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Mineral metabolism and aging: the FGF-23 enigma

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Abstract

Purpose of review—Our understanding of the regulation of mineral ion homeostasis is rather stagnant, and until recently the regulation of phosphate homeostasis was thought to be a passive process mediated largely by the well known calciotrophic hormones PTH and $1,25(OH)_2D_3$. This article summarizes the emerging trends that **i**) show an active regulation of phosphate homeostasis by FGF-23, a process that appears to be fairly independent of calcium homeostasis, and **ii**) how altered mineral ion metabolism might affect the aging process.

Recent findings—A major breakthrough in FGF-23 biology has been achieved by the demonstration of strikingly similar physical and biochemical phenotypes of *Fgf-23* knockout and *klotho* hypomorph mice, which eventually led to the identification of klotho as a cofactor in FGF-23 and its receptor interactions. A new regulatory pathway has been defined, where FGF23, in presence of klotho has shown to activate downstream signaling events, by phosphorylation of FGF receptor substrate-2α, ERK, and Egr-1. Furthermore, FGF-23 has emerged as a counter regulatory hormone that influences 1α(OH)ase and NaPi2a activities in the kidney to regulate phosphate homeostasis. Finally, studies also point towards a role of DMP1 in influencing the regulation of phosphate homeostasis, in coordination with FGF-23.

Summary—Recent *in vivo* mouse genetic studies have expanded our understanding of the biochemical and molecular pathways involved in phosphate homeostasis, and linked FGF-23 to the genesis of such regulation. A clear understanding of the molecular interactions of essential calcium and phosphate regulating factors will enhance our understanding of the coordinated regulation of mineral ion metabolism, and will help us to redefine the molecular pathology of age-associated lesions accompanied by abnormal mineral ion metabolism, that include but are not limited to vascular calcifications, and osteoporosis.

Keywords

Fibroblast growth factor 23; Klotho; Vitamin-D; Mineral ion homeostasis; Ageing

Introduction

Maintaining normal calcium and phosphate homeostasis is crucial for various essential biological activities that include but are not limited to energy metabolism, signaling activities, and normal skeletal growth, development and function; altered mineral metabolism can affect the functionality of almost any tissue or organ system. In spite of its wide biological importance and significance to maintain normal physiologic functions, it is not yet clearly understood how normal mineral ion homeostasis is coordinately regulated. Identification of fibroblast growth factor-23 (FGF-23), as a “phosphatonin” in the year 2000 has significantly enhanced our

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understanding of mineral metabolism [1,2]. A major breakthrough of how FGF-23 exerts its bioactivities has been achieved by the recent demonstration of strikingly similar physical and biochemical phenotypes of *Fgf-23* knockout and *klotho* hypomorph mice [3] which helped in the identification of klotho as a cofactor in FGF-23 and its receptor interactions [4,5].

Though much progress has been made in recent years in terms of identification of the mineral ion regulating molecules [6–11], it is not yet clear how they interact with each other to coordinately regulate mineral ion homeostasis. For instance, it is presumed that proximal tubules are the most likely site where FGF-23 could exert its bioactivities to regulate and maintain phosphate homeostasis, but the exact mode of action and interaction of FGF-23 with other calcium and phosphate regulating molecules in the kidney is not yet clear. The autocrine, paracrine, or endocrine functions of FGF-23, and how it interacts or influences molecules that are also involved in maintaining mineral ion homeostasis including *transient receptor potential cation channel, subfamily V, member 5* (TRPV5), sodium-phosphate co-transporter 2a (NaPi2a), $Calbindin_{28k}$, Na^{+}/Ca^{2+}-exchanger (NCX1), and plasma membrane ATPase (PMCA1b) in the kidney requires additional studies [11]. Another important aspect that needs further clarification is the interrelationship among FGF-23, klotho and vitamin-D [12].

Fibroblast growth factor 23

FGF-23 is a 30 kDa-secreted protein that is processed by a pro-convertase type enzyme into two smaller fragments of approximately 18 kDa (amino fragment) and 12 kDa (carboxy fragment); the exact functions of these fragments are not clear, and of intense focus of research. FGF-23 is mainly produced in bone by osteocytes [13]. Although the *in vivo* phosphaturic effect of the full-length synthetic FGF-23 protein is well-documented, the precise *in vivo* role of C-terminal and N-terminal fragments of FGF-23 needs additional studies [14]. Since the canonical FGF receptor (FGFR) binding domain is not present in C-terminal fragments of FGF-23, any *in vivo* activity by this fragment would suggest the existence of a novel receptor, in addition to the known classic FGF receptor signaling pathway [15].

Altered regulation of FGF-23 has direct relevance to human diseases; gain-of-function mutations of FGF-23 have shown to be associated with autosomal dominant hypophosphatemic rickets (ADHR) [1]; these mutations have been shown to prevent proteolytic cleavage of the FGF-23 protein, with net effect being phosphate wasting and skeletal defects, perhaps due to enhanced biologic activities of FGF-23. Similarly, increased serum level of FGF-23 in patients with oncogenic osteomalacia (OOM) is believed to be the causative factor for tumor-induced phosphate-wasting [16]. Jonsson et al. demonstrated that a markedly elevated serum level of FGF-23 is associated with renal phosphate wasting in patients with OOM. Patients affected by X-linked hypophosphatemia (XLH), which is caused by inactivating mutations of the gene encoding the phosphate regulating gene with homologies to endopeptidases on the X chromosome (PHEX), exhibit increased FGF-23 serum levels leading to phosphate wasting and osteomalacia in these individuals [17]. Recent studies have found the presence of inactivating mutations in the dentin matrix protein 1 (*DMP1*) gene in patients affected with autosomal recessive hypophosphatemia (ARHP), a rare genetic disorder with essentially similar clinical features as those seen in patients with OOM, XLH and ADHR [18,19]; such clinical similarities are thought to be due to increased circulating intact FGF-23 protein [18, 19]. DMP1 belongs to the Small Integrin-Binding Ligand N-linked Glycoprotein (SIBLING) family, and is thought to be involved in osteoblastic/odontoblastic differentiation, expression of osteocalcin, and extracellular mineralization [20,21]. The other members of SIBLING family include bone sialoprotein (BSP), osteopontin, dentin sialophosphoprotein (DSPP) and matrix extracellular phosphoglycoprotein (MEPE). Genetic ablation of *Dmp1* in mice results in increased circulating Fgf-23 serum levels, possibly due to overexpression of the *Fgf-23* gene by osteocytes [19]. It is, therefore, likely that the clinical features in patients with ARHP and

the phenotypes of *Dmp1* null mice are due to increased activity of FGF-23. Phosphate wasting and skeletal mineral deposition defects are also the two main phenotypes of *FGF-23* transgenic mice [22–24], with close resemblance to the symptoms described in patients with XLH, OOM, ADHR and ARHP.

In contrast to the transgenic animals, the phenotype of *Fgf-23* null animals mimics patients with familial tumoral calcinosis (FTC), a human disorder characterized by ectopic calcifications and elevated serum levels of phosphate due to loss-of-function mutations in the *FGF-23* gene [25,26]. Recently missense mutations in the gene encoding the glycosyltransferase polypeptide GalNAc-T3, which is involved in initiation of *O*-glycosylation, have shown to be associated with hyperostosis-hyperphosphatemia syndrome (HHS) and FTC [27,28]. Both FTC and HHS are autosomal recessive disorders characterized by unusually normal or elevated serum levels of vitamin-D, hyperphosphatemia, and ectopic calcifications. The fact that two different genes can cause the same human disease suggests a possible role for GalNAc-T3-mediated glycosylation in controlling FGF-23 activity. Studies have shown that the secretion of FGF-23 is indeed dependent on *O*-glycosylation, as GalNAc-T3 can glycosylate the subtilisin-like proprotein convertase (SPC) signal sequence of FGF-23 at Thr^{178} [29].

Interestingly, the phenotypes of *Fgf-23* knockout mice [3] that include but are not limited to infertility, kyphosis, atherosclerosis, skin atrophy, muscle wasting, T-cell dysregulation, pulmonary emphysema, altered mineral ion metabolism, rickets, and shortened lifespan are essentially very similar to the phenotypes of *klotho* ablated mice [30,31], and such observations in *Fgf-23* null mice have facilitated the identification of a novel FGF-23 signaling pathway that also involves klotho.

FGF-23 and receptor interaction

FGF-23 is a distinct member of FGF family and contains a pro-convertase processing site. Unlike other members of the FGF family, whether FGF-23 also needs heparin-like molecules to activate the fibroblast growth factor receptor (FGFR) system is an ongoing area of research [15]; but FGF-23, in presence of klotho could activate downstream signaling molecules, as determined by activation or phosphorylation of FGF receptor substrate-2a, extracellular signal-regulated kinase (ERK) and early growth response element-1 (Egr-1) [32]; it has been shown that cells exposed to FGF-23 underwent ERK phosphorylation and increased their abundance of Egr-1 protein only after transfection with klotho [4,5]. The full length and/or the extracellular domain of the klotho protein could bind to various FGFRs [5] which have signal-transducing extracellular ligand-binding domains and an intracellular tyrosine kinase domain.

The klotho gene encodes a single-pass transmembrane protein; the extracellular domain of Klotho protein consists of two homologous domains that share sequence homology to the β-glucosidase of bacteria and plants. The klotho extracellular domain does not directly bind to FGF-23, but it enhances FGF-23 binding to its receptor complex with much higher affinity than to FGF receptor alone, implicating klotho as a cofactor in FGF23-FGFR interaction and subsequent signaling [5]. Urakawa *et al.* from their experiments claimed that only FGFR1(IIIc), in combination with klotho, could induce significant FGF23 signaling, while basic FGF, a universal ligand for FGFRs could generate downstream signaling without klotho, therefore speculating a possibility of klotho to selectively convert FGFR1(IIIc) into a specific FGF23 receptor [4]. Our understanding of FGF-23 and its receptor interactions will pave the way to determine downstream FGF-23 signaling events, identify the FGF-23 responsive genes and, more importantly their collective functions in the regulation of mineral ion metabolism, and their yet to be clarified functions in the aging process.

Possible role of FGF-23 in the premature aging of *klotho* mutant mice

Aging is a complex biological process that includes multi-organ, multi-system pathologies [33–36]. Suitable mammalian models for aging with a wide range of age-associated pathologies are desirable to study molecular mechanisms of human aging. *Klotho* mutant mice have shown to generate multiple premature aging-like features, including shortened lifespan, impaired sexual maturation leading to infertility, kyphosis, atherosclerosis, extensive soft tissue calcifications, skin atrophy, muscles wasting, T-cell dysregulation, pulmonary emphysema, osteopenia, abnormal mineral ion metabolism, and impaired vitamin-D homeostasis; the inactivation of the gene that caused such extensive premature aging-like features was perceived as a novel aging gene, and was named *klotho*, after one of the three Fates who spins the thread of life [30,31]. However, recent studies suggest that premature aging-like features in *klotho* mutant mice are not the primary cause of klotho inactivation but rather the consequence of altered mineral metabolism due to lack of Fgf-23 activity in these mice [37]. Despite extremely high serum levels of Fgf-23 (about 2,000 fold higher) in *klotho* ablated mice, Fgf-23 is unable to induce phosphaturia in these animals [4]. Furthermore, deletion of *Fgf-23*, a molecule that is structurally different from klotho could induce identical phenotypes, as found in the *klotho* ablated mice [12]. It is, therefore, obvious that the strikingly similar physical, biochemical and morphological phenotypes of these mice, are either due to absence (*Fgf-23* null mice) or inability (*klotho* mutant mice) of Fgf-23 to exert its bioactivities [38]. Yet again, the underlying mechanisms of premature aging-like features of the *klotho* mutants are due to inability of Fgf-23 to exert its function in absence of klotho that leads to hypervitaminosis-D and altered mineral metabolism. That raises a very important question: if premature aging-like features and short lifespan of *klotho* null mice are due to the consequences of Fgf-23 inactivity, and not due to direct effects of inactivation of klotho, then how the lifespan of klotho transgenic mice are greatly increased?

How factors regulating mineral ion metabolism affect aging?

The roles of the factors that regulate mineral ion metabolism and affect aging need careful long-term studies. One of the mineral ion regulating molecules that has get particular attention in affecting aging process is vitamin-D; low levels of vitamin-D, that range from chronic hypovitaminosis, insufficiency to deficiency in elderly individuals are associated with osteoporosis or gradual loss of bone and thereby making the elderly individuals more vulnerable to fracture-related complications. Despite molecular understanding of vitamin-D synthesis [39], and apparently obvious pathological effects of its deficiency on skeletal mineralization, the randomized clinical trials of vitamin-D supplementation have produced differential outcomes. For instance, prophylaxis supplementation of vitamin-D to the elderly individuals (75 years or older), by providing a yearly intramuscular injection of ergocalciferol (150,000–300,000 IU) for 5 years did not produce any statistical difference, either in terms of occurrence of fractures or in total mortality between vitamin-D treated or untreated individuals [40]. Dawson-Hughes and coworkers [41] also failed to detect reduction in hip fractures in vitamin-D (cholecalciferol) and calcium supplemented elderly patients, although the investigators did find less non-vertebral fracture [41]. In a separate study conducted on 669 postmenopausal women, after adjustment for age, there was no difference in the risk of vertebral and non-vertebral fractures in women with 25-hydroxyvitamin-D level below 75 nmol/l or below 50 nmol/l, compared to women with higher vitamin-D levels; also there were no significant changes in bone mineral density [42]. In a relatively recent study with 36,282 postmenopausal women receiving calcium (daily dose of 1000 mg of calcium carbonate) and vitamin D supplements (daily dose of 400 IU of vitamin D_3) or placebo pills showed a modest benefit in preserving bone mass and only prevented hip fractures in certain age groups of women, but did not prevent other types of fractures or colorectal cancer [43]. During the study a fracture rate of 14 per 10,000 cases per year in the supplemented group compared to 16 per

10,000 per year in the placebo group (such reduction was not statistically significant) were noted; moreover, such vitamin-D and calcium supplementation was also associated with an about 17% increased rate of kidney stone formation [43].

Conversely, the uncontrolled consumption of vitamin-D supplements by the elderly individuals (53 to 73 years old) with osteoporosis has shown to induce occult vitamin-D intoxication, and the resultant effect being diminished bone mass; discontinuation of use of dietary supplements resulted in the normalization of serum levels of $25(OH)_2D$ leading to the recovery of the bone mineral density (annual increase of 1.9% ± 0.6%) and the normalization of the ratio of urinary calcium to creatinine [44]; a 3-year follow-up phase showed that the increase in bone mineral density persisted after initial recovery [44].

The observations that hypervitaminosis-D is associated with hypercalciuria and bone loss in the affected patients are comparable to the results obtained in some of the experimental studies with excessive vitamin-D activities; for instance, genetically altered mice that were ablated for the sodium phosphate co-transporters 2a (NaPi2a) have very high serum levels of $1,25(OH)_2D_3$, and exhibit hypercalciuria and skeletal anomalies [45]. Similarly, despite hypervitaminosis-D [46–48], such skeletal changes as osteopenia and/or osteomalacia (one would expect in vitamin-D deficiency state) are found both in *Fgf-23* and *klotho* deficient mice [3,12,13,31,47].

Hypervitaminosis-D and premature aging in *Fgf-23* and *klotho* mutant mice

Both *Fgf-23* and *klotho* deficient mice have increased renal expression of the *1α-hydroxylase [1α(OH)ase]* gene, accompanied by significantly elevated serum levels of $1,25(OH)_2D_3$ [13, 46–48]; such hypervitaminosis-D was associated with infertility, kyphosis, atherosclerosis, skin atrophy, muscle wasting, T-cell dysregulation, pulmonary emphysema, hyperphosphatemia, rickets, and shortened lifespan in both the mutant mice. A significant rescue of these phenotypes has been achieved either by reducing vitamin-D activities or by genetically ablating vitamin-D activities from *Fgf-23* and *klotho* ablated mice [12,48]. Reducing vitamin-D activities in *klotho* ablated mice by providing a vitamin-D deficient diet prevented formation of ectopic calcifications, gain of fertility, and most importantly prolonged survival, suggesting that the premature aging-like features in *klotho* mutant mice are the consequence of increased activity of vitamin-D [48]. In the same line, when vitamin-D activities were genetically ablated from *Fgf-23* null mice by deleting the *1α(OH)ase* gene, most of the phenotypes in *Fgf-23* null mice were either reversed or rescued in *Fgf-23*$^{-/-}$*/1α(OH)ase*$^{-/-}$ compound mutants [12,49]; in these compound mutants, the ectopic calcifications and generalized atrophic changes were rescued. It is, therefore, reasonable to conclude that most of the premature aging-like phenotypes in *Fgf-23* and *klotho* deficient mice are due to altered mineral ion homeostasis partly driven by hypervitaminosis-D; such increased vitamin-D activities are most likely the consequence of lack of activity of its counter regulatory hormone, i.e., Fgf-23 [3,12,50,51].

By reducing vitamin-D activities in *klotho* or *Fgf-23* ablated mice, the hyperphosphatemia was reversed, and the resultant effect being the disappearance of ectopic calcifications, and other premature aging-like features [3,48]. In the same line, both *TRPV-5* and *NaPi2a* knockout mice also have hypervitaminosis-D [11,45], but do not show widespread premature aging-like features, as noted in *klotho* or *Fgf-23* ablated mice, possibly because *TRPV-5* and *NaPi2a* null mice do not develop hyperphosphatemia. It, therefore, needs to be reemphasized that altered mineral ion homeostasis in form of hyperphosphatemia is the most important cause that leads to extensive soft tissue calcifications and other phenotypes of *Fgf-23* and *klotho* deficient mice.

Mechanisms of hyperphosphatemia in *Fgf-23* and *klotho* mutant mice

The sodium-dependent phosphate co-transport system in the kidney is mostly composed of the type 2a and type 2c cotransporters, localized in the apical surface of the proximal tubular epithelial cells; in the same line, type 2b cotransporters are present in the apical membrane of the intestinal epithelial cells [52]. The renal type 2a cotransporters are the major determinant that regulates plasma and urinary phosphate balance. NaPi2a levels in the apical surface of the proximal tubular cells is increased by the activity of $1,25(OH)_2D_3$, and is decreased by the activity of FGF-23, and PTH [52,53]. Similarly, the intestinal phosphate transport activity and NaPi2b levels are upregulated by the activity of $1,25(OH)_2D_3$ [54]. Of relevance, high phosphate diet decreases NaPi2a, and low phosphate diet increases NaPi2a levels.

Genomic ablation of the *Fgf-23* gene from mice leads to increased renal expression of NaPi2a (Figure-1) [13]; a similar increase in renal expression of NaPi2a was also found in klotho mutant mice [55]. It is mostly believed that increased activity of NaPi2a leads to increased reuptake of phosphate, leading to hyperphosphatemia that is consistently noted in these mutant mice. Low level of PTH is likely to facilitate apical presence of NaPi2a in both *Fgf-23* and *klotho* mutant mice, and thereby increased reabsorption of phosphate. Recent studies have provided genetic evidence to show that the upregulation of NaPi2a in *Fgf-23*$^{-/-}$ mice is not an epiphenomenon; ablation of NaPi2a from *Fgf-23*$^{-/-}$ mice reversed hypersphosphatemia to hypophosphatemia by 6 weeks of age in *NaPi2a*$^{-/-}$*/Fgf-23*$^{-/-}$ mice, and thereby providing a genetic evidence of role of NaPi2a in regulating renal phosphate homeostasis in *Fgf-23*$^{-/-}$ mice [13]. Similar studies will determine whether analogous phenomenon is also present in klotho mutant mice or not.

Clinical relevance of *in vivo* experimental mouse studies

From the experimental studies, it is reasonable to speculate that hypervitaminosis-D, in a hyperphosphatemic microenvironment, could facilitate induction of certain pathologic changes, including soft tissue and vascular calcifications that are mostly noted in the elderly population. A phenomenon appears to be very similar to the adverse effects of long-term use of active vitamin-D metabolites in patients with chronic kidney disease (CKD) with hyperphosphatemia; such treatment could facilitate abnormal calcification to increase the risk of cardiovascular death of CKD patients. Although the etiological diversity, along with multi-stage, multi-factorial events of CKD [56–58] make it clinically difficult to pinpoint one single risk factor, studies have suggested that excessive vitamin-D contribute to risk of increased serum accumulation of calcium, and incidence of calcification, which is associated with reduced survival and morbidity [59,60]. Abnormal tissue calcification is associated with almost half of the cardiovascular deaths among patients undergoing dialysis. Furthermore, continuous use of high calcium dialysate and prolonged administration of vitamin-D are the main underlying cause of tumoral calcinosis in patients with chronic renal failure [61]. Milliner et al. found that the probability of calcinosis was higher in patients receiving vitamin-D therapy [59], and withdrawal of vitamin-D therapy from the patients with tumoral calcinosis could markedly regress the lesion [62], emphasizing that reducing vitamin-D could ameliorate ectopic calcification. It is, however, necessary to mention that in an observational study with 242 patients undergoing hemodialysis, treatment with the vitamin-D analog (alfacalcidol) resulted in a significantly lower number of cardiovascular death when compared to the untreated group of patients [63]. Analyzing available information, the survival benefits and risk of vitamin-D therapy to CKD patients are not clear and an extremely debatable issue [64–66], although the direct impact of vitamin-D on mineral metabolism and calcification is beyond any doubt.

Even though the vitamin-D induced calcification in the disease state might not be comparable to the natural aging, there might be, however, similarities in the underlying mechanisms [67], as molecules involved in the mineral ion metabolism in normal and disease state are similar and a selective group of genes.

Conclusion

In this brief article, based on recent findings, we have presented our views on effects of altered mineral ion metabolism in generating features that are more likely to be present during aging. We also discussed why we believe that premature aging-like features of *klotho* mutant mice are actually due to the inability of Fgf-23 to exert its function in these mice. Moreover, lack of Fgf-23 activity in *Fgf-23* and *klotho* mutant mice eliminates the physiologic counter regulation of Fgf-23 and vitamin-D, leading to hypervitaminosis-D and hyperphosphatemia in these mutants and consequently induces most of the premature aging-like features. FGF-23, by affecting the activities of vitamin-D and altering mineral ion homeostasis might play a major role in the aging process that include but are not limited to senile osteoporosis and vascular calcifications. Finally, in this article, we wanted to highlight two important aspects related to aging: **1)** can factors regulating mineral ion homeostasis affect the aging process? The *in vivo* results of *Fgf-23* and *klotho* mutant mice clearly suggest such possibilities; that raises another important question **2)** is altered mineral ion metabolism a cause or a consequence of the aging process? The existing observations suggest that some of the age-associated pathologies are actually caused by altered mineral ion metabolism. Further *in vivo* studies will explain how factors coordinately maintain normal mineral ion homeostasis (Figure-2); at this stage, FGF-23 appears to be a very specific messenger that is produced in bone and conveys signals from the bone to the kidney and possibly to the parathyroid gland to regulate mineral metabolism. Our understanding of the physiologic regulation of mineral ion homeostasis will help us to explain how altered regulation of factors controlling mineral ion homeostasis could adversely affect the aging process.

Abbreviations

1α(OH)ase	1α-hydroxylase
ADHR	Autosomal dominant hypophosphatemic rickets
ARHP	autosomal recessive hypophosphatemia
CKD	Chronic kidney disease
DMP1	Dentine matrix protein 1
Egr-1	Early growth response element-1
ERK	Extracellular signal-regulated kinase
FGF	Fibroblast growth factor
FGFR	Fibroblast growth factor receptor
FTC	Familial tumoral calcinosis
HHS	Hyperostosis-hyperphosphatemia syndrome
NaPi	Sodium-phosphate cotransporter
OOM	Oncogenic osteomalacia
SIBLING	Small Integrin-Binding Ligand N-linked Glycoprotein
TRPV-5	Transient receptor potential cation channel, subfamily V, member 5

XLH	X-linked hypophosphatemia

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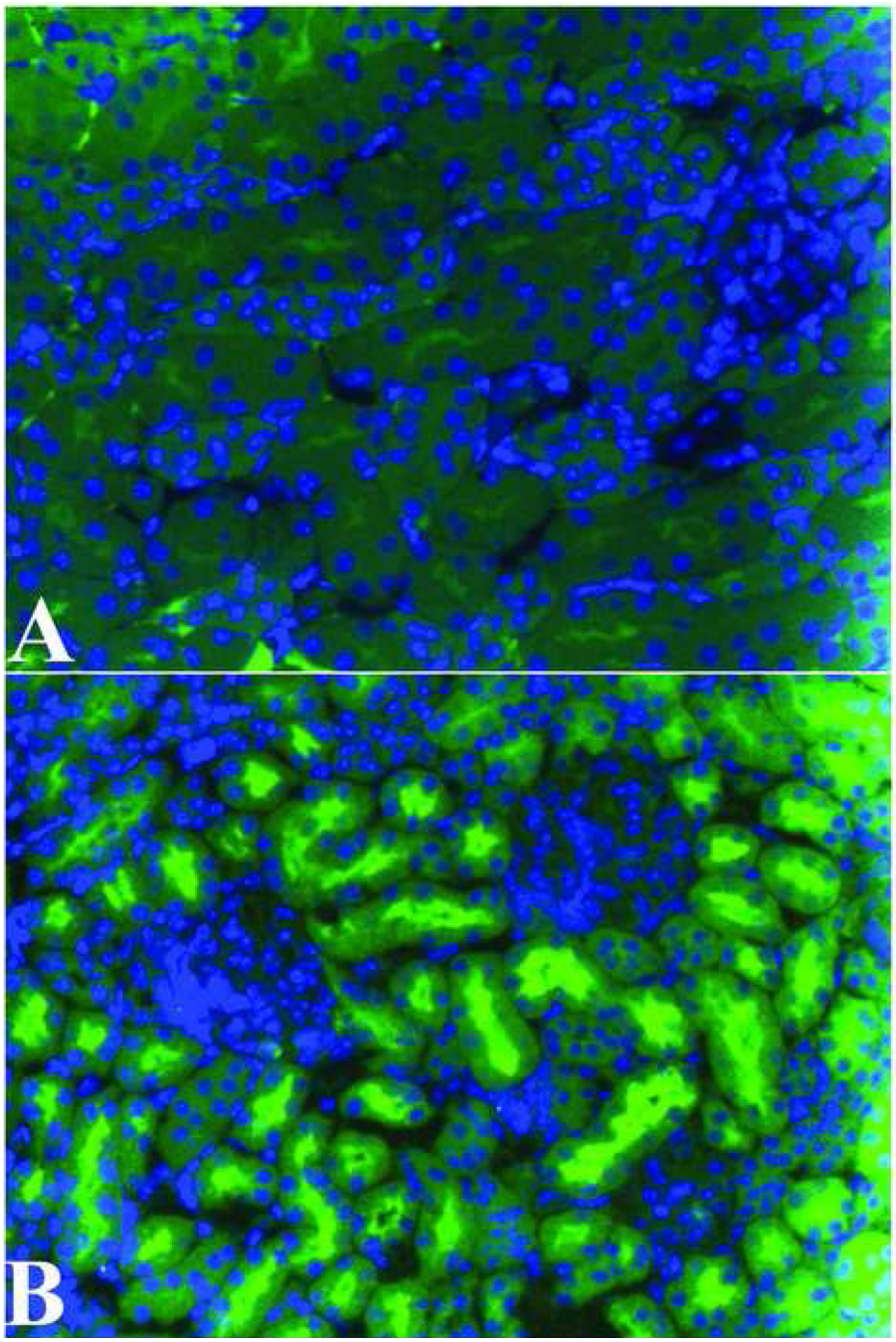

Figure-1.

Immunolocalization of NaPi2a in control (**A**) and *Fgf-23* null mice kidney (**B**). Note a markedly increased expression of NaPi2a in the luminal side of the proximal tubular epithelial cells of *Fgf-23* null mice (**B**). Increased expression of NaPi2a causes increased renal uptake of phosphate, leading to hyperphosphatemia in *Fgf-23* null mice.

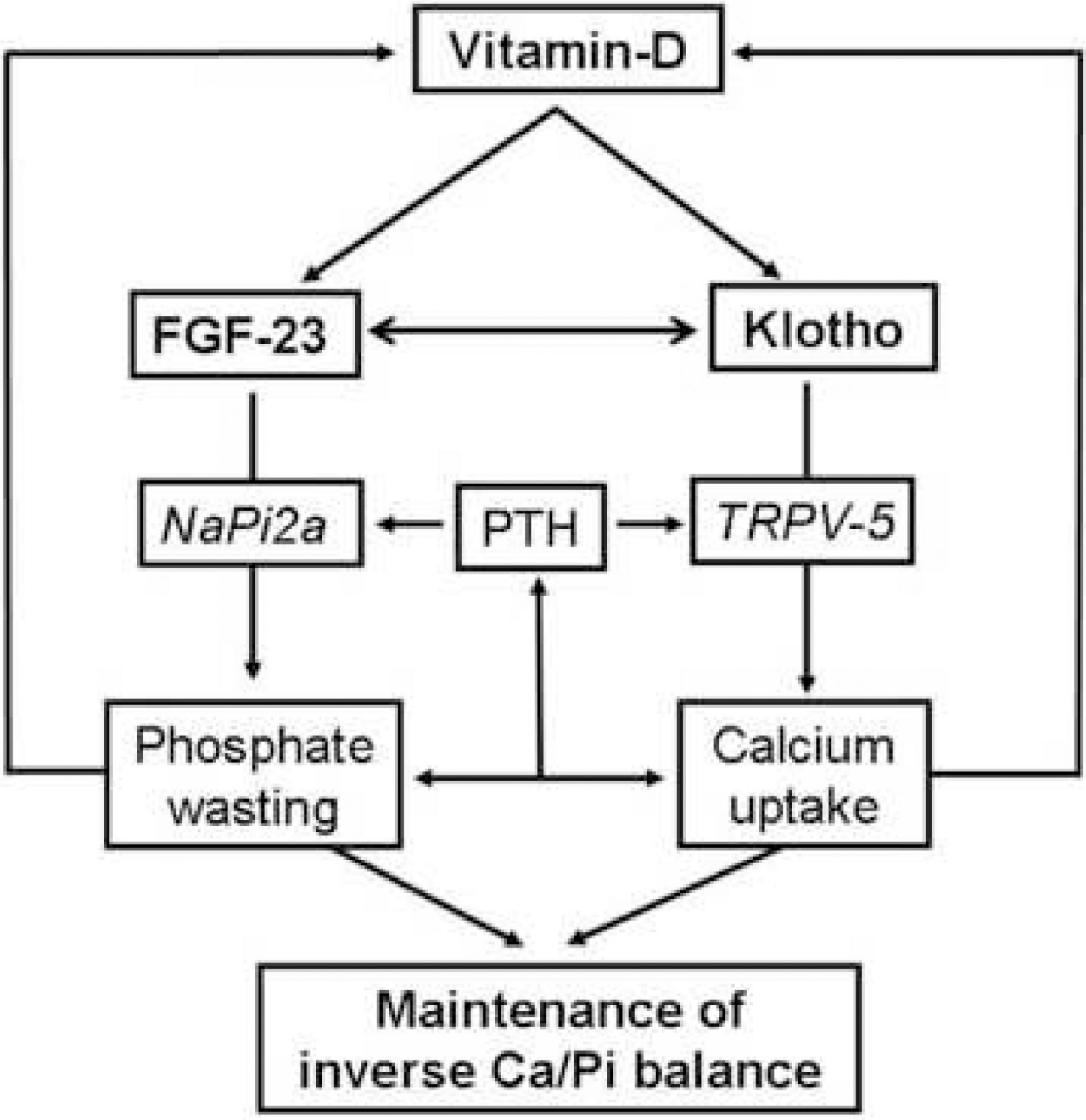

Figure-2.

Simplified schematic diagram of renal regulation of calcium (Ca) and phosphate (Pi) homeostasis. Vitamin-D could exert stimulatory effects on klotho, FGF-23 and TRPV-5 [48, 68,69]; TRPV-5 could induce renal calcium uptake through involving calbindin-D28k [70], while, FGF-23 could induce renal phosphate wasting through influencing the renal NaPi system [13,71], and thereby maintaining physiologic inverse Ca/Pi balance. Serum level of Ca/Pi has direct and indirect feedback through PTH on vitamin-D activities. Klotho by influencing biological activities of both TRPV-5 [72] and FGF-23 [4] could delicately fine tune the physiologic balance of Ca/Pi. There are other molecules that play important roles in regulation of Ca/Pi homeostasis, but to keep the diagram simple, we just cited the most essential factors.