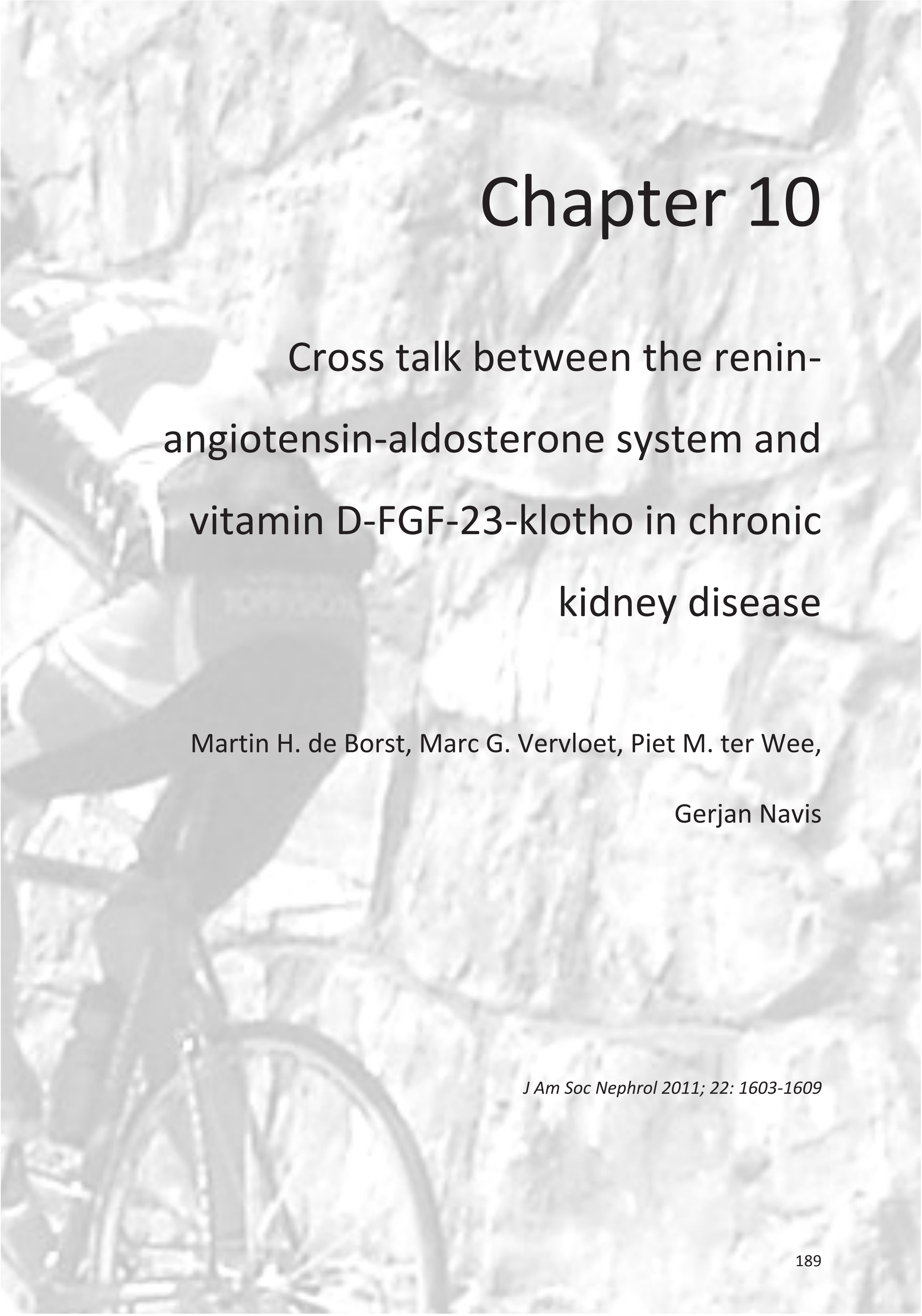

Chapter 10

Cross talk between the renin-angiotensin-aldosterone system and vitamin D-FGF-23-klotho in chronic kidney disease

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Abstract

There is increasingly evidence that the interactions between vitamin D, fibroblast growth factor-23 (FGF-23), and klotho form an endocrine axis for calcium and phosphate metabolism, and derangement of this axis contributes to the progression of renal disease. Several recent studies also demonstrate negative regulation of the renin gene by vitamin D. In chronic kidney disease (CKD), low levels of calcitriol due to the loss of 1-alpha hydroxylase increase renal renin production. Activation of the renin-angiotensin-aldosterone system (RAAS), in turn, reduces renal expression of klotho, a crucial factor for proper FGF-23 signaling. The resulting high FGF-23 levels suppress 1-alpha hydroxylase, further lowering calcitriol. This feedback loop results in vitamin D deficiency, RAAS activation, high FGF-23 levels and renal klotho deficiency, all of which associate with progression of renal damage. Here we review current evidence for an interaction between the RAAS and the vitamin D-FGF-23-klotho axis as well as its possible implications for progression of CKD.

Introduction

The renin-angiotensin-aldosterone system (RAAS) plays a pivotal role in renal progression and its complications. Accordingly, RAAS blockade has been the cornerstone of renoprotective interventions. Vitamin D deficiency is also traditionally recognized as a key factor in the bone and mineral disturbances of chronic kidney disease (CKD) and vitamin D supplementation is standard treatment for many renal patients. As reviewed elsewhere,[1] vitamin D interacts with the more recently identified moieties, fibroblast growth factor 23 (FGF-23) and klotho. As such, vitamin D, FGF-23, and klotho represent an endocrine axis involved in the regulation of calcium and phosphate metabolism.

Besides having effects on mineral metabolism, vitamin D deficiency is also associated with progressive renal disease and with mortality in CKD[2,3]. In line with these observations, the use of vitamin D analogues associates with a survival advantage in dialysis patients,[4] and preclinical and clinical data indicate that vitamin D analogues have additional renoprotective effects in addition to RAAS blockade,[5-8] supporting their clinical relevance. Multiple mechanisms may be involved in the protective effects of vitamin D, including autocrine anti-inflammatory and anti-fibrotic effects, as well as a suppressive effect on the RAAS. Several lines of evidence support the impact of vitamin D on RAAS activity at the clinical, pathophysiological, and molecular level. The other way round, RAAS activity influences the vitamin D-FGF-23-klotho axis. Recent studies show that angiotensin II reduces renal expression of klotho, which in turn modulates FGF-23-signaling and 1-alpha hydroxylase, the enzyme converting calcidiol to calcitriol. As derangement of the vitamin D-FGF-23-klotho axis associate with cardiovascular complications in several studies, the interactions of this axis with the RAAS may have therapeutic implications in CKD patients, regarding both renal and cardiovascular outcomes.

Modulation of the RAAS by vitamin D

The first clinical studies suggesting an inverse relationship between calcitriol and renin levels were published two decades ago[9,10] and were recently confirmed in a large cohort study[11]. Vitamin D deficiency, defined as calcidiol levels below 15 ng/mL, associates with reduced renal plasma flow responses to infused angiotensin II, suggesting endogenous intrarenal RAAS activation in vitamin D deficient subjects,[12] and intervention with calcitriol decreases plasma renin and angiotensin II levels in hemodialysis patients with secondary hyperparathyroidism[13].

Several mechanistic studies confirming negative regulation of the renin gene by calcitriol have been published by the group of Li *et al.*, who demonstrated increased renin gene expression in kidneys of vitamin D receptor (VDR)-null mice, accompanied by increased plasma angiotensin II levels, hypertension, and cardiac hypertrophy[14]. In wild-type mice, conversely, treatment with calcitriol reduces renal renin production. The negative regulation of renin by calcitriol seems independent of calcium and PTH[15]. On a molecular level, calcitriol binds to the VDR and subsequently blocks formation of the CRE-CREB-CBP complexes in the promoter region of the renin gene, reducing its level of expression[16].

Together, the associations found in clinical studies and the supporting mechanistic studies make it plausible that vitamin D deficiency could indeed contribute to an inappropriately activated RAAS, as a mechanism for progression of CKD and/or cardiovascular disease. This may well be relevant for therapeutic purposes. Pharmacological blockade of the RAAS is the main therapeutic modality in CKD, and despite its proven efficacy, renoprotection is usually far from complete[17]. Several lines of evidence indicate that persistent RAAS-activity, either by incomplete pharmacological blockade or related to the reactive rise in renin during therapy, can hamper its therapeutic efficacy. This is suggested by the added antiproteinuric effect of renin inhibition to AT1 receptor blockade[18]. These findings hypothesize that treatment with a vitamin D receptor agonist on top of conventional RAAS-blockade, would give additional renoprotection through its negative regulation of renin.

In line with this notion, several experimental studies confirm that the renoprotective effects of vitamin D at least in part through the suppression of renal renin expression[5,7]. In a recent randomized controlled trial, paricalcitol given in addition to RAAS blockade further reduces albuminuria compared to RAAS blockade alone in patients with diabetic nephropathy, although it remains unclear whether this therapeutic benefit was obtained by an effect on renal renin activity[19]. Vitamin D analogues may also have cardioprotective effects in association with suppression of renin in kidney and heart[20,21]. Whether paricalcitol reduces left ventricular hypertrophy in stage III/IV CKD patients is currently under investigation in the PRIMO study (ClinicalTrials.gov Identifier: NCT00497146).

Interactions between vitamin D and other RAAS components have been studied as well. Aldosterone acts through the mineralocorticoid receptor, which belongs to the same superfamily of nuclear receptors as the VDR. Therefore, crosstalk between these receptors and their agonists could potentially exist, but this has not been studied so far. Mice that are genetically deficient for klotho, a protein associated with downregulation of 1-alpha hydroxylase and thus limited production of calcitriol, show excessive levels of calcitriol but also hyperaldosteronism, which is similarly reversed by a vitamin D-deficient diet[22]. Although these findings suggest a possible interaction between vitamin D and aldosterone synthesis, it is uncertain whether hyperaldosteronism is a direct consequence of hypervitaminosis D. Data from *in vitro* studies do not support positive regulation of aldosterone synthesis by vitamin D, as treatment of cultured adrenocortical cells with calcitriol reduce aldosterone levels[23]. In VDR null mice, although there seems to be a trend towards increased aldosterone levels, the elevation is not significant as compared to wild-type mice,[24] which is in contrast with the strong down-regulation of renal renin,[5] suggesting the effect on aldosterone may in fact be through renin. Treatment of spontaneous hypertensive rats (SHR) with cholecalciferol also reduces plasma aldosterone levels, but here also a direct suppressive effect on renin transcription cannot be excluded[25]. Vice versa, aldosterone may potentiate the effects of calcitriol, as demonstrated in cultured renal thick ascending limb cells[26]. In this study, calcitriol negatively regulates HCO_3^- absorption in the rat medullary thick ascending limb which may contribute to net urine acid and/or calcium excretion. Addition of aldosterone potentiated the effects of calcitriol through an ERK-dependent, non-genomic pathway. This implicates a crosstalk between the mineralocorticoid receptor and VDR may indeed be present, but understudied.

Whether vitamin D modulates the expression of angiotensin II receptors is unknown. The only study on this subject reports that in adipocytes, vitamin D down-regulates expression of the AT1 receptor in a dose-dependent manner,[27] but to our knowledge these findings have never been replicated in other cell types.

In conclusion, both clinical and mechanistic studies suggest that calcitriol, through the VDR, has a negative regulatory role on renin gene transcription. Whether vitamin D also interacts with other RAAS components is unclear. Correcting vitamin D deficiency may have renal and cardioprotective effects, at least in part through suppression of the RAAS. The direct suppressive effect of calcitriol on the renin gene raises the question whether a feedback loop exists; that is, if the RAAS also influences vitamin D metabolism. Recent data suggest that indeed activation of vitamin D, through klotho and 1-alpha hydroxylase, could be affected by angiotensin II.

Possible effects of the RAAS on klotho, FGF-23, and vitamin D

The possible regulation of vitamin D metabolism by the RAAS is less well defined than the opposite regulation of the renin gene by calcitriol. Although there is no evidence that any RAAS component directly influences the enzyme 1-alpha hydroxylase or VDR, indirect effects of angiotensin II on 1-alpha hydroxylase, through klotho and FGF-23, may play a role.

Negative regulation of klotho by angiotensin II.

Accumulating data suggests that angiotensin II negatively regulates renal klotho expression[28-31]. In an animal model, Mitani *et al.* demonstrated down-regulation of renal klotho expression in response to angiotensin II infusion. Klotho down-regulation also followed infusion of angiotensin II in a non-pressor dose[28]. The down-regulation of klotho was angiotensin II type 1 (AT1) receptor-dependent, since it is completely abolished by losartan and not by hydralazine. Intriguingly, subsequent restoration of klotho abundance in the kidney by gene transfer improved angiotensin II-induced proteinuria, suggesting that non-pressor-driven angiotensin II-induced proteinuria at least in part depends on loss of klotho.

In cultured tubular epithelial cells, angiotensin II-induced AT1-receptor-mediated down-regulation of klotho was confirmed[29]. In a recent elegant study, Yoon *et al.* demonstrated in a mouse model that salt restriction, a well-known RAAS-activating intervention, reduced klotho expression, which was reversed by losartan[30]. The same study revealed that 195hosphaturia-induced damage associates with down-regulation of renal klotho in association with up-regulation of renal RAAS activation; addition of losartan completely prevented the loss of klotho expression[30].

Several other animal models characterized by an activated RAAS including SHR, non-insulin-dependent diabetic nephropathy (Otsuka Long-Evans Tokushima Fatty rats) and 5/6 nephrectomy all demonstrate downregulation of renal klotho[32]. The fact that renal klotho expression is also reduced in the DOCA salt rat model, characterized by RAAS suppression and renal damage, suggests that also other factors such as tubular injury contribute to down-regulation of klotho in renal damage. Studies in patients suggest reduced renal klotho expression per nephron in kidney sections from CKD patients as compared to control kidneys, but whether this is associated with RAAS activation is unclear[33]. Interestingly, calcitriol has been shown to enhance renal klotho expression *in vivo,*[34] possibly as a consequence of reduced RAAS activation.

Mechanisms of klotho down-regulation.

The mechanisms of klotho down-regulation by angiotensin II are incompletely understood. Although direct negative regulation through the AT1 receptor is possible, other factors such as oxidative stress may contribute. Like angiotensin II, oxidative stress itself can cause down-regulation of renal klotho[31,35]. Subsequent administration of a free radical scavenger not only prevented klotho down-regulation induced by oxidative stress, but also by infusion of angiotensin[31]. It is well established that angiotensin II influences oxidative stress through the NADPH oxidase system[36-38]. Conversely, inhibitors of the RAAS ameliorate the production of reactive oxygen species,[39] which could at least in part explain the effects of RAAS inhibitors on klotho expression.

Interestingly, tumor necrosis factor alpha converting enzyme (TACE or ADAM17), which is up-regulated in the presence of vitamin D deficiency,[40] may be involved in cleavage of the extracellular domain of klotho[41]. Moreover, angiotensin II itself also up-regulates TACE,[42] providing another mechanism for klotho down-regulation. It may be through this mechanism, through TACE, that even distant inflammation down-regulates renal klotho[43]. These preliminary data suggest that klotho function could be affected by vitamin D deficiency through proteolytic activity of TACE, especially under inflammatory conditions.

Consequences of down-regulated klotho and FGF-23 resistance.

The klotho gene encodes two proteins from five exons: membrane bound klotho (mKlotho; molecular weight 130 kDa) and secreted klotho (sKlotho; 80 kDa), which is a product of alternative splicing[44,45]. A third form of klotho, a cleavage product of the extracellular domain of mKlotho, is referred to as cut-Klotho (cKlotho)[45]. Although under physiological conditions the klotho gene is expressed only in selected tissues, including distal tubular segments of the kidney, klotho null mice have the phenotype of generalized aging[46]. The similarity between klotho and FGF-23 mice led to the discovery that klotho is mandatory for FGF-23 signaling by modification of the low-affinity FGF receptor (FGFR1), leading to the high-affinity receptor that comprises the membrane-bound FGFR1/klotho complex[47,48]. Only mKlotho, and not one of the circulating forms, can form an FGF-23 receptor from FGFR1[49]. As a consequence of klotho down-regulation, the high-affinity FGFR1/klotho complex is reduced, inducing FGF-23-resistance. Thus, by its effect on klotho, angiotensin II could theoretically be involved in induction of FGF-23 resistance. In CKD, FGF-23 resistance abates fractional excretion of phosphate, leading to further hyperphosphatemia and thus providing another trigger for FGF-23 release[50]. As a consequence of high FGF-23 levels, vitamin D activation is suppressed. Administration of recombinant FGF-23 to normal mice reduces renal expression of 1-alpha hydroxylase and increases renal CYP24A1, resulting in low calcitriol levels[51]. This effect is mediated by extracellular signal-regulated kinase (ERK)[52]. Both FGF-23 and klotho null mice display increased expression of 1-alpha hydroxylase[53,54]. This suggests that klotho, in concert with FGF-23, participates in an inhibitory feedback loop that results in the suppression of calcitriol synthesis[34]. High levels of FGF-23 suppress activation of vitamin D and are associated with progression of CKD,[55] left ventricular mass and geometry,[56,57] atherosclerosis,[58] and mortality in dialysis patients[59]. Clinical data suggest that the mechanisms underlying the association between FGF-23 and vascular complications are multiple, including suppression of vitamin D activation, reduced fetuin-A levels, and endothelial dysfunction, partially through asymmetrical dimethyl arginine (ADMA)[60]. Moreover, higher FGF23 is associated with proteinuria throughout several ranges of CKD,[60] independently of levels of active vitamin D[61].

Besides its role as part of the FGF-23 receptor complex, the β-glucuronidase activity of klotho is important in stabilizing the abundance of the TRPV5 in the apical membrane of tubular cells[54,62]. Loss of renal klotho in CKD[33] may thus lead to renal calcium loss, providing another impulse for vitamin D activation. In addition, overexpression of klotho provides renoprotection in mouse[63] and rat[64] models of renal damage, suggesting that renal loss of klotho as observed in CKD may result in renal damage.

Finally, klotho appears to have distant effects, especially on the vasculature. Although it is possible that the originally described phenotype of premature aging in klotho-deficient mice[46] is the consequence of disturbed regulation of calcium, phosphate, and vitamin D metabolism in the kidney, several recent reports suggests that klotho has specific functions at distant tissues. Klotho, for example, protects endothelial cells against oxidative-stress induced apoptosis[65,66]. An elegant study demonstrated that klotho-deficient mice have endothelial dysfunction, which could be restored by parabiosis with wild-type mice[67]. At least part of these beneficial effects may be mediated by inhibition of insulin/IGF-1 signaling, thereby improving resistance against oxidative stress[68]. Recently it was shown that klotho is involved in intracellular calcium handling in endothelial cells through the VEGF receptor and TRPC-1, and thus protects these cells from loss of integrity through apoptosis[69]. The resemblance of the abovementioned observations with the effects of RAAS activation suggests that a part of the clinical picture as observed in state of RAAS activation in CKD might in fact be due to klotho deficiency.

Can the vitamin D-FGF-23-klotho axis be monitored and targeted?

The data summarized here suggest a relationship between RAAS activation, low renal klotho levels, and high FGF-23 levels in CKD patients, all of which associate with adverse outcomes. Future studies are needed to address whether adequate renal klotho, as measured for instance by fractional phosphate excretion, vitamin D, or FGF23 levels, (possibly) circulating klotho, or a combination of these factors, could be a therapeutic intervention. Targeting klotho deficiency in CKD, which can be achieved by optimizing RAAS blockade and correction of vitamin D deficiency, may further reduce cardiovascular disease and progression of kidney injury.

Conclusions

Emerging evidence demonstrates the negative regulation of the RAAS by calcitriol, providing renoprotective effects of vitamin D analogues in addition to RAAS blockade in CKD. A growing number of studies support suppression of 1-alpha hydroxylase by angiotensin II through renal down-regulation of klotho and subsequent FGF-23 resistance (summarized in Figure 1, left panel). Besides its effects on vitamin D metabolism, high levels of FGF-23 or FGF-23 resistance due to klotho deficiency are associated with endothelial dysfunction, cardiovascular morbidity and mortality, and progression of CKD[70]. In CKD, loss of capacity for excreting phosphate by reduced nephron mass and loss of klotho due to RAAS activation and tubulointerstitial damage both further enhance circulating levels of FGF-23 and reduce levels of active vitamin D (Figure 1, right panel). Further derangement of these interconnected axes may well contribute to the cardiovascular complications of CKD.

RAAS inhibition and supplementation of vitamin D deficiency are well-established interventions for prevention of progressive renal function loss and its extrarenal complications, as recommended by current guidelines^{71} (http://www.nice.org.uk/cg73). Interestingly, these interventions appear to share a part of their beneficial effects by

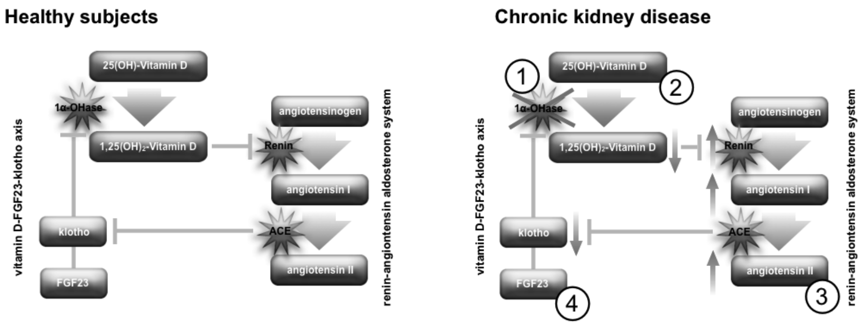

Figure 1:

Cross-talk between vitamin D (red), FGF-23-Klotho (yellow) and the RAAS (blue) in healthy subjects and patients with chronic kidney disease. In the normal situation (left panel), active vitamin D (1,25(OH)$_2$ vitamin D) generated by renal 1-alpha hydroylase, suppresses renal renin production. When the RAAS is not activated (low angiotensin II), renal klotho levels are sufficient to allow normal function of the FGF-23 receptor. Therefore levels of FGF-23, a negative regulator of 1-alpha hydroxylase, are normal under these conditions. In chronic kidney disease (right panel), the RAAS, vitamin D, FGF-23 and klotho are concertedly disturbed. (1) Activity of 1α-hydroxylase is reduced due to nephron loss and high FGF-23 in CKD, (2) leading to reduced production of 1,25(OH)$_2$-vitamin D, which in turn upregulated renal renin production. (3) The subsequent higher levels of angiotensin II cause renal klotho loss and (4) disrupted FGF-23 signaling, impairing 199hosphaturia and rising FGF-23 levels. RAAS activation, vitamin D deficiency, high FGF-23 levels and low renal klotho have all been associated with adverse renal outcome in CKD.

Interrupting the vicious cycle of increasing FGF23 resistance due to klotho deficiency. However, the protection against renal progression and its extrarenal complications by current therapy is far from complete, prompting for improvement of treatment strategies. Currently, management of blood pressure and proteinuria by RAAS blockade, and vitamin D supplementation are independent components of the treatment regimen. Their separate and combined impact on the FGF-23-klotho axis is not monitored, nor is it a treatment target. It would be attractive to hypothesize that targeting optimization of this axis, particularly optimal levels of renal klotho, could enhance therapeutic efficacy, either by adapting titration strategies for currently available drugs, or by novel agents.

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References

1. Razzaque MS: The FGF23-klotho axis: Endocrine regulation of phosphate homeostasis. Nat Rev Endocrinol 5: 611-619, 2009

2. Melamed ML, Astor B, Michos ED, Hostetter TH, Powe NR, Muntner P: 25-hydroxyvitamin D levels, race, and the progression of kidney disease. J Am Soc Nephrol 20: 2631-2639, 2009

3. Wolf M, Shah A, Gutierrez O, Ankers E, Monroy M, Tamez H, Steele D, Chang Y, Camargo CA,Jr, Tonelli M, Thadhani R: Vitamin D levels and early mortality among incident hemodialysis patients. Kidney Int 72: 1004-1013, 2007

4. Teng M, Wolf M, Lowrie E, Ofsthun N, Lazarus JM, Thadhani R: Survival of patients undergoing hemodialysis with paricalcitol or calcitriol therapy. N Engl J Med 349: 446-456, 2003

5. Zhang Y, Kong J, Deb DK, Chang A, Li YC: Vitamin D receptor attenuates renal fibrosis by suppressing the renin-angiotensin system. J Am Soc Nephrol 21: 966-973, 2010

6. Zhang Y, Deb DK, Kong J, Ning G, Wang Y, Li G, Chen Y, Zhang Z, Strugnell S, Sabbagh Y, Arbeeny C, Li YC: Long-term therapeutic effect of vitamin D analog doxercalciferol on diabetic nephropathy: Strong synergism with AT1 receptor antagonist. Am J Physiol Renal Physiol 297: F791-801, 2009

7. Tan X, He W, Liu Y: Combination therapy with paricalcitol and trandolapril reduces renal fibrosis in obstructive nephropathy. Kidney Int 76: 1248-1257, 2009

8. Zhang Z, Zhang Y, Ning G, Deb DK, Kong J, Li YC: Combination therapy with AT1 blocker and vitamin D analog markedly ameliorates diabetic nephropathy: Blockade of compensatory renin increase. Proc Natl Acad Sci U S A 105: 15896-15901, 2008

9. Burgess ED, Hawkins RG, Watanabe M: Interaction of 1,25-dihydroxyvitamin D and plasma renin activity in high renin essential hypertension. Am J Hypertens 3: 903-905, 1990

10. Resnick LM, Muller FB, Laragh JH: Calcium-regulating hormones in essential hypertension. relation to plasma renin activity and sodium metabolism. Ann Intern Med 105: 649-654, 1986

11. Tomaschitz A, Pilz S, Ritz E, Grammer T, Drechsler C, Boehm BO, Marz W: Independent association between 1,25-dihydroxyvitamin D, 25-hydroxyvitamin D and the renin-angiotensin system: The ludwigshafen risk and cardiovascular health (LURIC) study. Clin Chim Acta 411: 1354-1360, 2010

12. Forman JP, Williams JS, Fisher ND: Plasma 25-hydroxyvitamin D and regulation of the renin-angiotensin system in humans. Hypertension 55: 1283-1288, 2010

13. Park CW, Oh YS, Shin YS, Kim CM, Kim YS, Kim SY, Choi EJ, Chang YS, Bang BK: Intravenous calcitriol regresses myocardial hypertrophy in hemodialysis patients with secondary hyperparathyroidism. Am J Kidney Dis 33: 73-81, 1999

14. Li YC, Kong J, Wei M, Chen ZF, Liu SQ, Cao LP: 1,25-dihydroxyvitamin D(3) is a negative endocrine regulator of the renin-angiotensin system. J Clin Invest 110: 229-238, 2002

15. Kong J, Qiao G, Zhang Z, Liu SQ, Li YC: Targeted vitamin D receptor expression in juxtaglomerular cells suppresses renin expression independent of parathyroid hormone and calcium. Kidney Int 74: 1577-1581, 2008

16. Yuan W, Pan W, Kong J, Zheng W, Szeto FL, Wong KE, Cohen R, Klopot A, Zhang Z, Li YC: 1,25-dihydroxyvitamin D3 suppresses renin gene transcription by blocking the activity of the cyclic AMP response element in the renin gene promoter. J Biol Chem 282: 29821-29830, 2007

17. de Jong PE, & Navis G: Proteinuria lowering needs a multifactorial and individualized approach to halt progression of renal disease. Nat Clin Pract Nephrol 4: 654-655, 2008

18. Parving HH, Persson F, Lewis JB, Lewis EJ, Hollenberg NK, AVOID Study Investigators: Aliskiren combined with losartan in type 2 diabetes and nephropathy. N Engl J Med 358: 2433-2446, 2008

19. de Zeeuw D, Agarwal R, Amdahl M, Audhya P, Coyne D, Garimella T, Parving HH, Pritchett Y, Remuzzi G, Ritz E, Andress D: Selective vitamin D receptor activation with paricalcitol for reduction of albuminuria in patients with type 2 diabetes (VITAL study): A randomised controlled trial. Lancet 376: 1543-1551, 2010

20. Kong J, Kim GH, Wei M, Sun T, Li G, Liu SQ, Li X, Bhan I, Zhao Q, Thadhani R, Li YC: Therapeutic effects of vitamin D analogs on cardiac hypertrophy in spontaneously hypertensive rats. Am J Pathol 177: 622-631, 2010

21. Bodyak N, Ayus JC, Achinger S, Shivalingappa V, Ke Q, Chen YS, Rigor DL, Stillman I, Tamez H, Kroeger PE, Wu-Wong RR, Karumanchi SA, Thadhani R, Kang PM: Activated vitamin D attenuates left ventricular abnormalities induced by dietary sodium in dahl salt-sensitive animals. Proc Natl Acad Sci U S A 104: 16810-16815, 2007

22. Fischer SS, Kempe DS, Leibrock CB, Rexhepaj R, Siraskar B, Boini KM, Ackermann TF, Foller M, Hocher B, Rosenblatt KP, Kuro-O M, Lang F: Hyperaldosteronism in klotho-deficient mice. Am J Physiol Renal Physiol 299: F1171-7, 2010

23. Lundqvist J, Norlin M, Wikvall K: 1alpha,25-dihydroxyvitamin D3 affects hormone production and expression of steroidogenic enzymes in human adrenocortical NCI-H295R cells. Biochim Biophys Acta 1801: 1056-1062, 2010

24. Simpson RU, Hershey SH, Nibbelink KA: Characterization of heart size and blood pressure in the vitamin D receptor knockout mouse. J Steroid Biochem Mol Biol 103: 521-524, 2007

25. Santos Rde S, Vieira da Costa VA, Vianna LM: Cholecalciferol treatment changes urinary sodium-potassium ratio and plasma aldosterone of spontaneously hypertensive rats. Clin Chim Acta 376: 253-254, 2007

26. Good DW, George T, Watts BA,3rd: Aldosterone potentiates 1,25-dihydroxyvitamin D3 action in renal thick ascending limb via a nongenomic, ERK-dependent pathway. Am J Physiol Cell Physiol 285: C1122-30, 2003

27. Morris KL, & Zemel MB: 1,25-dihydroxyvitamin D3 modulation of adipocyte glucocorticoid function. Obes Res 13: 670-677, 2005

28. Mitani H, Ishizaka N, Aizawa T, Ohno M, Usui S, Suzuki T, Amaki T, Mori I, Nakamura Y, Sato M, Nangaku M, Hirata Y, Nagai R: In vivo klotho gene transfer ameliorates angiotensin II-induced renal damage. Hypertension 39: 838-843, 2002

29. Zhou Q, Lin S, Tang R, Veeraragoo P, Peng W, Wu R: Role of fosinopril and valsartan on klotho gene expression induced by angiotensin II in rat renal tubular epithelial cells. Kidney Blood Press Res 33: 186-192, 2010

30. Yoon HE, Ghee JY, Piao S, Song JH, Han DH, Kim S, Ohashi N, Kobori H, Kuro-O M, Yang CW: Angiotensin II blockade upregulates the expression of klotho the anti-ageing gene, in an experimental model of chronic cyclosporine nephropathy. Nephrol Dial Transplant 2010

31. Saito K, Ishizaka N, Mitani H, Ohno M, Nagai R: Iron chelation and a free radical scavenger suppress angiotensin II-induced downregulation of klotho, an anti-aging gene, in rat. FEBS Lett 551: 58-62, 2003

32. Aizawa H, Saito Y, Nakamura T, Inoue M, Imanari T, Ohyama Y, Matsumura Y, Masuda H, Oba S, Mise N, Kimura K, Hasegawa A, Kurabayashi M, Kuro-o M, Nabeshima Y, Nagai R: Downregulation of the klotho gene in the kidney under sustained circulatory stress in rats. Biochem Biophys Res Commun 249: 865-871, 1998

33. Koh N, Fujimori T, Nishiguchi S, Tamori A, Shiomi S, Nakatani T, Sugimura K, Kishimoto T, Kinoshita S, Kuroki T, Nabeshima Y: Severely reduced production of klotho in human chronic renal failure kidney. Biochem Biophys Res Commun 280: 1015-1020, 2001

34. Tsujikawa H, Kurotaki Y, Fujimori T, Fukuda K, Nabeshima Y: Klotho, a gene related to a syndrome resembling human premature aging, functions in a negative regulatory circuit of vitamin D endocrine system. Mol Endocrinol 17: 2393-2403, 2003

35. Mitobe M, Yoshida T, Sugiura H, Shirota S, Tsuchiya K, Nihei H: Oxidative stress decreases klotho expression in a mouse kidney cell line. Nephron Exp Nephrol 101: e67-74, 2005

36. Sachse A, & Wolf G: Angiotensin II-induced reactive oxygen species and the kidney. J Am Soc Nephrol 18: 2439-2446, 2007

37. Zuo Z, Lei H, Wang X, Wang Y, Sonntag W, Sun Z: Aging-related kidney damage is associated with a decrease in klotho expression and an increase in superoxide production. Age (Dordr) 2010

38. Griendling KK, Minieri CA, Ollerenshaw JD, Alexander RW: Angiotensin II stimulates NADH and NADPH oxidase activity in cultured vascular smooth muscle cells. Circ Res 74: 1141-1148, 1994

39. Doran DE, Weiss D, Zhang Y, Griendling KK, Taylor WR: Differential effects of AT1 receptor and Ca2+ channel blockade on atherosclerosis, inflammatory gene expression, and production of reactive oxygen species. Atherosclerosis 195: 39-47, 2007

40. Dusso A, Arcidiacono MV, Yang J, Tokumoto M: Vitamin D inhibition of TACE and prevention of renal osteodystrophy and cardiovascular mortality. J Steroid Biochem Mol Biol 121: 193-198, 2010

41. Chen CD, Podvin S, Gillespie E, Leeman SE, Abraham CR: Insulin stimulates the cleavage and release of the extracellular domain of klotho by ADAM10 and ADAM17. Proc Natl Acad Sci U S A 104: 19796-19801, 2007

42. Lautrette A, Li S, Alili R, Sunnarborg SW, Burtin M, Lee DC, Friedlander G, Terzi F: Angiotensin II and EGF receptor cross-talk in chronic kidney diseases: A new therapeutic approach. Nat Med 11: 867-874, 2005

43. Thurston RD, Larmonier CB, Majewski PM, Ramalingam R, Midura-Kiela M, Laubitz D, Vandewalle A, Besselsen DG, Muhlbauer M, Jobin C, Kiela PR, Ghishan FK: Tumor necrosis factor and interferon-gamma down-regulate klotho in mice with colitis. Gastroenterology 138: 1384-94, 1394.e1-2, 2010

44. Imura A, Iwano A, Tohyama O, Tsuji Y, Nozaki K, Hashimoto N, Fujimori T, Nabeshima Y: Secreted klotho protein in sera and CSF: Implication for post-translational cleavage in release of klotho protein from cell membrane. FEBS Lett 565: 143-147, 2004

45. Matsumura Y, Aizawa H, Shiraki-Iida T, Nagai R, Kuro-o M, Nabeshima Y: Identification of the human klotho gene and its two transcripts encoding membrane and secreted klotho protein. Biochem Biophys Res Commun 242: 626-630, 1998

46. Kuro-o M, Matsumura Y, Aizawa H, Kawaguchi H, Suga T, Utsugi T, Ohyama Y, Kurabayashi M, Kaname T, Kume E, Iwasaki H, Iida A, Shiraki-Iida T, Nishikawa S, Nagai R, Nabeshima YI: Mutation of the mouse klotho gene leads to a syndrome resembling ageing. Nature 390: 45-51, 1997

47. Kurosu H, Ogawa Y, Miyoshi M, Yamamoto M, Nandi A, Rosenblatt KP, Baum MG, Schiavi S, Hu MC, Moe OW, Kuro-o M: Regulation of fibroblast growth factor-23 signaling by klotho. J Biol Chem 281: 6120-6123, 2006

48. Urakawa I, Yamazaki Y, Shimada T, Iijima K, Hasegawa H, Okawa K, Fujita T, Fukumoto S, Yamashita T: Klotho converts canonical FGF receptor into a specific receptor for FGF23. Nature 444: 770-774, 2006

49. Farrow EG, Davis SI, Summers LJ, White KE: Initial FGF23-mediated signaling occurs in the distal convoluted tubule. J Am Soc Nephrol 20: 955-960, 2009

50. Vervloet MG, van Ittersum FJ, Buttler RM, Heijboer AC, Blankenstein MA, Ter Wee PM: Effects of dietary phosphate and calcium intake on fibroblast growth factor-23. Clin J Am Soc Nephrol 6: 383-389, 2011

51. Shimada T, Hasegawa H, Yamazaki Y, Muto T, Hino R, Takeuchi Y, Fujita T, Nakahara K, Fukumoto S, Yamashita T: FGF-23 is a potent regulator of vitamin D metabolism and phosphate homeostasis. J Bone Miner Res 19: 429-435, 2004

52. Perwad F, Zhang MY, Tenenhouse HS, Portale AA: Fibroblast growth factor 23 impairs phosphorus and vitamin D metabolism in vivo and suppresses 25-hydroxyvitamin D-1alpha-hydroxylase expression in vitro. Am J Physiol Renal Physiol 293: F1577-83, 2007

53. Nakatani T, Sarraj B, Ohnishi M, Densmore MJ, Taguchi T, Goetz R, Mohammadi M, Lanske B, Razzaque MS: In vivo genetic evidence for klotho-dependent, fibroblast growth factor 23 (Fgf23) -mediated regulation of systemic phosphate homeostasis. FASEB J 23: 433-441, 2009

54. Alexander RT, Woudenberg-Vrenken TE, Buurman J, Dijkman H, van der Eerden BC, van Leeuwen JP, Bindels RJ, Hoenderop JG: Klotho prevents renal calcium loss. J Am Soc Nephrol 20: 2371-2379, 2009

55. Fliser D, Kollerits B, Neyer U, Ankerst DP, Lhotta K, Lingenhel A, Ritz E, Kronenberg F, MMKD Study Group, Kuen E, Konig P, Kraatz G, Mann JF, Muller GA, Kohler H, Riegler P: Fibroblast growth factor 23 (FGF23) predicts progression of chronic kidney disease: The mild to moderate kidney disease (MMKD) study. J Am Soc Nephrol 18: 2600-2608, 2007

56. Mirza MA, Larsson A, Melhus H, Lind L, Larsson TE: Serum intact FGF23 associate with left ventricular mass, hypertrophy and geometry in an elderly population. Atherosclerosis 207: 546-551, 2009

57. Kirkpantur A, Balci M, Gurbuz OA, Afsar B, Canbakan B, Akdemir R, Ayli MD: Serum fibroblast growth factor-23 (FGF-23) levels are independently associated with left ventricular mass and myocardial performance index in maintenance hemodialysis patients. Nephrol Dial Transplant 2010

58. Mirza MA, Hansen T, Johansson L, Ahlstrom H, Larsson A, Lind L, Larsson TE: Relationship between circulating FGF23 and total body atherosclerosis in the community. Nephrol Dial Transplant 24: 3125-3131, 2009

59. Gutierrez OM, Mannstadt M, Isakova T, Rauh-Hain JA, Tamez H, Shah A, Smith K, Lee H, Thadhani R, Juppner H, Wolf M: Fibroblast growth factor 23 and mortality among patients undergoing hemodialysis. N Engl J Med 359: 584-592, 2008

60. Yilmaz MI, Sonmez A, Saglam M, Yaman H, Kilic S, Demirkaya E, Eyileten T, Caglar K, Oguz Y, Vural A, Yenicesu M, Zoccali C: FGF-23 and vascular dysfunction in patients with stage 3 and 4 chronic kidney disease. Kidney Int 78: 679-685, 2010

61. Vervloet MG, Van Zuilen AD, Blankestijn PJ, Ter Wee PM, Wetzels JF: Fibroblast growth factor 23 is associated with proteinuria [Abstract]. J Am Soc Nephrol, 21 186A,2010

62. 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 310: 490-493, 2005

63. Haruna Y, Kashihara N, Satoh M, Tomita N, Namikoshi T, Sasaki T, Fujimori T, Xie P, Kanwar YS: Amelioration of progressive renal injury by genetic manipulation of klotho gene. Proc Natl Acad Sci U S A 104: 2331-2336, 2007

64. Wang Y, & Sun Z: Klotho gene delivery prevents the progression of spontaneous hypertension and renal damage. Hypertension 54: 810-817, 2009

65. Ikushima M, Rakugi H, Ishikawa K, Maekawa Y, Yamamoto K, Ohta J, Chihara Y, Kida I, Ogihara T: Anti-apoptotic and anti-senescence effects of klotho on vascular endothelial cells. Biochem Biophys Res Commun 339: 827-832, 2006

66. Kuro-o M: Klotho as a regulator of oxidative stress and senescence. Biol Chem 389: 233-241, 2008

67. Saito Y, Yamagishi T, Nakamura T, Ohyama Y, Aizawa H, Suga T, Matsumura Y, Masuda H, Kurabayashi M, Kuro-o M, Nabeshima Y, Nagai R: Klotho protein protects against endothelial dysfunction. Biochem Biophys Res Commun 248: 324-329, 1998

68. Yamamoto M, Clark JD, Pastor JV, Gurnani P, Nandi A, Kurosu H, Miyoshi M, Ogawa Y, Castrillon DH, Rosenblatt KP, Kuro-o M: Regulation of oxidative stress by the anti-aging hormone klotho. J Biol Chem 280: 38029-38034, 2005

69. Kusaba T, Okigaki M, Matui A, Murakami M, Ishikawa K, Kimura T, Sonomura K, Adachi Y, Shibuya M, Shirayama T, Tanda S, Hatta T, Sasaki S, Mori Y, Matsubara H: Klotho is associated with VEGF receptor-2 and the transient receptor potential canonical-1 Ca2+ channel to maintain endothelial integrity. Proc Natl Acad Sci U S A 107: 19308-19313, 2010

70. Wolf, M: Forging forward with 10 burning questions on FGF23 in kidney disease. J Am Soc Nephrol 21**:** 1427-35, 2010

71. Kidney Disease: Improving Global Outcomes (KDIGO) CKD-MBD Work Group: KDIGO clinical practice guideline for the diagnosis, evaluation, prevention, and treatment of chronic kidney disease-mineral and bone disorder (CKD-MBD). Kidney Int Suppl (113): S1-130, 2009.