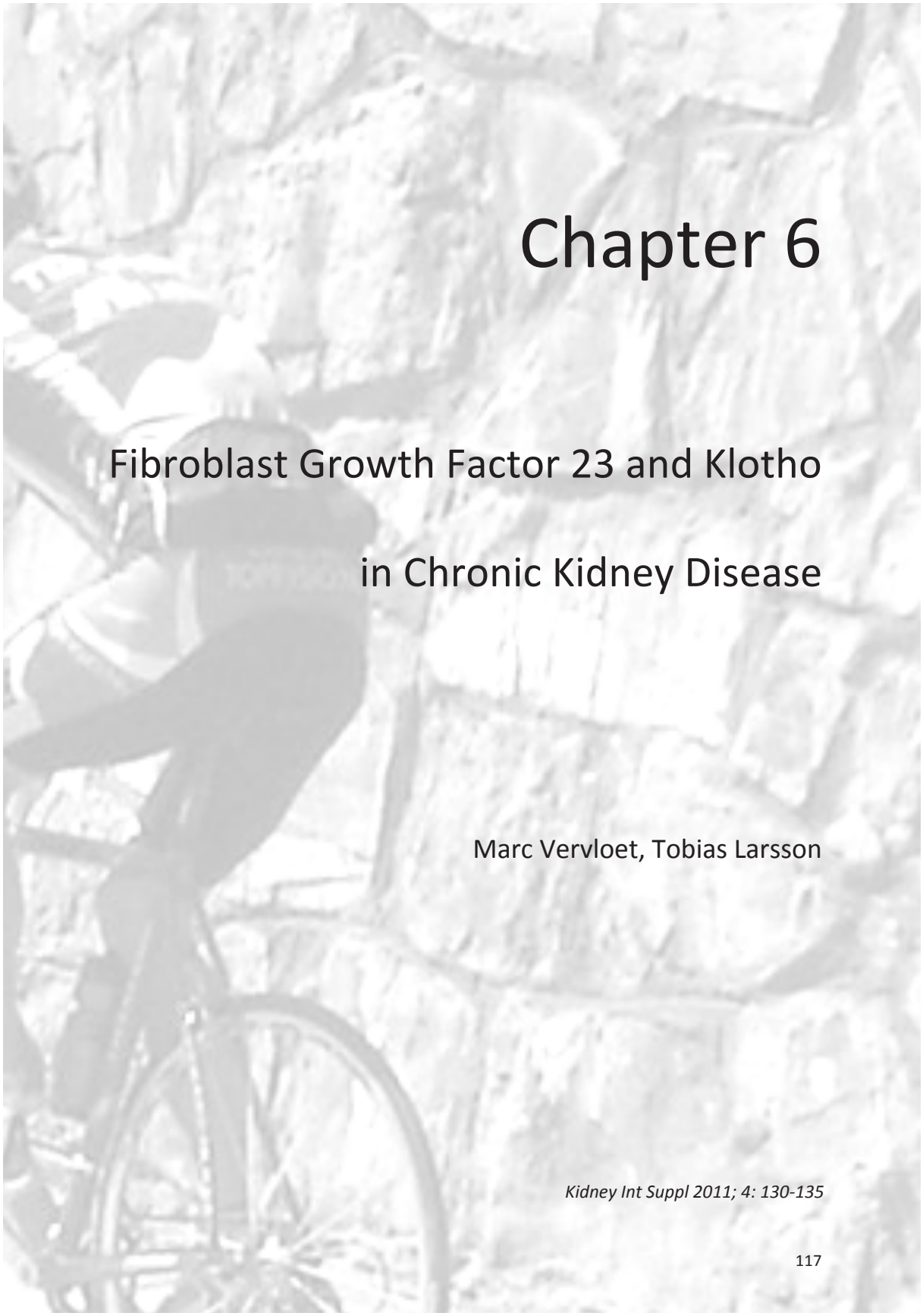

Chapter 6

Fibroblast Growth Factor 23 and Klotho in Chronic Kidney Disease

Marc Vervloet, Tobias Larsson

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Abstract

The well established increased cardiovascular risk that is a hallmark of chronic kidney disease (CKD) has directed research to metabolic changes that are typical of CKD. Epidemiological data point to derangements of mineral metabolism to be involved in this risk profile. Subsequently newly discovered humoral factors, like fibroblast growth factor- 23 (FGF23) that are involved in mineral- and vitamin D homeostasis, turned out to be associated with clinical outcome, independently of the minerals they regulate. Additional proteins involved in FGF23 signaling, such as Klotho, subsequently appeared to have FGF23-independent effects as well. In this review the discovery, mode of action and clinical implications of these new players is outlined.

Introduction

Identification of fibroblast growth factor-23 (FGF23) has in many ways revolutionized our current understanding of mineral metabolism. It was initially discovered through attempts to identify the predicted existence of 'phosphatonins', i.e. phosphate-regulating hormones. Summarizing the accomplishments made by many different researchers, FGF23 was found to be the primary cause of autosomal dominant hypophoshatemic rickets[1] as well as an ectopically over-produced phosphaturic factor in patients with tumor-induced osteomalacia[2] Structurally, FGF23 was the twenty-third member of the FGF family to be discovered with approximately 25-30% homology to other FGFs. The first 24 amino acids of the N-terminus function as a signal peptide for its transport from the Golgi network to the extracellular space and it is consequently a circulating factor. The C-terminus is distinct from other FGFs providing unique characteristics in terms of glycosylation and receptor activation. Two arginines located at residue 176 and 179, respectively, provide a consensus site for proteolytic cleavage by furin-like enzymes that inactivate and degrade the active FG23 protein[3].

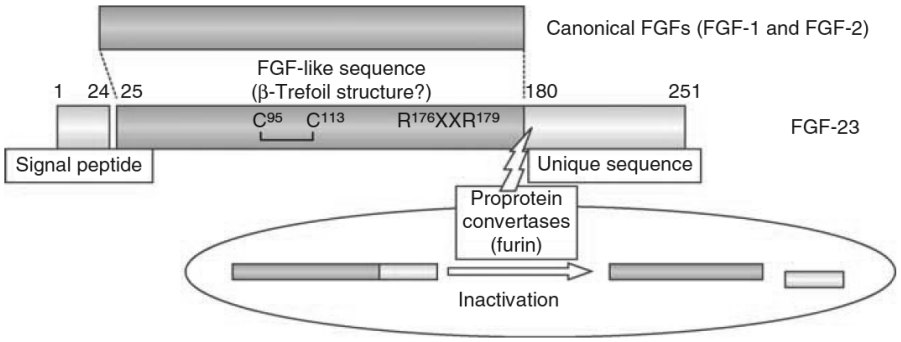


Figure 1: Structure of fibroblast growth factor (FGF)-23. *The diagram shows its FGF-like domain, the cleavage site and the signal peptide. Reproduced with permission from Yamashita; Structural and biochemical properties of FGF-23. Ther Apher Dial 2005;9: 313-318.*

Mechanisms and regulation

There is striking concordance between the phenotypic changes found in patients with primary or secondary FGF23 excess and/or deficiency and data from the extensive animal and *in vitro* studies. Collectively, FGF23 is a potent negative regulator of circulating phosphate and 1,25-dihydroxy vitamin D_3 ($1,25(OH)_2D_3$) levels[4,5]. FGF23 induces phosphaturia and lowers serum phosphate level through reduction and internalization of the sodium-phosphate co-transporters Npt2a and Npt2c in the kidney proximal tubules[6,7]. Further, FGF23 directly suppresses renal 1-alpha-hydroxylase, leading to decreased conversion of 25-hydroxyvitamin D_3 ($25(OH)D_3$) to its active metabolite $1,25(OH)_2D_3$[8]. Another role of FGF23 in vitamin D metabolism is to enhance the degradation pathway of vitamin D through stimulation of the 24-hydroxylase. In the context of physiology, more recent studies have also convincingly shown that FGF23, at least in the short term, directly decreases the transcript level and secretion of parathyroid hormone (PTH)[9,10]. The role of FGF23 in regulation of PTH and secondary hyperparathyroidism in CKD is currently under intense investigation (see below).

Whereas the main target of FGF23 is the kidney, the tissue source of FGF23 is primarily bone, more specifically osteocytes and osteoblasts[11,12]. This further underscores the fact that bone, beyond its capacity to store minerals and provide mechanical support, is a highly active

endocrine organ. Further, there is robust evidence for the presence of a previously unidentified bone-kidney axis. The interplay between bone and kidney is not farfetched given that the kidney is the main determinant of circulating phosphate levels and actively participates in maintaining calcium homeostasis, providing the skeleton with sufficient minerals to form hydroxyapatite crystals at the mineralization front.

Because FGF23 holds promise as a biomarker for patient outcome (see below), especially in patients with chronic kidney disease (CKD), it is important to understand its mode of regulation. The most rapid stimuli for FGF23 expression both *in vitro* and *in vivo* is $1,25(OH)_2D_3$, evoking a response in serum FGF23 level within three to four hours after intravenous administration[13]. This completes a feedback loop between vitamin D and FGF23, and FGF23 can in that sense be viewed as a counter-regulatory hormone for vitamin D. As a result, the decline in vitamin D level that occurs already in the initial phase of CKD can likely be attributed to a rise in FGF23 rather than a reduced renal mass *per se*.

FGF23 production is also promoted by high dietary phosphate intake[14-18] as well as chronic hyperphosphatemia, although rapid changes in serum phosphate concentrations may not invoke acute increments in FGF23. One hypothesis is that FGF23 responds to the net phosphate balance rather than the serum phosphate level, but experimental data supporting this hypothesis is weak. Further, the complete chain of events from high dietary phosphate intake and hyperphosphatemia to increased FGF23 synthesis in bone is currently unknown.

It also stands clear that vitamin D and phosphate regulate FGF23 through independent pathways, because mice lacking the vitamin D receptor are still highly responsive to high dietary phosphate intake[19]. As a final remark, the response in FGF23 elicited by dietary phosphate intake in humans is much weaker than in rodents.

Despite the fact that FGF23 belongs to the FGF family, in which all members signal through one or several of the known FGF-receptors, it has been difficult to unveil the 'true' FGF23-receptor both *in vivo* and *in vitro*. A major breakthrough came from studies by Urakawa et al, who demonstrated that type I membrane-bound alpha-Klotho (Klotho) directly binds to FGF-receptor 1c, converting it into a specific FGF23-receptor[20].

Accordingly, FGF23 is dependent on Klotho to induce FGF-receptor signaling. More specifically, the expression pattern of Klotho defines the tissue specificity of FGF23. The importance of Klotho in FGF23 signaling is evidenced by *Klotho* null mice, which harbor nearly an identical biochemical phenotype compared to *Fgf23* knockout mice, despite

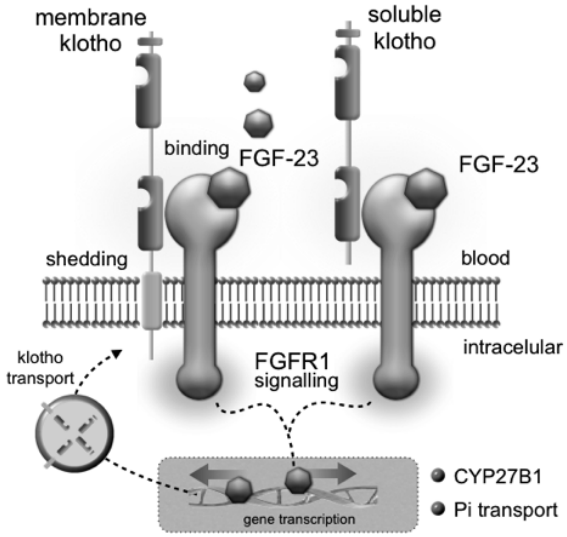
exceptionally high circulatory FGF23 levels[21,22]. There are however still controversies and unresolved issues around FGF23 receptor signaling. First, Klotho expression in kidney is largely confined to the distal tubules, whereas renal phosphate reabsorption occurs in the proximal tubules. It is currently unclear how FGF23 signaling in distal tubules modifies phosphate reabsorption in proximal tubules. Second, there is an ongoing debate on whether high levels of FGF23, as present in many patients with advanced CKD, could induce unspecific 'off-target' (i.e. Klotho-independent) FGF-receptor signaling.

FGF23 and Klotho are likely to play important roles in the pathophysiology of secondary hyperparathyroidism. Although FGF23 in the short-term suppress PTH secretion, chronically high exposure of FGF23 may override this effect by lowering the systemic levels of $1,25(OH)_2D_3$ and attenuate parathyroid vitamin D receptor signaling. Equally important, it was recently demonstrated that FGF23 reduce the expression level of Klotho and that parathyroid Klotho expression in surgically removed human parathyroid adenomas declines in parallel with loss of renal function[23]. This is a plausible explanation for the parathyroid FGF23 'resistance' observed both in CKD patients and in rodent models of experimentally induced renal failure[24].

In summary, the discovery of FGF23 and Klotho has led to significant advances in our understanding of mineral metabolism. This knowledge is now gradually being translated into the clinic with many potential implications, including the endorsement of FGF23 as a predictive biomarker and the possibility of FGF23/Klotho as a novel therapeutic target.

Klotho

Klotho was discovered in 1997 in a mouse strain with a phenotype consistent with premature aging as the principle hallmark[25]. Klotho has a short trans-membrane domain and two large extracellular domains[26], as shown in figure 2. Klotho exist as a membrane-bound and two circulating forms, one being the shed-product of the membrane form, the other a truncated form derived from the same gene by alternative splicing.



Besides its above-described role as cofactor in FGF23 signaling as shown in figure 2, Klotho harbors at least two important additional functions[27]. First it turned out that Klotho has enzymatic activity, that resides on the extra cellular domain[28]. The latter is of importance, because Klotho retains this enzymatic activity, even when it is shed from the plasma membrane. This is in contrast to the role of Klotho as co-factor in FGF23, where

Figure 2: The fibroblast growth factor (FGF) -23 receptor. *In light blue FGFR-1. In dark blue, left side, membrane bound klotho; right-sided in dark blue, solble or shed klotho. The red hexagonal structure depicts FGF-23. FGF-23 signaling is established when FGFR-1 and klotho colocalize, as shown on the left side. It is unknown whether signal transduction can occer with soluble klotho (right-sided). In the kidney, signaling leads to downregulation of CYP27B1, and retrieval of phosphate transporters from the luminal membrane of proximal tubular cells. (figure provided by J Hoenderop, dept of Physiology, University Medical Centre, Nijmegen, The Netherlands)*

the membrane-bound form appears to be necessary. The best-described role of this enzymatic activity of Klotho is its influence on the transient receptor potential V5 (TRPV5) at the luminal side of the renal tubules[29]. The glucuronidase-like activity of Klotho modulates the sugar moieties leading to sustained retention of these highly important calcium transporters in the kidney. In this way Klotho promotes reabsorption of ultra-filtrated calcium and prevents calciuria[30]. This calcium retaining effect of Klotho in the healthy kidney is yet another mechanism used to prevent over-activation of native vitamin D, besides its suppressive role on vitamin D metabolism mediated by FGF23-signaling. Active vitamin D in turn up-regulates Klotho expression, closing a feedback loop between vitamin D and Klotho[31]. Very recently it was shown that Klotho, besides its involvement in calcium handling in the kidney also has direct effects on NaPi2a, the principle phosphate transporter in the

proximal tubule[32]. Remarkable about the latter finding is that this effects is located on the proximal tubule, while most investigator found the distal tubule to be the most prominent site of Klotho expression. The other remarkable fact is that the Klotho-induced phosphaturic effects were also observed in *Fgf23* knockout mice, indicating a direct effect on NaPi2a. A third recognized mode of action of Klotho is its protective effect against oxidative stress. This was demonstrated by Kuro-o (who discovered Klotho in 1997) and co-workers[33,34]. They showed that the shed and circulating form of Klotho activates FOXO, leading to enhanced expression of superoxide dismutase.

Future research will likely unveil additional intriguing effects of Klotho. Most recently, Klotho appeared to be involved in endothelial integrity[35], possibly explaining previous findings that Klotho is involved in endothelial-dependent vasodilatation[36,37]. The final potentially important observation is the fact that Klotho is expressed in the sinoatrial node and its decreased expression leads to SA node malfunction and premature death[38]. All these findings are likely to be most relevant to CKD patients as both renal expression and circulating levels of Klotho are reduced in this vulnerable population[39].

Clinical aspects of FGF23

Giving its central role in regulating phosphate levels, its influence on vitamin D metabolism and the regulatory role of FGF23 on PTH secretion, the obvious question arises how important FGF23 is clinically? In early CKD FGF23 appears to be beneficial, compensating for reduced phosphate excretory capacity, by increasing fractional excretion of phosphate. Although phosphate retention is also an stimulus for PTH secretion, in early stage CKD, the rise in FGF23 is more pronounced that that of PTH, possibly because of the inhibitory effects of FGF23 on PTH[40;41] A trade-off of this more pronounced increase of FGF23 may be a reduction in levels of $1,25(OH)_2D_3$ by the mechanism described above. As CKD progresses, the efficacy of FGF23 to induce phosphaturia declines, due to at least two mechanisms. First, the loss of functioning nephrons reduces the amount of phosphate being ultrafiltrated, and second, lowered renal Klotho expression dismantles the FGF23-receptor, leading to higher phosphate reabsorption per nephron. For these reasons it is expected that in advanced CKD FGF23 could be an indicator for dismal outcome. Indeed this has been shown for more advanced CKD, showing that FGF23 independently predicted progression of disease[42;43]. In

that study[44], the predictive value of FGF23 for disease progression was only surpassed by proteinuria. Despite its independency in predicting disease progression, FGF23 levels and proteinuria are positively associated across several ranges of CKD, as recently demonstrated (ASN 2010, PO325). The most convincing argument of the clinical meaning of FGF23 comes from the analysis of a large cohort of dialysis patients, in which an independent association between FGF23 and mortality was demonstrated

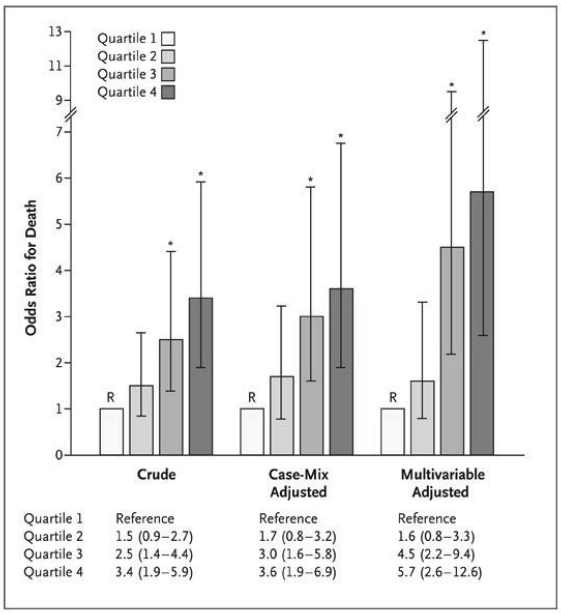


Figure 3: Odds ratios for death in hemodialysis patients in relation to quartiles of FGF23 levels. *Both unadjusted and fully adjusted models demonstrate a strong and graded association. Reproduced from Gutierrez et al permission[45].*

(figure 3)[45]. Even after correcting for established predictors of mortality, the hazard ratio's for the higher ranges of FGF23 outranked these others. The fact that correcting for phosphate level, PTH and vitamin D use did not mitigate predictive value of FGF23 for dismal outcome is remarkable, because these parameters were thought to be in the same causal pathway to clinical events as FGF23. This could mean that FGF23 is a much more sensitive marker for, for instance, phosphate burden, or induces harm itself. In support for the first hypothesis, FGF23 has in large observational studies of elderly subjects with normal or only mildly impaired renal function been associated with vascular dysfunction[46], total atherosclerotic burden[47] and left ventricular hypertrophy[48]. Further, FGF23 is also predictor of fracture risk in this population, another central feature of the CKD-MBD syndrome[49]. Arguments for the latter comes from a recent study indicating that FGF23 influences flow mediated vasodilation in CKD stage 3 and 4 patients[50]. Additional arguments, albeit indirect, come from recent research indicating FGF23 as an factor associated with left ventricular

mass index (LVMI) independent from BNP[51], myocardial performance[52], and coronary artery disease[53].

If FGF23 indeed turns out to be either a sensitive biomarker of phosphate load, or has some different pathological effect in advanced stages of CKD, the next clinical question would be: Is it modifiable? From a theoretical point of view, as pointed out above, possibilities to lower FGF23 would be to lower levels of active vitamin D, to lower PTH or to lessen phosphate burden. Indeed parathyroidectomy has been shown to induce a significant decline if FGF23[54], possibly because of the direct effect of PTH on FGF23 production[55]. Lowering dietary phosphate intake decreases FGF23 in healthy volunteers with a lag time[56], but this approach is not likely to be sufficient in CKD. The use of phosphate binders has shown inconsistent results as FGF23 lowering agents. Sevelamer declined FGF23 in a dose dependent manner in an experimental model of uremia[57], and this was confirmed clinically after 6 weeks of treatment[58]. Use of calcium-based binder therapy did not change FGF23 significantly in that study. These findings were confirmed in a cohort of 72 hemodialysis patients followed for a year[59]. Use of lanthanumcarbonate however, while significantly reducing 24-hour urine phosphate excretion indicating adequate phosphate binding, did not change FGF23[60]. Lowering levels of $1,25(OH)_2D_3$ in CKD as a way to decline FGF23 is not an attractive option for obvious reasons[61].

Currently, there is no evidence supporting that FGF23 is a modifiable risk factor that leads to improvement of clinical outcomes such as mortality. Prospective trials are underway that study changes in vascular function after FGF23 targeted interventions. If intervening in FGF23 levels is executed by targeting phosphate levels or burden, it will be problematic to discern the effects of a lower FGF23 level from the effect of more stringent phosphate control. To examine the direct effects of FGF23 clinically either blocking agents or targeting FGF23 producing cells, either osteocytes in bone or ectopic production from bone cells in the vessel wall in the presence of calcified arterial lesions[62] may provide useful information.

From the convincing epidemiological association between FGF23 and mortality in dialysis patients[63] an enigma emerges: How is it possible that a hormone like FGF23 has such an impact on outcome, while its main target organ, i.e. the kidney, is non-functioning? Of course, the parathyroid is another target for FGF23, but in CKD PTH-suppression by FGF23 is abolished by parathyroid resistance[64,65]. While it is likely that all FGF23 actions require the presence of the fibroblast growth factor receptor 1c (FGFR1c) and membrane klotho[66],

theoretically, other tissues expressing klotho and FGFR1c might be unidentified targets for FGF23. Recently both klotho and FGFR1 were shown to be present in human aortic smooth muscle cell (ASN 2010, Th-PO126), but at present it is unclear whether this is membrane-bound or soluble klotho, and whether actual signal transduction by FGF23 can occur at this site. Nevertheless, the arterial wall as target tissue for FGF23 action is an attractive concept, giving the central role of vascular disease in CKD-associated morbidity. Alternatively, indirect effects of FGF23 on the arterial wall are also plausible. These could be mediated by phosphate itself or by a reduction in Klotho level, given that FGF23 suppress Klotho[67] and recent data suggest that it is implicated in the process of arterial calcification[68].

In conclusion, data pointing to FGF23 and Klotho being active players in the burden of CKD are ever increasing. Both epidemiological data and pathophysiological mechanisms have set the stage for targeted intervention in the clinical setting. These new apparently important factors in CKD provide hope for improved future targeted therapy in CKD.

References

1. Autosomal dominant hypophosphataemic rickets is associated with mutations in FGF23. Nat Genet 2000; 26: 345-348

2. Shimada T, Mizutani S, Muto T *et al*. Cloning and characterization of FGF23 as a causative factor of tumor-induced osteomalacia. Proc Natl Acad Sci U S A 2001; 98: 6500-6505

3. White KE, Carn G, Lorenz-Depiereux B, et-Pages A, Strom TM, Econs MJ. Autosomal-dominant hypophosphatemic rickets (ADHR) mutations stabilize FGF-23. Kidney Int 2001; 60: 2079-2086

4. Shimada T, Mizutani S, Muto T *et al*. Cloning and characterization of FGF23 as a causative factor of tumor-induced osteomalacia. Proc Natl Acad Sci U S A 2001; 98: 6500-6505

5. Shimada T, Kakitani M, Yamazaki Y *et al*. Targeted ablation of Fgf23 demonstrates an essential physiological role of FGF23 in phosphate and vitamin D metabolism. J Clin Invest 2004; 113: 561-568

6. Bai X, Miao D, Li J, Goltzman D, Karaplis AC. Transgenic mice overexpressing human fibroblast growth factor 23 (R176Q) delineate a putative role for parathyroid hormone in renal phosphate wasting disorders. Endocrinology 2004; 145: 5269-5279

7. Larsson T, Marsell R, Schipani E *et al*. Transgenic mice expressing fibroblast growth factor 23 under the control of the alpha1(I) collagen promoter exhibit growth retardation, osteomalacia, and disturbed phosphate homeostasis. Endocrinology 2004; 145: 3087-3094

8. Shimada T, Hasegawa H, Yamazaki Y *et al*. FGF-23 is a potent regulator of vitamin D metabolism and phosphate homeostasis. J Bone Miner Res 2004; 19: 429-435

9. Krajisnik T, Bjorklund P, Marsell R *et al*. Fibroblast growth factor-23 regulates parathyroid hormone and 1alpha-hydroxylase expression in cultured bovine parathyroid cells. J Endocrinol 2007; 195: 125-131

10. Ben-Dov IZ, Galitzer H, Lavi-Moshayoff V *et al*. The parathyroid is a target organ for FGF23 in rats. J Clin Invest 2007; 117: 4003-4008

11. Liu S, Guo R, Simpson LG, Xiao ZS, Burnham CE, Quarles LD. Regulation of fibroblastic growth factor 23 expression but not degradation by PHEX. J Biol Chem 2003; 278: 37419-37426

12. Riminucci M, Collins MT, Fedarko NS *et al*. FGF-23 in fibrous dysplasia of bone and its relationship to renal phosphate wasting. J Clin Invest 2003; 112: 683-692

13. Liu S, Tang W, Zhou J *et al*. Fibroblast growth factor 23 is a counter-regulatory phosphaturic hormone for vitamin D. J Am Soc Nephrol 2006; 17: 1305-1315

14. 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 2010;

15. Ferrari SL, Bonjour JP, Rizzoli R. Fibroblast growth factor-23 relationship to dietary phosphate and renal phosphate handling in healthy young men. J Clin Endocrinol Metab 2005; 90: 1519-1524

16. Nishida Y, Taketani Y, Yamanaka-Okumura H *et al*. Acute effect of oral phosphate loading on serum fibroblast growth factor 23 levels in healthy men. Kidney Int 2006; 70: 2141-2147

17. Isakova T, Gutierrez OM, Smith K *et al*. Pilot study of dietary phosphorus restriction and phosphorus binders to target fibroblast growth factor 23 in patients with chronic kidney disease. Nephrol Dial Transplant 2010;

18. Antoniucci DM, Yamashita T, Portale AA. Dietary phosphorus regulates serum fibroblast growth factor-23 concentrations in healthy men. J Clin Endocrinol Metab 2006; 91: 3144-3149

19. Yu X, Sabbagh Y, Davis SI, Demay MB, White KE. Genetic dissection of phosphate- and vitamin D-mediated regulation of circulating Fgf23 concentrations. Bone 2005; 36: 971-977

20. Urakawa I, Yamazaki Y, Shimada T *et al*. Klotho converts canonical FGF receptor into a specific receptor for FGF23. Nature 2006; 444: 770-774

21. Kuro-o M, Matsumura Y, Aizawa H *et al*. Mutation of the mouse klotho gene leads to a syndrome resembling ageing. Nature 1997; 390: 45-51

22. Shimada T, Urakawa I, Yamazaki Y *et al*. FGF-23 transgenic mice demonstrate hypophosphatemic rickets with reduced expression of sodium phosphate cotransporter type IIa. Biochem Biophys Res Commun 2004; 314: 409-414

23. Krajisnik T, Olauson H, Mirza MA *et al*. Parathyroid Klotho and FGF-receptor 1 expression decline with renal function in hyperparathyroid patients with chronic kidney disease and kidney transplant recipients. Kidney Int 2010; 78: 1024-1032

24. Galitzer H, Ben-Dov IZ, Silver J, Naveh-Many T. Parathyroid cell resistance to fibroblast growth factor 23 in secondary hyperparathyroidism of chronic kidney disease. Kidney Int 2010; 77: 211-218

25. Kuro-o M, Matsumura Y, Aizawa H *et al*. Mutation of the mouse klotho gene leads to a syndrome resembling ageing. Nature 1997; 390: 45-51

26. Shiraki-Iida T, Aizawa H, Matsumura Y *et al*. Structure of the mouse klotho gene and its two transcripts encoding membrane and secreted protein. FEBS Lett 1998; 424: 6-10

27. Torres PU, Prie D, Molina-Bletry V, Beck L, Silve C, Friedlander G. Klotho: an antiaging protein involved in mineral and vitamin D metabolism. Kidney Int 2007; 71: 730-737

28. Tohyama O, Imura A, Iwano A *et al*. Klotho is a novel beta-glucuronidase capable of hydrolyzing steroid beta-glucuronides. J Biol Chem 2004; 279: 9777-9784

29. 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; 310: 490-493

30. 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; 310: 490-493

31. 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 2003; 17: 2393-2403

32. Hu MC, Shi M, Zhang J *et al*. Klotho: a novel phosphaturic substance acting as an autocrine enzyme in the renal proximal tubule. FASEB J 2010; 24: 3438-3450

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

34. Yamamoto M, Clark JD, Pastor JV *et al*. Regulation of oxidative stress by the anti-aging hormone klotho. J Biol Chem 2005; 280: 38029-38034

35. Kusaba T, Okigaki M, Matui A *et al*. 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 2010; 107: 19308-19313

36. Nagai R, Saito Y, Ohyama Y *et al*. Endothelial dysfunction in the klotho mouse and downregulation of klotho gene expression in various animal models of vascular and metabolic diseases. Cell Mol Life Sci 2000; 57: 738-746

37. Nakamura T, Saito Y, Ohyama Y *et al*. Production of nitric oxide, but not prostacyclin, is reduced in klotho mice. Jpn J Pharmacol 2002; 89: 149-156

38. Takeshita K, Fujimori T, Kurotaki Y *et al*. Sinoatrial node dysfunction and early unexpected death of mice with a defect of klotho gene expression. Circulation 2004; 109: 1776-1782

39. Kuro O. Phosphate and Klotho. Kidney Int 2011;

40. Krajisnik T, Bjorklund P, Marsell R *et al*. Fibroblast growth factor-23 regulates parathyroid hormone and 1alpha-hydroxylase expression in cultured bovine parathyroid cells. J Endocrinol 2007; 195: 125-131

41. Ben-Dov IZ, Galitzer H, Lavi-Moshayoff V *et al*. The parathyroid is a target organ for FGF23 in rats. J Clin Invest 2007; 117: 4003-4008

42. Fliser D, Kollerits B, Neyer U *et al*. Fibroblast growth factor 23 (FGF23) predicts progression of chronic kidney disease: the Mild to Moderate Kidney Disease (MMKD) Study. J Am Soc Nephrol 2007; 18: 2600-2608

43. Titan SM, Zatz R, Graciolli FG *et al*. FGF-23 as a Predictor of Renal Outcome in Diabetic Nephropathy. Clin J Am Soc Nephrol 2010;

44. Fliser D, Kollerits B, Neyer U *et al*. Fibroblast growth factor 23 (FGF23) predicts progression of chronic kidney disease: the Mild to Moderate Kidney Disease (MMKD) Study. J Am Soc Nephrol 2007; 18: 2600-2608

45. Gutierrez OM, Mannstadt M, Isakova T *et al*. Fibroblast growth factor 23 and mortality among patients undergoing hemodialysis. N Engl J Med 2008; 359: 584-592

46. Mirza MA, Larsson A, Lind L, Larsson TE. Circulating fibroblast growth factor-23 is associated with vascular dysfunction in the community. Atherosclerosis 2009; 205: 385-390

47. Mirza MA, Hansen T, Johansson L *et al*. Relationship between circulating FGF23 and total body atherosclerosis in the community. Nephrol Dial Transplant 2009; 24: 3125-3131

48. 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 2009; 207: 546-551

49. Mirza MA, Karlsson MK, Mellstrom D *et al*. Serum fibroblast growth factor-23 (FGF23) and fracture risk in elderly men. J Bone Miner Res 2010;

50. Yilmaz MI, Sonmez A, Saglam M *et al*. FGF-23 and vascular dysfunction in patients with stage 3 and 4 chronic kidney disease. Kidney Int 2010; 78: 679-685

51. Negishi K, Kobayashi M, Ochiai I *et al*. Association between fibroblast growth factor 23 and left ventricular hypertrophy in maintenance hemodialysis patients. Comparison with B-type natriuretic peptide and cardiac troponin T. Circ J 2010; 74: 2734-2740

52. Kirkpantur A, Balci M, Gurbuz OA *et al*. 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;

53. Kanbay M, Nicoleta M, Selcoki Y *et al*. Fibroblast growth factor 23 and fetuin A are independent predictors for the coronary artery disease extent in mild chronic kidney disease. Clin J Am Soc Nephrol 2010; 5: 1780-1786

54. Sato T, Tominaga Y, Ueki T *et al*. Total parathyroidectomy reduces elevated circulating fibroblast growth factor 23 in advanced secondary hyperparathyroidism. Am J Kidney Dis 2004; 44: 481-487

55. Lavi-Moshayoff V, Wasserman G, Meir T, Silver J, Naveh-Many T. PTH increases FGF23 gene expression and mediates the high-FGF23 levels of experimental kidney failure: a bone parathyroid feedback loop. Am J Physiol Renal Physiol 2010; 299: F882-F889

56. 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 2010;

57. Nagano N, Miyata S, Abe M *et al*. Effect of manipulating serum phosphorus with phosphate binder on circulating PTH and FGF23 in renal failure rats. Kidney Int 2006; 69: 531-537

58. Oliveira RB, Cancela AL, Graciolli FG *et al*. Early control of PTH and FGF23 in normophosphatemic CKD patients: a new target in CKD-MBD therapy? Clin J Am Soc Nephrol 2010; 5: 286-291

59. Cancela AL, Oliveira RB, Graciolli FG *et al*. Fibroblast growth factor 23 in hemodialysis patients: effects of phosphate binder, calcitriol and calcium concentration in the dialysate. Nephron Clin Pract 2011; 117: c74-c82

60. Isakova T, Gutierrez OM, Smith K *et al*. Pilot study of dietary phosphorus restriction and phosphorus binders to target fibroblast growth factor 23 in patients with chronic kidney disease. Nephrol Dial Transplant 2010;

61. Vervloet MG, Twisk JW. Mortality reduction by vitamin D receptor activation in end-stage renal disease: a commentary on the robustness of current data. Nephrol Dial Transplant 2009; 24: 703-706

62. Voigt M, Fischer DC, Rimpau M, Schareck W, Haffner D. Fibroblast growth factor (FGF)-23 and fetuin-A in calcified carotid atheroma. Histopathology 2010; 56: 775-788

63. Gutierrez OM, Mannstadt M, Isakova T *et al*. Fibroblast growth factor 23 and mortality among patients undergoing hemodialysis. N Engl J Med 2008; 359: 584-592

64. Krajisnik T, Olauson H, Mirza MA *et al*. Parathyroid Klotho and FGF-receptor 1 expression decline with renal function in hyperparathyroid patients with chronic kidney disease and kidney transplant recipients. Kidney Int 2010; 78: 1024-1032

65. Lafage-Proust MH. Does the downregulation of the FGF23 signaling pathway in hyperplastic parathyroid glands contribute to refractory secondary hyperparathyroidism in CKD patients? Kidney Int 2010; 77: 390-392

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

67. Krajisnik T, Olauson H, Mirza MA *et al*. Parathyroid Klotho and FGF-receptor 1 expression decline with renal function in hyperparathyroid patients with chronic kidney disease and kidney transplant recipients. Kidney Int 2010; 78: 1024-1032

68. Hu MC, Shi M, Zhang J *et al*. Klotho deficiency causes vascular calcification in chronic kidney disease. J Am Soc Nephrol 2011; 22: 124-136