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, Lakhesis 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. This klotho knockout 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 it has opened up an amazing understanding of the role of Klotho and mineral homeostasis in the pathogenesis of cardiovascular disease.

**Klotho**

**A Master Regulator of Cardiovascular Disease?**

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Klotho is a 130-kDa transmembrane protein that is predominantly expressed in the distal tubule of the kidney but also in multiple other tissues. The extracellular domain is also cleaved and secreted into the blood, urine, and cerebrospinal fluid, and thus there is both tissue Klotho and secreted (soluble) Klotho. In the kidney, Klotho serves as a coreceptor for fibroblast growth factor 23 (FGF-23), the major phosphatonin, and receptor activation leads to increased urinary excretion of phosphorus. Klotho also stimulates calcium reabsorption in the distal tubule by preventing endocytosis, stabilizing the major calcium channels, and the transient receptor potential cation channel subfamily V (TRPV5 and TRPV6). Thus, Klotho may work with FGF-23 to increase urinary phosphorus content, but it also ensures that the urine with high phosphorus does not also have high calcium (thus preventing supersaturation of the urine). Both FGF-23 and Klotho are stimulated by 1,25-dihydroxyvitamin D (1,25(OH)2D; calcitriol), and both FGF-23 and Klotho inhibit renal 1α-hydroxylase (CYP27B1), thereby decreasing the conversion of 25-hydroxyvitamin D to calcitriol to complete endocrine feedback loop. Thus, Klotho joins the ranks of FGF-23, parathyroid hormone, 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 overexpressing Klotho. In cultured vascular smooth muscle cells (VSMCs) from Klotho+/− mice, there was downregulation of the smooth muscle cell marker sm-22 and upregulation of the osteoblast marker Runx2 and sodium phosphate cotransporters Pit1 and Pit2, suggesting that Klotho deficiency predisposed cells to transform into osteblast-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 α. They also confirmed the results of Hu et al in human VSMC, demonstrating that decreased Klotho (by small interfering RNA) leads to increased calcification. Finally, they demonstrate that upregulation of Klotho through vitamin D receptor activation by calcitriol or paricalcitol restores Klotho and FGF-23 signaling and inhibits vascular calcification. These 2 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 FGF-23 responsiveness is protective. This raises the question as to whether the elevations in FGF-23 observed in patients with kidney disease 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, because 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 used 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 pathway and decreases tumor necrosis factor α-induced intracellular adhesion molecule-1, vascular cell adhesion molecule-1, and nuclear factor κB 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 FGF-23 also increases cardiomyocyte hypertrophy in a Klotho-independent manner, and elevations of FGF-23 are linked to progression of left ventricular hypertrophy in patients with early stage kidney disease.12 Thus, FGF-23 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 chronic kidney disease (CKD), at stage 2 (estimated glomerular filtration rates of 60–90 mL/min).13,14 In rat models of hypertension (salt-sensitive hypertensive rats, spontaneously hypertensive rats), acute myocardial infection, and ischemia-induced CKD, there is also downregulation 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.15 The urinary excretion of Klotho is decreased with progressive CKD,6 suggesting that 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 function16 or show increased levels in kidney disease.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 epidemiological studies? Albuminuria with or without CKD is a known cardiovascular risk factor.18 Elevated blood levels of FGF-23 and phosphorus are associated with increased mortality in patients with CKD and on dialysis,19,20 and FGF-23 levels are increased in patients with albuminuria even with normal kidney function.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 with22 and without23 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 FGF-23/Klotho signaling at the kidney to phosphaturia, and/or failure of appropriate FGF-23/Klotho signaling in the vasculature?

Phosphorus is a known direct vascular toxin in vitro, in vivo animal studies, and in humans20 but elevations in blood phosphorus may only arise late in the course of CKD when there is a failure in the FGF-23/Klotho signaling pathways. Yet, as noted above, elevations in phosphorus appear to be required to get arterial calcification. In contrast, FGF-23 is elevated early in the course of CKD. So is elevated FGF-23 a risk factor owing to direct effects, or because it signifies that there is a lack of responsiveness because of Klotho deficiency? Is decreased Klotho in the kidney and arteries a risk factor owing to direct effects, or because it signifies that there is a lack of responsiveness because of Klotho deficiency? Are studies demonstrating reduced mortality with the administration of calcitriol and its analogs in patients on dialysis24 because of 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 owing to inflammation be a cardiovascular risk factor independent of kidney disease? As the science unfolds, the interactions become more complex (Figure). We still do not understand the inciting or initiating factor that links disordered mineral metabolism and abnormal hormonal levels of FGF-23, parathyroid hormone, calcitriol, and now Klotho. But 1 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.

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**References**


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