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Vitamin D and secreted Klotho: a long-awaited panacea for vascular calcification?

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Chronic kidney disease (CKD) is characterized by accelerated vascular calcification, which may in part be caused by deficiency of the anti-aging factor Klotho. Lau *et al.* demonstrate that administration of active vitamin D and its analog decreases aortic calcification in association with increases in two potent calcification inhibitors—the secreted form of Klotho and vascular osteopontin. These data might provide a new perspective on the association of active vitamin D with improved survival in patients with CKD.

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Vascular calcification is one of the major complications of chronic kidney disease (CKD) and contributes to an increased risk of cardiovascular morbidity and mortality in this population. Emerging evidence suggests that vascular calcification is not just a passive process of mineral precipitation but a pathobiological process that shares many features with skeletal osteogenesis and involves various calcification inducers and inhibitors.[1] Recently, epidemiological studies of CKD patients demonstrated a significant survival benefit associated with the use of active vitamin D—the mainstream therapy for secondary hyperparathyroidism—independently of the traditional effects on mineral metabolism.[2] A plausible explanation for these findings is provided by experimental studies showing several nonclassical actions of vitamin D, including effects on the immune and inflammatory system, the renin–angiotensin system, and cell growth and differentiation. In addition, recent data suggest that a physiological dose of active vitamin D may have protective effects against vascular calcification, although a supraphysiological dose of active vitamin D induces massive vascular calcification in experimental uremia.[3]

Klotho, originally identified as an anti-aging factor, is a 130-kDa single-pass transmembrane protein that is predominantly expressed in the kidney, parathyroid gland, and choroid plexus.[4] Klotho functions as an obligate cofactor for fibroblast growth factor-23 (FGF23) and mediates the effects of FGF23 to induce urinary phosphate excretion, suppress renal production of 1,25-dihydroxyvitamin D, and inhibit synthesis and secretion of parathyroid hormone.[5] The extracellular domain of Klotho can be secreted into blood, urine, and cerebrospinal fluid and functions as a humoral factor. Recent experimental data showed that secreted Klotho enhances calcium reabsorption in the distal tubule, promotes phosphaturia in the proximal tubule,[6] and inhibits phosphate uptake by vascular smooth muscle cells.[7] Interestingly, systemic expression of Klotho is decreased in humans and animals with CKD, and it is suggested that Klotho deficiency may contribute to development of vascular calcification as well as resistance to FGF23 in the kidney and parathyroid gland in CKD.[5]

Whether the putative protective effect of active vitamin D against vascular calcification is mediated by secreted Klotho or other calcification-related factors is the subject of the study by Lau *et al.*[8] (this issue). Using a uremic mouse model of extensive arterial medial calcification generated by partial renal ablation and a high-phosphate (1.5%) diet on the calcification-prone DBA/2J background, the investigators examined the effect of calcitriol or its analog paricalcitol on vascular calcification. They revealed that both types of active vitamin D, in doses equivalent to those given to patients with CKD, increases serum and urinary levels of secreted Klotho and expression of the calcification inhibitor osteopontin in aortic medial cells, and ameliorates aortic medial calcification (Figure 1). These results suggest that pharmacological doses of active vitamin D may have beneficial effects on vascular calcification by increasing secreted Klotho and arterial medial osteopontin in the setting of CKD.

Although the results of the study by Lau *et al.*[8] might provide a new perspective on the association of active vitamin D with improved survival in patients with CKD,[2] their data should be interpreted with caution. First, the administration of calcitriol or paricalcitol resulted in paradoxical reductions in serum phosphate and FGF23 and no effects on serum calcium and parathyroid hormone. Perhaps these findings may in part be explained by the high dietary phosphate content used in the study, which might have overcome the inhibitory effect of active vitamin D on parathyroid hormone secretion and the potent phosphaturic action of secreted Klotho that was increased by active vitamin D. As the investigators acknowledge, this experimental model might be reasonable for examining the effect

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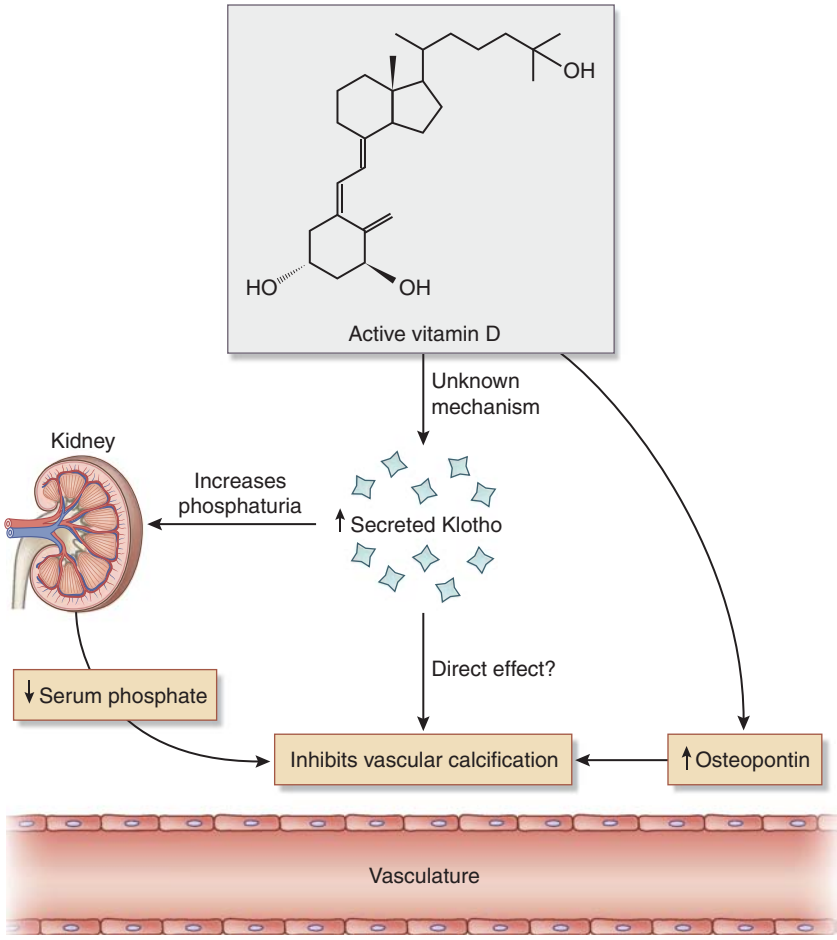

Figure 1 | Proposed mechanisms of inhibition of vascular calcification by active vitamin D. Active vitamin D inhibits vascular calcification by two possible pathways: (1) increasing secreted Klotho, which in turn enhances phosphaturia and directly inhibits phosphate uptake by vascular smooth muscle cells; and (2) increasing expression of the calcification inhibitor osteopontin in aortic medial cells.

of active vitamin D on secreted Klotho and vascular calcification independently of changes in serum calcium and parathyroid hormone. However, since the observed changes are largely different from those in patients with CKD, additional studies are required to determine whether the results of this animal study are true of patients with CKD in the real world.

Second, it remains unclear how the secreted form of Klotho in blood and urine was increased by active vitamin D. To elucidate this mechanism, the investigators analyzed systemic expression of Klotho in detail. However, they found that Klotho expression in the kidney was not upregulated by active vitamin D in their experimental model, in contrast to previous studies.[9] Similarly, no upregulation of Klotho expression was observed in other organs, including the parathyroid, in mice treated with active vitamin D. These data raise the possibility that the increased secreted Klotho following administration of active vitamin D may be caused by accelerated shedding of Klotho without changes in membrane-bound Klotho expression, although the source of secreted Klotho and the mechanism of regulation of Klotho cleavage are largely unknown. A recent study of hemodialysis patients has shown that treatment with cinacalcet hydrochloride resulted in only small and transient reductions in serum secreted Klotho despite significant alterations in mineral and bone metabolism.[10] Future studies should investigate the mechanism by which shedding of membrane-bound Klotho is regulated and the involvement of active vitamin D in this process.

Third, the study by Lau *et al.*[8] does not provide evidence that increased secreted Klotho actually contributed to the protective effects of active vitamin D against vascular calcification in their model. Recent *in vitro* studies demonstrated direct effects of secreted Klotho protein on vascular smooth muscle cell calcification through suppression of sodium-dependent phosphate transport into cells,[7] but the *in vivo* significance of these effects remains unknown. Because secreted Klotho functions as a phosphaturic hormone independently of the effect of FGF23,[6] the observed amelioration of vascular calcification may be attributable to the phosphaturic effect of secreted Klotho. Lau *et al.*[8] also demonstrated upregulated expression of osteopontin, a potent local inhibitor of vascular calcification, following administration of active vitamin D. Again, however, it remains to be determined whether the upregulation of osteopontin by active vitamin D contributed to the improvement of vascular calcification in this mouse model.

Finally, it is important to point out that the protective or detrimental effect of active vitamin D on vascular calcification has been a controversial issue, presumably because of the differences in the experimental model or the dose or type of active vitamin D used.[3] This suggests the possibility of a therapeutic window for active vitamin D to exert protective actions against vascular calcification, which may vary according to the type of active vitamin D. In this regard, additional studies are needed to determine whether the effects of active vitamin D on secreted Klotho and arterial medial osteopontin differ according to the dose over a much wider range than examined in the study by Lau *et al.*[8] In addition, it would be important to investigate whether supplementation of native vitamin D to correct vitamin D deficiency, which occurs commonly in patients with CKD, results in modulation of secreted Klotho and other calcification-related factors and whether these effects

translate into protective effects against vascular calcification.

Despite these caveats, the study by Lau *et al.*[8] is important, as it adds a new dimension to the beneficial effects of active vitamin D on the vasculature in patients with CKD—who are in a state of Klotho deficiency.[7] The results of this study highlight the need to determine whether an optimal dose of active vitamin D is a panacea for vascular calcification in patients with CKD and, if so, whether secreted Klotho is involved in this beneficial effect of active vitamin D. Clearly, there is still much to learn about the role of vitamin D and secreted Klotho in the pathogenesis of vascular calcification in CKD, and much more work is needed to translate the findings of this study into clinical practice in patients with CKD.

DISCLOSURE
The authors declared no competing interests.

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Autophagy protects proximal tubular cells from injury and apoptosis

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Autophagy is upregulated during ischemia–reperfusion (IR)-induced and cisplatin-induced acute kidney injury (AKI). Proximal tubule-specific *Atg7* knockout mice exhibited increased renal injury compared with wild-type mice following cisplatin- and IR-induced AKI. Inhibition of autophagy by chloroquine aggravated AKI, whereas upregulation of autophagy by rapamycin recovered lost renal function and histology, further indicating a protective role of autophagy in AKI. These findings reported by Jiang *et al.* will provide stimulus to further examine the role and mechanism of the enhancement of autophagy in AKI.

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Autophagy is a catabolic process of degradation of long-lived proteins, cellular macromolecules, and intracellular organelles through the lysosomal hydrolases. In this process, a double-membrane structure known as an autophagosome sequesters and delivers cytoplasmic contents to the lysosome for degradation. Free amino acids and fatty acids generated on degradation of cellular components are recycled to synthesize new proteins and bioenergetic supplies of the cell. Thus, under normal physiological conditions, basal autophagy plays a homeostatic role that maintains cellular homeostasis and quality control. Autophagy is induced in response to stress conditions including cell starvation, growth factor deprivation, hypoxia, and oxidant injury. Under stress conditions, autophagy induction is generally considered to play an adaptive role that ensures cell survival. The molecular machinery involved in autophagosome formation is composed of evolutionarily conserved Atg-related proteins originally identified in yeast.[1] The formation of an autophagosome is initiated by several autophagic protein complexes, including the unc-51-like kinase 1 or 2 (ULK1 or ULK2) complex, the class III phosphatidylinositol 3-kinase complex, Atg12–Atg5–Atg16 conjugation, and lipidation of microtubule-associated protein 1 light chain 3 (LC3) with phosphatidylethanolamine to form LC3-II[1] (Figure 1). The elongation and expansion steps in autophagosome formation involve two ubiquitin-like proteins, Atg12 and Atg8/LC3 (Figure 1). The conjugation of Atg12 to Atg5 is catalyzed by Atg7 and Atg10 (E1- and E2-like enzymes, respectively) to form covalently linked Atg12–Atg5. Following formation of the Atg12–Atg5 conjugate, Atg16L non-covalently associates with this conjugate to produce the Atg12–Atg5–Atg16 multimeric complex.[1] At present, there is great interest in studying autophagy under various

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