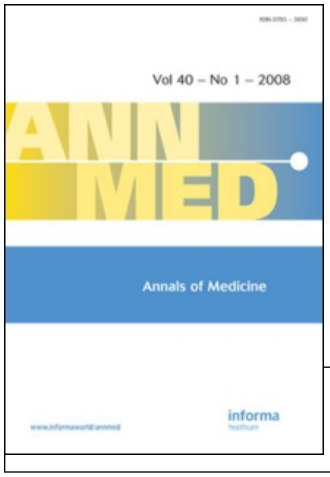

Annals of Medicine
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713699451

Calcium and phosphate homeostasis: Concerted interplay of new regulators

Kirsten Y. Renkema [a]; R. Todd Alexander [a]; René J. Bindels [a]; Joost G. Hoenderop [a]
[a] Department of Physiology, Nijmegen Centre for Molecular Life Sciences, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands

First Published on: 09 November 2007

To cite this Article: Renkema, Kirsten Y., Alexander, R. Todd, Bindels, René J. and Hoenderop, Joost G. (2007) 'Calcium and phosphate homeostasis: Concerted interplay of new regulators', Annals of Medicine, 40:2, 82 — 91

To link to this article: DOI: 10.1080/07853890701689645
URL: http://dx.doi.org/10.1080/07853890701689645

Annals of Medicine. 2008; 40: 82–91

informa
healthcare

REVIEW ARTICLE

Calcium and phosphate homeostasis: Concerted interplay of new regulators

KIRSTEN Y. RENKEMA, R. TODD ALEXANDER, RENÉ J. BINDELS & JOOST G. HOENDEROP

Department of Physiology, Nijmegen Centre for Molecular Life Sciences, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands

Abstract
Calcium (Ca^{2+}) and phosphate (P_i) are essential to many vital physiological processes. Consequently the maintenance of Ca^{2+} and P_i homeostasis is essential to a healthy existence. This occurs through the concerted action of intestinal, renal, and skeletal regulatory mechanisms. Ca^{2+} and P_i handling by these organs is under tight hormonal control. Disturbances in their homeostasis have been linked to pathophysiological disorders including chronic renal insufficiency, kidney stone formation, and bone abnormalities. Importantly, the kidneys fine-tune the amount of Ca^{2+} and P_i retained in the body by altering their (re)absorption from the glomerular filtrate. The ion transport proteins involved in this process have been studied extensively. Recently, new key players have been identified in the regulation of the Ca^{2+} and P_i balance. Novel regulatory mechanisms and their implications were introduced for the antiaging hormone klotho and fibroblast growth factor member 23 (FGF23). Importantly, transgenic mouse models, exhibiting disturbances in Ca^{2+} and P_i balance, have been of great value in the elucidation of klotho and FGF23 functioning. This review highlights the current knowledge and ongoing research into Ca^{2+} and P_i homeostasis, emphasizing findings from several relevant knockout mouse models.

Key words: *1,25-Dihydroxyvitamin-D_3, calcium, fibroblast growth factor 23, klotho, phosphate, sodium-phosphate cotransporter, TRPV5, TRPV6*

Introduction

The tight control of plasma calcium (Ca^{2+}) and phosphate (P_i) levels is essential to the performance of many vital physiological functions. Muscle contraction, blood clotting and neuronal excitation all require Ca^{2+}, whereas P_i is vital to intracellular signaling, as a component of membrane lipids and to build the backbone of DNA. Moreover, significant elements of bone are Ca^{2+} and P_i (1,2). Several organs contribute to the exquisite regulation of Ca^{2+} and P_i homeostasis by facilitating intestinal absorption, bone (de)mineralization, and renal excretion/reabsorption of both ions. Regulation of these processes occurs by a number of hormones. The biologically active form of vitamin D (1,25-dihydroxyvitamin D_3), parathyroid hormone (PTH), and calcitonin have been extensively studied in this regard. More recently, fibroblast growth factor member 23 (FGF23) and klotho have been identified as new players essential to the regulation of Ca^{2+} and P_i homeostasis (3–5). Remarkably, large alterations in dietary P_i and Ca^{2+} intake produce only small alterations in the circulating levels of these ions due to the combined action of these signaling molecules on bone, intestinal, and renal Ca^{2+} and P_i transport (2,6,7). At times these regulatory processes are overwhelmed producing disturbances in Ca^{2+} and P_i homeostasis. Patients with chronic renal insufficiency (CRI) highlight pathophysiology complicated by altered Ca^{2+} and P_i handling. These individuals have a decreased glomerular filtration rate, a decreased circulating plasma 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) level and consequently develop secondary hyperparathyroidism (8). This review highlights the physiological mechanisms governing Ca^{2+} and P_i transport, two intimately

Correspondence: Joost G. J. Hoenderop, 286 Physiology, Radboud University Nijmegen Medical Centre, P.O. Box 9101, NL-6500 HB Nijmegen, The Netherlands. Fax: +31-24-3616413. E-mail: j.hoenderop@ncmls.ru.nl

ISSN 0785-3890 print/ISSN 1365-2060 online
DOI: 10.1080/07853890701689645

related processes, and the clinical implications of their altered regulation.

Key messages

- Maintenance of normal phosphate (P_i) and calcium (Ca^{2+}) homeostasis is crucial to many vital physiologic processes including cellular signaling, DNA structure, bone mineralization, muscle contraction, blood clotting, and neuronal excitation.
- Fibroblast growth factor member 23 (FGF23), klotho, parathyroid hormone, and 1,25-dihydroxyvitamin-D_3 are key regulators of Ca^{2+} and P_i homeostasis. Their concerted action alters (re)absorption from intestine, kidney, and bone via recently identified pathways.
- The concerted interplay of klotho and FGF23 contributes substantially to the regulation of renal P_i handling and may provide a therapeutic target for chronic renal insufficiency-related hyperphosphatemia and other disorders involving disturbances of P_i balance.

Physiology of Ca^{2+} and P_i homeostasis

Ingested Ca^{2+} and P_i are absorbed by different segments of the small intestine. The active absorption of sodium (Na^+) throughout the entire course of the intestine results in a large net water absorption. This mainly occurs in the small intestine, where Ca^{2+} and P_i are concomitantly taken up in a passive, paracellular manner, down their concentration gradient. The active transcellular absorption of P_i occurs predominately from the ileum (9), whereas active Ca^{2+} transport takes place largely from the duodenum (10).

The vast majority of whole body Ca^{2+} and P_i is stored as the mineral hydroxylapatite in the skeleton. In blood 45% of Ca^{2+} is present in a free, ionized form, 45% is bound to proteins, and a small fraction, 10%, forms complexes with anions including citrate, sulphate, and phosphate. Plasma P_i is present in its inorganic form and as a component of several organic substances including sugars, phosphoproteins, and high-energy phosphates. The tightly regulated renal elimination of electrolytes, including Ca^{2+} and P_i, maintains a near constant plasma level of ions and significantly contributes to whole body homeostasis. Free Ca^{2+} and P_i are filtered by the glomerulus and gain entry into the renal tubule. In contrast to the gastrointestinal system, P_i is largely absorbed in a transcellular, Na^+-dependent manner in the proximal tubule (PT). This process is dependent on the electrochemical gradient present for Na^+ (11). Renal Ca^{2+} absorption occurs paracellularly in the PT and the thick ascending limb of Henle (TAL). A small (10%–15%) highly regulated amount of Ca^{2+} is actively absorbed from the distal convoluted tubule (DCT) and the connecting tubule (CNT) (10,11). A diverse array of ion transport proteins, localized to the apical and basolateral membranes of intestinal and renal epithelia, mediate this active transport of Ca^{2+} and P_i. The entry of these ions, from lumina into the cytosol of the specific epithelium, is tightly regulated and represents the rate-limiting step in transcellular Ca^{2+} and P_i (re)absorption (10).

Ca^{2+} and P_i transport proteins

The ability of the small intestine to actively absorb Ca^{2+} and P_i is hormonally regulated and occurs from the duodenum and ileum, respectively. Active intestinal Ca^{2+} absorption begins with its luminal entry mediated by the epithelial Ca^{2+} channel, TRPV6 (12). Ca^{2+} is subsequently shuttled to the basolateral side of the cell via the Ca^{2+}-binding protein, calbindin-D_{9K}. This process is completed by transport of Ca^{2+} back into the bloodstream via the plasma membrane Ca^{2+}-adenosine triphosphatase (PMCA1b) (13).

The Na^+-dependent P_i cotransporter type IIb (NaPi-IIb) is localized to the brush border of ileum. There it is responsible for active P_i transport from the intestinal lumen into the blood (9,14). NaPi-IIb is also present, although to a significantly decreased extent, in duodenum and jejunum, consistent with less overall active P_i reabsorption from these locales (9). The mechanism and identity of the molecules responsible for the extrusion of P_i back into the blood is not known at present.

After intestinal absorption into the blood, Ca^{2+} and P_i are filtered across the glomerulus into the tubular lumen of the nephron. Along the course of the nephron passive, paracellular Ca^{2+} absorption occurs from the PT and TAL. A three-step process mediates the active Ca^{2+} reabsorption mechanism from the DCT and CNT. First, Ca^{2+} enters the cell via the epithelial Ca^{2+} channel TRPV5 (and to a lesser extent TRPV6). Then Ca^{2+} diffuses through the cytosol via calbindin-D_{28K}, to the basolateral side of the cell. There, Ca^{2+} is extruded into the peritubular capillary by the Na^+-Ca^{2+}-exchanger (NCX1) and PMCA1b (10). Active P_i absorption occurs in the PT of the kidney via the

Na^+-dependent P_i cotransporters, NaPi-IIa and NaPi-IIc. Both display high expression levels in the early segments (S1 and S2) of the PT (2,11,15–17). Figure 1 summarizes the transcellular pathways of Ca^{2+} (A) and P_i (B) transport in kidney and intestine.

Hormonal regulation of transcellular Ca^{2+} (re)absorption

Exquisite regulation of TRPV5 and TRPV6 activity is essential to whole body Ca^{2+} homeostasis. Here, we review the regulatory effects of the classically described hormones: PTH, $1,25(OH)_2D_3$, and calcitonin on active Ca^{2+} transport processes. Later we will discuss the newly identified regulators of Ca^{2+} homeostasis: tissue kallikrein (TK), the antiaging hormone klotho, and estrogen. Central to this process is the Ca^{2+}-sensing receptor (CaSR), which is expressed in the parathyroid gland where it senses blood Ca^{2+} levels (18,19). In response to changes in blood Ca^{2+} concentrations, the CaSR regulates the release of PTH into the circulation (Figure 2). Specifically, hypocalcemia inhibits the CaSR that in turn promotes the secretion of PTH. This hormone stimulates Ca^{2+} mobilization from bone and the conversion of inactive vitamin D to $1,25(OH)_2D_3$ by the renal cytochrome P450 enzyme 25-hydroxyvitamin D_3-1α-hydroxylase (1αOHase) (20). Increased $1,25(OH)_2D_3$ levels activate vitamin D receptor (VDR)-mediated gene transcription, resulting in an increased transcription of Ca^{2+} transport proteins. Ultimately this results in the stimulation of Ca^{2+} (re)absorption (3,21–24). Hypercalcemia has the opposite effect. This state activates the CaSR, thereby inhibiting PTH release and stimulating the secretion of calcitonin from the parafollicular cells of the thyroid. Calcitonin decreases osteoclast-mediated bone resorption and promotes lowering the blood Ca^{2+} concentration (25).

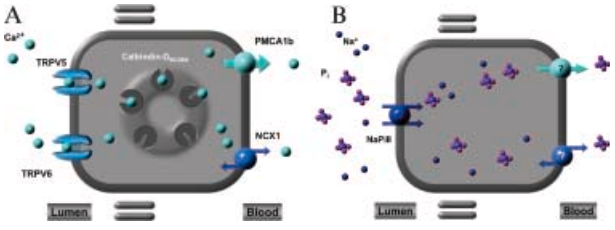

Figure 1. Transcellular Ca^{2+} and P_i transport. Active Ca^{2+} and P_i transport across renal and intestinal epithelium. A: Ca^{2+} influx is mediated by TRPV5 in the renal distal convoluted and connecting tubules and TRPV6 in the duodenum. Subsequently, Ca^{2+} is transported across the cell to the basolateral membrane by Ca^{2+}-binding proteins calbindin-D_{28K} (kidney) and calbindin-D_{9K} (intestine). Extrusion into the blood takes place via the plasma membrane Ca^{2+}-ATPase (PMCA1b) in the intestine and sodium-Ca^{2+} exchanger (NCX1) and PMCA1b in the kidney. B: The ileal brush border, sodium-dependent P_i cotransporter, NaPi-IIb, mediates entry of P_i into enterocytes. In the proximal tubules, NaPi-IIa and NaPi-IIc are responsible for P_i entry into the tubular epithelial cells. The transport mechanisms involved in basolateral extrusion of P_i into blood are unknown both in the intestine and kidney.

Estrogen contributes importantly to Ca^{2+} homeostasis by promoting bone mineralization and stimulating renal TRPV5 expression. This results in increased active Ca^{2+} reabsorption, independently of $1,25(OH)_2D_3$ (26–28). PTH, $1,25(OH)_2D_3$, and estrogen exert their effect on renal-mediated Ca^{2+} handling by altering, at the transcriptional level, the expression of Ca^{2+} transporters (7). This has been confirmed, *in vivo*, where a direct stimulatory effect of PTH on renal Ca^{2+} transporter expression levels was demonstrated in PTH-supplemented parathyroidectomized rats (29), and where the dietary supplementation of Ca^{2+} and the administration of estrogen to ovariectomized rats was shown to alter renal TRPV5 expression, all independently of $1,25(OH)_2D_3$ (7,23,28).

The TK knockout ($TK^{-/-}$) mouse displays significant renal Ca^{2+} wasting compared to control mice, implicating an important role for TK in the regulation of active Ca^{2+} reabsorption (30). In 2006, Gkika et al. described the molecular mechanism through which TK stimulates TRPV5-mediated active Ca^{2+} reabsorption. TK, a proteolytic enzyme that is secreted into the tubular fluid by renal epithelial cells, activates the bradykinin receptor stimulating the phospholipase C/1,2-diacylglycerol/protein kinase C (PLC/DAG/PKC) pathway. This results in increased plasma membrane expression of TRPV5, possibly due to an inhibition of TRPV5 endocytosis. Regardless of the exact mechanism, TK increases TRPV5-mediated Ca^{2+} transport (31).

Recently the antiaging hormone klotho has also been implicated in the regulation of the Ca^{2+} balance. Klotho knockout ($klotho^{-/-}$) mice display hypercalcemia, hyperphosphaturia, hypercalciuria, and other manifestations resembling aging (32). Chang et al. demonstrated that klotho stimulates TRPV5-mediated Ca^{2+} transport *in vitro*, revealing a novel regulatory mechanism of active Ca^{2+} transport (33). Klotho is a type I transmembrane protein with β-glucuronidase activity. This activity is contained in the extracellular domain that can be secreted into the blood, urine, and cerebrospinal fluid (32,34). Klotho hydrolyzes extracellular N-linked oligosaccharides present on TRPV5, entrapping the channel in the plasma membrane. This stimulatory effect of klotho on TRPV5 substantiates the important role of

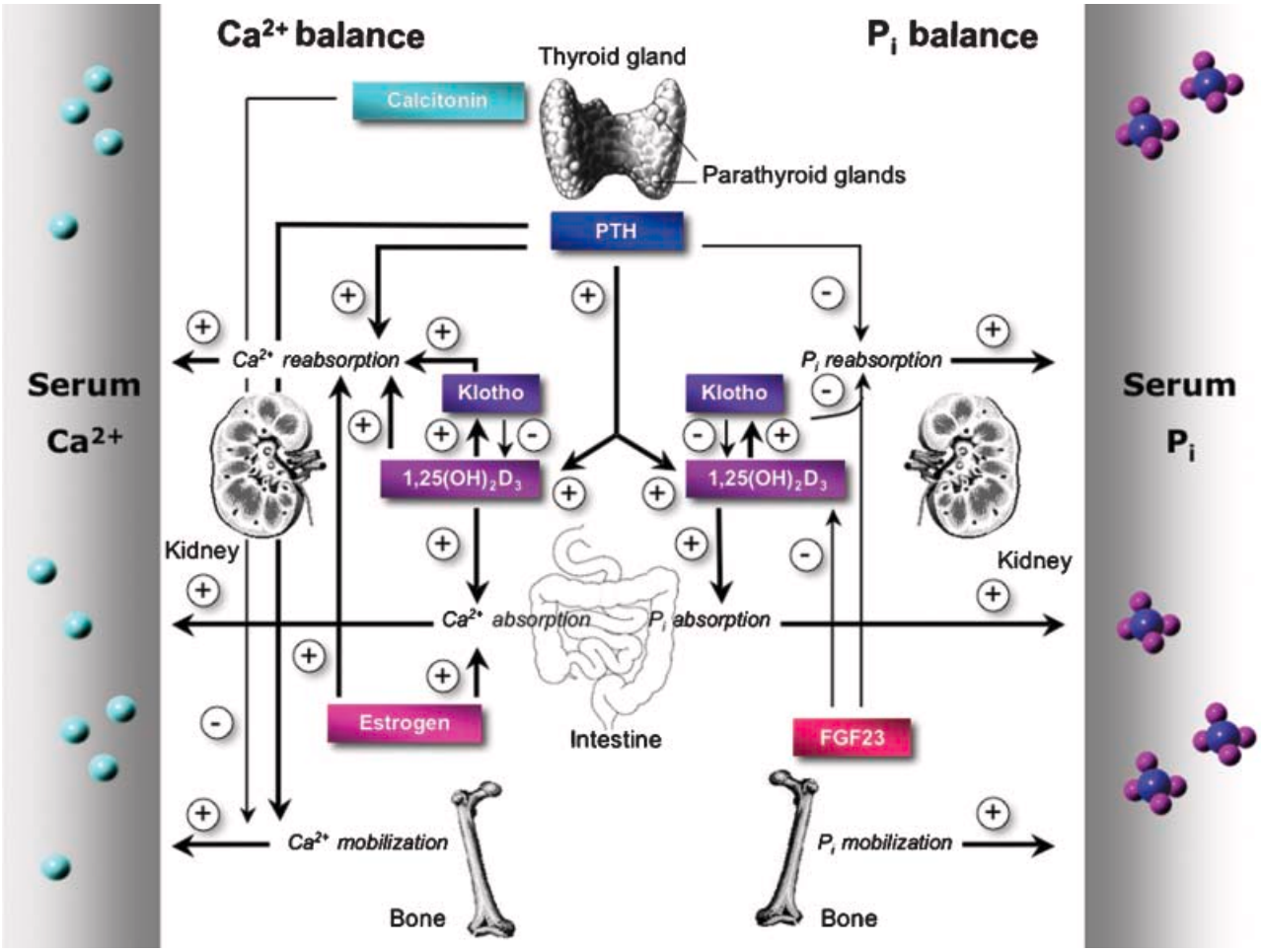

Figure 2. Key players in Ca^{2+} and P_i homeostasis. The concerted interplay of intestinal uptake, reabsorption in kidney, and bone (de)mineralization establishes the maintenance of a normal Ca^{2+} and P_i balance. The calcium sensing receptor (CaSR), present in the thyroid gland, senses blood Ca^{2+} levels and triggers the secretion of the calcitropic hormones parathyroid hormone (PTH) and calcitonin. Ovarian-produced estrogen stimulates Ca^{2+} reabsorption also. Similar hormones are involved in the regulation of P_i balance. Blood P_i levels are controlled by PTH, $1,25(OH)_2D_3$, klotho, and fibroblast growth factor member 23 (FGF23). A negative feedback mechanism prevents the accumulation of FGF23 and klotho since 1αOHase-mediated $1,25(OH)_2D_3$ production is inhibited by FGF23 and klotho.

this antiaging hormone in Ca^{2+} homeostasis, specifically by altering the luminal membrane Ca^{2+} permeability of the DCT and the cortical collecting duct (CCD) (33). Alternatively, the mechanism of hypercalciuria observed in klotho$^{-/-}$ mice has been suggested to be the result of decreased proximal Ca^{2+} absorption because of a decreased Na^+K^+-ATPase activity (35).

Hormonal regulation of transcellular P_i (re)absorption

Our understanding of the hormonal control of P_i balance is rapidly evolving. Klotho and FGF23 were recently identified as new key players involved in P_i regulation, adding to what had already been delineated about the classical PTH/$1,25(OH)_2D_3$ regulatory pathway. PTH, $1,25(OH)_2D_3$, and dietary P_i intake are known to alter P_i (re)absorption (36). An increased intestinal NaPi-IIb expression can be stimulated by either $1,25(OH)_2D_3$ and/or low P_i intake, ultimately resulting in elevated blood P_i (6). Conversely, PTH decreases blood P_i levels by inhibiting P_i reabsorption from the PT. This occurs via an acute redistribution of NaPi-IIa proteins from the brush border to intracellular lysosomes, targeting them for degradation. The overall effect of PTH is therefore to lower blood P_i levels by stimulating P_i excretion (37).

Recently, FGF23 has been labeled a phosphatonin, due to its role in P_i homeostasis (5,38–40). Injection of FGF23 into mice results in hypophosphatemia by stimulating renal P_i excretion. This is achieved by decreasing the expression of renal NaPi-IIa and NaPi-IIc and inhibiting 1αOHase production. The role of FGF23 in human Ca^{2+} and P_i

homeostasis has been firmly established by genetic linkage analysis. Inactivating mutations in FGF23 have been identified in patients with tumoral calcinosis causing hyperphosphatemia, and activating mutations have been shown to cause autosomal dominant hypophosphatemic rickets (ADHR) (41–44). FGF23 is a secreted, transmembrane protein produced in bone osteoblasts (45,46), which can bind to and activate FGF receptors (FGFRs) (47,48). Further, the involvement of klotho in FGF23 signaling was suggested as klotho$^{-/-}$ mice and FGF23 knockout (FGF23$^{-/-}$) mice exhibit many common phenotypic features including hyperphosphatemia (32,38,49). This was confirmed in 2006, when Kurosu et al. demonstrated that klotho forms a complex with FGFRs. This new complex binds FGF23 with a higher affinity than either the FGFR or klotho alone (50). Two separate groups extended this observation by showing that direct binding of klotho to the FGFR1(IIIc) subtype converts this receptor into a specific FGF23 receptor (48,51). In addition, Ogawa et al. demonstrated that another member of the klotho family, β-klotho, can function as a cofactor in FGF21 signaling, through a similar interaction as with klotho and FGF23 (52). FGF21 is expressed predominantly in the liver and is a metabolic regulator of glucose uptake in adipocytes, consequently it provides a potential therapeutic target in diabetes mellitus (53). Other FGF family members have been implicated in varying signal transduction pathways throughout the body (54). A recent report revealed that FGF7 is overexpressed in tumors associated with renal P_i wasting and osteomalacia (55). FGF7 inhibits P_i transport in renal epithelial cells, although the exact molecular mechanisms involved still need to be elucidated. Further investigation is necessary to determine whether other FGF superfamily members are involved in the regulation of Ca^{2+} and P_i homeostasis.

Hypercalcemia and hypocalcemia

Disturbances in both serum and whole body Ca^{2+} levels can cause severe pathological conditions, the etiology of which is both complex and variable. Hypercalcemia can result from Ca^{2+} hyperabsorption from the gastrointestinal (GI) tract, decreased urinary excretion, or an increased resorption from bone. Elevated serum PTH levels, secondary to hyperparathyroidism or a hypophosphatemic state, will cause increased Ca^{2+} absorption from the GI tract. Increased Ca^{2+} loss from bone is caused by elevated PTH and/or $1,25(OH)_2D_3$ levels or skeletal metastasis, while severe dehydration will increase serum Ca^{2+} concentration without altering the total amount in blood. Symptoms and findings of hypercalcemia include fatigue, electrocardiogram abnormalities, nausea, vomiting, constipation, anorexia, abdominal pain, hypercalciuria, and consequently kidney stone formation. Treatment of hypercalcemia depends on the severity of the abnormality and ranges from dietary adaptation to the administration of calcimimetic compounds that activate the CaSR in the parathyroid glands, reducing blood PTH levels (56). Hypocalcemia can result in muscle cramping, depression, psychosis, and seizures. Causes include decreased Ca^{2+} absorption due to a poor intake, $1,25(OH)_2D_3$ deficiency or resistance, lack of sunlight, decreased bone resorption, a complication of thyroid surgery (i.e. parathyroidectomy), or renal Ca^{2+} wasting. Oral Ca^{2+} and $1,25(OH)_2D_3$ supplementation and ultraviolet light exposure are the current treatments for hypocalcemia.

Hypercalciuria

Hypercalciuria is a risk factor for renal Ca^{2+} stone formation and therefore contributes to this significant health and socioeconomic problem (57,58). In the United States (US) more than 5% of the population will develop a clinically significant episode of kidney stone disease in their lifetime of which the economic impact is approximately $2 billion annually (59,60). Hypercalciuria has been classified into at least three different forms. Absorption hypercalciuria is due to Ca^{2+} hyperabsorption from the GI tract, renal hypercalciuria is the result of a defect in renal Ca^{2+} reabsorption, whereas resorptive hypercalciuria manifests urinary Ca^{2+} wasting secondary to increased bone degradation. Other than these three types of hypercalciuria, the largest group of patients with this disorder lack an explanation for their increased Ca^{2+} excretion and have been classified as having idiopathic hypercalciuria. Individuals with a family history of nephrolithiasis are themselves prone to develop kidney stones. This strongly suggests that genetic factors are involved in the pathogenesis of idiopathic hypercalciuria (61). There is a myriad of potential disturbances in Ca^{2+} homeostasis that can cause hypercalciuria. Thus, hypercalciuria may be monogenic, polygenic, or multifactorial in its etiology. As TRPV5 as well as TRPV6 gene ablation in mice leads to severe forms of hypercalciuria, these channels were proposed as candidate genes for hypercalciuria (1,62). To date, mutation analyses of the TRPV5-encoding gene has not revealed a primary role for TRPV5 in autosomal dominant

idiopathic hypercalciuria (63). However, the involvement of TRPV5 or TRPV6 in hypercalciuria has not been definitively excluded. Specific single nucleotide polymorphisms (SNPs) or combinations of SNPs in TRPV5/TRPV6 may modulate channel activity, and might therefore be responsible for altered renal Ca^{2+} excretion (64). Further investigation is necessary to identify mutations in TRPV5 and TRPV6 associated with disease.

Hyperphosphatemia and hypophosphatemia

Autosomal dominant hypophosphatemic rickets (ADHR) is a hereditary disorder characterized by bone malformation and renal P_i wasting. Activating mutations in the FGF23 gene have been identified in these patients (41,65). Related diseases include X-linked hypophosphatemia (XLH) and hereditary hypophosphatemic rickets with hypercalciuria (HHRH); both are characterized by disturbances in P_i homeostasis. Tumor-induced osteomalacia (TIO) is characterized by hypophosphatemia secondary to renal P_i wasting and reduced blood $1,25(OH)_2D_3$ levels (66,67). Whilst the molecular identity of all the molecules responsible for these clinical disorders have yet to be identified, in a few instances genes involved in the maintenance of P_i homeostasis have been implicated other than FGF23 (68,69). Hypophosphatemia causes decreased bone mineralization and subsequently bone fragility, pain, rickets, and growth retardation (69). Treatment is aimed at the replacement of P_i and/or $1,25(OH)_2D_3$.

Deactivating mutations in FGF23 cause hyperphosphatemic tumoral calcinosis, characterized by hypervitaminosis D and increased intestinal and renal P_i absorption (43). Consistent with this, $FGF23^{-/-}$ mice exhibit severe hyperphosphatemia further substantiating the regulatory role of FGF23 in P_i homeostasis (49). In humans, renal insufficiency, malignancy, drug abuse, or hypoparathyroidism can lead to hyperphosphatemia. Treatment is with P_i binders or directed at the primary cause.

CRI

CRI is the progressive loss of renal function evinced by a decreasing glomerular filtration rate. Clinical symptoms and findings include hypertension, edema, hyperkalemia, hypocalcemia secondary to a decreased serum $1,25(OH)_2D_3$ level, secondary hyperparathyroidism, and hyperphosphatemia. Disorders with disturbances in both Ca^{2+} and P_i homeostasis, of which CRI is just one, can lead to severe bone, cardiovascular, and other systemic diseases. CRI patients ultimately develop end stage renal disease and require renal replacement therapy, i.e. dialysis or kidney transplantation. In order to minimize the burden of this disease it is imperative to attempt normalization of the Ca^{2+} and P_i balance in these patients (70).

Elevated blood FGF23 levels are detectable in CRI patients with secondary hyperparathyroidism. This is thought to represent a compensatory mechanism in an attempt to excrete excess P_i and thus lower blood P_i levels. The $klotho^{-/-}$ mice exhibit increased blood FGF23 levels as well, possibly due to their apparent hyperphosphatemia (71,72). As klotho has been implicated in the stimulation of TRPV5-mediated Ca^{2+} reabsorption and in the regulation of FGF23-mediated P_i reabsorption, both klotho and FGF23 may play significant roles in the pathogenesis, treatment options and prediction of the prognosis of CRI (73–75).

Lessons from TRPV5 and TRPV6 knockout mice

The generation and characterization of TRPV5 knockout ($TRPV5^{-/-}$) mice confirmed this epithelial Ca^{2+} channel to be the gatekeeper of active Ca^{2+} reabsorption (1). $TRPV5^{-/-}$ mice display severe hypercalciuria compared to control ($TRPV5^{+/+}$) mice that are normocalcemic. The knockout mice display hypervitaminosis D and upregulation of intestinal TRPV6 and calbindin-D_{9K}, as a compensatory mechanism for their severe renal Ca^{2+} wasting. Cross-breeding of $TRPV5^{-/-}$ and 1αOHase knockout ($1\alpha OHase^{-/-}$) mice was performed in order to address the role of the increased blood $1,25(OH)_2D_3$ levels in these animals. TRPV5/1αOHase double knockout mice have decreased intestinal TRPV6 and calbindin-D_{9K} expression, hypocalcemia, and severe bone abnormalities compared to $TRPV5^{-/-}$ mice. These findings support the notion that the hypervitaminosis D observed in $TRPV5^{-/-}$ mice is responsible for the upregulation of intestinal Ca^{2+} transport proteins, and the consequent intestinal Ca^{2+} hyperabsorption, likely as compensation for the renal Ca^{2+} leak (76). Notably, $TRPV5^{-/-}$ mice have acidic urine and are polyuric relative to their control littermates (1). Both of these symptoms would act to promote the excretion of large amounts of Ca^{2+} without it being precipitated in the collecting ducts. $TRPV5^{-/-}$ mice display significant hyperphosphaturia, predisposing them to an increased risk of Ca^{2+} phosphate precipitation. The molecular mechanism responsible for the renal P_i leak in $TRPV5^{-/-}$ mice remains unknown.

The TRPV5$^{-/-}$ mice show a greatly diminished expression of calbindin-D_{28K}. To assess whether the absence of TRPV5 or the downregulation of calbindin-D_{28K} was responsible for the phenotype observed in the TRPV5$^{-/-}$ animals, double knockout mice, for both TRPV5 and calbindin-D_{28K}, were generated. These mice do not display a further increase in their Ca^{2+} loss relative to TRPV5$^{-/-}$ mice, confirming that TRPV5 and not calbindin-D_{28K} is the rate-limiting transporter in active Ca^{2+} reabsorption (77).

In vivo studies of specific knockout mice models significantly contribute to our knowledge of renal and intestinal Ca^{2+} handling. Recently, Bianco et al. generated the TRPV6 knockout (TRPV6$^{-/-}$) mouse in order to assess the role of this channel in intestinal Ca^{2+} absorption *in vivo* and its involvement in other organ systems (62). Surprisingly, these mice display skin abnormalities due to a decreased Ca^{2+} content in their epidermis and have impaired fertility. Consistent with known TRPV6 localization the knockout animals exhibit defective intestinal Ca^{2+} absorption as well as significant hypercalciuria and decreased bone mineralization compared to control littermates (62). Further, TRPV6$^{-/-}$ mice have secondary hyperparathyroidism and hypervitaminosis D such that they are normocalcemic. These findings indicate that TRPV6 is central to $1,25(OH)_2D_3$-regulated Ca^{2+} absorption and overall Ca^{2+} homeostasis. Finally, the exhibition of abnormalities in multiple organs in TRPV6$^{-/-}$ mice underlines the importance of this channel in other body tissues. While TRPV5 clearly functions as a gatekeeper of active Ca^{2+} reabsorption from the lumen of DCT and CNT, these recent findings suggest that TRPV6 may be an important regulator of Ca^{2+} transport during embryonic and placental development. Maintenance of normal Ca^{2+} levels is crucial for physiological functioning of the uterus and placenta, including both smooth muscle contraction and embryo implantation. Recently, Lee et al. demonstrated that estrogen regulates TRPV6 expression levels during pregnancy, which is important for normal uterine function (78). Studies of TRPV6$^{-/-}$ mice during embryogenesis and development will be important to elucidate the exact role of TRPV6 and of its contribution to Ca^{2+} homeostasis during different stages of organogenesis, especially in the intestine and kidney.

Insights into P_i and Ca^{2+} homeostasis from FGF23 and klotho knockout mice

The recent generation of FGF23$^{-/-}$ mice, by two independent research groups, confirmed a central role for this hormone in P_i homeostasis (38,49). The FGF23$^{-/-}$ mice display hyperphosphatemia, hypervitaminosis D, growth retardation and limb deformation. Crossing the FGF23$^{-/-}$ mice with 1αOHase-deficient animals rescued much of this phenotype (79). Specifically the atherosclerosis and ectopic skeletal and soft tissue calcifications observed in the FGF23$^{-/-}$ animals are absent from the FGF23/1αOHase double knockout mice. The double knockout animals also have increased survival. Of note the phenotype is not rescued completely, the FGF23/1αOHase double knockout mice have a further reduction in bone mineral density in comparison to the FGF23$^{-/-}$ mice. Together these findings strongly suggest that the aging-like features, disturbed electrolyte levels, and impaired skeletogenesis in FGF23$^{-/-}$ mice are the result of increased blood $1,25(OH)_2D_3$ levels (79,80). To further elucidate the role of $1,25(OH)_2D_3$ action in FGF23$^{-/-}$ mice, Hesse and co-workers cross-bred FGF23$^{-/-}$ mice with VDR knockout (VDR$^{-/-}$) mice (81). FGF23/VDR double knockout mice display a similar phenotype to the VDR$^{-/-}$ mice, suggesting that the aging-like symptoms in FGF23$^{-/-}$ mice depend upon intact signaling through the VDR (82). It is known that FGF23 expression is itself regulated by dietary P_i and $1,25(OH)_2D_3$, suggesting a regulatory feedback mechanism in which $1,25(OH)_2D_3$ stimulates FGF23 production, that in turn inhibits 1αOHase, thereby decreasing circulating $1,25(OH)_2D_3$ levels (83,84).

Klotho$^{-/-}$ mice display a phenotype similar to FGF23$^{-/-}$ mice, which includes hyperphosphatemia, hypervitaminosis D and bone abnormalities (32). The fact that the interplay of klotho and FGFRs is essential for FGF23 functioning and the finding that both klotho$^{-/-}$ and FGF23$^{-/-}$ mouse strains display comparable symptoms indicates that this newly identified klotho/FGF23 regulatory pathway is essential to P_i homeostasis (48,50). Klotho$^{-/-}$ mice also demonstrate other phenotypic features not described in FGF23$^{-/-}$ mice, including pulmonary, neuronal, and skin disorders, implying that klotho may interact with other FGFRs throughout the body (32,50). How klotho causes these additional phenotypic features and whether other FGF family members mediate them requires further investigation.

Outlook

The work described in this review has greatly increased our knowledge of Ca^{2+} and P_i homeostasis; however, several questions remain. Identification of the antiaging hormone klotho as a modulator of TRPV5 activity and its interaction with

FGFRs permitting increased FGF23 signaling have been major breakthroughs in the understanding of both Ca^{2+} and P_i handling. An important tool in the elucidation of these research questions has been the use of knockout animal models. In this regard, it remains a challenge to determine the exact role of $1,25(OH)_2D_3$ in both klotho- and FGF23-mediated signaling. It will be through further investigation of these newly identified regulatory pathways, including klotho, FGF23, and $1,25(OH)_2D_3$, that a clearer understanding of how the body controls Ca^{2+} and P_i balance will be achieved. This knowledge will be crucial for the manipulation of either klotho or FGF23 to a therapeutic end point.

Acknowledgements

This work was financially supported in part by grants from the Dutch Kidney Foundation (C03.6017 and C06.2170), the Netherlands Organization for Scientific Research (Zon-Mw 016.006.001, NWO-ALW 814.02.001, NWO-CW 700.55.302), and the Dutch Stomach-Intestine-Liver foundation (MWO 03-19). J. Hoenderop and R. T. Alexander are supported by an EURYI and a CIHR Clinician-Scientist award, respectively. We thank the members of our laboratory for valuable discussion and advice.

References

1. Hoenderop JG, van Leeuwen JP, van der Eerden BC, Kersten FF, van der Kemp AW, Merillat AM, et al. Renal Ca2+ wasting, hyperabsorption, and reduced bone thickness in mice lacking TRPV5. J Clin Invest. 2003;112:1906–14.
2. Takeda E, Yamamoto H, Nashiki K, Sato T, Arai H, Taketani Y. Inorganic phosphate homeostasis and the role of dietary phosphorus. J Cell Mol Med. 2004;8:191–200.
3. Hurwitz S. Homeostatic control of plasma calcium concentration. Crit Rev Biochem Mol Biol. 1996;31:41–100.
4. Torres PU, Prie D, Molina-Bletry V, Beck L, Silve C, Friedlander G. Klotho: An antiaging protein involved in mineral and vitamin D metabolism. Kidney Int. 2007;71:730–7.
5. Shimada T, Hasegawa H, Yamazaki Y, Muto T, Hino R, Takeuchi Y, et al. FGF-23 is a potent regulator of vitamin D metabolism and phosphate homeostasis. J Bone Miner Res. 2004;19:429–35.
6. Capuano P, Radanovic T, Wagner CA, Bacic D, Kato S, Uchiyama Y, et al. Intestinal and renal adaptation to a low-Pi diet of type II NaPi cotransporters in vitamin D receptor- and 1alphaOHase-deficient mice. Am J Physiol Cell Physiol. 2005;288:C429–34.
7. van Abel M, Hoenderop JG, van der Kemp AW, van Leeuwen JP, Bindels RJ. Regulation of the epithelial Ca^{2+} channels in small intestine as studied by quantitative mRNA detection. Am J Physiol Gastrointest Liver Physiol. 2003;285:G78–85.
8. Slatopolsky E, Delmez JA. Pathogenesis of secondary hyperparathyroidism. Am J Kidney Dis. 1994;23:229–36.
9. Radanovic T, Wagner CA, Murer H, Biber J. Regulation of intestinal phosphate transport. I. Segmental expression and adaptation to low-P_i diet of the type IIb Na^+-P_i cotransporter in mouse small intestine. Am J Physiol Gastrointest Liver Physiol. 2005;288:G496–500.
10. Hoenderop JG, Nilius B, Bindels RJ. Calcium absorption across epithelia. Physiol Rev. 2005;85:373–422.
11. Murer H, Hernando N, Forster I, Biber J. Proximal tubular phosphate reabsorption: molecular mechanisms. Physiol Rev. 2000;80:1373–409.
12. Peng JB, Chen XZ, Berger UV, Vassilev PM, Tsukaguchi H, Brown EM, et al. Molecular cloning and characterization of a channel-like transporter mediating intestinal calcium absorption. J Biol Chem. 1999;274:22739–46.
13. Hoenderop JG, Bindels RJ. Epithelial Ca2+ and Mg2+ channels in health and disease. J Am Soc Nephrol. 2005;16:15–26.
14. Hilfiker H, Hattenhauer O, Traebert M, Forster I, Murer H, Biber J. Characterization of a murine type II sodium-phosphate cotransporter expressed in mammalian small intestine. Proc Natl Acad Sci U S A. 1998;95:14564–9.
15. Collins JF, Ghishan FK. Molecular cloning, functional expression, tissue distribution, and in situ hybridization of the renal sodium phosphate (Na+/Pi) transporter in the control and hypophosphatemic mouse. FASEB J. 1994;8:862–8.
16. Hernando N, Gisler SM, Pribanic S, Deliot N, Capuano P, Wagner CA, et al. NaPi-IIa and interacting partners. J Physiol. 2005;567:21–6.
17. Forster IC, Hernando N, Biber J, Murer H. Proximal tubular handling of phosphate: A molecular perspective. Kidney Int. 2006;70:1548–59.
18. Chang W, Pratt S, Chen TH, Nemeth E, Huang Z, Shoback D. Coupling of calcium receptors to inositol phosphate and cyclic AMP generation in mammalian cells and Xenopus laevis oocytes and immunodetection of receptor protein by region-specific antipeptide antisera. J Bone Miner Res. 1998;13:570–80.
19. Riccardi D, Park J, Lee WS, Gamba G, Brown EM, Hebert SC. Cloning and functional expression of a rat kidney extracellular calcium/polyvalent cation-sensing receptor. Proc Natl Acad Sci U S A. 1995;92:131–5.
20. Brown EM, Gamba G, Riccardi D, Lombardi M, Butters R, Kifor O, et al. Cloning and characterization of an extracellular Ca2+-sensing receptor from bovine parathyroid. Nature. 1993;366:575–80.
21. Muller D, Hoenderop JG, Merkx GF, van Os CH, Bindels RJ. Gene structure and chromosomal mapping of human epithelial calcium channel. Biochem Biophys Res Commun. 2000;275:47–52.
22. Weber K, Erben RG, Rump A, Adamski J. Gene structure and regulation of the murine epithelial calcium channels ECaC1 and 2. Biochem Biophys Res Commun. 2001;289:1287–94.
23. Hoenderop JG, Dardenne O, Van Abel M, Van Der Kemp AW, Van Os CH, St Arnaud R, et al. Modulation of renal Ca2+ transport protein genes by dietary Ca2+ and 1,25-dihydroxyvitamin D3 in 25-hydroxyvitamin D3-1alpha-hydroxylase knockout mice. FASEB J. 2002;16:1398–406.
24. Hoenderop JG, Muller D, Van Der Kemp AW, Hartog A, Suzuki M, Ishibashi K, et al. Calcitriol controls the epithelial calcium channel in kidney. J Am Soc Nephrol. 2001;12:1342–9.
25. Fudge NJ, Kovacs CS. Physiological studies in heterozygous calcium sensing receptor (CaSR) gene-ablated mice confirm

that the CaSR regulates calcitonin release in vivo. BMC Physiol. 2004;4:5.
26. Prince RL. Counterpoint: estrogen effects on calcitropic hormones and calcium homeostasis. Endocr Rev. 1994;15: 301–9.
27. Young MM, Nordin BE. The effect of the natural and artificial menopause on bone density and fracture. Proc R Soc Med. 1969;62:242.
28. Van Abel M, Hoenderop JG, Dardenne O, St Arnaud R, Van Os CH, Van Leeuwen HJ, et al. 1,25-dihydroxyvitamin D3-independent stimulatory effect of estrogen on the expression of ECaC1 in the kidney. J Am Soc Nephrol. 2002;13:2102–9.
29. van Abel M, Hoenderop JG, van der Kemp AW, Friedlaender MM, van Leeuwen JP, Bindels RJ. Coordinated control of renal Ca2+ transport proteins by parathyroid hormone. Kidney Int. 2005;68:1708–21.
30. Picard N, Van Abel M, Campone C, Seiler M, Bloch-Faure M, Hoenderop JG, et al. Tissue kallikrein-deficient mice display a defect in renal tubular calcium absorption. J Am Soc Nephrol. 2005;16:3602–10.
31. Gkika D, Topala CN, Chang Q, Picard N, Thebault S, Houillier P, et al. Tissue kallikrein stimulates Ca2+ reabsorption via PKC-dependent plasma membrane accumulation of TRPV5. EMBO J. 2006;25:4707–16.
32. Kuro-o M, Matsumura Y, Aizawa H, Kawaguchi H, Suga T, Utsugi T, et al. Mutation of the mouse klotho gene leads to a syndrome resembling ageing. Nature. 1997;390:45–51.
33. Chang Q, Hoefs S, van der Kemp AW, Topala CN, Bindels RJ, Hoenderop JG. The beta-glucuronidase klotho hydrolyzes and activates the TRPV5 channel. Science. 2005;310:490–3.
34. Imura A, Iwano A, Tohyama O, Tsuji Y, Nozaki K, Hashimoto N, et al. Secreted Klotho protein in sera and CSF: implication for post-translational cleavage in release of Klotho protein from cell membrane. FEBS Lett. 2004;565:143–7.
35. Imura A, Tsuji Y, Murata M, Maeda R, Kubota K, Iwano A, et al. alpha-Klotho as a regulator of calcium homeostasis. Science. 2007;316:1615–8.
36. Katai K, Miyamoto K, Kishida S, Segawa H, Nii T, Tanaka H, et al. Regulation of intestinal Na^+-dependent phosphate co-transporters by a low-phosphate diet and 1,25-dihydroxyvitamin D_3. Biochem J. 1999;343Pt 3, 705–12.
37. Bacic D, Lehir M, Biber J, Kaissling B, Murer H, Wagner CA. The renal Na+/phosphate cotransporter NaPi-IIa is internalized via the receptor-mediated endocytic route in response to parathyroid hormone. Kidney Int. 2006;69:495–503.
38. Shimada T, Kakitani M, Yamazaki Y, Hasegawa H, Takeuchi Y, Fujita T, et al. Targeted ablation of Fgf23 demonstrates an essential physiological role of FGF23 in phosphate and vitamin D metabolism. J Clin Invest. 2004;113:561–8.
39. Riminucci M, Collins MT, Fedarko NS, Cherman N, Corsi A, White KE, et al. FGF-23 in fibrous dysplasia of bone and its relationship to renal phosphate wasting. J Clin Invest. 2003;112:683–92.
40. Berndt TJ, Schiavi S, Kumar R. "Phosphatonins" and the regulation of phosphorus homeostasis. Am J Physiol Renal Physiol. 2005;289:F1170–82.
41. ADHR Consortium, Autosomal dominant hypophosphataemic rickets is associated with mutations in FGF23. Nat Genet. 2000;26:345–8.
42. White KE, Carn G, Lorenz-Depiereux B, Benet-Pages A, Strom TM, Econs MJ. Autosomal-dominant hypophosphatemic rickets (ADHR) mutations stabilize FGF-23. Kidney Int. 2001;60:2079–86.
43. Benet-Pages A, Orlik P, Strom TM, Lorenz-Depiereux B. An FGF23 missense mutation causes familial tumoral calcinosis with hyperphosphatemia. Hum Mol Genet. 2005;14:385–90.
44. Larsson T, Yu X, Davis SI, Draman MS, Mooney SD, Cullen MJ, et al. A novel recessive mutation in fibroblast growth factor-23 causes familial tumoral calcinosis. J Clin Endocrinol Metab. 2005;90:2424–7.
45. Masuyama R, Stockmans I, Torrekens S, Van Looveren R, Maes C, Carmeliet P, et al. Vitamin D receptor in chondrocytes promotes osteoclastogenesis and regulates FGF23 production in osteoblasts. J Clin Invest. 2006;116:3150–9.
46. Mirams M, Robinson BG, Mason RS, Nelson AE. Bone as a source of FGF23: regulation by phosphate? Bone. 2004;35:1192–9.
47. Yu X, Ibrahimi OA, Goetz R, Zhang F, Davis SI, Garringer HJ, et al. Analysis of the biochemical mechanisms for the endocrine actions of fibroblast growth factor-23. Endocrinology. 2005;146:4647–56.
48. Urakawa I, Yamazaki Y, Shimada T, Iijima K, Hasegawa H, Okawa K, et al. Klotho converts canonical FGF receptor into a specific receptor for FGF23. Nature. 2006;444:770–4.
49. Sitara D, Razzaque MS, Hesse M, Yoganathan S, Taguchi T, Erben RG, et al. Homozygous ablation of fibroblast growth factor-23 results in hyperphosphatemia and impaired skeletogenesis, and reverses hypophosphatemia in Phex-deficient mice. Matrix Biol. 2004;23:421–32.
50. Kurosu H, Ogawa Y, Miyoshi M, Yamamoto M, Nandi A, Rosenblatt KP, et al. Regulation of fibroblast growth factor-23 signaling by klotho. J Biol Chem. 2006;281:6120–3.
51. Drueke TB, Prie D. Klotho spins the thread of life—what does Klotho do to the receptors of fibroblast growth factor-23 (FGF23)? Nephrol Dial Transplant. 2007;22:1524–6.
52. Ogawa Y, Kurosu H, Yamamoto M, Nandi A, Rosenblatt KP, Goetz R, et al. betaKlotho is required for metabolic activity of fibroblast growth factor 21. Proc Natl Acad Sci U S A. 2007;104:7432–7.
53. Kharitonenkov A, Shiyanova TL, Koester A, Ford AM, Micanovic R, Galbreath EJ, et al. FGF-21 as a novel metabolic regulator. J Clin Invest. 2005;115:1627–35.
54. Powers CJ, McLeskey SW, Wellstein A. Fibroblast growth factors, their receptors and signaling. Endocr Relat Cancer. 2000;7:165–97.
55. Carpenter TO, Ellis BK, Insogna KL, Philbrick WM, Sterpka J, Shimkets R. Fibroblast growth factor 7: an inhibitor of phosphate transport derived from oncogenic osteomalacia-causing tumors. J Clin Endocrinol Metab. 2005;90:1012–20.
56. Nemeth EF, Steffey ME, Hammerland LG, Hung BC, Van Wagenen BC, DelMar EG, et al. Calcimimetics with potent and selective activity on the parathyroid calcium receptor. Proc Natl Acad Sci U S A. 1998;95:4040–5.
57. Lloyd SE, Pearce SH, Fisher SE, Steinmeyer K, Schwappach B, Scheinman SJ, et al. A common molecular basis for three inherited kidney stone diseases. Nature. 1996;379:445–9.
58. Clark JY, Thompson IM, Optenberg SA. Economic impact of urolithiasis in the United States. J Urol. 1995;154:2020–4.
59. Stamatelou KK, Francis ME, Jones CA, Nyberg LM, Curhan GC. Time trends in reported prevalence of kidney stones in the United States: 1976–1994. Kidney Int. 2003;63:1817–23.
60. Pearle MS, Calhoun EA, Curhan GC. Urologic diseases in America project: urolithiasis. J Urol. 2005;173:848–57.

61. Curhan GC, Willett WC, Rimm EB, Stampfer MJ. Family history and risk of kidney stones. J Am Soc Nephrol. 1997;8:1568–73.
62. Bianco SD, Peng JB, Takanaga H, Suzuki Y, Crescenzi A, Kos CH, et al. Marked disturbance of calcium homeostasis in mice with targeted disruption of the Trpv6 calcium channel gene. J Bone Miner Res. 2007;22:274–85.
63. Muller D, Hoenderop JG, Vennekens R, Eggert P, Harangi F, Mehes K, et al. Epithelial Ca2+ channel (ECAC1) in autosomal dominant idiopathic hypercalciuria. Nephrol Dial Transplant. 2002;17:1614–20.
64. Akey JM, Swanson WJ, Madeoy J, Eberle M, Shriver MD. TRPV6 exhibits unusual patterns of polymorphism and divergence in worldwide populations. Hum Mol Genet. 2006;15:2106–13.
65. Econs MJ, McEnery PT. Autosomal dominant hypophosphatemic rickets/osteomalacia: clinical characterization of a novel renal phosphate-wasting disorder. J Clin Endocrinol Metab. 1997;82:674–81.
66. Econs MJ, Drezner MK. Tumor-induced osteomalacia—unveiling a new hormone. N Engl J Med. 1994;330:1679–81.
67. Cai Q, Hodgson SF, Kao PC, Lennon VA, Klee GG, Zinsmiester AR, et al. Brief report: inhibition of renal phosphate transport by a tumor product in a patient with oncogenic osteomalacia. N Engl J Med. 1994;330:1645–9.
68. Sabbagh Y, Jones AO, Tenenhouse HS. PHEXdb, a locus-specific database for mutations causing X-linked hypophosphatemia. Hum Mutat. 2000;16:1–6.
69. Tieder M, Modai D, Samuel R, Arie R, Halabe A, Bab I, et al. Hereditary hypophosphatemic rickets with hypercalciuria. N Engl J Med. 1985;312:611–7.
70. Hsu YJ, Hoenderop JG, Bindels RJ. TRP channels in kidney disease. Biochim Biophys Acta. 2007;1772:928–36.
71. Shigematsu T, Kazama JJ, Yamashita T, Fukumoto S, Hosoya T, Gejyo F, et al. Possible involvement of circulating fibroblast growth factor 23 in the development of secondary hyperparathyroidism associated with renal insufficiency. Am J Kidney Dis. 2004;44:250–6.
72. Pande S, Ritter CS, Rothstein M, Wiesen K, Vassiliadis J, Kumar R, et al. FGF-23 and sFRP-4 in chronic kidney disease and post-renal transplantation. Nephron Physiol. 2006;104:23–32.
73. Miyamoto K, Ito M, Segawa H, Kuwahata M. Molecular targets of hyperphosphataemia in chronic renal failure. Nephrol Dial Transplant. 2003;18Suppl 3:iii79–80.
74. Locatelli F, Cannata-Andia JB, Drueke TB, Horl WH, Fouque D, Heimburger O, et al. Management of disturbances of calcium and phosphate metabolism in chronic renal insufficiency, with emphasis on the control of hyperphosphataemia. Nephrol Dial Transplant. 2002;17:723–31.
75. Fliser D, Kollerits B, Neyer U, Ankerst DP, Lhotta K, Lingenhel A, et al. Fibroblast Growth Factor 23 (FGF23) Predicts Progression of Chronic Kidney Disease: The Mild to Moderate Kidney Disease (MMKD) Study. J Am Soc Nephrol. 2007;18:2600–8.
76. Renkema KY, Nijenhuis T, van der Eerden BC, van der Kemp AW, Weinans H, van Leeuwen JP, et al. Hypervitaminosis D mediates compensatory Ca2+ hyperabsorption in TRPV5 knockout mice. J Am Soc Nephrol. 2005;16:3188–95.
77. Gkika D, Hsu YJ, van der Kemp AW, Christakos S, Bindels RJ, Hoenderop JG. Critical role of the epithelial Ca2+ channel TRPV5 in active Ca2+ reabsorption as revealed by TRPV5/calbindin-D28K knockout mice. J Am Soc Nephrol. 2006;17:3020–7.
78. Lee GS, Jeung EB. Uterine TRPV6 expression during estrous cycle and pregnancy in a mouse model. Am J Physiol Endocrinol Metab. 2007;293:E132–8.
79. Razzaque MS, Sitara D, Taguchi T, St-Arnaud R, Lanske B. Premature aging-like phenotype in fibroblast growth factor 23 null mice is a vitamin D-mediated process. FASEB J. 2006;20:720–2.
80. Sitara D, Razzaque MS, St Arnaud R, Huang W, Taguchi T, Erben RG, et al. Genetic ablation of vitamin D activation pathway reverses biochemical and skeletal anomalies in Fgf-23-null animals. Am J Pathol. 2006;169:2161–70.
81. Erben RG, Soegiarto DW, Weber K, Zeitz U, Lieberherr M, Gniadecki R, et al. Deletion of deoxyribonucleic acid binding domain of the vitamin D receptor abrogates genomic and nongenomic functions of vitamin D. Mol Endocrinol. 2002;16:1524–37.
82. Hesse M, Frohlich LF, Zeitz U, Lanske B, Erben RG. Ablation of vitamin D signaling rescues bone, mineral, and glucose homeostasis in Fgf-23 deficient mice. Matrix Biol. 2007;26:75–84.
83. Saito H, Maeda A, Ohtomo S, Hirata M, Kusano K, Kato S, et al. Circulating FGF-23 is regulated by 1alpha,25-dihydroxyvitamin D3 and phosphorus in vivo. J Biol Chem. 2005;280:2543–9.
84. Barthel TK, Mathern DR, Whitfield GK, Haussler CA, Hopper HA 4th, Hsieh JC, et al. 1,25-Dihydroxyvitamin D3/VDR-mediated induction of FGF23 as well as transcriptional control of other bone anabolic and catabolic genes that orchestrate the regulation of phosphate and calcium mineral metabolism. J Steroid Biochem Mol Biol. 2007;103:381–8.