Klotho, a protein highly expressed in the kidney, has been also prevalent in patients with chronic kidney disease (CKD).

Ectopic calcification of the vasculature comes with age and is also prevalent in patients with chronic kidney disease (CKD). Klotho, a protein highly expressed in the kidney, has been identified as a novel anti-aging factor,1,2 and Klotho-deficient animals develop medial vascular calcification.3 Importantly, patients with CKD are deficient in Klotho, suggesting that lack of this protein may be a key factor driving calcification. To date, the mechanisms whereby Klotho protects against vascular calcification have focused on its role as an obligate co-factor for fibroblast growth factor 23 (FGF23) signaling in regulating phosphate and vitamin D metabolism. However, emerging evidence suggests that Klotho may also exert direct effects on vascular smooth muscle cells (VSMC) and act as a novel circulating inhibitor of vascular calcification.

Klotho is named after the Greek Goddess of Fate, who spins the thread of life. Mice deficient in Klotho are progeric and display tissue-specific, age-associated defects, including medial vascular calcification.3 Klotho is highly expressed in the kidney and an essential co-factor for the phosphatonin FGF23, which is expressed in bone. Klotho-FGF23 signaling promotes renal phosphate excretion through sodium phosphate (NaPi) co-transporter 2a channels, thereby lowering blood phosphate levels, and downregulates the expression of 1α-hydroxylase to suppress production of active 1,25-dihydroxyvitamin D. Defects in either FGF23 or Klotho cause a combination of metabolic disturbances, including hyperphosphatemia, hypercalcemia, and hypervitaminosis D.4–6

FGF23 levels are upregulated in early CKD, possibly as an adaptive response to promote phosphate excretion. However, with progressive CKD, Klotho expression significantly decreases and signaling is disabled.7,8 Importantly, the resultant hyperphosphatemia, a universal feature of advanced CKD, is a potent inducer of VSMC calcification in vitro and in vivo9,10 and is independently associated with increased cardiovascular mortality.11

To date, accumulated evidence has led to the contention that Klotho exerts all of its effects on vascular calcification indirectly through its actions on these key metabolic processes. Animal studies show that the life span and the aging-like phenotypes of Klotho or FGF23-deficient mice can be mostly rescued by a vitamin D–deficient diet or by resolving hyperphosphatemia with dietary or genetic manipulation. Thus, as in CKD, vascular calcification in these mice is thought to be due to VSMC damage resulting from chronically high phosphate and, to a lesser extent, dysregulated calcium (Ca) and vitamin D levels.4

However, an exciting new study published in this issue of JASN puts a different slant on these observations.12 Hu et al.12 set out to explore whether Klotho itself was a direct inhibitor of VSMC calcification. Using genetically manipulated mice expressing varying levels of Klotho and in vitro tools, they provide evidence that Klotho may also exert direct effects on VSMCs, and this may be an additional mechanism for protection against calcification.

The first key observation made in this study was that murine CKD induced by 5/6 nephrectomy was associated with a marked reduction in plasma, urine, and kidney Klotho and that calcification progressed in these animals in the same way as in Klotho haploinsufficient animals. The decrease in urinary Klotho in patients with CKD7 led them to hypothesize that Klotho deficiency is responsible for the calcification observed in CKD. To test this further, they induced CKD in mice expressing various levels of Klotho. As expected, they found that Klotho deficiency exacerbated the effects of CKD on vascular calcification, whereas increased Klotho expression ameliorated these effects. Not surprising, Klotho deficiency was associated with elevated parathyroid hormone, increased 1,25-dihydroxyvitamin D, and
elevated circulating inorganic phosphate (Pi), whereas overexpression of Klotho normalized these factors, supporting the conclusion from previous studies that vascular calcification is due to systemic perturbations of Pi and calciotropic hormones.4

However, when they divided the mice into subgroups on the basis of their genetic Klotho status and then correlated tissue Ca content with plasma creatinine (Cr) and Pi levels, they found for a given Pi or Cr level that animals with the highest Klotho levels had the lowest Ca content. This relationship was most dramatic in the kidney, where Klotho is known to have direct effects on organ function; however, there was also a modest relationship in the aorta suggesting for the first time that Klotho may have direct effects on the vasculature.

To test this idea further, the authors examined the phenotype of VSMCs in the vessel wall of animals with CKD and high, normal, or low levels of Klotho. Animals lacking Klotho showed upregulated expression of the phosphate transporters Pit1/2 and the key osteogenic transcription factor Runx2. This suggested that in the absence of Klotho, upregulation of Pit1/2 increases Pi transport into VSMCs and this drives osteogenic conversion, a key event in the calcification process.10 However, this observation alone is not strong evidence for direct effects of Klotho on VSMC calcification. Calcification itself can drive VSMC osteogenic differentiation; therefore, such differences may be attributable to different levels of aortic Ca deposition in the different animal models.13,14 More convincing evidence was provided by in vitro studies in which the addition of recombinant soluble Klotho protein to VSMCs was able to decrease high Pi–induced calcification. This inhibition was mediated through suppression of Na-dependent Pi transport into VSMCs, potentially through normalization of mRNA levels encoding Pit1/2.

Although tantalizing, this article poses more questions than it answers. First, it does not address how Klotho mediates its effect on VSMCs. Klotho exists in two forms that have distinct functions. The full-length membrane-bound form acts to convert FGF receptors to high-specificity FGF23 receptors.15 The secreted form, produced by cleavage and release of the extracellular domain, acts as a humoral factor and is emerging as a key regulator of ion transport and insulin signaling linked to its influence. Klotho has also been implicated as a protective factor in apoptosis, an essential event in the initiation of calcification.13 However, apoptosis was not examined either in vitro or in vivo to ascertain whether levels of Pi–induced cell death differed in the presence or absence of Klotho.

The in vitro studies of Hu et al.12 were also limited by their use of a rat cell line (A10) that is unlikely to model human VSMCs in vitro or in vivo. For example, Ca can induce VSMC calcification and Klotho can regulate Ca transport through modulation of TRPV5 Ca channels and the Na/K-ATPase.4,16 Although Ca transport was tested and shown to be unchanged in A10 cells treated with Klotho, this needs to be interpreted with caution. VSMCs within the vessel wall are contractile cells with complex mechanisms to maintain Ca homeostasis, and these mechanisms will not be mimicked in noncontractile, proliferative VSMCs in vitro.19

More important, a major function of Klotho is as an aging suppressor, and studies of the same mouse models used by Hu et al. demonstrated that Klotho confers resistance to oxidative stress. The mechanisms potentially involve insulin/IGF signaling through FOXO transcription factors. In future studies, it will be essential to examine the levels of reactive oxygen species, oxidative DNA damage, and activation of these signaling pathways in VSMCs and in animal models of CKD in the context of altered Klotho expression. Moreover, senescent or aging VSMCs exhibit increased osteogenic differentiation and calcification potential.20 The transformed A10 cell line will not undergo senescence; therefore, studies investigating whether Klotho treatment can alter the osteogenic differentiation potential of aged human VSMCs are essential. Finally, Klotho has been identified as a soluble regulator of Wnt signaling in mesenchymal stem cells. Importantly, human VSMCs have many features in common with mesenchymal stem cells, and Wnt signaling is implicated in the osteogenic conversion of VSMCs.19,21 Again, further experiments in appropriate model systems might include testing whether Klotho can block calcification if Wnt signaling is disabled.

In summary, this study is likely to provide impetus for more mechanistic studies investigating potential direct protective effects of Klotho on the vasculature and on establishing whether Klotho is a robust biomarker for early CKD. Clearly, these studies will have implications not only for the utility of Klotho therapy in CKD but also in age-related vascular decline associated with calcification.

DISCLOSURES
C.S. has received speaker’s honoraria from Shire, Amgen, and Genzyme.

REFERENCES
2. Yang HC, Deleuze S, Zuo Y, Potthoff SA, Ma LJ, Fogo AB: The


Targeting Complement C5 in Atypical Hemolytic Uremic Syndrome

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Hemolytic uremic syndrome (HUS) is defined as the combination of microangiopathic hemolytic anemia (MAHA), acute renal failure, and thrombocytopenia. It occurs most often during outbreaks of diarrheal food poisoning with enteropathogenic strains of *Escherichia coli* such as O157:H7 and others that produce a shiga-like toxin. Less commonly, HUS may be associated with other infections, such as HIV and *Streptococcus pneumoniae*, various drugs such as calcineurin inhibitors and chemotherapeutic agents, and mucin-secreting adenocarcinomas.

Atypical HUS (aHUS) accounts for approximately 10% of all cases of HUS. The clinical manifestations are indistinguishable from diarrhea-associated HUS without the bloody diarrhea. Whereas diarrhea-associated HUS generally recovers after a period of supportive care, aHUS often recurs, may lead to end-stage renal failure and death in a high proportion of cases, and frequently recurs after renal transplantation. aHUS may be sporadic or familial. It usually presents in childhood but may occur at any age.

Endothelial injury leading to platelet aggregation and thrombotic microangiopathy (TMA), particularly in glomerular capillaries, underlies all forms of HUS. In diarrhea-associated HUS, endothelial damage is caused by the catalytic subunit of the shiga-like toxin, which inhibits protein synthesis by cleaving 28S ribosomal RNA. In contrast, unregulated activation of complement accounts for at least 50% of cases of aHUS.

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