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-o³). 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 tubule¹³,⁴) and parathyroid gland.⁵

The phenotypes of Klotho−/− and Fgf23−/− mice are very similar, involving premature aging and abnormal mineral metabolism.¹⁶ 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).

Kidney¹ and parathyroid⁵ 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)₂D₃ synthesis;⁵ in the parathyroid, FGF-23 suppresses parathyroid hormone secretion.⁵,¹⁰,¹¹
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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)2D3 levels remained elevated.17 Several other genetic and dietary interventions that rescue Klotho−/− and/or Fgf23−/− phenotypes18–20 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

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 mice16 and Fgf23−/− mice.17

which may also contribute to the ability of FGF-23 to reduce 1,25(OH)2D3 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 binds to Klotho/FGF receptor and shuts off the PTH promoter. FGF-23, fibroblast growth factor-23; PTH, parathyroid hormone.

Figure 1 | Endocrine regulation of phosphate metabolism.

Circulating 1,25(OH)2D3 turns on the FGF-23 promoter in bone. 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)2D3. PTH affects these renal promoters in a reverse manner to FGF-23, leading to 1,25(OH)2D3 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 phenotypes17–20

| Intervention                                           | Direction of change in serum levels |
|--------------------------------------------------------|-------------------------------------|
| Low-phosphate diet                                      | Phosphate 1,25(OH)2D3 Calcium       |
| 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)2D3, and phosphate as CKD progresses. When Klotho expression first decreases, FGF-23 increases, lowering circulating 1,25(OH)2D3, which depresses Klotho expression further and increases PTH expression. Increased PTH induces further FGF-23 increases, causing large decreases in 1,25(OH)2D3 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.

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)2D3 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 CKD24 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.  
Median Klotho mRNA levels in healthy kidney tissue represented slightly >8% of the level of glyceraldehyde-3-phosphate dehydrogenase, a housekeeping mRNA. 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. More sensitive assays by multiple reaction monitoring using mass spectrometry are currently in progress.

Reducing serum FGF-23 increases serum 1,25(OH)2D3 and renal Klotho expression. Parathyroidectomy is expected to reduce FGF-23 production, which in turn increases 1,25(OH)2D3 synthesis and then renal Klotho expression. Vitamin D administration, peroxisome proliferator-activated receptor-γ agonists, or angiotensin II inhibitors also increase Klotho expression.

Large serum FGF-23 increases during CKD progression 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 availability decreases. In the normal kidney, Klotho expression is abundant and a small amount of FGF-23 effectively inhibits NaPi2a in proximal tubules to allow phosphate excretion in normal and Fgf23−/− mice. 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.

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.

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, 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 reabsorption and feedback inhibition of 1,25(OH)2D3 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 Ardelx. MK has a patent with the Japanese patent filling number H10-529809; Title: Novel polypeptide, novel DNA and novel antibody (Klotho).

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REFERENCES
1. Kuro-o M, Matsumura Y, Aizawa H et al. Mutation of the mouse klotho gene leads to a syndrome resembling ageing. Nature 1997; 390: 45–51.
2. Kurosu H, Yamamoto M, Clark JD et al. Suppression of aging in mice by the hormone Klotho. Science 2005; 309: 1829–1833.
3. Kuro-o M. Overview of the FGF23-Klotho axis. Pediatr Nephrol 2010; 25: 583–590.
4. Hu MC, Shi M, Zhang J et al. Klotho: a novel phosphaturic substance acting as an autocrine enzyme in the renal proximal tubule. FASEB J 2010; 24: 3438–3450.
5. Ben-Dov IZ, Galitzer H, Lavi-Moshayoff V et al. The parathyroid is a target organ for FGF23 in rats. Clin Invest 2007; 117: 4003–4008.
6. Razzaque MS, Sitara D, Taguchi T et al. Premature aging-like phenotype in fibroblast growth factor 23 null mice is a vitamin D-mediated process. FASEB J 2006; 20: 720–722.
7. Kurosu H, Ogawa Y, Miyoshi M et al. Regulation of fibroblast growth factor 23-signal by klotho. J Biol Chem 2006; 281: 6120–6123.
8. Urakawa I, Yamazaki Y, Shimada T et al. Klotho converts canonical FGF receptor into a specific receptor for FGF23. Nature 2006; 444: 770–774.
9. Shimada T, Hasegawa H, Yamazaki Y et al. FGF23 is a potent regulator of vitamin D metabolism and phosphate homeostasis. J Bone Miner Res 2004; 19: 429–435.
10. Silver J, Naveh-Many T, FGF23 and the parathyroid glands. Pediatr Nephrol 2010; 25: 2241–2245.
11. Krajniak T, Bjorklund P, Marsell R et al. Fibroblast growth factor-23 regulates parathyroid hormone and 1α-hydroxylase expression in cultured bovine parathyroid cells. J Endocrinol 2007; 195: 125–131.
12. Liu S, Tang W, Zhou J et al. Fibroblast growth factor 23 is a counter-regulatory phosphaturic hormone for vitamin D. J Am Soc Nephrol 2006; 17: 1305–1315.
13. Silve C, Beck L. Is FGF23 the long sought after phosphaturic factor phosphatonin? Nephrol Dial Transplant 2002; 17: 958–961.
14. Saij F, Shizaki K, Shimada S et al. Regulation of fibroblast growth factor 23 production in bone in uremic rats. Nephron Physiol 2009; 111: 59–66.
15. Tsujikawa H, Kurotaki Y, Fujimori T et al. Klotho, a gene related to a syndrome resembling human premature aging, functions in a negative regulatory circuit of vitamin D endocrine system. Mol Endocrinol 2003; 17: 2393–2403.
16. Morishita K, Shirai A, Kubota M et al. The progression of aging in klotho mutant mice can be modified by dietary phosphorus and zinc. J Nutr 2001; 131: 3182–3188.
17. Stubbins JR, Liu S, Tang W et al. Role of hyperphosphatemia and 1,25-dihydroxyvitamin D in vascular calcification and mortality in fibroblastic growth factor 23 null mice. J Am Soc Nephrol 2007; 18: 2116–2124.
18. Ohnishi M, Nakatani T, Lanske B et al. Reversal of mineral homeostasis and soft-tissue calcification of klotho knockout mice by deletion of vitamin D 1α-hydroxylase. Kidney Int 2009; 75: 1166–1172.
19. Hesse M, Fröhlich LF, Zeitz U et al. Ablation of vitamin D signaling rescues bone, mineral, and glucose homeostasis in Fgf-23 deficient mice. Matrix Biol 2007; 26: 75–84.
20. Ohnishi M, Nakatani T, Lanske B et al. In vivo genetic evidence for suppressing vascular and soft-tissue calcification through the reduction of serum phosphate levels, even in the presence of high serum calcium and 1,25-dihydroxyvitamin D levels. Circ Cardiovasc Genet 2009; 2: 583–590.
21. Ohnishi M, Razzaque MS. Dietary and genetic evidence for phosphate toxicity accelerating mammalian aging. FASEB J 2010; 24: 3562–3571.
22. United States Renal Data System. USRDS 2010 Annual Data Report: Atlas of Chronic Kidney Disease and End-Stage Renal Disease in the United States. National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases: Bethesda, MD. http://www.usrds.org/atlas.htm (accessed 30 November 2010).
23. Brancaccio D, Bellasi A, Cozzolino M et al. Arterial accelerated aging in dialysis patients: the clinical impact of vascular calcification. Curr Vasc Pharmacol 2009; 7: 374–382.
24. Hu MC, Shi M, Zhang J et al. Klotho deficiency causes vascular calcification in chronic kidney disease. J Am Soc Nephrol 2011; 22: 124–136.
25. O'Brien SP, Boulanger JH, Liu S et al. Decline in Klotho expression precedes FGF23 and PTH induction in the Jck mouse, a progressive genetic model of CKD-MBD [Abstract F-FC224]. J Am Soc Nephrol 2009; 20: 54A.
26. Koh N, Fujimori T, Nishiguchi S et al. Severely reduced production of klotho in human chronic renal failure kidney. Biochem Biophys Res Commun 2001; 280: 1015–1020.
27. Yamazaki Y, Imura A, Urakawa I et al. Establishment of sandwich ELISA for soluble alpha-Klotho: measurement: age-dependent change of soluble alpha-Klotho level in healthy subjects. Biochem Biophys Res Commun 2010; 398: 513–518.
28. Zhang H, Li Y, Fan Y et al. Klotho is a target gene of PPAR-γ. Kidney Int 2008; 74: 732–739.
29. Mitani H, Ishizaka N, Aizawa T et al. In vivo klotho gene transfer ameliorates angiotensin II-induced renal damage. Hypertension 2002; 39: 838–843.
30. Saito K, Ishizaka N, Mitani H et al. Iron chelation and a free radical scavenger suppress angiotensin II-induced downregulation of klotho, an anti-aging gene, in rat. FEBS Lett 2003; 551: 58–62.
31. Luizierio O, Isakova T, Rhein L et al. Fibroblast growth factor-23 mitigates hyperphosphatemia but accentuates calcitriol deficiency in chronic kidney disease. J Am Soc Nephrol 2005; 16: 2205–2215.
32. Haruna Y, Kashiwara N, Sato M et al. Amelioration of progressive renal injury by genetic manipulation of Klotho gene. Proc Natl Acad Sci USA 2007; 104: 2331–2336.
33. Sugiuira H, Yoshida T, Tsuchiya K et al. Klotho reduces apoptosis in experimental ischaemic acute renal failure. Nephrol Dial Transplant 2005; 20: 2636–2645.
34. Chen CD, Podvin S, Gillespie E et al. Insulin stimulates the cleavage and release of the extracellular domain of Klotho by ADAM10 and ADAM17. Proc Natl Acad Sci USA 2007; 104: 19796–19801.
35. Bloch L, Sineshchekova O, Reichenbach D et al. In vivo klotho deficiency causes vascular toxicity accelerating mammalian aging. Kidney Int 2009; 75: 1166–1172.
36. Lu P, Boros S, Chang Q et al. In vivo analysis of renal outer medullary potassium channel and renal K+ excretion by klotho. Mol Pharmacol 2009; 76: 38–46.
37. Huang CL. Regulation of ion channels by secreted Klotho: mechanisms and implications. Kidney Int 2010; 77: 855–860.

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