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Phosphate and Klotho

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Klotho **is a putative aging suppressor gene encoding a single-pass transmembrane co-receptor that makes the fibroblast growth factor (FGF) receptor specific for FGF-23. In addition to multiple endocrine organs, *Klotho* is expressed in kidney distal convoluted tubules and parathyroid cells, mediating the role of FGF-23 in bone–kidney–parathyroid control of phosphate and calcium. *Klotho*$^{-/-}$ mice display premature aging and chronic kidney disease-associated mineral and bone disorder (CKD-MBD)-like phenotypes mediated by hyperphosphatemia and remediated by phosphate-lowering interventions (diets low in phosphate or vitamin D; knockouts of 1α-hydroxylase, vitamin D receptor, or NaPi cotransporter). CKD can be seen as a state of hyperphosphatemia-induced accelerated aging associated with Klotho deficiency. Humans with CKD experience decreased Klotho expression as early as stage 1 CKD; Klotho continues to decline as CKD progresses, causing FGF-23 resistance and provoking large FGF-23 and parathyroid hormone increases, and hypovitaminosis D. Secreted Klotho protein, formed by extracellular clipping, exerts FGF-23-independent phosphaturic and calcium-conserving effects through its paracrine action on the proximal and distal tubules, respectively. We contend that decreased Klotho expression is the earliest biomarker of CKD and the initiator of CKD-MBD pathophysiology. Maintaining normal phosphate levels with phosphate binders in patients with CKD with declining Klotho expression is expected to reduce mineral and vascular derangements.**

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Klotho, named after an ancient Greek goddess of fate, is a putative aging suppressor gene. A defect in *Klotho* gene expression in mice confers penetrant phenotypes resembling human premature aging syndromes,[1] whereas Klotho overexpression confers longevity exceeding the wild type.[2] Pathology in *Klotho*$^{-/-}$ mice includes osteopenia and calcifications (vascular and ectopic) resembling chronic kidney disease-associated mineral and bone disorder (CKD-MBD), in addition to short lifespan and senescent changes in the heart, lungs, thymus, gonads, skin, muscles, hearing, and motor neurons (reviewed by Kuro-o[3]). The *Klotho* gene encodes a single-pass transmembrane protein expressed predominantly in the kidney (intensely in the distal convoluted tubule (DCT) and to a lesser extent in the proximal tubule[1,3,4]) and parathyroid gland.[5]

The phenotypes of *Klotho*$^{-/-}$ and *Fgf23*$^{-/-}$ mice are very similar, involving premature aging and abnormal mineral metabolism.[1,6] Both mutants share the senescent phenotypes of short lifespan, growth retardation, hypogonadism, early thymic involution, skin and muscle atrophy, osteoporosis, and emphysema, and deranged mineral metabolism phenotypes including vascular calcification, hyperphosphatemia, hypercalcemia, hypoglycemia, and hypervitaminosis D. These similarities point to the involvement of Klotho and fibroblast growth factor (FGF)-23 in a common physiological pathway.

OBJECTIVE

This review article will discuss the involvement of Klotho in phosphate metabolism in CKD-MBD and propose the hypothesis that Klotho deficiency is the earliest biomarker of CKD.

THE KLOTHO PROTEIN AS OBLIGATE FGF-23 CO-RECEPTOR

Canonical FGF receptors (FGFRs), which require cofactors for specific binding and signal transduction, are expressed in multiple tissues. Most FGFs use heparan sulfate as a cofactor facilitating their binding to FGFRs.[3] Endocrine FGFs, however, including FGF-23, use other cofactors (or co-receptors).[3] Klotho protein is a co-receptor specific for FGF-23 (refs 7, 8).

Kidney[1] and parathyroid[5] Klotho expression identifies these organs as high-affinity FGF-23 endocrine targets. The Klotho/FGFR complex thus mediates FGF-23 participation in the bone–kidney–parathyroid endocrine axis. In the kidney, FGF-23 acting on Klotho/FGFR suppresses phosphate reabsorption and $1,25(OH)_2D_3$ synthesis;[9] in the parathyroid, FGF-23 suppresses parathyroid hormone secretion,[5,10,11]

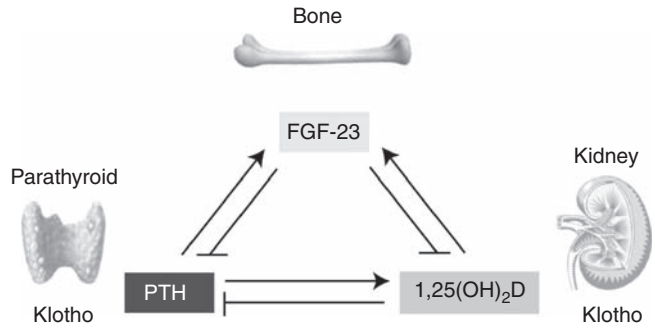

Figure 1 | Endocrine regulation of phosphate metabolism. Circulating $1,25(OH)_2D_3$ turns on the FGF-23 promoter in bone cells. Secreted FGF-23 binds to renal cell Klotho/FGF receptor to turn off the 1α-hydroxylase promoter and turn on the 24-hydroxylase promoter, resulting in net inactivation of conversion of vitamin D to $1,25(OH)_2D_3$. PTH affects these renal promoters in a reverse manner to FGF-23, leading to $1,25(OH)_2D_3$ production. In the parathyroid, FGF-23 binds to Klotho/FGF receptor and shuts off the PTH promoter. FGF-23, fibroblast growth factor-23; PTH, parathyroid hormone.

Table 1 | Effects on mineral metabolism of interventions rescuing *Klotho*$^{-/-}$ and *Fgf23*$^{-/-}$ mouse phenotypes[17–20]

	Direction of change in serum levels		
Intervention	Phosphate	$1,25(OH)_2D_3$	Calcium
Low-phosphate diet	↓	↑	↑
1α-Hydroxylase knockout	↓	↓	↓
Vitamin D receptor knockout	↓	↑	↓
Na–Pi cotransporter IIa knockout	↓	↑	↑
Low-vitamin D diet	↓	↓	↓

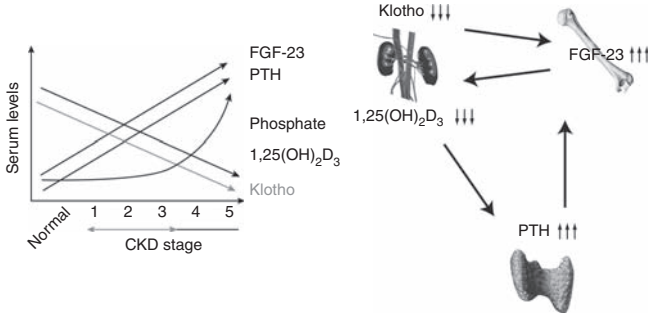

Figure 2 | Changes in Klotho protein, FGF-23, PTH, $1,25(OH)_2D_3$, and phosphate as CKD progresses. When Klotho expression first decreases, FGF-23 increases, lowering circulating $1,25(OH)_2D_3$, which depresses Klotho expression further and increases PTH expression. Increased PTH induces further FGF-23 increases, causing large decreases in $1,25(OH)_2D_3$ and large increases in PTH. This cycle results in hyperphosphatemia in late stages of CKD. CKD, chronic kidney disease; FGF-23, fibroblast growth factor-23; PTH, parathyroid hormone.

which may also contribute to the ability of FGF-23 to reduce $1,25(OH)_2D_3$ synthesis. Thus, FGF-23 is both a phosphaturic hormone and the counter-regulatory hormone to vitamin D in the bone–kidney–parathyroid endocrine axis, and Klotho is required for the effects of FGF-23 (ref. 12; Figure 1). Both these FGF-23 actions promote negative phosphate balance. Thus, FGF-23 can be identified as 'phosphatonin', the bone-generated humoral phosphaturic factor postulated more than 10 years ago.[13] Secretion of FGF-23 by bone is induced by phosphate and $1,25(OH)_2D_3$(9) and possibly by parathyroid hormone.[10,14] *Klotho* gene expression is inducible by $1,25(OH)_2D_3$ (ref. 15).

PATHOGENESIS OF HYPERPHOSPHATEMIA IN *KLOTHO*$^{-/-}$ MUTANTS

Phosphate pathophysiology mediates complex aging-like phenotypes in mice with defects in the Klotho–FGF-23 system. Low-phosphate diet improves the aging-like phenotypes of *Klotho* mutant mice[16] and *Fgf23*$^{-/-}$ mice.[17] Mutant homozygotes consuming 1.03 g phosphorus/100 g diet had typical mutant phenotypes. Male homozygotes consuming 0.4 g phosphorus/100 g diet expressed the Klotho protein in their kidneys and resumed normal spermatogenesis.[16] Female homozygotes required zinc supplementation as well as phosphorus restriction for phenotypic rescue.[16] Phosphate restriction corrected CKD-MBD-like FGF-23-null phenotypes (hyperphosphatemia, vascular calcifications, and mortality) even though serum calcium and $1,25(OH)_2D_3$ levels remained elevated.[17] Several other genetic and dietary interventions that rescue *Klotho*$^{-/-}$ and/or *Fgf23*$^{-/-}$ phenotypes[18–20] have lowered serum phosphate as their only common denominator (Table 1). Phosphate retention may thus accelerate aging and/or age-related diseases in mice and humans.[21]

Patients with CKD are far more likely to die of cardiovascular disease than to live to require dialysis.[22] CKD-related cardiovascular disease is substantially fueled by hyperphosphatemia and can be seen as phosphate-related accelerated cardiovascular aging.[23]

A KLOTHO-CENTRIC VIEW OF CKD

It may be hypothesized that CKD represents a state of accelerated aging associated with Klotho deficiency and phosphate retention, and that Klotho deficiency is the earliest biomarker of CKD and the initiator of CKD-related mineral dysregulation.

Klotho expression declines progressively in CKD as FGF-23 expression increases progressively; high serum phosphate and parathyroid hormone and low $1,25(OH)_2D_3$ accompany these changes (Figure 2). The first measurable decline in urinary secreted Klotho expression (as detected by western blotting of concentrated urine samples, normalized to the same creatinine content) occurs as early as stage 1 CKD[24] and is potentially an early clinical marker of nascent acute renal damage. Klotho decline precedes FGF-23 increase as CKD develops in *Jck* mice, a cystic kidney disease model of early progressive CKD.[25] Renal Klotho expression assays (mRNA measurement by RNAse protection, protein measurement by western blotting, and immunohistochemistry)

in human kidney specimens from dialysis patients or controls showed that dialysis patients expressed renal membrane Klotho at only 5–15%, most often $<5\%$, of control levels.[26] Median Klotho mRNA levels in healthy kidney tissue represented slightly $>8\%$ of the level of glyceraldehyde-3-phosphate dehydrogenase, a housekeeping mRNA.[26] A sandwich enzyme-linked immunosorbent assay for secreted Klotho in serum also exists and has shown that circulating secreted Klotho in healthy adults ranges from 239 to 1266 pg/ml, decreasing with advancing age and increasing calcemia and increasing with phosphatemia levels.[27] More sensitive assays by multiple reaction monitoring using mass spectrometry are currently in progress.

Reducing serum FGF-23 increases serum $1,25(OH)_2D_3$ and renal Klotho expression. Parathyroidectomy is expected to reduce FGF-23 production,[14] which in turn increases $1,25(OH)_2D_3$ synthesis and then renal Klotho expression. Vitamin D administration,[15] peroxisome proliferator-activated receptor-γ agonists,[28] or angiotensin II inhibitors[29,30] also increase Klotho expression.

Large serum FGF-23 increases during CKD progression[31] are efforts to maintain FGF-23 signaling as receptor availability decreases. In the normal kidney, Klotho expression is abundant and a small amount of FGF-23 effectively induces phosphaturia. As CKD progresses, ever-increasing serum FGF-23 acts on a resistant kidney with ever-fewer functional nephrons, each of which expresses less Klotho than in a healthy kidney. Serum FGF-23 increases in an attempt to maintain normophosphatemia, but also suppresses $1,25(OH)_2D_3$ synthesis.[31] Ultimately, total phosphate excretion can no longer keep pace and serum phosphate increases.

Klotho is a renoprotective factor; when overexpressed it exerts a beneficial effect on mouse glomerulonephritis[32] and acute kidney injury[33] models. Decline in renal Klotho expression precedes both FGF-23 overexpression and hyperphosphatemia, and may represent the initiating event of CKD.

Thus far, we have discussed modulation of the bone–kidney–parathyroid endocrine and phosphaturic axis by renal and parathyroid cell-surface Klotho. However, Klotho also exists as a secreted form produced by clipping the extracellular part of the molecule.[34,35] No form of Klotho protein without FGFR can bind to FGF-23 with high affinity. Secreted Klotho is found in blood, urine, and cerebrospinal fluid,[36] and acts as an FGF-23-independent phosphaturic hormone.[4]

SECRETED KLOTHO, PHOSPHATE EXCRETION, AND RENAL PARACRINE SIGNALING

Proximal tubules are where FGF-23 suppresses phosphate reabsorption and $1,25(OH)_2D_3$ synthesis. However, Klotho is expressed most intensely in renal DCT and only weakly in proximal tubules. Two mechanisms for proximal tubule FGF-23 activity are possible:[3] either FGF-23 acts directly on the proximal tubules and Klotho's function in the DCT is unexplained, or FGF-23 acts through Klotho on the DCT to induce a paracrine signal to proximal tubules.[37]

The extracellular portion of Klotho protein is clipped by membrane proteases ADAM10 and ADAM17 (ref. 34) and BACE1 (ref. 35) and secreted into blood, urine, and cerebrospinal fluid.[36] Secreted Klotho inhibits the sodium/phosphate transporters NPT2a, NPT2c, and NPT3 (refs 4, 24) and activates ion channels TRPV5 (ref. 38), TRPV6 (ref. 39), and ROMK1 (ref. 40).

Secreted Klotho exerts phosphaturic effects independently of FGF-23. Intravenously administered secreted Klotho induces phosphaturia in normal and *Fgf23*$^{-/-}$ mice.[4] NaPi2a mediates proximal tubule phosphate reabsorption (70–80% of total phosphate reabsorption); studies on brush border membrane vesicles from proximal tubule cells show that secreted Klotho inactivates NaPi2a.[4]

Secreted Klotho conserves serum calcium and reduces calciuria. Some 70% of calcium reabsorption occurs in the proximal tubule and 15% in the DCT (utilizing TRPV5; ref. 41). Whole-cell patch-clamp experiments show that secreted Klotho activates the calcium channel TRPV5 (ref. 38), which is responsible for DCT calcium reabsorption.[41]

We hypothesize that FGF-23 suppresses renal phosphate reabsorption and promotes calcium reabsorption by promoting the secretion of Klotho from DCT cells. Klotho entering the luminal fluid inhibits NaPi2a in proximal tubules to allow phosphate excretion and activates TRPV5 in distal tubules to reabsorb calcium. Secreted Klotho is present in the luminal fluid of proximal tubules,[4] but how it is transported into the proximal tubular lumen is not yet known.

CONCLUSIONS

Renal and parathyroid Klotho co-receptors make FGFR specific for FGF-23, the humoral phosphatonin secreted by bone. In the kidney, Klotho mediates phosphate excretion and feedback inhibition of $1,25(OH)_2D_3$ synthesis in response to FGF-23. Klotho deficiency causes hyperphosphatemia and accelerated aging phenotypes, which are prevented in animals by resolving phosphate retention.

CKD and its complications, including CKD-MBD and vascular calcification, represent accelerated aging triggered by Klotho deficiency. Klotho expression begins declining early in CKD and may precede both hyperphosphatemia and FGF-23 upregulation. Further research is needed to determine whether Klotho decline or increased FGF-23 drives the vicious cycle of phosphate pathology in CKD.

Secreted Klotho, an FGF-23-independent phosphaturic hormone, regulates renal sodium/phosphate cotransporters and calcium and potassium ion channels. We hypothesize that FGF-23 induces secretion of Klotho from DCT cells, and secreted Klotho is a paracrine signal to proximal tubule cells to inhibit phosphate reabsorption and stimulate calcium reabsorption. Decreased urinary secreted Klotho may reflect decreased renal Klotho expression and is one of the earliest biomarkers of CKD.

It is concluded that phosphate retention induces complex aging-like phenotypes. Thus, maintaining normal phosphate levels with phosphate binders in patients with CKD with

declining Klotho expression is expected to reduce mineral and vascular derangements.

DISCLOSURE

MK has received research grant support from Genzyme Corporation, the National Institutes of Health, the Texas Higher Education Coordinating Board, and from Ardelyx. MK has a patent with the Japanese patent filing number H10-529809; Title: Novel polypeptide, novel DNA and novel antibody (Klotho).

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