# Kidney and Aging: More Than What You Think You Know

**Internal Medicine Grand Rounds**

**June 4, 2010**

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Daughters of Zeus and Themis


*Klotho* spins the thread of life
*Lakhesis* measures the length
*Atropos* cuts the thread

This is to acknowledge that Dr. Huang has not disclosed any financial interests or other relationships with commercial entities related directly or indirectly to this program. Dr. Huang will not discuss off-label use in this presentation.

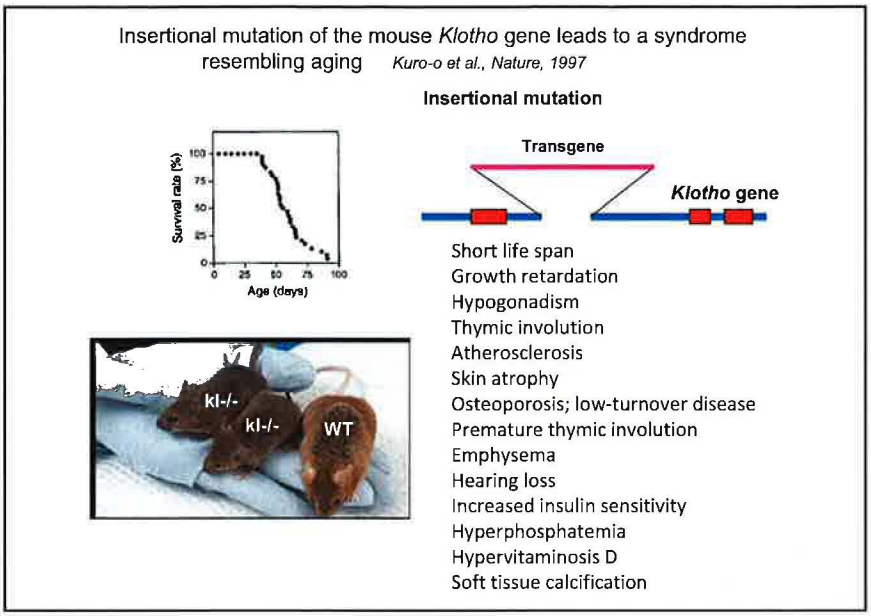
Professor of Medicine

Clinical interests: fluid & electrolyte disorders; general consultative nephrology

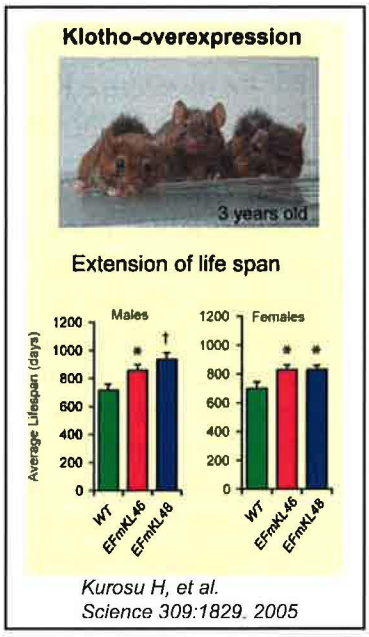
Research interests: genetic diseases of ion transport disorders; hypertension; WNK kinases signaling pathway in the regulation of cardiovascular development and ion transport; anti-aging hormone Klotho.

## Introduction

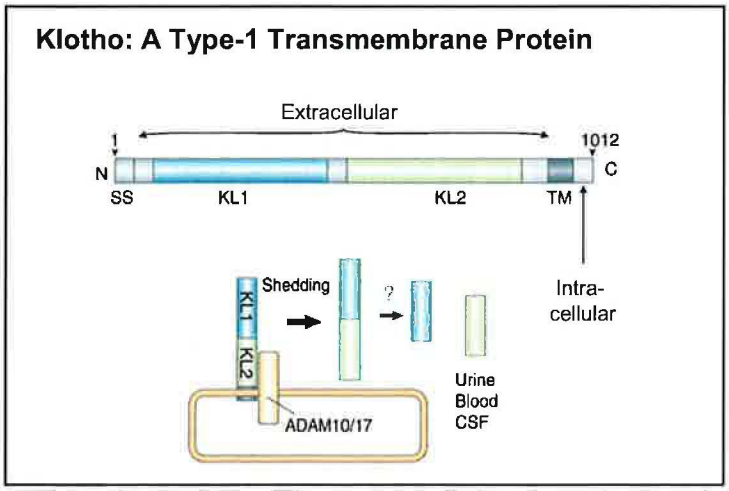
Mice with disruption in the *Klotho* gene display multiple phenotypes closely resembling human aging including shortened lifespan, muscle and skin atrophy, pulmonary emphysema, osteopenia, infertility, hyperphosphatemia, and vascular and soft tissue calcification (Kuro-o et al., 1997).




In support of its function in aging suppression, overexpression of Klotho in mice extends their life span (Kurosu et al., 2005).



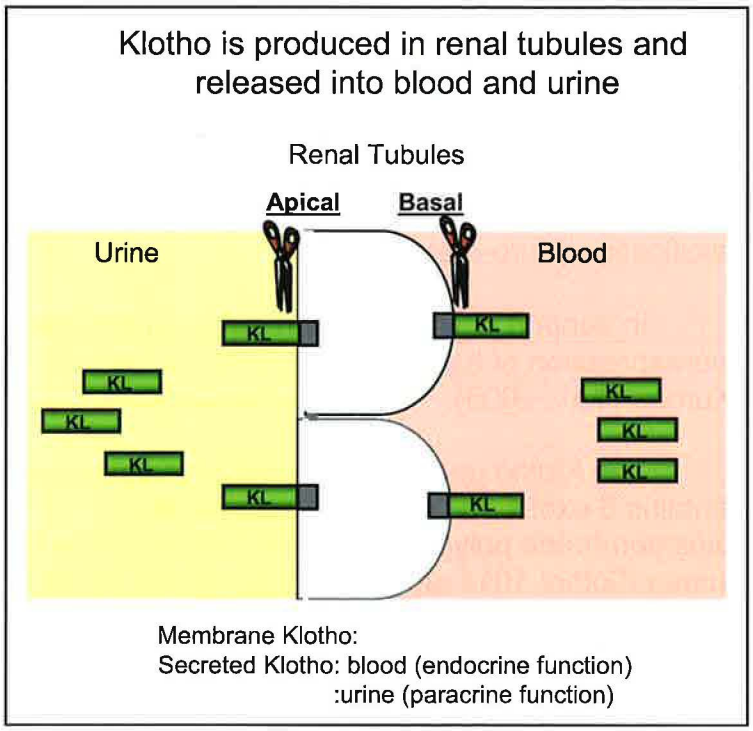

The *Klotho* gene, in mice and humans as well, contains 5 exons and encodes a single-pass transmembrane polypeptide of 1012 amino acids (for human Klotho; 1014 amino acids for mouse Klotho) (Kuro-o et al., 1997). Klotho protein is predominantly expressed in the kidney, parathyroid glands, and the epithelial cells of choroid plexus of the brain.




The majority of amino acids in the Klotho peptide (~980 residues) reside in the amino-terminal extracellular domain, which is followed by 21 amino acids membrane-spanning domain, and a 11 amino acid short intracellular carboxyl terminus. The extracellular domain consists of two internal repeat sequences of 440 amino acids, named KL1 and KL2, respectively. The linker

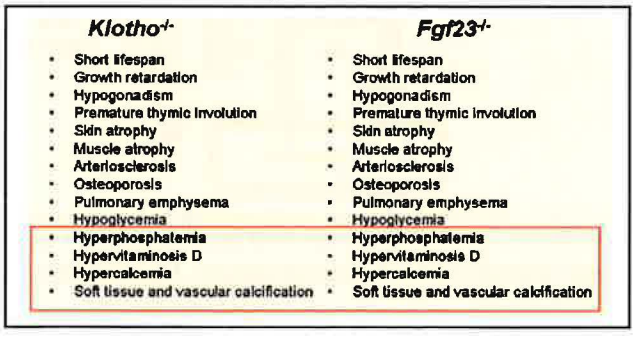
region between the two internal repeats contains a stretch of 4 basic amino acids (Lys-Lys-Arg-Lys) that forms a potential site for proteolytic cleavage. A secreted form of Klotho consisting of the full-length extracellular domain is detected in the blood, urine, and cerebrospinal fluid (Kurosu et al., 2005; Imura et al., 2004). A recent report shows that the extracellular domain is cleaved by the metalloproteases ADAM10 and ADAM17 and that insulin stimulates the cleavage of Klotho (Chen et al., 2007). Separate KL1 and KL2 fragments are found in the urine, likely a result of cleavage of the full-length extracellular domain at the linker region by proteases.

The membrane-bound full-length Klotho and secreted extracellular domain of Klotho have distinct functions. Membrane Klotho interacts with fibroblast growth factor (FGF) receptors to form high affinity receptors for FGF23. Secreted Klotho functions as an autocrine/paracrine and endocrine hormone. As an autocrine/paracrine hormone, Klotho is secreted from renal tubules into urine and regulates the surface abundance of the ion transporters at the apical membrane. As an endocrine hormone, Klotho is released into blood circulation and regulates the intracellular signaling or function of membrane proteins in other organs (Huang, 2010).



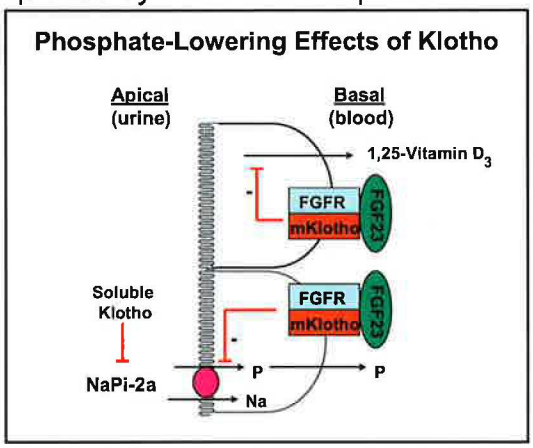

**Role of Klotho in Mineral Metabolism and Its Relationship to Anti-Aging**

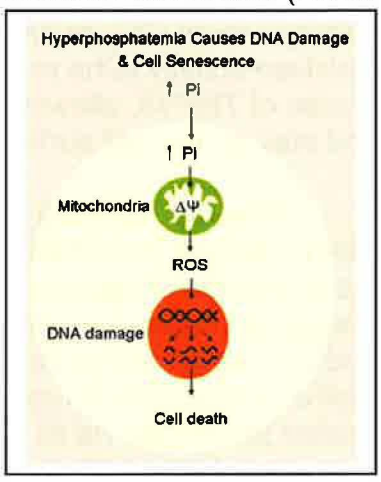
Studies have shown that Klotho has many important functions, including anti-insulin/insulin-like growth factor (Kuroso et al., 2005), reduction of oxidative stress (Yamamoto et al., 2005), antagonism of Wnt signaling pathway (Liu et al., 2007), regulation of cell proliferation (Medici et al., 2008) and regulation of mineral metabolism (calcium, phosphate and vitamin D, etc) (Razzaque & Lanske, 2007)



One of the consequences of Klotho deficiency is hyperphosphatemia. Increasing evidence indicates that the premature aging resulting from Klotho deficiency is related to deranged phosphate metabolism (Kurosu & Kuro-o, 2008). Mice lacking FGF23, a bone-derived phosphaturic hormone, exhibit phenotypes identical to Klotho-deficient mice, including hyperphosphatemia, increased levels of 1,25-vitamin $D_3$, and premature aging (Razzaque & Lanske, 2007).

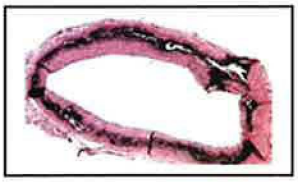
Both membrane-bound Klotho and secreted Klotho are involved in the regulation of phosphate metabolism. Membrane-bound Klotho interacts with FGF receptors to form high affinity receptors for FGF23 (Kurosu et al., 2006). Activation of FGFR-Klotho co-receptor complexes by FGF23 in the proximal tubule of the kidney decreases serum phosphorus via two mechanisms. First, it reduces the expression of Na-dependent phosphate transporters on the luminal membrane of the proximal tubules to decrease renal phosphate reabsorption (Razzaque, 2009). Second, it suppresses the synthesis of 1,25-vitamin $D_3$, which leads to decreased phosphate absorption from the gastrointestinal tract (Yoshida et al., 2002). The absolute requirement of Klotho for FGFR function explains why Klotho deficiency and FGF23 deficiency have the same phenotype. Additionally, secreted Klotho directly inhibit $Na^+$-dependent phosphate transporters via an FGF23-independent mechanism (Hu et al., 2010). Thus, Klotho deficiency and FGF23-deficiency cause hyperphosphatemia by increasing intestinal phosphate absorption and increasing renal phosphate reabsorption.



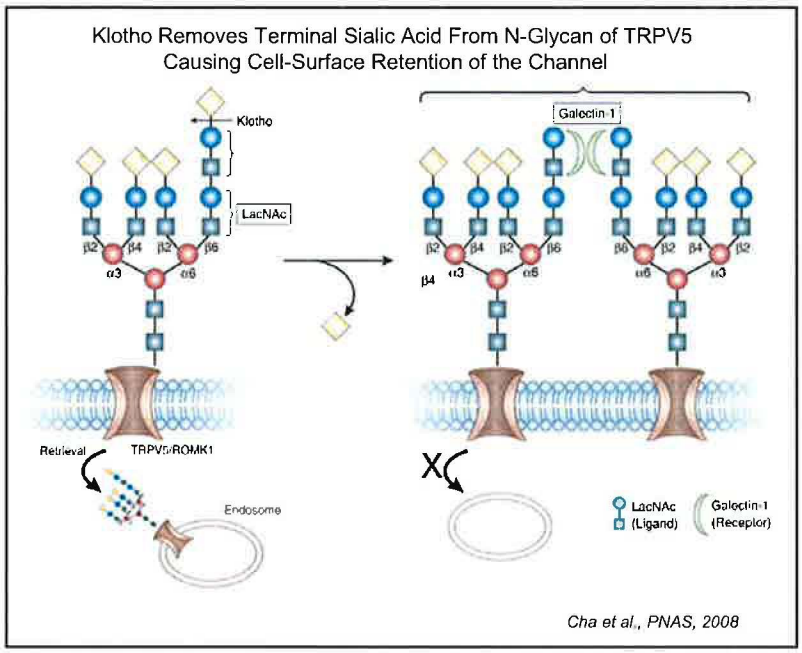

Hyperphosphatemia can accelerate aging via several mechanisms. High serum phosphate levels positively correlate with a pattern of intracellular insulin signaling and unfavorable cell metabolism associated with aging (Kuro-o, 2010). An increase in cellular phosphate entry increases mitochondrial membrane potential and cellular oxidative stress, which is closely associated with cell senescence (Kuro-o, 2010).

In addition, hyperphosphatemia promotes vascular calcification, which among other things leads to organ senescence. In support of the idea that hyperphosphatemia leads to accelerated aging, dietary



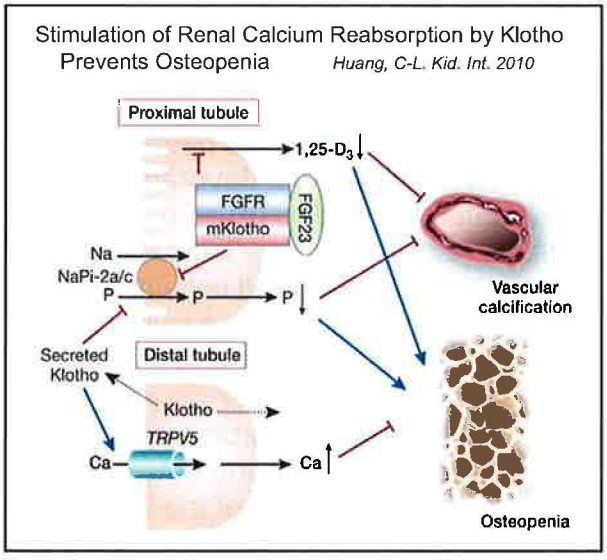
phosphate restriction prevents vascular calcification and rescues premature aging in Klotho-deficient mice as well as in FGF23-deficient mice (Kuroso & Kuro-o, 2008; Razzaque & Lanske, 2007). Thus, the effect of Klotho to extend life is, at least in part, related to its ability to promote renal phosphate excretion and maintain a low phosphate balance.

Low phosphate balance over a long period of time via the effect of FGF 23 leads to bone diseases, as seen in the conditions of acquired (tumor-induced osteomalacia) or congenital excess of FGF23 (autosomal-dominant hypophosphatemic rickets) (Razzaque, 2009). In contrast, Klotho does not cause bone disease notwithstanding its similar effect on phosphate homeostasis.




Recent studies show that Klotho stimulates renal $Ca^{2+}$ channel TRPV5 to increase renal $Ca^{2+}$ reabsorption (Chang et al., 2005). The effect is through its sialidase activity (Cha et al., 2008). Klotho removes terminal sialic acid from N-glycan of TRPV5, allowing the channel to bind to extracellular lectin galectin-1 and stay on the cell surface.

The $Ca^{2+}$-sparing effect of Klotho (absent in FGF23) counteracts the deleterious effects of low phosphate balance on bone. In addition, as a cofactor to FGF 23, Klotho participates in regulating aging-related processes via its effect on phosphate homeostasis. As shown, binding of FGF23 to the membrane Klotho and FGFR co-receptor complex leads to inhibition of the synthesis of 1,25-vitamin $D_3$ and inhibition of the expression of $Na^+$-dependent phosphate cotransporter



(NaPi-2a and/or -2c) in the apical membrane of the proximal tubule. In addition, secreted Klotho directly inhibits NaPi-2a/2c. The overall phosphate-lowering effects of Klotho protect against vascular calcification and cellular aging while at the same time, secreted Klotho stimulates renal $Ca^{2+}$ reabsorption and prevents the development of osteomalacia.associated with low phosphate balance (Huang, 2010).

## Klotho and Human Aging

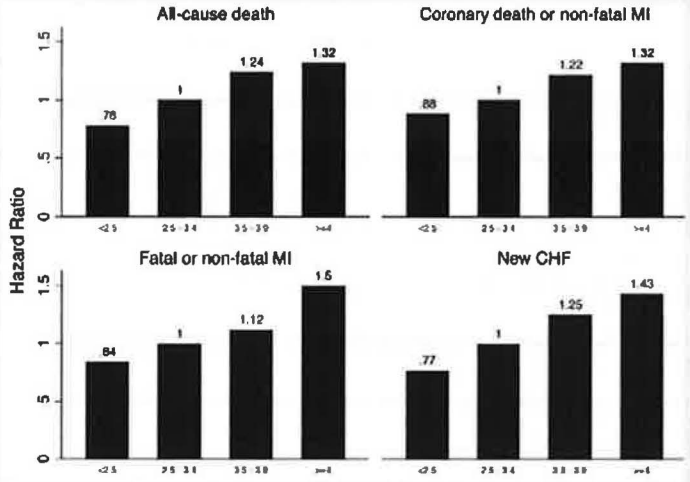
The long life span of human precludes definitive longitudinal studies on effect of low phosphate on human aging processes. The relationship between phosphate and life span however can be ascertained from studies of diseased population with reduced life span.

**Phosphate and Human Aging**

Post hoc analysis of data from the Cholesterol And Recurrent Events (CARE) study
*Tonelli et al., Circulation, 2005*

Randomized trial of pravastatin on prevention of all-cause and cardiac death
Men and women who had acute 3-20 months prior to randomization
21-75 years of age
LDL 115-174 mg/dL
On pravastatin 40 mg vs placebo daily
375 of 4159 participant died during 60 months follow-up

4127 of 4159 study individuals have baseline serum phosphate levels
Stratified into serum phosphate < 2.5; 2.5-3.4; 3.5-3.9; ≥ 4 mg/dL




Hazard ratio (HR) adjusted for age, sex, race, smoking, diabetic, glucose, w/r ratio, eGFR, Hg, albumin, aspirin, LVEF

Analysis reveals that adjusted hazard ratio for all-cause and several cardiac-related deaths all positively correlated with increasing serum phosphate levels.

## Klotho and Human Aging

Many studies have found association of *KLOTHO*

**Klotho and Human Aging**

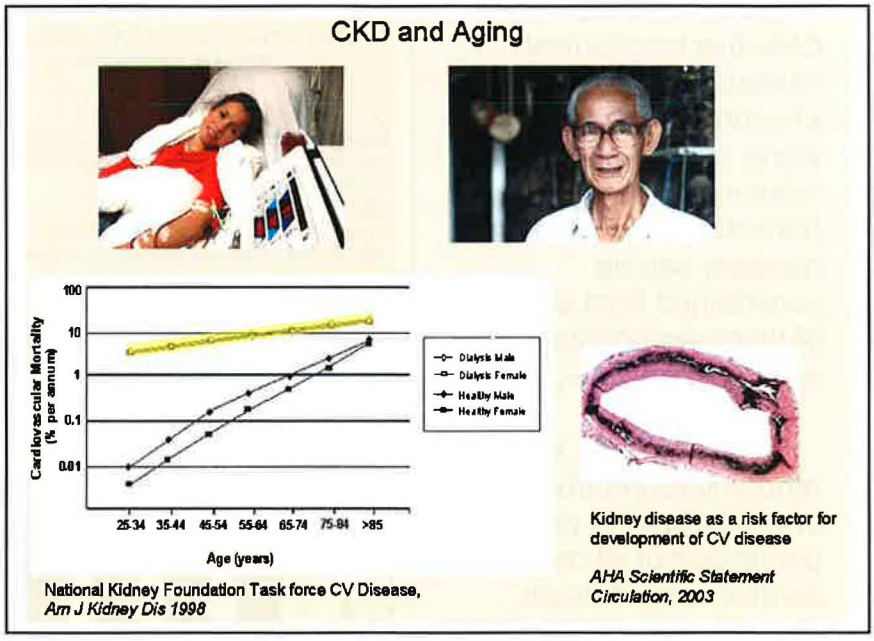
Many studies have found association of *KLOTHO* gene polymorphism with phenotypes of aging or longevity

Homozygous missense mutation in human KLOTHO causes severe hyperphosphatemia and tumoral calcinosis
*Ichikawa et al., JCI, 2007*

The serum level of Klotho decreases with aging
*Xiao et al. Chin Med J. 117: 742-747, 2004*

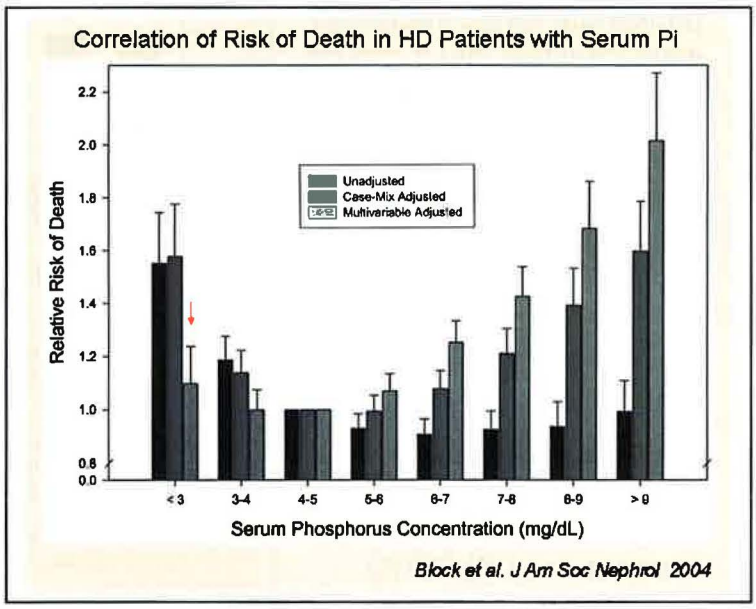
Urinary Klotho levels decrease in parallel with declining eGFR in CKD patients
*Hu et al, Submitted*

gene polymorphism with phenotypes of aging or longevity. Ichikawa et al (2007) reported a 13 year-old girl with homozygous missense in human *KLOTHO* gene who developed severe hyperphosphatemia and metastatic calcification in blood vessels and soft tissues. One study has reported that serum level of Klotho in human decreases with aging (Xiao et al., 2004). However, there is no consensus on the validity of the assay for serum Klotho. Dr. Orson Moe's lab has used western blot analysis standardized against the recombinant purified Klotho to analyze Klotho in human urine and reported that urinary Klotho levels decrease in parallel with declining eGFR in normal and CKD patients (Hu et al., submitted). The kidney is the main organ for production of Klotho. Since nephron mass and GFR decline with aging, these results also support the idea that the abundance of Klotho in the kidney (and hence in circulation and urine) declines with aging.




## Chronic Kidney Disease (CKD) and Aging

Despite dialysis, the mortality of end-stage renal disease (ESRD) patients is strikingly high. The main cause of mortality in kidney diseased patients is cardiovascular (Sarnak et al., 2003). At the moment, the cardiovascular mortality of a 30 year-old individual on dialysis is close to that of a 75 year-old (Levey et al., 1998). The risk of CV death in HD patients is strongly correlated with serum phosphate levels.




As shown in the study by Block et al (2004), the adjusted relative risk to death from CV causes increases progressively from serum phosphate 3 mg/dl to >9 mg/dl.

The higher CV morbidity and mortality from higher phosphate is not limited to dialysis patients. The relationship also exists in earlier stages of CKD and with serum phosphate levels at the "normal range" (2.5-4.5 mg/dl).

Kestenbaum et al (2005) studied the relationship between serum phosphate levels and mortality risk among veterans with CKD from 8 VA hospitals in the Pacific Northwest. The mean age of subjects studied is 71.8 year old. The mean level of estimated creatinine clearance (eCrCl) is 47.2 ml/min (median 45.1 ml/min). Serum phosphate levels are separated into 5 groups from 2.5 to 5 mg/dl, with 0.5 mg/dl increment for each group.

Serum Phosphate Levels and Mortality Risk Among People with Chronic Kidney Disease

*Kestenbaum et al., JASN, 2005*

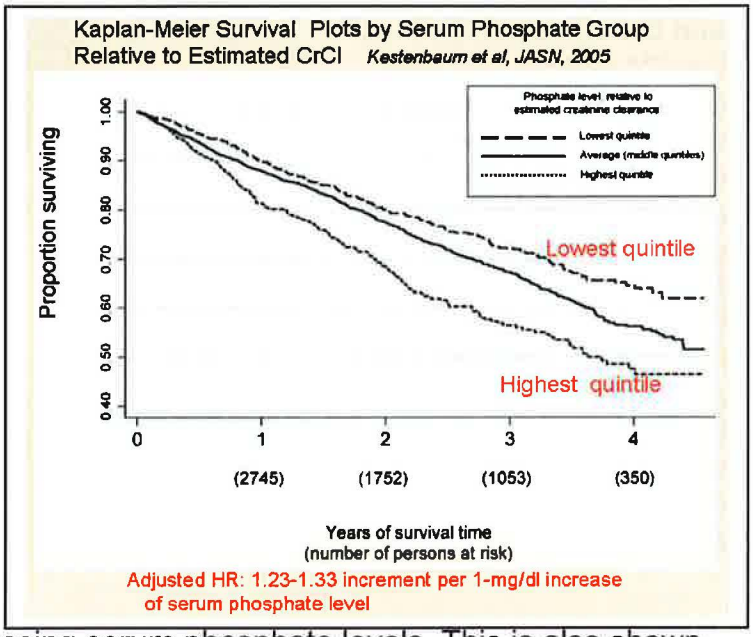
A retrospective cohort study from 8 VA hospitals in Pacific Northwest
Two abnormal Cr measurement >6 months apart 1999-2002
HD and transplant patient excluded
95,619 veterans reviewed; 3,490 subjects with CKD and serum phosphate

*Table 4.* Mortality rates and Cox regression results by phosphate category

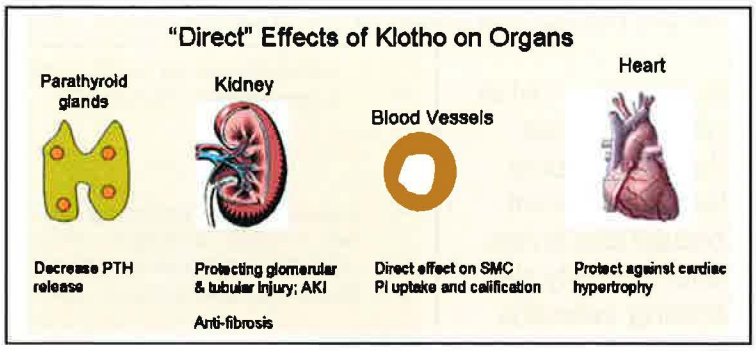
| Serum phosphate level | | No. of Patients | Crude Mortality Rate per 1000 Person-Years (No. of Deaths) | Adjusted HR[a] (95% CI) |
|---|---|---|---|---|
| mg/dl | mmol/L | | | |
| <2.5 | <0.81 | 201 | 101.7 (54) | 0.95 (0.69–1.32) |
| 2.5–2.999 | 0.81–0.9699 | 684 | 102.6 (180) | Reference |
| 3.0–3.499 | 0.97–1.1299 | 1098 | 125.1 (327) | 1.15 (0.95–1.39) |
| 3.5–3.999 | 1.13–1.2899 | 887 | 162.7 (309) | 1.32 (1.09–1.61) |
| 4.0–4.499 | 1.29–1.4499 | 388 | 192.8 (144) | 1.34 (1.05–1.71) |
| 4.5–4.999 | 1.45–1.6199 | 141 | 256.9 (62) | 1.83 (1.33–2.51) |
| ≥5.0 | ≥1.62 | 91 | 304.7 (38) | 1.90 (1.30–2.79) |

[a]Adjusted for age, race, gender, prevalent diabetes, ischemic heart disease, cerebrovascular disease, congestive heart failure, acute renal failure, calcium intake from medications, hemoglobin, serum calcium, the inverse of baseline creatinine, time-averaged creatinine (area under the curve), slope of creatinine, and maximal creatinine concentration during the baseline period (mode

As expected, serum phosphate levels are inversely correlated with eCrCl. For example, the mean eCrCl for the group of serum phosphate <2.5 mg/dl and of 4.5-4.999 are 50.4 ml/min and 39.5 ml/min, respectively. Serum phosphate levels among individual of the same eCrCl, however, are quite variable. Analysis reveals hazard ratio for mortality, adjusted for many variables including CrCl, is positively correlated with increasing serum phosphate levels. This is also shown in Kaplan-Meier survival plots where lowest quintle of serum phosphate relative to estimated CrCl has better survival than the middle 3 quintles and the highest quintle.



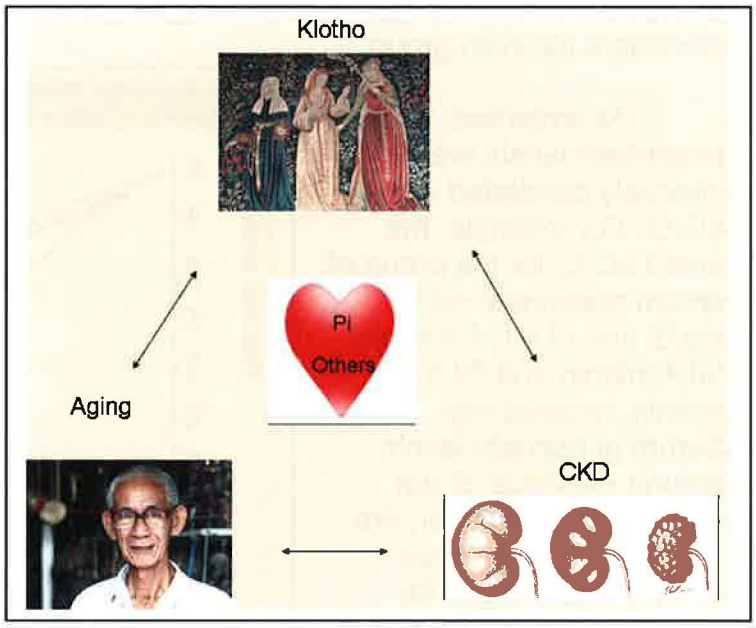
## Effects of Klotho on Organs Independent of Serum Phosphate




Klotho has also been shown to decrease PTH release by the glands (Ben-Dov et al., 2007). The mechanisms involve both decreased mRNA transcript and decreased secretion. This effect is believed to be mediated by membrane Klotho and requires FGF receptors. The effect of protecting kidneys in tubular and glomerular injuries has also been reported (Haruna et al., 2007), though the mechanism remains unknown. Direct effects on CV systems have also been suggested, but require further studies.

## Summary and Conclusion

Like patients with chronic diseases of major organs, CKD patents age and die early. The resemblance between effects of aging and the effects of CKD on the CV system suggests that the two processes share common linkage. The recent discovery of Klotho and advances on the understanding of its functions suggest that Klotho is an important missing link between CKD and aging. Center to the heart of the link is deranged metabolism of phosphate. Other functions of Klotho are certainly also important.



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