Cardiovascular Risk and Mineral Bone Disorder in Patients with Chronic Kidney Disease

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Abstract
The term chronic kidney disease-mineral bone disorder has been coined recently to highlight that the disturbed mineral and bone metabolism is a major contributor to vascular calcification and finally cardiovascular disease. This syndrome is characterized by clinical, biochemical and/or histological findings, i.e. i) biochemical alterations in the homeostasis of calcium, phosphate and their key player parathyroid hormone (PTH), Fibroblast growth factor-23 (FGF-23), klotho and vitamin-D, ii) the occurrence of vascular and/or soft tissue calcification, and iii) an abnormal bone structure and/or turnover. Apart from the combined and synergistic action of "traditional" and uremia-related risk factors, promoters and inhibitors of calcification have to be considered as well. This review will focus on the disturbed mineral metabolism as the triggering force behind distortion of vascular integrity and cardiovascular malfunction in CKD patients.

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Introduction
Chronic kidney disease (CKD) is associated with a severely increased risk of cardiovascular morbidity and mortality [1-6]. Numerous structural and functional alterations of the cardiovascular system, e.g. endothelial dysfunction, arterial stiffening, left ventricular hypertrophy (LVH) and remodeling of the vessel wall with hyperplasia
and calcification occur early in the course of CKD (stage 2-4 CKD) and contribute to the overt risk of ischemic cardiovascular disease (CVD) and sudden cardiac death [6-14]. While an impaired renal function has the potential to aggravate "traditional" risk factors like hypertension, dyslipidaemia, inflammation, and oxidative stress, the concomitant deterioration of mineral homeostasis and thus also bone metabolism is probably the key player leading to accelerated CVD [8]. To highlight the central role of mineral metabolism for both, cardiovascular and skeletal integrity, the term chronic kidney disease-mineral bone disorder (CKD-MBD) was coined recently [15]. This syndrome is characterized by clinical, biochemical and/or histological findings, i.e. 1) biochemical alterations in the homeostasis of calcium, phosphate and their key player parathyroid hormone (PTH), Fibroblast growth factor-23 (FGF-23), klotho and vitamin-D, 2) the occurrence of vascular and/or soft tissue calcification, and 3) an abnormal bone structure and/or turnover. Apart from the combined and synergistic action of "traditional" and uremia-related risk factors, promoters and inhibitors of calcification have to be considered as well. This review will focus on the disturbed mineral metabolism in CKD patients as the triggering force behind distortion of vascular integrity and cardiovascular malfunction in these patients.

Vascular calcification

Calcification can occur either in the tunica intima or tunica media (arteriosclerosis or Mönckeberg’s sclerosis) of the vessels. The former is due to intima-media thickening and the formation of atherosclerotic plaques with injury of the intima, local inflammation, dyslipidaemia with subsequent accumulation of foam cells as the main triggering events [16]. Atherosclerosis is restricted to large and medium-sized conduit arteries and has been linked mainly to "traditional" risk factors like smoking, obesity, dyslipidaemia, hypertension and aging. By contrast, media calcification can occur in arteries of any size, is a typical age-related process and has been associated with increased stiffness and reduced cushioning function [17, 18]. In patients with diabetes mellitus and/or chronic kidney disease, both forms of calcification are present but are usually not distinguished in epidemiological studies, since non-ambiguous discrimination is hardly achievable unless vessel specimens for histological evaluation are available [19]. Although the initial events leading to either intima or media calcification differ, both reflect an active and highly regulated process closely resembling endochondral and/or intramembrane bone formation. Furthermore, an inverse association between bone mineralization and vascular mineral burden has been noted in both, the general population and in CKD patients (calcification paradox) [18, 20].

Mineral metabolism and chronic kidney disease

Maintenance of calcium and phosphate homeostasis within rather narrow limits (i.e. 0.8 - 1.4 mmol/l for P and 2.2 - 2.6 mmol/l for Ca) is of great physiological importance. Phosphate is required for ATP generation, formation of phospholipids and nucleic acids, cellular signaling, and maintenance of pH. Likewise, calcium is critically involved in several physiological processes including neuronal and endothelial signaling, muscle contraction, and blood clotting. Approximately 85% of total body phosphate and > 98% of total body calcium are stored in the skeleton, mainly in the form of hydroxyapatite (Ca_{10}(PO_{4})_{6}(OH)_{2}) [21-26]. In adults bone remodeling leads to a continuous flow of calcium (~ 500 mg/day) and phosphate (~ 300 mg/day) out from and into the skeleton [23, 26]. Numerous epidemiological studies pointed to hyperphosphatemia, hypercalcemia, and an elevated calcium x phosphate serum product as the main causes of vascular calcification, even in the general population and although the effects of these aberrations are most prominent in patients with end-stage renal disease [27-42] (for a critical review on this topic see also [43]).
PTH, calcitriol, FGF-23 and klotho form the regulatory system together with their related feedback loops ("bone-parathyroid-kidney axis") for mineral metabolism. Within this circuit the skeleton serves not only as an active mineral repository and endocrine target but also as source of FGF-23 (Figure 1).

A declining GFR is paralleled by a declining renal formation of calcitriol and a subsequently impaired maintenance of calcium homeostasis. A low extracellular calcium level is the main activator of the calcium-sensing receptor (CaSR) in the parathyroid. Activation of the CaSR leads to release of PTH, which exerts its effect via binding to the G-protein coupled PTH receptor (PTHR1) in bone and kidney, i.e. the key regulators of mineral metabolism. In bone, PTH increases the activity of osteoblasts and indirectly of osteoclasts, which in turn stimulates the skeletal release of calcium and phosphate [44]. At the same time, renal phosphate reabsorption is diminished via internalization inhibition of the sodium dependent phosphate cotransporter NaPi-IIa and NaPi-IIc in the proximal tubules while calcium reabsorption is stimulated by activation of calcium channels, e.g. TRPV5 [25, 45, 46]. Likewise, the PTH-mediated stimulation of the renal 1α-hydroxylase (Cyp27b1) leads to synthesis of 1,25 dihydroxyvitamin D₃ (calcitriol). Calcitriol does not only stimulate gastrointestinal calcium and phosphate absorption but also inhibits PTH synthesis [46-48]. In the skeleton, calcitriol transcriptionally activates expression and secretion of FGF-23 in osteoblasts and induces (in cooperation with PTH) the expression of RANK-ligand at the surface of osteoblasts. This finally stimulates osteoclastogenesis and bone turnover. FGF-23, whether induced by calcitriol or hyperphosphatemia, reduces tubular phosphate reabsorption and enhances renal phosphate excretion via inactivation of NaPi-IIa. Furthermore, it inhibits PTH synthesis and renal 1α-hydroxylase (Cyp27b1) while it stimulates the catabolic 24-hydroxylase (Cyp24A1) [46, 49-52]. Thus, FGF-23 regulates its own expression and closes negative feedback loops between bone, kidney and the parathyroid glands, respectively [46]. However, the complex interaction between FGF-23 and PTH has not been completely understood yet, and whether the increased PTH serum levels in CKD patients are cause or consequence of elevated FGF-23
levels remains to be clarified [46, 49, 53-56]. A recent study with healthy volunteers on the effects of a 24h infusion of PTH indicated, that FGF-23 secretion is mitigated before calcitriol concentrations increase with subsequent (re-) stimulation of FGF-23 synthesis [57]. In the parathyroid, chronic uremia has been suggested to cause relative resistance to FGF-23 [58, 59].

Under physiological conditions, the endocrine effects of FGF-23 are not only dependent on the presence of FGF-receptors (FGF-R) but also on the protein klotho, which ensures high affinity binding of FGF-23 [49, 51, 60].

Klotho, named after a Greek goddess who spins the thread of life, is a large (120 - 135 kDa) type-I membrane protein with a single transmembrane domain near the cytoplasmatic C-terminus which is supposed to anchor the protein to the membrane. It is expressed mainly in the distal convoluted renal tubules, the parathyroid and the epithelium of the choroid plexus in the brain [49, 61, 62]. Proteolytic cleavage releases the extracellular domain into the circulation and this can be detected in blood, urine and cerebrospinal fluid [63-66]. Secreted klotho was shown to act presumably independent from FGF-23 as a humoral factor with pleiotropic activities, e.g. suppression of oxidative stress, inhibition of intracellular insulin- and insulin-like growth factor-1 signaling, regulation of endothelial production of nitric oxide and calcium homeostasis [56, 67, 68]. The latter is of special interest as it puts an additional layer of complexity to the control of mineral metabolism. In particular, phosphate reabsorption occurs in the proximal tubules, while expression of klotho and thus FGF-23 signaling occurs mainly in the distal tubules. It remains to be elucidated whether shedded klotho or an yet unknown mediator signals between distal and proximal tubules [50, 69]. Furthermore, expression and secretion of klotho decreases with age and a recent large population-based study revealed an inverse association with CVD [70].

However, it is still a matter of discussion whether or not klotho-independent effects of FGF-23 exist and our understanding of klotho and FGF-23 signaling is based mainly on data from genetically engineered mice (for review see [71]).

**CKD-MBD effects on the cardiovascular system**

A decline of renal function affects mineral metabolism at the excretory (resorption and excretion of Ca and P) and regulatory level (bone-parathyroid-kidney axis). The endocrine regulation of mineral metabolism as described above brings the interplay of calcitriol, PTH, FGF-23 and klotho into focus, especially in view of the declining kidney function. In fact, calcitriol deficiency together with the secondary hyperparathyroidism (sHPT) and hyperphosphatemia was thought for years to be the main culprit of cardiovascular disease in CKD patients. The identification of FGF-23 as the main regulator of phosphate homeostasis and as a reliable predictor for mortality risk in CKD patients has changed this view considerably within the last decade [72-74]. Thus, cardiovascular remodeling related to CKD is hardly the result of single but complex metabolic aberrations.

In fact, a strong positive association between FGF-23 serum levels, impaired vasoreactivity, atherosclerosis, left ventricular mass index and left ventricular function was seen independently from renal function and mineral status [75-79]. Furthermore, we were able to detect FGF-23 within calcified carotid atheromas derived from patients with preserved renal function, and observed a positive association between the FGF-23 serum concentration and the degree of calcification [80]. Whether this reflects local synthesis as a consequence of osteogenic transformation or accumulation of bone derived FGF-23 remains to be elucidated.

The causative role of FGF-23 was confirmed by in-vivo and in-vitro experiments, and as mentioned above there is evidence for klotho-independent effects of FGF-23. In particular, injection of FGF-23 into rodent models with preserved or impaired renal function (5/6 nephrectomy, 5/6-Nx) caused klotho-independent left ventricular hypertrophy which was attenuated by an FGF-Receptor antagonist [81]. In a more recent study the effects of
antibody-mediated neutralization of FGF-23 was evaluated in a rat model of CKD-MBD. For this purpose, rats underwent 5/6-Nx and were started on normal or high-phosphate diet three weeks before onset of treatment to firmly establish CKD-MBD with sHPT, calcitriol deficiency, hyperphosphatemia and elevated levels of FGF-23. On the one hand, the results met the expectations, i.e. sustained reduction of sHPT, an increase of calcitriol serum levels and an improvement of high-turnover bone disease [82]. On the other hand, neutralization of FGF-23 resulted in a dose-dependent increase of serum phosphate with concomitant aortic calcification and increased risk of mortality. Apart from differences in the experimental settings, these two experiments support, that FGF-23 acts on its own as a pathogenic factor and that increasing FGF-23 concentrations most likely reflect adaptation to CKD [72].

In fact, elevated levels of FGF-23 are already seen in patients with early stages of CKD (GFR 60-90 ml/min/1.73 m²), i.e. when serum phosphate is still within the normal range and sHPT is rather mild. In dialysis patients FGF-23 serum concentrations were shown to be clearly associated with vascular calcification and adverse cardiovascular outcome [83-90]. Although these findings are in favor of a causative role of FGF-23 in the development and progression of cardiovascular disease, a recent study on the progression of aortic arch calcification during a 5-year interval in non-diabetic patients on chronic hemodialysis (n=127) revealed an inverse association with the baseline serum concentration of FGF-23 [91]. By contrast, a negative association between FGF-23 serum levels and intensity of peripheral calcification was seen in a rather small cohort of diabetic (n=32) and non-diabetic hemodialysis patients (n=56) [85]. These apparently contradictory results may simply reflect the poor standardization of assays used for the determination of FGF-23 serum concentrations [73]. But perhaps even more important, this could indicate that not a single factor but rather a whole and individually mixed cocktail contributes to cardiovascular disease. In this regard, one should keep in mind that PTH and klotho targets cardiovascular cells as well and that klotho deficiency contributes to vascular calcification in-vitro and in-vivo [73, 92-94].

Hyperphosphatemia as a consequence of reduced renal excretion, sHPT, and an elevated calcium x phosphate serum product have been identified as the culprits of accelerated vascular calcification in CKD patients [95-99]. This notion is strengthened by the recently reported association between hyperphosphatemia and aortic calcification secondary to neutralization of FGF-23 in established CKD-MBD [82]. Several elegant studies with vascular smooth muscle cells (VSMC) have shown that graded concentrations of calcium and phosphate are capable to induce osteogenic/chondrogenic differentiation including matrix vesicle release [100-103]. Likewise, immunohistochemical investigations and in-vivo studies confirmed that vascular calcification closely resembles the mechanisms of endochondral or intramembranous bone formation, i.e. markers of osteoblasts, chondrocytes and also osteoclasts were readily detectable [80, 104-111]. Apart from high extracellular phosphate and/or calcium concentrations triggering the osteo-/chondrogenic differentiation of VSMCs, degradation of extracellular matrix, the release of apoptotic bodies and apatite containing vesicles contribute to initiation and progression of vascular calcification [26]. In CKD patients therapeutic interventions, e.g. nutrition, prescription of calcium-free and/or calcium-containing phosphate binders, vitamin D metabolites, calcimimetics, choice of calcium concentration in dialysates, are directed to normalize both, the hormonal regulation and the deteriorated mineral metabolisms. One should keep in mind however, that these measures can add fuel to the fire instead of breaking the vicious circle. Especially, sub- and supraphysiological levels of calcitriol have been associated with poor cardiovascular outcome and increased vascular calcification [112-118]. Although recent animal experiments using calcimimetics in 5/6-Nx rats reduced aortic calcification subsequent to high dose calcitriol and pointed even to a regression of vascular calcification, the available data from a large international placebo-controlled mulitcenter trial are less clear [119-122].
Calcification inhibitors

Osteogenic transdifferentiation of VSMC is now widely accepted as a predominant mechanism of vascular calcification. In addition, the role of mineralization inhibitors (e.g. pyrophosphate (PPi), osteopontin (OPN), osteoprotegerin (OPG), matrix γ-carboxyglutamic acid protein (matrix gla-protein, MGP) and fetuin-A) has to be considered as well. Especially, VSMC are able to mineralize extracellular matrix via release of matrix vesicles without switching the phenotype when the concentration of mineralization inhibitors are inadequately low or even zero [123-128]. Extracellular PPi interferes with formation of hydroxyapatite nanocrystals and PPi is hydrolysed via a tissue-nonspecific alkaline phosphatase (TNAP). Under conditions of chronic uremia, diminished circulating concentrations of PPi are paralleled by an increase of TNAP activity [129-133]. Recent studies with uremic rats indicate that administration of PPi is able to revert this mechanism and to prevent vascular calcification [134, 135].

The matrix molecule OPN is a negative regulator of calcification, which is expressed by macrophages, VSMC and endothelial cells. On the one hand it is able to activate osteoclasts via signaling through the αvβ3 integrin pathway and on the other hand it may directly bind to apatite crystals thereby interfering with the mineralization process [136-139].

OPG is an important modulator of osteogenesis, i.e. the coordinated differentiation of mesenchymal progenitors and hemopoietic stem cells into osteoblasts and osteoclasts, respectively. This process requires both, the presence of macrophage colony stimulating factor (M-CSF) and binding of RANK (receptor activator of nuclear factor-κB), which is expressed at the surface of the pre-osteoclast, to RANKL at the surface of osteoblasts. Binding of OPG to RANKL inhibits this interaction and thus differentiation of osteoclasts. While RANK and RANKL are virtually undetectable in healthy vessel walls, OPG is constitutively expressed in the vessel wall and the circulation, thereby preventing activation of osteoclastogenesis [140, 141]. Whereas overexpression of OPG results in osteopetrosis, diminished concentrations of OPG lead to osteoporosis and vascular calcification in animal models [105, 142, 143]. However, a paradoxical positive association between vascular calcification and OPG serum concentrations has been described in CKD patients [144, 145].

MGP is a potent ubiquitous extracellular inhibitor of vascular calcification in-vivo. It is a small (10 kDa) protein, was initially isolated from bone, and is synthesized predominately by VSMCs and chondrocytes in response to an increase of local calcium levels. The protein exists in an inactive (undecarboxylaed) and an active form, which requires vitamin K-dependent γ-glutamyl carboxylation. The anti-calcific capacity of MGP drops significantly, when γ-carboxylation of glutamic acid residues is impaired, i.e. under conditions of oral anticoagulation therapy or vitamin K-deficiency. Animal studies have shown that blocking of vitamin K lead to rapid and extreme vascular calcification and highlight the functional relevance of this post-translational protein modification [123, 146-149]. In line with this, patients with an inborn lack of mature MGP (Keutel syndrom) suffer from excessive arterial calcification [127]. It has been suggested, that MGP prevents vascular calcification not only via high-affinity binding of calcium, which relies on phosphorylation rather than γ-carboxylation, but also as an antagonist of bone morphogenetic protein-2 (BMP-2) [101, 150]. In plasma, γ-carboxylated MGP is carried by fetuin-A and as such part of the fetuin-A-mineral complex [151]. Antibodies specific for uncaboxylated and γ-carboxylated MGP allowed to distinguish between active and inactive forms of this important calcification inhibitor [152]. With this approach an inverse association between vascular calcification and the circulating concentrations of uncaboxylated MGP were shown in patients with preserved and reduced kidney function [152-154].

Fetuin-A (also known as α2-Heremans-Schmidt glycoprotein; α2-HS) is constitutively produced in the liver, belongs to the cystatine superfamily of cysteine protease inhibitors, and has long been known as a negative acute phase protein [155-158]. It serves a multitude of biological functions as diverse as antagonizing growth factor mediated signaling, ensuring orthotopic and preventing ectopic mineralization [159-161]. In particular, circulating
fetuin-A acts as a scavenger and cargo for basic calcium phosphates (i.e. acts as calcification inhibitor), preventing formation and precipitation of microcrystals within the circulation [162, 163]. At the same time, fetuin-A enables mineralization of protein scaffolds, accessible for apatite crystals [164-166]. Although this has been formally proven only in in-vitro studies using demineralized bone or purified bone- and tendon-specific collagen type I, the same mechanisms are likely to operate in vascular calcification, which usually occurs late during the course of the disease. In terms of vascular remodeling, multiple rounds of injury and inflammation may alter structure and properties of the extracellular matrix, i.e. mineral-loaded fetuin-A will adhere to the vessel wall and eventually release its cargo instead of being cleared from the circulation [160]. In such a situation, matrix mineralization is initiated and may progress independently from professional mineralizing cells, although these are required to establish a well organized and highly crystalline mineral phase rather than amorphous precipitation. In a recent study, we clearly showed co-localization of fetuin-A and smooth muscle cell actin (SMA) at sites of tissue remodeling [80]. Both, elevated and reduced serum concentrations of fetuin-A, are increasingly recognized as risk markers of cardiovascular and metabolic disease in the general population and in CKD patients, respectively. In particular, elevated serum concentrations of fetuin-A were identified as risk marker for diabetes, metabolic syndrome, myocardial infarction and stroke in the general population [167-169]. In contrast, advanced vascular calcification in adults suffering from end-stage renal disease has been associated with diminished fetuin-A serum concentrations, which in turn were identified as an inflammation-related predictor of cardiovascular and all-cause mortality in dialysis patients [170, 171]. Likewise, children on dialysis therapy showed significantly reduced fetuin-A serum concentrations as well [172, 173]. Thus far, several antibody-based assays for the quantitative determination of fetuin-A are available. Although the results are almost always given in absolute units (i.e. g/l), one should keep in mind that apart from poor standardization of the calibrants, the effects of posttranslational fetuin-A modifications like phosphorylation, glycosylation or even binding to other serum compounds including calciumphosphate nanocrystals may interfere with antibody binding [174, 175]. Especially, increasing calcification stress is paralleled by an increase of mineral-loaded fetuin-A, which may escape from quantitative immunological detection [176-179]. High-speed centrifugation of serum was shown in principle to separate free and mineral-loaded fetuin-A [176, 180]. Despite being at first glance a rather simple procedure and direct approach to assess calcification stress more precisely, standardization of the pre-analytical procedures (clotting time, storage of serum samples with respect to time and temperature, centrifugation parameters etc.) are of outmost importance. In addition, a nanoparticle-based test for determination of the overall propensity for calcification in serum has been reported recently [181].

Conclusion

Multiple metabolic aberrations in chronic kidney disease contribute to accelerated cardiovascular disease with vascular calcification presenting the hard end of CKD-MBD rather than the beginning. Once firm vascular calcifications are present these are likely to contribute to the progression of disease and to increase the risk of mortality. It might be close to impossible to accuse only one of the factors outlined above as the main culprit, especially in patients with advanced CKD. During the last couple of years vitamin-D has been replaced by FGF-23 as the main denominator of the deranged mineral and bone metabolism in CKD patients. The promotion of FGF-23 from a biomarker of impaired phosphate handling into a pathogenic factor on its own was justified by the ability of FGF-23 to directly induce LVH in rodents [81]. However, targeting FGF-23 serum concentrations in a rat model of CKD improved shHPT and renal osteodystrophy at the expense of increased vascular calcification and mortality [82]. Taken together, the optimal FGF-23 serum level relative to renal function
remains unknown. Systemic adaptation to a declining renal function is complex, and regardless of the hormonal circuits controlling mineral homeostasis prevention of sustained hyperphosphatemia appears crucial to minimize vascular calcification [72]. Monitoring of mineral metabolism in all of the facets discussed above strongly relies on sensitive and standardized methods.

Conflict of Interests
None

References


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