Klotho: A Master Regulator of Cardiovascular Disease?

Running title: Moe; Vascular klotho deficiency

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Finding the mechanism that will make us live a long and healthy life is the goal of medical research. How appropriate that Klotho, the Greek goddess of life is involved in vascular disease. Klotho is one of the Greek Moirai, the goddesses of fate who controlled the ultimate destiny of man: death. Klotho spins the thread of life, Lakhes measured the thread of life, and Atropos cut the thread of life. For this reason, the gene that seemed to control aging was named Klotho when first discovered by Kuro-o et al in 1997\(^1\). This klotho knock out ‘aging’ mouse suffered from early demise, infertility, arteriosclerosis and arterial calcification, osteoporosis, hyperphosphatemia, emphysema and skin atrophy. This landmark discovery has not (yet) extended our life span, but has opened up an amazing understanding of the role of Klotho and mineral homeostasis in the pathogenesis of cardiovascular disease.

Klotho is a 130 kDa transmembrane protein that is predominantly expressed in the distal tubule of the kidney but also in multiple other tissues\(^1\). The extracellular domain is also cleaved and secreted into the blood, urine and cerebrospinal fluid\(^2\) and thus there is both tissue Klotho and secreted (soluble) Klotho. In the kidney, Klotho serves as a co-receptor for fibroblast growth factor 23 (FGF23), the major phosphatonin, and receptor activation leads to increased urinary excretion of phosphorus\(^3\). Klotho also stimulates calcium reabsorption in the distal tubule by preventing endocytosis (stabilizing) the major calcium channels, transient receptor potential cation channel subfamily V (TRPV5 and TRPV6)\(^4\). Thus Klotho may work with FGF23 to increase urinary phosphorus content but also ensures that the urine with high phosphorus does not also have high calcium (and thus preventing supersaturation of the urine). Both FGF23 and Klotho are stimulated by \(1,25(\text{OH})_2\text{D}\), and both FGF23 and Klotho stimulate renal 1-alpha hydroxylase (CYP27B1), thereby decreasing the conversion of \(25(\text{OH})\text{D}\) to \(1,25(\text{OH})_2\text{D}\) (calcitriol) to complete endocrine feedback loop\(^5\). Thus, Klotho joins the ranks of
FGF23, PTH, and calcitriol in a series of feedback loops that ensure optimal concentrations of calcium and phosphorus in bone and blood.

Klotho is also involved in the pathogenesis of arterial calcification. Hu and colleagues found increased arterial calcification in Klotho+/- mice with superimposed kidney disease and the opposite in mice over-expressing Klotho. In cultured vascular smooth muscle cells (VSMC) from Klotho+/- mice, there was downregulation of the smooth muscle cell marker sm-22 and upregulation of the osteoblast marker Runx2 and sodium phosphate co-transporters Pit1 and Pit2 suggesting that Klotho deficiency predisposed cells to transform into osteoblast like cells that are ready to initiate mineralization in response to phosphate uptake. Again, Klotho overexpression reversed these findings. In this issue of Circulation, Lim et al confirm these findings in human VSMC, demonstrating for the first time that Klotho, and FGF receptor 1 and 3 are expressed in human arteries, with downregulation in response to phosphorus and tumor necrosis factor alpha (TNFα). They also confirmed the results of Hu et al in human VSMC demonstrating that decreased Klotho (by siRNA) leads to increased calcification. Finally, they demonstrate that upregulation of Klotho through vitamin D receptor activation by calcitriol or paricalcitol, restores Klotho, FGF23 signaling and inhibits vascular calcification. These two studies suggest that Klotho is protective against vascular calcification by preventing differentiation of the VSMC to an osteoblast like phenotype, and in the study by Lim suggest that restoration of FGF23 responsiveness is protective. This raises the question as to whether the elevations in FGF23 observed in kidney disease patients are reflective of underlying phosphorus levels or are a manifestation of resistance due to Klotho deficiency. It should also be emphasized that phosphorus may still be a requisite for arterial calcification, as calcification only occurred in the setting of kidney disease in the mouse studies by Hu in which blood levels of phosphorus were
elevated. In addition the in vitro work by Lim also utilized a high phosphorus media.

In addition to this important role in vascular calcification, Klotho also is involved in vascular health through other mechanisms. In Klotho heterozygous mutant mice, there is impaired endothelial dependent vasodilation. In cultured endothelial cells, the addition of Klotho protects against endothelial cell apoptosis through the mitogen-activated protein kinase (MAPK) pathway and decreases tumor necrosis factor alpha induced intracellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and NF-kappaB activation. Klotho transfection of cultured vascular smooth muscle cells also reduced intracellular superoxide production and decreased angiotensin II oxidative stress. Thus Klotho is also involved in endothelial cell function and inflammation of both endothelial and VSMC. In addition to these apparent direct effects of Klotho, it should also be noted that FGF23 also increases cardiomyocyte hypertrophy in a Klotho independent manner, and elevations of FGF23 are linked to progression of left ventricular hypertrophy in patients with early stage kidney disease. Thus, FGF23 and Klotho appear to have broad cardiovascular effects.

In human kidney biopsies and animal models, decreased Klotho expression is found very early in the course of CKD, at stage 2, (estimated glomerular filtration rates of 60 to 90ml/min). In rat models of hypertension (salt sensitive hypertensive rats, spontaneously hypertensive rats), acute myocardial infection, and ischemia induced CKD, there is also down regulation of Klotho, but later in the course of disease implying that the Klotho downregulation is a consequence of kidney injury rather than a cause of either kidney or cardiovascular disease. The urinary excretion of Klotho is decreased with progressive CKD suggesting it may be an early marker of kidney disease or ischemic insults. However, current assays for circulating levels of Klotho do not appear to correlate with residual renal function or show increased levels
in kidney disease\textsuperscript{17}. Whether this difference reflects blood assay problems, or differences in the regulation of cellular Klotho versus circulating Klotho is not yet known.

So how does this exploding field of investigation relate to clinical epidemiologic studies? Albuminuria with or without CKD is a known cardiovascular risk factor\textsuperscript{18}. Elevated blood levels of FGF23 and phosphorus are associated with increased mortality in patients with CKD and on dialysis\textsuperscript{19,20} and FGF23 levels are increased in patients with albuminuria even with normal kidney function\textsuperscript{21}. In patients with phosphorus levels in the upper quartile of the normal range and no known kidney disease, there is increased cardiovascular disease and mortality in patients with \textsuperscript{22} and without \textsuperscript{23} known cardiovascular disease. Are the observational studies identifying phosphorus as a risk factor due to direct effects of phosphorus on the cardiovascular system or due to a failure of appropriate FGF23/Klotho signaling at the kidney to induce phosphaturia, and/or failure of appropriate FGF23/Klotho signaling in the vasculature? Phosphorus is a known direct vascular toxin in vitro, in vivo animal studies, and in humans\textsuperscript{20} but elevations in blood phosphorus may only arise late in the course of CKD when there is a failure in the FGF23/Klotho signaling pathways. Yet, as noted above, elevations in phosphorus appear to be required to get arterial calcification. In contrast, FGF23 is elevated early in the course of CKD. So is elevated FGF23 a risk factor due to direct effects, or because it signifies that there is a lack of responsiveness due to Klotho deficiency? Is decreased Klotho in the kidney and arteries a risk factor because it is an indicator of kidney disease or calcitriol deficiency? Are studies demonstrating reduced mortality with the administration of calcitriol and its analogs in patients on dialysis\textsuperscript{24} due to a direct effect from the upregulation of Klotho in the vasculature? Is Klotho deficiency simply a biomarker of early kidney disease, or can a reduction in Klotho due to inflammation be a cardiovascular risk factor independent of kidney disease? As the science
unfolds, the interactions become more complex. We still do not understand the inciting or initiating factor that links disordered mineral metabolism and abnormal hormonal levels of FGF23, PTH, calcitriol and now Klotho. But one thing is for sure, Klotho is a key factor in these interactions. Maybe Greek mythology was correct- Klotho does spin the secret of life, or at least a life free of cardiovascular disease.

In patients with kidney disease, and possibly in patients with inflammation but no kidney disease, there is decreased tissue Klotho in the kidney and arteries (Figure 1). In the kidney, the decreased Klotho leads to an inability of FGF23 to induce phosphaturia due to the need for Klotho to serve as a receptor to FGF23 in the kidney. In arteries, the decreased Klotho leads to an inability of FGF23 to prevent cellular differentiation to osteoblast like cells. As a result there is resistance to FGF23 even in the presence of high circulating levels. The decreased phosphorus excretion and progressive rise in phosphorus also leads to transformation of VSMC to osteoblast like cells and provides a substrate for the mineral deposits. These cells then take up phosphorus (and calcium) to induce vascular calcification. Finally, inflammation and decreased Klotho induces endothelial dysfunction which further worsens cardiovascular disease.

**Conflict of Interest Disclosures:** Dr. Moe has consulting agreements and grants from Amgen, Genzyme, Shire and Litholink.

**References:**


Figure Legend:

Figure 1. Cardiovascular effects of decreased Klotho