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Role of Klotho in Aging, Phosphate Metabolism, and CKD

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Abstract

The klotho gene (*KL*) was first identified as a putative aging-suppressor gene that extended lifespan when over-expressed, and accelerated aging-like phenotypes when disrupted in mice. It encodes a single-pass transmembrane protein and is expressed predominantly in kidney, where it functions as an obligate co-receptor for fibroblast growth factor 23 (FGF-23). FGF-23 is a bone-derived hormone that suppresses phosphate reabsorption and 1,25 dihydroxyvitamin D₃ (vitamin D) synthesis in the kidney. Klotho is also expressed in the parathyroid gland, where FGF-23 decreases expression and secretion of parathyroid hormone, further suppressing vitamin D synthesis in kidney. Thus, FGF-23 functions as a phosphaturic hormone as well as a counter-regulatory hormone for vitamin D, thereby inducing negative phosphate balance. Mice lacking either FGF-23 or klotho exhibit hyperphosphatemia in addition to developing multiple aging-like phenotypes, which can be rescued by resolving phosphate retention. These findings have unveiled an unexpected link between aging and phosphate. In patients with chronic kidney disease (CKD), phosphate retention is universally seen and has been associated with increased mortality risk. CKD patients exhibit high serum FGF23 levels together with reduced klotho expression in the kidney and parathyroid, rendering FGF23 and klotho as potential biomarkers and therapeutic targets for CKD. The klotho protein serves not only as a co-receptor for FGF23 but also functions as a humoral factor. Klotho's extracellular domain is released into blood and urine by ectodomain shedding and exerts various functions independently of FGF23, including regulation of multiple ion channels and transporters. Decrease in urinary klotho protein levels has been identified as one of the earliest biomarkers of CKD progression. This review will focus on the current understanding of klotho protein function with an emphasis on its potential involvement in the pathophysiology of CKD.

Keywords

Klotho; FGF23; Phosphate; Vitamin D; PTH; chronic kidney disease

BACKGROUND

Chronic kidney disease (CKD) includes a state of progressive decline of kidney function over months or years caused by various disease processes or by natural aging. Prevalence of CKD is approaching epidemic proportions worldwide. More than 26 million Americans, or

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13% of total population, have CKD.¹ Notably, the vast majority of CKD patients die prematurely not from kidney failure, but due to early onset of common age-related diseases like cardiovascular disease, cancer, and infection.² CKD patients also suffer many aging-like symptoms, including hypogonadism, skin atrophy, osteopenia, and cognition impairment.

Hyperphosphatemia has been recognized as an important mortality risk factor in patients with CKD.^{3,4} An important feature of hyperphosphatemia is that it usually becomes evident only in advanced stages of CKD, when the disease is largely irreversible and destined to progress to end-stage renal disease. Epidemiological studies have shown that increased fibroblast growth factor 23 (FGF-23), increased parathyroid hormone (PTH), and decreased 1,25 dihydroxyvitamin D₃ precede hyperphosphatemia during CKD progression⁵, indicating that dysregulation of phosphate metabolism ensues in early-stage CKD long before there is an actual increase in blood phosphate levels.

CASE VIGNETTE

A 68 year-old woman was referred for symptoms of poor appetite, lower extremity edema, and progressive worsening of kidney function for 3 months. She had been diagnosed with diabetes 10 years earlier, and treated with oral hypoglycemics (rosiglitazone). Upon examination, her blood pressure was at 130/70 mmHg and she was currently on anti-hypertensive therapy with amlodipine, valsartan, and carvedilol. She had bilateral lower limb edema to the level just above her ankles. Her blood chemistry tests revealed the following: serum creatinine 2.98 mg/dL (263.4 μ mol/L), serum urea nitrogen 50 mg/dL (17.9 mmol/L), eGFR 16.6 ml/min/1.73 m² (0.28 mL/s/1.73 m²; calculated using the 4-variable MDRD [Modification of Diet in Renal Disease] Study equation), glucose of 127 mg/dL (7.05 mmol/L), phosphate level of 5.8 mg/dL, calcium 9.5 mg/dL (2.37 mmol/L), 25 hydroxyvitamin D 18 ng/mL (44.93 nmol/L), and PTH level of 199 pg/mL (199 ng/L). The urinalysis showed protein (2+) with an albumin-creatinine ratio of 1.96 g/g. Ultrasound revealed bilaterally enlarged kidneys with increased cortical echogenicity. The patient was diagnosed with CKD stage 4, possibly related to longstanding diabetes, and recommended a strict dietary regimen of low sodium, phosphate, and protein and balanced fluid intake. Additionally she was prescribed medications including loop diuretics and angiotensin-converting enzyme inhibitor to control her blood pressure and to reduce leg edema and proteinuria. The patient was also prescribed calcium carbonate to control her hyperphosphatemia.

Following 3 months of therapy, her lower limb edema had gradually subsided and her serum and urine tests showed modest decrease of phosphate 4.9 mg/dL, calcium 8.4 mg/dL (2.1 mmol/L), creatinine 2.65 mg/dL (234.3 μ mol/L), serum urea nitrogen of 35 mg/dL (12.5 mmol/L), eGFR 18 ml/min/1.73 m² (0.3 mL/s/1.73 m²) and urine albumin-creatinine ratio 1.2 g/g. After 6 months of dietary and medication therapy, she had shown significant improvement in her proteinuria and a stabilization of the progressive kidney damage. At her last office visit, her phosphate was 4.8 mg/dL and PTH was 183 pg/mL (183 ng/L).

Although serum FGF-23 levels were not measured, patients with CKD stage 4 usually exhibit high serum FGF-23⁵. However, FGF-23 cannot exert its phosphaturic activity in advanced CKD, resulting in hyperphosphatemia. What is the mechanism of FGF-23 resistance in CKD? What is the relation between FGF-23 and other phosphate regulating hormones (ie, PTH and 1,25 dihydroxyvitamin D₃)? Recent advances of our knowledge on the FGF-23-klotho system may provide a clue to the answer for these questions and suggest novel targets of therapeutic interventions in CKD.

PATHOGENESIS

Age-Associated Decline of Kidney Function

Due to improved health care, the world has witnessed a reduction in early mortality and an increase in longevity which has resulted in an aging population⁶. One characteristic hallmark of aging is the consequent decline in the physiological functions of various tissues and organ systems. In particular, the kidneys are highly susceptible to aging and their progressive structural and functional damage is quite dramatic and severe when compared to any other organ systems⁷. The major structural damage to the kidneys with age is exemplified as arterial intimal and interstitial fibrosis, glomerulosclerosis, and tubular atrophy⁸. Renal fibrosis usually begins as tubulointerstitial injury that can be potentially detrimental to essential functional aspects such as glomerular filtration rate and renal blood flow. Renal fibrosis is a final common pathology of kidney aging and chronic kidney disease, often resulting from the inflammatory response to kidney damages by various causes, including age-related oxidative stress, hypertension, diabetes, and chronic glomerulonephritis. During this process, inflammatory cells such as macrophages infiltrate the interstitial space and secrete cytokines and growth factors such as transforming growth factor β 1 (TGF β 1), which promote the phenotypic transformation of residing epithelial or non-epithelial cells into myofibroblasts, leading to interstitial fibrosis and structural damages in the kidney.⁹ Though young kidneys have significant regenerative capabilities, aged kidneys fail to reverse such fibrotic changes and ultimately develop fibrosis and atrophy due to reduced cellular regeneration and increased apoptosis⁷. A dramatic reduction of renal mass, especially in the renal cortex, has been observed in individuals over the age of 80 years as compared to those younger than 39 years old¹⁰. Furthermore the renal cortex is subject to significant increase of glomerulosclerosis, especially in individuals in the 8th decade¹¹.

The most widely-used biomarker for kidney function as a function of age is glomerular filtration rate (GFR)¹². At birth the GFR is low and reaches adult levels of 140 mL/min/1.73 m² at the age of 20 years. A decline in GFR ensues after the age of 40 and has been calculated to drop by the rate of 8 mL/min/1.73 m² every decade thereafter¹³. A GFR of less than 60 mL/min/1.73 m² is considered as a state of chronic kidney disease with a high risk of kidney failure, cardiovascular disease and death¹⁴. GFR is usually measured from serum creatinine concentration¹⁵. An age-related reduction in GFR is consequent to the decrease in urinary creatinine clearance and reduced muscle mass. Changes in renal blood flow are also observed with age, where a level of 600 mL/min is maintained until the age of 40, then starts to decline about 10% each decade¹⁶.

Klotho, Phosphate, and Aging

Klotho (named after the goddess in Greek mythology who spun the thread of life) was discovered in 1997 as a gene that is mutated in a mouse strain that exhibited multiple aging-like phenotypes and a shortened lifespan¹⁷. The klotho mutant mouse suffered from multiple disorders resembling aging, including hypogonadism, growth retardation, accelerated thymic involution, skin atrophy, sarcopenia, vascular calcification, osteopenia, aging lung, cognition impairment¹⁸, hearing loss¹⁹, and motor neuron degeneration²⁰ among others. In contrast, transgenic mice that overexpress klotho exhibited a 20-30% increase in their life span²¹; thus, the klotho gene (*KL*) has been regarded as an aging-suppressor gene. The klotho gene encodes a single-pass transmembrane protein and is expressed predominantly in the renal tubular cells¹⁷.

In patients with autosomal dominant hypophosphatemic rickets²², gain-of-function mutations in the *FGF-23* gene have been identified that confer resistance to proteolytic degeneration by protease(s) yet to be identified. Consequently, these patients have high

blood FGF-23 levels. FGF-23, which is secreted from bone, acts on the kidney to promote phosphaturia²³. The fact that mice lacking FGF-23 develop impaired renal phosphate excretion and hyperphosphatemia²⁴ further confirmed the notion that FGF-23 functions as a phosphaturic hormone.

Unexpectedly, FGF-23-deficient mice develop not only hyperphosphatemia but also aging-like phenotypes closely resembling those observed in *klotho*-deficient mice²⁵, in whom hyperphosphatemia associated with vascular calcification also becomes manifest^{17, 26}. Thus, FGF-23-deficient mice and *klotho*-deficient mice turned out to be phenotypically identical. These observations unveiled the relation between FGF-23 and *klotho*: *klotho* forms a constitutive binary complex with FGF receptors and functions as an obligatory co-receptor for FGF-23^{27, 28}. Accordingly, FGF-23 and *klotho* have emerged as fundamental factors in the recently identified bone-kidney endocrine axis responsible for phosphate homeostasis.

Importantly, the aging-like phenotypes observed in mice lacking FGF-23 or *klotho* can be rescued by reversing hyperphosphatemia using various genetic and dietary interventions, including low phosphate diet, low vitamin D diet, and knockout of the genes indispensable for vitamin D action or renal phosphate reabsorption^{8, 25, 26, 29-31}. In addition, dietary phosphate overload induced the aging-like phenotypes in *Klotho*^{-/-} mice rescued by blocking renal phosphate reabsorption³². These observations indicate that phosphate retention is predominantly responsible for the aging-like phenotypes, implying a potential link between aging and phosphate.

Endocrine Regulation of Phosphate Metabolism

A balance among dietary absorption from intestine, mobilization from bone, and excretion from the kidney to urine determines blood phosphate level³³. Several endocrine factors regulate phosphate metabolism. In addition to their well-known role as calcium metabolism regulators,³⁴ vitamin D and parathyroid hormone (PTH) also participate in the regulation of phosphate metabolism. Vitamin D's active form (1,25 dihydroxyvitamin D₃), which is synthesized in the kidney, increases intestinal absorption of dietary calcium and phosphate. It also stimulates osteoclastogenesis thereby promoting mobilization of calcium and phosphate from bone (the body's main reservoir for both) consequently increasing their levels in the blood. Changes in the extracellular levels of calcium are sensed by the calcium sensing-receptors of the parathyroid gland to regulate the synthesis and secretion of PTH³⁵. PTH can subsequently act on the bone to increase efflux of calcium and phosphate, and the kidney to increase vitamin D synthesis. Vitamin D in turn suppresses PTH production and secretion, thus closing a negative feedback loop. One critical feature of PTH is that, unlike vitamin D, it does not induce net positive phosphate balance because PTH simultaneously induces phosphaturia³⁶. Thus, it had been thought until quite recently that phosphate metabolism was regulated indirectly by calcium-regulating hormones (vitamin D and PTH).

Identification of FGF-23 as a phosphaturic hormone has transformed the classical view on endocrine regulation of phosphate homeostasis³⁷. FGF-23 acts on the kidney to 1) induce phosphaturia by reducing expression and/or cell-surface amount of type 2a sodium-dependent phosphate co-transporters (NaPi-2a) in renal proximal tubular cells and 2) suppress vitamin D synthesis by reducing expression of 1 α -hydroxylase, the enzyme that converts the inactive precursor 25 hydroxyvitamin D₃ to 1,25 dihydroxyvitamin D₃. Consequently, FGF-23 causes a decrease in serum 1,25 dihydroxyvitamin D₃ levels, which leads to reduced intestinal phosphate absorption. By functioning as both a phosphaturic hormone and as a counter-regulatory hormone for vitamin D, FGF-23 therefore induces a negative phosphate balance³⁸⁻⁴⁰. Because 1,25 dihydroxyvitamin D₃ up-regulates FGF-23 expression and increases blood FGF-23 levels⁴¹, there exists a negative feedback loop between FGF-23 and vitamin D.

Klotho primarily is expressed in distal convoluted tubules, suggesting that this site is the primary target of FGF-23 action. However, FGF-23 suppresses NaPi-2a and 1 α -hydroxylase, which are expressed in proximal tubules. Two possible explanations for this discrepancy are considered: 1) Because a low level of klotho is detected in the proximal tubules⁴², FGF-23 may directly act on proximal tubules to suppress NaPi-2a and 1 α -hydroxylase. 2) FGF-23 could act on the distal convoluted tubules to make them secrete a paracrine factor that acts on adjacent proximal tubules and suppresses NaPi-2a and 1 α -hydroxylase. A recent study showed that injection of FGF-23 activated the FGF signaling pathway only in distal convoluted tubules where klotho is predominantly expressed⁴³, which supported the second possibility. A putative paracrine factor secreted from the distal convoluted tubules remains to be identified.

Recent studies have shown that parathyroid glands express klotho endogenously, and act as another major target of FGF-23⁴⁴. Indeed, FGF-23 suppresses PTH expression and secretion and thus functions as a counter-regulatory hormone for PTH as well. Because parathyroidectomy reduces blood FGF-23 levels in uremic rats⁴⁵, PTH may promote FGF-23 expression and/or secretion, suggesting the existence of another negative feedback loop between FGF-23 and PTH (Fig 1).

Phosphate metabolism in CKD

A universal characteristic of patients with CKD is their high serum phosphate levels, which epidemiologic studies have established as an independent mortality risk factor in these patients⁴⁶. Compared with CKD patients with lower serum phosphate levels (<6.5 mg/dL), those with hyperphosphatemia (≥ 6.5 mg/dL) have higher risk for death caused by a number of diseases including coronary artery disease⁴.

Recent studies point to the notion that deranged phosphate metabolism in CKD patients may be attributed to the dysregulation of the FGF-23-klotho endocrine axes (Fig 2)⁴⁷. With loss of functioning nephrons during CKD progression, each nephron is required to excrete an increasing amount of phosphate to maintain normal serum phosphate levels. This can be attained by increasing serum FGF-23 levels, which is an early sign of changes in phosphate metabolism during CKD progression^{5, 48}. However, because FGF-23 is a counter-regulatory hormone for vitamin D, an increase in its levels is at the expense of vitamin D synthesis. Diminishing vitamin D stimulates PTH secretion, leading to secondary hyperparathyroidism. In fact, decreases in serum 1,25 dihydroxyvitamin D₃ levels and increases in serum PTH levels are observed in early-stage CKD patients with normophosphatemia^{5, 49}. The fact that klotho expression in parathyroid is decreased in CKD patients⁵⁰⁻⁵³ may explain why high FGF-23 fails to suppress PTH. High FGF-23 and low vitamin D may further reduce klotho expression in kidney and parathyroid tissue in CKD patients, because FGF-23 and vitamin D can down-regulate and up-regulate klotho expression, respectively^{26, 54}. Declines in klotho expression exacerbate FGF-23 resistance in the kidney and parathyroid gland, further increasing FGF-23, decreasing vitamin D, and increasing PTH. This vicious cycle may contribute to the pathophysiology of deranged phosphate metabolism in CKD patients. In advanced stages of CKD, increased FGF-23 can no longer compensate for dietary phosphate overload; this results in overt hyperphosphatemia, vitamin D deficiency, and development of parathyroid hyperplasia. The fact that patients with advanced stage CKD have extremely high levels of serum FGF-23 that is biologically active⁵⁵ has raised the possibility that the super-physiological levels of FGF-23 may activate FGF signaling in tissues that do not express klotho, which may then contribute to systemic complications in end-stage renal disease. However, mice lacking both klotho and FGF-23 (*Klotho*^{-/-} *Fgf23*^{-/-}), and mice lacking only klotho (*Klotho*^{-/-}), exhibited identical phenotypes⁵⁶ despite the extremely high serum FGF-23 levels in *Klotho*^{-/-} mice, which disputes this possibility.

RECENT ADVANCES

Secreted Klotho

Klotho proteins on the cell surface can be substrates for membrane-anchored proteases (α - and β -secretases)^{57, 58}, thus releasing the extracellular domain of klotho into the extracellular space. The extracellular domain of klotho can be detected in blood, urine, and cerebrospinal fluid.^{21, 59} Thus, klotho protein exists in two forms, membrane klotho and secreted klotho. Membrane klotho functions as an obligate co-receptor for FGF-23 as discussed above. Recent studies have revealed various functions of secreted klotho protein. Of note, secreted klotho cannot act as a soluble receptor for FGF-23, because it is klotho-FGFR complexes (and not klotho or FGFR alone) that can bind to FGF-23 with a high affinity²⁷.

Secreted klotho belongs to family 1 glycosidases and has homology to β -glucosidases of bacteria and plants and to lactase-phlorizin hydrolases in mammals¹⁷. These are enzymes that hydrolyze terminal β -glucosidic linkages in saccharides, glycoproteins, and glycolipids and release glucose. However, recombinant klotho protein does not exhibit β -glucosidase-like enzymatic activity, probably due to the fact that critical glutamate residues at the active center conserved in all members of β -glucosidases are replaced with alanine in klotho protein. Instead, weak β -glucuronidase activity has been detected in recombinant klotho protein *in vitro*⁶⁰; however, the physiological significance of this activity and the natural substrates for secreted klotho are not clear.

Secreted Klotho and Ion Channels/Transporters

Recent studies have demonstrated that secreted klotho activates members of the transient receptor potential (TRP) family of calcium channels, specifically TRPV5 and TRPV6.^{61, 62} TRPV5 is expressed in distal nephrons to mediate renal calcium ion reabsorption, whereas TRPV6 is expressed in intestinal epithelia to mediate intestinal calcium absorption. The mechanism by which secreted klotho activates TRPV5 is unique. Secreted klotho removes terminal sialic acids in the N-linked glycans of TRPV5 through its enzymatic activity on the cell surface, raising the possibility that a natural substrate for secreted klotho is not glucuronic acid but sialic acid. Removal of sialic acids exposes underlying galactose residues, which serve as ligands for galectin 1, a lectin abundant in extracellular matrices. Thus, secreted klotho induces interaction between galectin 1 and N-linked glycans of TRPV5, thereby entrapping the channel on the cell surface and preventing it from caveolae-mediated endocytosis. Treatment of TRPV5-expressing HEK293 cells with secreted klotho increases cell-surface amount of TRPV5 and Ca^{2+} current, which is mimicked by bacterial sialidase treatment and abolished by knocking down galectin 1 by siRNA or blocking it with antibody.⁶² Thus, secreted klotho may contribute to durable calcium channel activity and calcium reabsorption in the distal nephrons. A similar enzymatic function of secreted klotho is noted to regulate potassium channel activity in the kidney. Klotho treatment removes terminal sialic acid residues from N-linked glycans of ROMK1 (renal outer medullary K^{+} channel)⁶³. ROMK1 is expressed in distal nephrons and mediates potassium secretion into urine. Removal of terminal sialic acids exposes underlying galactose residues and induces interaction with galectin-1, thereby entrapping ROMK1 on the cell surface and preventing it from clathrin-mediated endocytosis. As a result, secreted klotho promotes renal potassium excretion. Secreted klotho was found to have another enzymatic function: direct regulation of phosphate transport in the proximal tubule of the kidney by deglycosylation of NaPi-2a. Deglycosylation of N-linked glycans renders NaPi-2a protein more susceptible to proteases residing in the proximal tubular brush border membrane, resulting in reduction in the number and activity of NaPi-2a, hence promoting phosphaturia independently of FGF-23⁶⁴. Thus, one possible hypothesis is that a putative paracrine factor discussed above that

mediates FGF-23 action (secreted from distal convoluted tubules in response to FGF-23 and suppresses NaPi-2a) may be secreted klotho. In other words, FGF-23 may function as a klotho-releasing hormone. This hypothesis also explains why *Fgf23*^{-/-} mice exhibit impaired phosphate excretion, though they have klotho. Multiple functions of membrane klotho and secreted klotho are summarized in Fig 3.

Secreted Klotho and CKD

Although there are practical difficulties in determining the amount of klotho expression in kidney in patients, it is possible to measure secreted klotho protein levels in blood and urine^{65, 66}. Recently, it was found that a progressive decrease in secreted klotho protein levels in urine occurs during CKD progression⁶⁶. Importantly, a decrease in urinary klotho becomes evident as early as CKD stages 1-2 in patients, rendering klotho as one of the most sensitive biomarkers for early detection of CKD. Because secreted klotho activates TRPV5 and inhibits NaPi-2a from the luminal side of the renal tubules, decrease in urinary levels of secreted klotho may decrease calcium reabsorption and increase phosphate reabsorption, potentially contributing to pathophysiology of CKD.

Klotho has been shown to function as a renoprotective factor in animal studies. Transgenic overexpression of klotho was observed to produce dramatic improvements in kidney function and morphological lesions in a mouse model of glomerulonephritis carrying a mutation in the *tensin2* gene⁶⁷. Injection of recombinant secreted klotho protein or delivery of a gene expressing secreted klotho by means of viral vector transduction was also effective in improving serum creatinine levels and reducing apoptosis in mouse model of ischemic acute renal failure.^{68, 69} Of note, klotho expression is down-regulated by Angiotensin II and enhanced by peroxisome proliferator-activated receptor γ (PPAR γ) agonists (such as rosiglitazone)^{70, 71}; observations that imply that the renoprotective effects of angiotensin-converting enzyme inhibitors and PPAR γ agonists may be in part attributed to their ability to preserve klotho expression and interrupt the vicious cycle discussed above.

SUMMARY

In reviewing the case vignette, provided that increasing demand for phosphate excretion per nephron triggers the vicious cycle that leads to increased FGF-23, decreased vitamin D, increased PTH, and decreased klotho (Fig 2), restriction of dietary phosphate intake (low phosphate diet) and administration of phosphate binder can be regarded as the most important interventions in this case. Because the vicious cycle is likely activated long before hyperphosphatemia occurs during CKD progression, phosphate restriction would have been more effective if started before serum phosphate levels increased, perhaps as soon as serum FGF-23 levels rose. Phosphate restriction alleviates phosphate overload per nephron and can disrupt the vicious cycle: phosphate restriction can reduce serum FGF-23 levels and increase vitamin D, which in turn increase klotho expression in kidney and parathyroid. Rosiglitazone and angiotensin-converting enzyme inhibitors may also up-regulate klotho expression. Increased klotho in kidney may improve FGF-23 sensitivity, which further reduce the amount of FGF-23 required for excreting a given amount of phosphate. Increased klotho in parathyroid may improve the ability of FGF-23 to suppress PTH; increased vitamin D also suppresses PTH.

CKD is an important and growing global health problem that reduces both the length and quality of life. Current diagnostic and therapeutic options are far from satisfactory in identifying patients with CKD, monitoring disease progression, evaluating response to interventions, and identifying improved treatment through new clinical trials. Klotho is a protein expressed in the kidney and parathyroid, where it acts as an obligate co-receptor for FGF-23. FGF-23 is a hormone that creates negative phosphate balance by functioning as

both a phosphaturic hormone and counter-regulatory hormone for vitamin D. Dysregulation of the endocrine axes mediated by FGF-23 and *klotho* are linked to progression of CKD and its complications, especially mineral metabolism disorders (including vascular calcification), rendering FGF-23 and *klotho* as novel biomarkers and therapeutic targets for CKD. Also, demonstrated renoprotective functions of *klotho* have created hope in it being used a therapeutic to improve kidney function. Because *klotho* protein levels in urine decline with the onset and progression of CKD,⁶⁶ *klotho* has potential to serve as a biomarker to diagnose early CKD, predict progression of kidney disease, and serve as surrogate marker in future clinical trials.

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10 years ago **Now** **Figure 1.**

Endocrine regulation of phosphate homeostasis. Ten years ago, two principal calcium-regulating hormones, 1,25 dihydroxyvitamin D₃ (vitamin D) and parathyroid hormone (PTH), were thought to regulate phosphate metabolism. PTH increases vitamin D synthesis in kidney (①). Vitamin D in turn decreases PTH (②), thereby closing a negative feedback loop. Now the fibroblast growth factor 23 (FGF-23)-klotho system has emerged as the principal phosphate-regulating endocrine axes. FGF23 is secreted from bone and acts on kidney to reduce vitamin D synthesis (③). Because vitamin D increases FGF23 expression in bone (④), a negative feedback loop exists between FGF-23 and vitamin D. FGF23 also acts on parathyroid to reduce PTH (⑤). Because PTH increases FGF-23 expression (⑥), there exists another negative feedback loop between PTH and FGF-23.

Figure 2.

Changes in phosphate-regulating factors during chronic kidney disease (CKD) progression. **(A)** Decrease in urinary klotho levels is probably the earliest change detectable in early stage CKD.^{66, 72} Increase in fibroblast growth factor 23 (FGF-23), decrease in vitamin D, and increase in parathyroid hormone (PTH) precede hyperphosphatemia during CKD progression. **(B)** A model that explains panel A. Decrease in klotho induces end-organ resistance to FGF-23, resulting in compensatory increase in FGF-23 to maintain normal serum phosphate levels. However, this is at the expense of vitamin D synthesis. Because vitamin D increases klotho gene expression, low vitamin D further reduces klotho expression. In addition, low vitamin D increases PTH and high PTH further stimulates FGF-23. Phosphate retention, which occurs later, further amplifies the abnormality in all four factors. This constellation thus forms a deterioration spiral of decreased klotho and increased FGF-23.

Figure 3.

Multiple functions of membrane klotho and secreted klotho. Membrane klotho functions as an obligate co-receptor to fibroblast growth factor 23 (FGF-23) in the kidney and parathyroid gland. This activity ensures a negative phosphate balance by 1) induction of phosphaturia and suppression of vitamin D synthesis in kidney and by 2) suppression of parathyroid hormone (PTH) expression and secretion in the parathyroid gland. Secreted klotho exists in luminal fluid of renal tubules as well as in urine and functions as an enzyme that modifies N-linked glycans of TRPV5 and ROMK1 (renal outer medullary K⁺ channel) on the apical membrane of renal distal tubules, which increases calcium reabsorption and potassium excretion. Secreted klotho also modifies N-linked glycans of type-2a Na-dependent phosphate co-transporters (NaPi-2a) on the apical brush border membrane of renal proximal tubules, which reduces phosphate reabsorption independently of FGF-23.