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COMMENTARIES

The Spinner Meets the Stone: Klotho and Mineral Metabolism

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Commentary on:

- Kurosu H, Yamamoto M, Clark JD, Pastor JV, Nandi A, Gurnani P, McGuinness OP, Chikuda H, Yamaguchi M, Kawaguchi H, Shimomura I, Takayama Y, Herz J, Kahn CR, Rosenblatt KP, Kuro-o M. Suppression of aging in mice by the hormone Klotho. *Science.* 2005 Sep 16;309(5742):1829-33.

- Chang Q, Hoefs S, van der Kemp AW, Topala CN, Bindels RJ, Hoenderop JG. The beta-glucuronidase klotho hydrolyzes and activates the TRPV5 channel. *Science*. 2005 Oct 21;310(5747):490-3.

We don't understand what makes us age, or how we regulate phosphate metabolism, but the two may be more closely related than we had imagined. We now know that FGF23 is the phosphatonin that causes renal phosphate wasting and decreased renal synthesis of $1,25(OH)_2D$ in diverse hypophosphatemic disorders (1;2). Conversely, *Fgf23*(-/-) mice display hyperphosphatemia and increased levels of $1,25(OH)_2D$ (3). Longevity has been linked to food intake and insulin action, and a recent paper in *Science* proposes that the mouse *klotho* gene, mutations in which are associated with premature aging, determines longevity by inducing insulin resistance (4). Startlingly, a poster presentation (5) at the recent 2005 Annual Meeting of the American Society for Bone and Mineral Research (ASBMR) shows that FGF23 and *klotho* are partners.

To understand how FGF23 acts in its principal target tissue, the kidney, Urakawa *et al.* (5) passed renal homogenates over an affinity chromatography column to which full-length FGF23 was linked, thereby identifying the klotho protein as a principal binding partner of FGF23. Although *klotho* was initially identified as a mutation that produces premature aging in the mouse (6), the *klotho* mouse was subsequently shown to have hyperphosphatemia, hypercalcemia and elevated $1,25(OH)_2D$ levels (7), a phenotype similar to that of the *Fgf23*(-/-) mouse. Direct comparison of the two mutant mouse strains showed virtually identical levels of serum calcium, phosphate, parathyroid hormone (PTH) and $1,25(OH)_2D$ (5). FGF23 levels were elevated to approximately 10,000 times normal in the *klotho* mouse, however, while they were undetectable in the *Fgf23*(-/-) mouse, implying that resistance to the action of FGF23 accounts for the *klotho* phenotype.

Urakawa *et al*. also reported direct biochemical evidence that klotho is required for FGF23 action. Human kidney cells had an easily demonstrable response to basic FGF, but *klotho* expression in the cells was necessary for a response to FGF23. Neutralizing antibodies to the klotho protein antagonized FGF23 action in a cell culture system. In addition, administration of the antibodies to normal mice induced apparent resistance to FGF23 *in vivo*, with an increase in serum phosphate and $1,25(OH)_2D$ concentrations, as well as in serum FGF23 levels.

The *klotho* mutation was originally produced in mice by random insertional mutagenesis. One of the resulting strains had a reduced lifespan, atherosclerosis, osteopenia, skin atrophy, impaired sexual maturation and pulmonary emphysema (6). Since these traits were viewed as evidence of premature

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aging, the mutation was named *klotho,* after one of the three Fates who spins the thread of life. The *klotho* gene was shown to encode a cell surface protein with a short cytoplasmic tail, whose extracellular domain consists of tandem duplicated copies of a β–glucosidase-like sequence, which can be released as a soluble form of klotho.

At least two models of the klotho-FGF23 interaction could explain their apparent epistatic relationship. Klotho could act as a co-ligand for FGF23, could modify FGF23, or both. If klotho binds to FGF23 and acts as a co-ligand, the klotho-binding sequence in FGF23 may reside in its 73 amino acid carboxyl-terminus. In this scenario, the C-terminal extension, which is unique to FGF23 in comparison to all other members of the FGF family, would direct FGF23 to receptors that control phosphate and vitamin D metabolism. These could be klotho receptors, since a high-affinity klotho binding site has recently been identified (4), or FGF receptors. The renal receptor by which FGF23 induces phosphaturia has not been identified, and by itself FGF23 binds with only modest affinity to known FGF receptors (8). It is also conceivable that, rather than simply binding FGF23, klotho has a glucosidase activity that is essential for FGF23 action. It has been reported that the klotho protein has weak β–glucuronidase activity (9). A glycotransferase encoded by the GALNT3 gene is predicted to O-glycosylate FGF23 (10) and thereby induce proper folding. One form of hereditary tumoral calcinosis is caused by a mutation in the GALNT3 gene. Could removal or modification of this sugar be required in order for FGF23 to act?

In another recent *Science* paper (11), Chang *et al*. report that klotho cleaves oligosaccharides from and thereby activates the transient receptor potential ion channel TRPV5. TRPV5 is an epithelial calcium channel that is found in the apical membrane of cells in the distal convoluted tubule and connecting tubule of the nephron (12) – the same nephron segments where *klotho* is expressed (6). Its relative, TRPV6, is the principal vitamin D-responsive calcium channel in the intestine (12). Soluble klotho protein increases calcium transport in cells that were transfected with the *TRPV5* gene, as well as primary cultures of rabbit connecting tubules and cortical collecting ducts. This effect is mimicked by addition of β–glucuronidase and blocked by an inhibitor of enzyme activity. Klotho cleaved labeled extracellular sugars from TRPV5 and increased the abundance of biotinylated TRPV5 on the cell surface. Neither klotho nor β–glucuronidase increased calcium transport by transporters lacking the N-linked glycosylation site. Thus, the β–glucuronidase activity of klotho activates calcium transport by removing extracellular N-linked sugars from the calcium channel and thereby increasing the display of the channel on the apical membrane. Klotho appears to enhance reclamation of calcium from the renal tubule and may also enhance intestinal calcium absorption through a similar effect on TRPV6.

Although klotho protein has been localized to the distal nephron (6), both phosphate reabsorption and renal synthesis of $1,25(OH)_2D$ take place in the proximal renal tubule. Perhaps soluble klotho derived from the distal nephron or other sources is a coligand with FGF23 for receptors in the proximal tubule. Alternatively, other messengers could carry the signal for phosphaturia from the distal to proximal nephron, a possibility that is consistent with the fact that it takes several hours to develop phosphaturia after FGF23 administration, while it only takes minutes for the same response to PTH. Thus, other phosphatonins such as frizzled related protein-4 could conceivably be downstream of FGF23 (13). In a tissue screen for responsiveness to FGF23, using EGR1 expression as the readout, kidney, pituitary and parathyroid responded to FGF23, but other tissues were negative (5). This pattern of responsiveness corresponds closely to tissue expression of the *klotho* gene (6). Yet the effects of both FGF23 deficiency and the *klotho* mutation involve many additional tissues. This raises the possibility that some features may be indirect consequences of FGF23 deficiency, a conclusion that was confirmed in two other abstracts at the 2005 ASBMR meeting.

To determine the role of vitamin D in the FGF23-deficient state (3), mice lacking vitamin D 1α-hydroxylase (14) or the vitamin D receptor (VDR) (15) were crossed with FGF23-deficient mice. The two double mutant strains had similar phenotypes. The hypercalcemia, nephrocalcinosis and renal failure of the *Fgf23*(-/-) mouse were absent, and these mice did not die prematurely. These phenotypes were expected, since hypercalcemia, nephrocalcinosis and renal failure seem to result from the combination of elevated $1,25(OH)_2D$ levels and hyperphosphatemia. Surprisingly, however, both double mutant strains had a phenotype that resembles the VDR knockout, with hypophosphatemia, hypocalcemia and rickets. Vitamin D action is thus required to manifest the hyperphosphatemia of the FGF23-null mouse. This could reflect a specific requirement for vitamin D to express hyperphosphatemia, or the combination of opposing phosphaturia from the secondary hyperparathyroidism of VDR-null mice and hyperphosphatemia from the absence of FGF23 action. The *Fgf23/Vdr* double mutant mice also did not develop hypoglycemia or hypocholesterolemia, suggesting these features of the *Fgf23*(-/-) phenotype, which are also found in *klotho* mice, can be explained by hypervitaminosis D.

Fgf23(-/-) mice have markedly reduced survival because the combination of hypercalcemia and hyperphosphatemia causes nephrocalcinosis and renal failure – their kidneys and other soft tissues turn to stone because the high calcium-phosphate product causes soft tissue calcification (3). It could be argued that the premature aging phenotype of *klotho* mice, which display similar calcium and phosphate levels, is simply a consequence of profoundly disturbed calcium-phosphate metabolism, with damage of many tissues by ectopic calcification. Recent studies of *klotho* mice, however, suggest that the effects of *klotho* are more complex. Kurosu *et al.* (4) showed that the life span of transgenic mice in which klotho is overexpressed is markedly increased -- by as much as 31% in males. Could this be a consequence of altered mineral metabolism? No studies of mineral metabolism were carried out to determine whether klotho overexpression in the transgenic mice enhanced the action of FGF23, possibly resetting thresholds for renal phosphate and vitamin D metabolism.

To account for the diverse tissue effects of *klotho* without invoking altered vitamin D metabolism, Kurosu *et al.* hypothesized that soluble klotho protein, which circulates at a concentration of 100 pM, directly affects the metabolism of diverse tissues (4). Injection of klotho protein induces hyperglycemia that is attributable to insulin resistance, and in male mice leads to IGF-1 resistance. This is consistent with the finding of hypoglycemia in the absence of either klotho or FGF23. Moreover, the rescue of hypoglycemia in *Fgf23/Vdr* double mutant mice (15) indicates that vitamin D has a role in hypoglycemia. Kurosu *et al*., however, report that klotho protein binds with high affinity to a saturable receptor in cultured hepatoma cells, and that addition of recombinant klotho protein to L6 cells blocks ligand-induced autophosphorylation of insulin and IGF1 receptors, as well as phosphorylation of the downstream signaling molecules insulin receptor substrate 1 (IRS-1) and IRS-2 (4). Does klotho protein directly inhibit signaling of insulin and IGF-1 receptors and increase signaling of FGF receptors? If so, it seems unlikely that the mechanism involves klotho protein as a coligand for insulin and IGF1 as well as FGF23.

Altered insulin signaling could account for features of the *klotho* phenotype. Kurosu *et al.* crossed *klotho* and *IRS-1*(+/-) mice and found that the atherosclerosis, ectopic calcification, skin atrophy, pulmonary emphysema and hypogonadism phenotypes of the klotho-deficient mice were improved (4). This is a somewhat unexpected result because *IRS-1*(+/-) mice have a subtle phenotype and are not resistant to insulin or IGF1 (16). Some of these problems (e.g., vascular and ectopic calcification) would be reversed by removing genes required for vitamin D responsiveness from *Fgf23*(-/-) mice (15;14); hence it seems plausible that altered IGF1 signaling improved the clinical features of the syndrome by affecting phosphate and vitamin D metabolism. Relationships between IGF1 and renal phosphate handling have long been

recognized (17), and these results suggest they may be more important than previously thought.

Klotho and *Fgf23* are both vitamin D-induced genes, and their coordinate expression leads to calcium retention (and possibly enhanced calcium absorption) and phosphate excretion, as well as downregulation of vitamin D activation. It seems that a new vitamin D homeostatic system has been uncovered by these recent results. More work will be required to determine the role of altered vitamin D and calcium-phosphate metabolism in the longevity effects of the *klotho* gene, which may also be involved in the longevity of humans (18). It is fascinating that aging, insulin resistance, and the metabolism of vitamin D and phosphate are linked by *klotho*. Stay tuned.

Conflict of Interest: The author has declared that no conflict of interest exists.

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