (1999) summarized the considerable advancements in understanding the intricate balance of systemic and local growth factors, peptides, and cytokines that are required to regulate the coupling of bone formation and resorption for both modeling and remodeling of bone tissue. Advancements in understanding of bone biology have continued over the past decade such that recent reviews now describe bone as an endocrine gland (Fukumoto and Martin, 2009; Prié and Friedlander, 2010). Synthesis and release of 2 proteins from bone, FGF23 and osteocalcin, provide sufficient justification to consider bone as an endocrine gland. Osteocalcin, a protein produced by osteoblast cells, acts on the pancreas to regulate insulin synthesis. The role of osteocalcin is beyond the focus of this review, but is of considerable interest for understanding glucose and energy metabolism. The synthesis and release of FGF23 in response to systemic signals involved in P homeostasis is described in the following sections.

THE FGF23, P, AND VITAMIN D AXIS FOR REGULATION OF P HOMEOSTASIS

Historically, more research efforts have focused on Ca rather than P homeostasis, although both minerals are interrelated and co-imlicated in most disorders of bone mineral metabolism. The focus on Ca was most likely driven by the limiting amount of Ca in human diets. Human deficiency symptoms, such as rickets, were initially associated with Ca and vitamin D deficiencies. A similar impetus has now allowed a focus on P homeostasis. Nakatani et al. (2009) inferred that the clinical and biological importance of P homeostasis was driven, in part, by a need to resolve human health concerns associated with acute hypophosphatemia (i.e., myopathy, cardiac dysfunction, and hematological abnormalities), chronic hypophosphatemia (i.e., impaired bone mineralization, rickets, and osteomalacia), and hyperphosphatemia (i.e., vascular and soft tissue calcification). Early efforts to resolve disorders of P wasting led to discoveries of several peptides. Collectively these peptides were referred to as phosphatonin (Berndt et al., 2005). Phosphatonin is a peptide that act directly on the kidney or other target tissues to induce changes in P homeostasis. As summarized in a review by Fukumoto and Martin (2009), 3 groups of researchers, using different approaches, were almost simultaneous in reporting the discovery of FGF23 as a peptide involved in P disorders. Subsequent research has clarified a central role of FGF23 in regulation of P homeostasis. Implications for the importance of P homeostasis and human health have been emphasized in recent reviews by Liu and Quares (2007), Kuro-o (2010), Prié and Friedlander (2010), and Bergwitz and Juppner (2010). The focus of this review is on systemic factors rather than the cellular signals involved. Detailed descriptions of the cellular signals involved in FGF23 responses to systemic signals are beyond the scope of this review. Recent reviews of the current understanding of these cellular signaling pathways are provided by Prié et al. (2009), Kuro-o (2010), and Ramon et al. (2010).

In a review on the regulation of P homeostasis, Berndt et al. (2005) used the term phosphatonin to describe several factors thought to be involved in regulation of P. At that time, only limited information was available to define functional roles of FGF23. Initial studies of FGF23, other FGF proteins, and related receptors were based on studies of rare disorders involving renal P wasting. Subsequent studies have established specific roles for FGF23 as a central, hormone-like regulator of P homeostasis. The endocrine hormone, FGF23, is a 32-kD peptide (251 AA) produced primarily in bone osteoblast and osteocyte cells. Detection of FGF23 mRNA has been reported in several tissues; however, expression in bone tissue dramatically exceeded expression in the other tissues (Mirams et al., 2004; Yoshiko et al., 2007). Additional support for an endocrine role of FGF23 was based on identification of specific receptors for FGF23 in target tissues, feedback mechanisms that regulate the release of FGF23 from bone tissue, and systemic signals required to elicit the release of FGF23 or that inhibit FGF23 responses.

Renal responses to FGF23 involve effects on both P transport proteins and the regulation of vitamin D activation. Renal reabsorption of P is regulated by 2 Na-P co-transport proteins, NaPi2a and NaPi2c, which are similar to the protein NaPi2b, which is involved in intestinal P uptake (Tenenhouse, 2005). In the proximal collecting tubules of kidneys, NaPi2a and NaPi2c proteins function to control reabsorption of P from the collecting tubule back into blood, thus increasing serum P concentration (Beck et al., 1998).
One of the first pivotal publications that provided support for the endocrine role of FGF23 on renal P transport was provided by Shimada et al. (2004a). Serum P concentration decreased at 9 and 13 h after a single intravenous injection of recombinant FGF23 into normal mice, but returned to normal by 24 h after the FGF23 injection. The time course for suppression of serum P was consistent with a downregulation of renal NaPi2a transporter and downregulation of renal 25(OH)D$_3$-1α hydroxylase mRNA. Serum concentrations of PTH were also suppressed at the same periods. Increased PTH is known to downregulate NaPi2a expression; thus, the responses to FGF23 injection appear to be independent of PTH action on NaPi2a expression. Suppression of serum 1α,25-(OH)$_2$D$_3$ concentrations were detected within 3 h after the FGF23 injection, but serum Ca concentrations remained normal for the entire period, implying a direct action of FGF23 on vitamin D activation. After the FGF23 injection, renal 25(OH)D$_3$-1α hydroxylase mRNA abundance decreased and 24(OH)D$_3$-hydroxylase mRNA increased. This inverse relationship between hydroxylase enzymes implied a decreased potential for synthesis of 1α,25-(OH)$_2$D$_3$ and an increased ability to degrade the active form of vitamin D, thus compromising vitamin D status. However, results that showed an increase in serum FGF23 concentrations within 4 h after injection of 1α,25-(OH)$_2$D$_3$ in mice (Shimada et al., 2004a) implied a feedback relationship between 1α,25-(OH)$_2$D$_3$ and FGF23.

Establishment of a central role for FGF23 in regulation of P homeostasis was based on the targeted ablation of the FGF23 gene to produce FGF23-null mice (Shimada et al., 2004b). Differences in BW and survival of FGF23-null and control mice were not evident until after 2 wk of age. After 2 wk, growth in FGF23-null mice was depressed by more than 50% of control lines and survival was limited to approximately 12 wk of age. Dramatic changes in bone histomorphometry of the FGF23-null mice compared with heterogenic or wild-type (WT) controls were noted in 7-wk-old mice. Percent bone volume was reduced and trabecular space increased more than 3-fold in FGF23-null mice. However, the number of osteoclast cells was decreased by approximately 5-fold and growth plate thickness was reduced. These bone traits are consistent with a suppressed bone formation, which was confirmed by the presence of bone calluses throughout the skeleton. Serum P concentrations increased approximately 30% at 10 d of age and serum Ca concentrations increased by approximately 1% in FGF23-null mice compared with control mice. Downregulation of vitamin D conversion to the active hormone would be expected in the traditional responses to conditions of increased serum Ca and P concentrations. However, in FGF23-null mice, serum 1α,25-(OH)$_2$D$_3$ concentrations were 2- to 4-fold greater than concentrations in control and heterozygote lines. Northern blot analysis revealed that increases in renal 25(OH)D$_3$-1α hydroxylase mRNA preceded increases in 24(OH)D$_3$-hydroxylase in FGF23-null mice. The results showing an earlier expression of 25(OH)D$_3$-1α hydroxylase activity rather than 24(OH)D$_3$-hydroxylase implies a direct link between FGF23 and regulation of vitamin D activation. In addition to an upregulation of active vitamin D, an increased renal P reabsorption was consistent with an upregulation of renal NaPi2a transport protein in the FGF23-null mice.

As discussed previously, FGF23 is directly involved in controlling the expression of enzymes required for the activation and degradation of renal vitamin D. Evidence for a feedback inhibition by 1α,25-(OH)$_2$D$_3$ on the upregulation of FGF23 expression in bone, and thus FGF23 serum concentrations, was shown in mice administered 1α,25-(OH)$_2$D$_3$ injections (Kolek et al., 2005).

Additional contributions to understanding the role of FGF23 as a systemic hormone involved in P homeostasis were provided in experiments designed by Perwad et al. (2005). Perwad et al. (2005) studied the role of FGF23 in regulation of renal 25(OH)D$_3$-1α hydroxylase activity by imposing changes in dietary P concentrations. To evaluate FGF23 regulation independent of PTH and NaPi2a P transport, the investigators compared responses in normal WT mice with responses in NaPi2a-null mice. The NaPi2a-null mice exhibited approximately 85% loss of renal brush-border Na-Pi co-transport activity (Beck et al., 1998), thus displaying a limited ability to reabsorb P. After feeding a vitamin D, Ca, and P-replete diet for 5 d, Perwad et al. (2005) fed mice diets with 0.02, 0.6, or 1.65% P for 5 d to determine FGF23 mRNA abundance in bone. Serum FGF23 concentrations increased in direct proportion to dietary P concentrations (0.02 to 1.65% P) in normal mice. A 7-fold increase in serum FGF23 concentration was observed over the range of dietary P concentrations. Likewise, serum P concentration increased with dietary P concentration in WT mice. Thus, serum concentrations of FGF23 were directly proportional to serum P concentrations. To assess whether the effects of dietary P on FGF23 were mediated by changes in renal transport, NaPi2a-null mice were also fed diets with varied concentrations (0.02 to 1.65%) of P. As with WT mice, the serum FGF23 concentrations increased in NaPi2a-null mice in response to increased dietary P concentrations. After adjustment for differences in serum P concentration between WT and NaPi2a-null mice, the relative responses of serum FGF23 concentration was directly proportional to serum P concentration in mice with or without the NaPi2a transporter. Bone FGF23 mRNA concentration increased in WT mice as dietary P increased. In WT mice, the renal 25(OH)D$_3$-1α-hydroxylase activity and mRNA abundance decreased as dietary P concentration increased from 0.02 to 1.0% P, but a plateau in activity was observed in WT mice fed diets with 1.0 and 1.65% P. The plateau in 1α-hydroxylase activity and mRNA abundance
may be related to an increase in 24(OH)D$_3$-hydroxylase activity. Abundance of 24(OH)D$_3$-hydroxylase mRNA increased directly with serum FGF23 concentration. Thus, serum P directly affects FGF23 expression and release from bone, which induces renal responses that regulate P homeostasis.

In the traditional Ca, PTH, and vitamin D axis of Ca homeostasis, PTH serves as a central hormone to regulate and respond to feedback signals involved in the axis. As discussed previously, PTH responses do not appear to alter responses to FGF23 signals. Injections of recombinant FGF23 suppressed serum PTH and PTH mRNA abundance in parathyroid tissues of rats (Ben-Dov et al., 2007). Both FGF and klotho receptors (a co-receptor involved in FGF receptor binding) were detected in parathyroid cells. The FGF23 suppression of PTH response explained earlier observations in which PTH was not released as expected and provided additional evidence to support the central role of FGF23 in P homeostasis. Direct effects of FGF23 on PTH release also helps to explain responses observed in secondary hyperparathyroidism and offers insights for procedures used to manage chronic kidney disease (Silver and Naveh-Many, 2009).

Single ablations of FGF23 or NaPi2a genes have demonstrated critical roles that the protein produced by each gene contributes to P homeostasis. The FGF23 protein suppresses NaPi2a gene expression, leading to severe hypophosphatemia in the FGF23-null mouse (Shimada et al., 2004b). Opposite responses, severe hypophosphatemia, were observed in the NaPi2a-null mouse (Beck et al., 1998). Thus, supposedly, FGF23 and NaPi2a proteins work in a feedback mechanism, mediated via serum P, to maintain P homeostasis. Evidence to support the feedback relationship between FGF23 and NaPi2a was provided in a study (Sitara et al., 2008) involving double knockout mice with deletion of genes that control both FGF23 and NaPi2a proteins. The double knockout mice exhibited a restored phenotype with BW and survival more similar to control mice than to mice with single ablations of either FGF23 or NaPi2a genes. The double knockout mice were able to maintain normal serum P concentrations, presumably, because animals were fed diets that met minimal Ca and P requirements. Thus, the counteracting FGF23 and NaPi2a proteins were not needed to maintain P homeostasis if the mice were provided diets without excess or deficient amounts of P. However, skeletal defects were still observed in the double knockout mice. Additional roles for FGF23 on skeletal growth, independent of renal function, were implied by these results.

A summary of the systemic role of FGF23 in P homeostasis is presented in Figures 2 and 3. Systemically, bone functions as an endocrine gland that responds to changes in serum P concentrations and modulates the release of FGF23. An increased circulation of FGF23 (Figure 2) acts on target tissues in the kidney to suppress expression of NaPi2a and NaPi2c transport proteins, thus decreasing renal P reabsorption. In kidneys, FGF23 also targets renal 25(OH)D$_3$-1α-hydroxylase activity to suppress the synthesis of 1α,25-(OH)$_2$D$_3$. Thus, FGF23 acts via regulation of 1α,25-(OH)$_2$D$_3$ to affect bone resorption and small intestine absorption of Ca and P, thus providing additional contributions to the renal maintenance of P homeostasis. The changes in 1α,25-(OH)$_2$D$_3$ also act as feedback signals to downregulate FGF23 expression. In parathyroid tissue, FGF23 inhibits PTH gene expression, and thus the release of PTH into circulation. However, controversy exists (Ramon et al., 2010) about the direct effects of FGF23 on the parathyroid gland vs. indirect effects mediated by 1α,25-(OH)$_2$D$_3$.

Common dietary formulations in animal nutrition involve limitations in P rather than Ca. If dietary P restrictions were imposed, a decrement in serum P concentrations would suppress FGF23 expression and release from bone. The decreased FGF23 concentrations (Figure 3) would then allow an upregulation of renal NaPi2a and NaPi2c expression, which would increase P reabsorption. Likewise, the decreased FGF23 concentrations would allow upregulation of renal 25-(OH)D$_3$-1α-hydroxylase activity and the increased release of 1α,25-(OH)$_2$D$_3$. Increased 1α,25-(OH)$_2$D$_3$ acts directly on the small intestine to stimulate Ca and P absorption and on bone to stimulate Ca and P resorption, thus maintaining P concentration.

**DISCUSSION**

The historical focus of research efforts on Ca homeostasis has been driven, in part, by a limited amount of Ca and abundance of P in human diets. Most ingredients used in nonruminant animal diets are limiting in Ca and P. In animal diets, Ca and P have been typically supplied in excess of requirements using supplements from inorganic sources with minor incentives to improve nutrient efficiency. Constraints on amounts of supplemental P have been driven by ingredient costs and environmental concerns. Thus P, not Ca, is typically more limited in diets for nonruminant animals. The introduction of phytase supplements has only exacerbated the need to understand P homeostasis. In the past decade, discovery of a novel pathway for P homeostasis offers opportunities to improve P efficiency without compromising skeletal growth and animal well-being. In this review, attempts were made to describe the initial research reports that led to the validation of the FGF23, P, and vitamin D axis for regulation of P homeostasis. Applications from this pathway offer opportunities to improve the efficiency of P use in animal agriculture and approaches to reduce skeletal problems associated with mineral nutrition.

Our challenge to explore alternate pathways for vitamin D-mediated P homeostasis was stimulated, in part, by an outbreak and subsidence of kyphosis (~20% incidence) in pigs produced by the University of Wisconsin.
The kyphosis outbreak at SRTC stimulated a series of trials that eventually led to a link between vitamin D and dietary Ca and P concentrations. The symptoms were initially observed in growing pigs at SRTC fed diets with marginal amounts of Ca and P as part of an undergraduate class laboratory project. Evidence to link the induction of kyphosis with marginal dietary deficiencies of vitamin D, Ca, and P were recently reported (Rortvedt et al., 2010). Traditional modes of action of vitamin D involve homeostatic regulation of Ca and P concentrations. The animal responses were not expected. Either marginal deficiencies of all 3 nutrients exacerbated response symptoms or the life-cycle phase in which the deficiencies were imposed were critical. To our knowledge, vitamin D has not been implicated with kyphosis. Thus, alternate mechanisms were explored including potential links to FGF23-mediated responses. To this date, no definitive links between kyphosis and FGF23 have been identified.

As with kyphosis, several practical issues have arisen in recent years associated with the mineral nutritional support of agricultural animals. These issues involved bone-related disorders and lameness problems that are not easily explained by traditional approaches. Production practices in the area of mineral nutrition have been pressured to minimize the use of supplemental P from inorganic sources based on concerns for environmental pollution. Sources of inorganic P supplements have been altered to reduce costs associated with processing raw phosphate ores and costs associated with transportation. Supplemental phytase has proven to be an effective alternate for inorganic P supplements, if proper management procedures are followed. Failure to adhere to strict feed manufacturing procedures renders the enzyme ineffective, resulting in P deficiencies for the animal. Pressure to reduce dietary P has resulted in a greater proportion of diets for nonruminant animals actually becoming marginal relative to the animal needs for adequate skeletal mineralization. Applying the FGF23, P, and vitamin D axis to improve efficiency
SUMMARY AND CONCLUSIONS

A previously unknown peptide hormone, FGF23, has been identified within the past decade. The hormone is produced in bone tissue and acts on the kidney as a primary target tissue. In the kidney, FGF23 downregulates P transporters to reduce renal P reabsorption and downregulates enzymes involved in the conversion of vitamin D into active forms. Applications from understanding the central role of FGF23 in P homeostasis offer opportunities for improving the efficiency of P use in animals.

LITERATURE CITED


