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Circulating Klotho levels: clinical relevance and relationship with tissue Klotho expression

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Klotho is a protein that exerts paracrine and endocrine functions. In chronic kidney disease (CKD), its expression is decreased in several tissues. This decrease probably plays important roles in various complications associated with CKD, in both a fibroblast growth factor-23 (FGF23)-dependent and an FGF23-independent manner. The clinical diagnosis of Klotho deficiency is not easy. The relevance of circulating Klotho levels, if any, needs to be adequately defined. Serum Klotho may not reflect tissue Klotho concentration.

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The unraveling of the respective roles of Klotho and fibroblast growth factor-23 (FGF23) occupies at present a major place in both experimental and clinical research devoted to the mineral and bone disorder of chronic kidney disease (CKD-MBD) and to disturbances of bone and mineral metabolism in other disease states.

Klotho, or more precisely α-Klotho, is a single-pass transmembrane protein that is expressed predominantly in kidney tubular epithelium, and to a lesser extent in the parathyroid gland and epithelial cells of the choroid plexus. The protein has a large extracellular amino-terminal domain and a small intracellular carboxy-terminal domain. The extracellular domain is made of two internal repeat sequences, named KL1 and KL2.[1] Circulating Klotho results either from direct secretion by the cell or from cleavage of the intracellular domain of the full-length protein by secretases. Both processes lead to 'soluble Klotho,' which is found in blood, urine, and cerebrospinal fluid.[2]

It was believed for some time that Klotho functioned mainly, if not uniquely, as a coreceptor for FGF23 binding to FGF receptor 1 (FGFR1), and that this cooperativity function was restricted to the ectodomain of membrane Klotho. Although FGFRs have a much lower affinity for the isolated Klotho ectodomain than for the full-length transmembrane form, the relative importance of membrane and soluble Klotho in this interaction remains elusive.[2] It is clearly established at present that Klotho exerts both FGF23-dependent and FGF23-independent, pleiotropic actions. Soluble Klotho acts as both a paracrine and an endocrine factor.[3]

Its paracrine functions include regulation of the surface abundance of the tubular Ca^{2+} channel TRPV5 and K^+ channel ROMK in that Klotho is a glycosidase removing sialic acids from N-glycan.[1] Notably, soluble Klotho also has been shown to inhibit sodium phosphate (NaPi) cotransporters in renal opossum kidney cells and in cell-free membrane vesicles *in vitro* in the absence of FGF23. Moreover, exogenously administered Klotho resulted in decreased renal expression of NaPi-2a and hypophosphatemia in FGF23-null mice *in vivo*, demonstrating that Klotho can induce hyperphosphaturia independently of FGF23.[4] Finally, soluble Klotho also inhibits intestinal NaPi-2b transporters, as shown in NaPi-2b-expressing oocytes.[5]

As a hormone, Klotho targets multiple remote tissues and organs. These actions comprise anti-aging and cardiovascular protective properties. Klotho reduces oxidative stress by the inhibition of insulin and insulin-like growth factor-1 signaling pathways. It enhances endothelial nitric oxide production and thereby improves endothelium-dependent vasodilatation.[6] It is an endogenous inhibitor of vascular calcification, as shown in recent studies *in vitro* and in CKD mice *in vivo*. Finally, it decreases cell-surface abundance of the Ca^{2+} channel TRPC6. This channel is expressed in cardiac, glomerular, and vascular smooth muscle cells and plays an important role in the regulation of vascular resistance and blood pressure.[1] Since upregulation of TRPC6 is implicated in the pathogenesis of cardiac hypertrophy and glomerulosclerosis, downregulation of its expression by Klotho should be protective against these disease conditions.

At the cellular level, the antioxidant actions of Klotho include the induction of resistance to H_2O_2 and suppression of

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lipid peroxidation through activation of the FOXO family of transcription factors and stimulation of manganese superoxide dismutase.[2] Klotho inhibits cell senescence and apoptosis. Further, Klotho protects against renal fibrosis.[2,7] It also promotes kidney regeneration after ischemia–reperfusion injury via the suppression of fibrosis-promoting growth factors, the preservation of stem cells, and the recovery of endothelial integrity and function, respectively.[2] Figure 1 reflects the present understanding of the interactions of Klotho and FGF23 with calcium, phosphate, parathyroid hormone (PTH), and vitamin D metabolism.

In CKD, the expression of Klotho is decreased in various tissues. In the human kidney, decreased Klotho expression occurs as early as in CKD stage 2. This decrease could be responsible, at least in part, for the early increase in serum FGF23.[8] In the parathyroid tissue of patients with more advanced stages of CKD, reduced Klotho expression, together with reduced FGFR1 expression, is believed to explain the resistance to the inhibitory action of FGF23.[9] The vascular smooth muscle of patients with CKD also displays a state of Klotho deficiency.[10] Klotho knockdown potentiates the development of accelerated calcification through a Runx2- and myocardin/serum response factor-dependent pathway, and restoration of Klotho expression in vascular cells allows FGF23 and calcitriol to exert anticalcifying effects.

Why is tissue Klotho decreased in CKD? Current explanations include hyperphosphatemia and hypercalcemia, a proinflammatory state, and uremic toxins as potential culprits. The latter can cause epigenetic modification of specific genes, potentially resulting from increased DNA methyltransferase expression and DNA hypermethylation. This hypothesis is based on the observation that uremic toxins such as indoxyl sulfate or *p*-cresyl sulfate stimulate DNA methyltransferase and decrease Klotho expression *in vitro*, and that specific inhibition of DNA methyltransferase activity increases Klotho expression.[11]

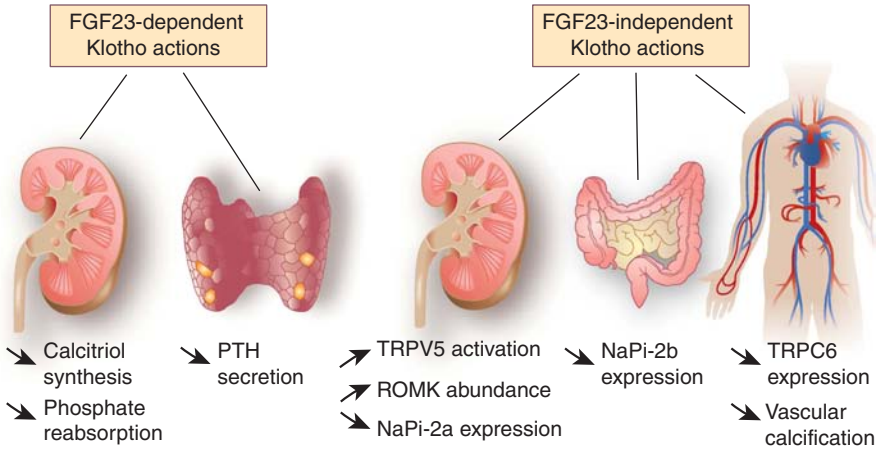

Figure 1 | Involvement of tissue and circulating Klotho in mineral metabolism: a schematic view. Calcitriol, 1,25-dihydroxyvitamin D; PTH, parathyroid hormone; TRPV5, highly selective renal Ca^{2+} entry channel; ROMK, renal K^{+} channel; NaPi-2a, renal sodium/phosphate cotransporter; NaPi-2b, intestinal sodium/phosphate cotransporter; TRPC6, ubiquitous Ca^{2+} entry channel.

An important question is whether in patients with CKD the concentration of soluble Klotho in the circulation reflects Klotho deficiency at the tissue level. Seiler *et al.*[12] (this issue) now address this question in detail. First of all, they point out that existing literature is inconclusive. Yamazaki *et al.*[13] found correlations of serum Klotho with age and serum levels of creatinine, phosphorus, and FGF23. They also observed higher levels in children than in adult people. Several cross-sectional, observational studies were carried out subsequently, with contradictory results as to a possible link between circulating Klotho and kidney function. Second, Seiler *et al.*[12] stress the fact that a reliable measurement of circulating Klotho has become available only recently, with the advent of a sensitive and specific enzyme-linked immunosorbent assay.[13] Third, they report their own findings in a large cohort of patients with CKD stages 2–4, with a follow-up of approximately 2 years. They find a significant correlation of plasma Klotho with age, but not with estimated glomerular filtration rate or other parameters of CKD-MBD, including plasma PTH, plasma FGF23, and fractional excretion of phosphorus (FePi). In contrast, they confirm previous observations of significant increases in these three parameters with declining kidney function, and significant correlations of circulating FGF23 with plasma PTH, plasma phosphorus, and FePi. Finally, by Cox regression analysis, plasma FGF23, plasma PTH, and FePi, but not plasma Klotho, are predictive of adverse patient outcomes (combined end point) in univariate analysis. FGF23, but not PTH or FePi, remains an independent predictor of adverse patient outcomes after adjustment for age, estimated glomerular filtration rate, and albuminuria.

The failure to observe an association between plasma Klotho and estimated glomerular filtration rate could be due to the fact that patients with late CKD stages were excluded from this analysis. In a recent study by Yokoyama *et al.*[14] serum Klotho was significantly lower in chronic hemodialysis patients than in healthy controls, and it displayed a positive correlation with serum phosphorus. The lack of a healthy control subject group in the study by Seiler *et al.*[12] somewhat complicates the interpretation of the results. It remains possible that CKD patients have lower plasma Klotho levels than healthy controls from CKD stage 2 onward, with no further decrease with the progression to CKD stages 3 and 4. In a recent experimental study, Hu *et al.*

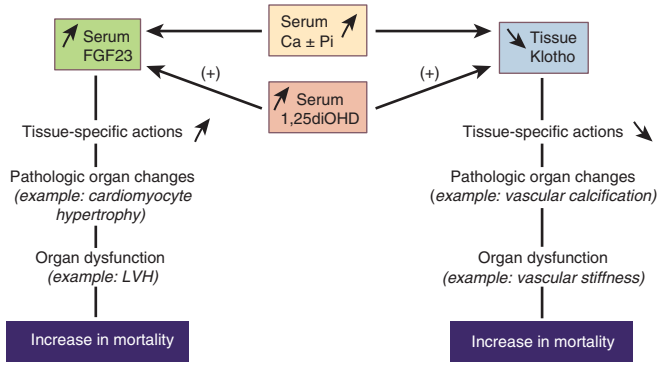

Figure 2 | Hypothetical scheme of the complex condition of FGF23 excess and Klotho deficiency in chronic kidney disease. Interplay with high serum calcium and phosphorus levels and the response to active vitamin D sterol administration via an increase in serum 1,25 dihydroxy vitamin D levels.[16] LVH, left ventricular hypertrophy.

found a decrease in urinary and plasma Klotho levels in mice with early CKD.[15] Notably, the normal circulating levels of Klotho reported by Yamazaki *et al.*[13] have been determined in serum, not in plasma, in healthy Asian volunteers, which may not precisely reflect plasma Klotho levels in European controls. It also is surprising that Seiler *et al.*[12] fail to identify circulating phosphorus as an independent predictor of adverse outcomes, in contrast to many previous reports both in patients with CKD and in the general population without known kidney impairment, where even only slight elevations of serum phosphorus were predictive of higher mortality risk. Seiler *et al.*[12] do not provide information on the vitamin D status of their patients. There might be a difference in plasma Klotho levels between vitamin D-replete and vitamin D-deficient CKD patients, since active vitamin sterols increase tissue Klotho expression.[10] Finally, the absence of changes in plasma Klotho with the progression of CKD and the absence of correlations with other biochemical parameters in the study by Seiler *et al.*[12] certainly do not preclude a potentially major role of Klotho at the organ and/or patient-outcome level. Other examples of a dissociation between tissue and plasma levels are tissue growth factors such as transforming growth factor (TGF)-α and TGF-β and bone morphogenetic proteins. Unfortunately, quantification of membrane-bound Klotho at the tissue or cellular level in humans is not feasible for large epidemiological studies, as Seiler *et al.*[12] point out. Therefore, further evaluation of circulating Klotho levels in the general population and in CKD patients is warranted.

In conclusion, on the basis of the present state of knowledge, Klotho measurement in the circulation does not appear to be a useful tool in patients with CKD, but additional studies are still needed. The precise understanding of the relative roles of FGF23-dependent and FGF23-independent Klotho actions also requires further study. Figure 2 shows a hypothetical scheme of the complex condition of FGF23 excess and Klotho deficiency in CKD, in interplay with high serum calcium and phosphorus levels and the response to active vitamin D sterol administration via an increase in serum 1,25 dihydroxy vitamin D concentration.

DISCLOSURE

Tilman B. Drüeke declares having received honoraria as adviser/consultant from Abbott, Amgen, Baxter, Chugai, FMC, Genzyme, KAI Pharmaceuticals, and Theraclion, and as speaker from Abbott, Amgen, Chugai, Genzyme, and Kirin; and grant/research support from Amgen, Baxter, and Shire. Ziad A. Massy declares having received honoraria as speaker from Abbott, Amgen, Chugai, FMC, and Genzyme, and grant/research support from Amgen, Baxter, and FMC.

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