Vascular calcification in chronic kidney disease: an update

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ABSTRACT

Cardiovascular calcification is both a risk factor and contributor to morbidity and mortality. Patients with chronic kidney disease (and/or diabetes) exhibit accelerated calcification of the intima, media, heart valves and likely the myocardium as well as the rare condition of calcific uremic arteriolopathy (calciphylaxis). Pathomechanistically, an imbalance of promoters (e.g. calcium and phosphate) and inhibitors (e.g. fetuin-A and matrix Gla protein) is central in the development of calcification. Next to biochemical and proteinaceous alterations, cellular processes are also involved in the pathogenesis. Vascular smooth muscle cells undergo osteochondrogenesis, excrete vesicles and show signs of senescence. Therapeutically, measures to prevent the initiation of calcification by correcting the imbalance of promoters and inhibitors appear to be essential. In contrast to prevention, therapeutic regression of cardiovascular calcification in humans has been rarely reported. Measures to enhance secondary prevention in patients with established cardiovascular calcifications are currently being tested in clinical trials.

Keywords: cardiovascular diseases, MGP, phenotype switch, vitamin K, VSMC

INTRODUCTION

Cardiovascular mortality increases progressively with advancing chronic kidney disease (CKD) and loss of renal function. In dialysis patients, it is the most frequent cause of death and indeed most patients beginning renal replacement therapy have abnormal cardiovascular function and dimensions. Traditional risk factors such as hypercholesterolaemia or arterial hypertension fail to adequately explain these observations, pointing to an added role of non-traditional risk factors such as uraemia, disordered mineral-bone disease, premature senescence of cardiovascular tissues, inflammatory and oxidative stress and others [1]. Here, we review the latest insights on cardiovascular calcifications in CKD, which become highly prevalent as CKD progresses and which are potent predictors of cardiovascular mortality in CKD patients [2].

It has been argued that cardiovascular calcification in CKD is merely a consequence of vascular inflammation and as such does not constitute a relevant treatment target [3]. However, inflammation is not a major feature of degenerative calcification, for example of the vascular media. In addition, measures that experimentally reduce CKD-associated vascular calcification and do not necessarily involve effects on inflammatory processes, translate into better survival [4]. However, we acknowledge that there is currently a paucity of data to confirm this in patients with CKD and of course the problem exists that therapeutic measures that affect calcification in patients usually have a broad array of actions and consequences. Thus, it may not be possible to conclusively answer this question in patients.

CLINICAL MANIFESTATIONS AND CONSEQUENCES

Two types of vascular calcification can be differentiated, both of which affect the majority of patients with long-standing CKD and particularly patients on dialysis: arterial media calcification (calcific arteriosclerosis or Mönckeberg’s sclerosis) and accelerated calcification of intimal plaque (calcific atherosclerosis) [2]. Calcific atherosclerosis may be the last step in classical atherosclerosis whereas medial calcification is mainly non-inflammatory and associated with duration of haemodialysis, calcium–phosphate disorders, diabetes and ageing [5]. Cardiac calcifications (i.e. myocardial or valvular calcifications) are not covered here and predominantly affect the aortic valve leaflets. A fourth, rare form of vascular calcification is calcific uremic arteriolopathy (calciphylaxis), which we have reviewed recently [6]. This disorder is of potential outstanding interest since it is considered a time-lapse example of more common calcification disorders potentially allowing for an easier identification of pathomechanisms contributing to calcification in general.
Irrespective of the affected vascular bed, the presence, amount and also progression of vascular calcification as detected by various non-invasive means helps identify patients at particular risk compared to those without or with low-grade calcification. There is an ongoing scientific debate about the true killer in CKD—calcification versus arterial stiffening [3, 7]—however, vascular calcification is without doubt associated with a dismal cardiovascular outcome in patients with CKD. There are no fixed boundaries between stiffening arterial disease and calcifying arterial disease in CKD. In fact, these closely related vascular pathologies both go along with reduced arterial elasticity, high pulse pressure and augmented pulse-wave velocity. The resultant cardiomyocyte hypertrophy contributes to the negative sequel of left-ventricular dysfunction and hypertrophy (LVH), which are among the most significant driving forces of cardiovascular risk in CKD patients [8]. Uraemic valvular disease, particularly as calcific aortic stenosis, further contributes to an increased afterload and therefore represents a highly prevalent trigger or aggravating factor for LVH. Uraemia-associated LVH is characterized by cardiomyocyte disarray and interstitial fibrosis [9] thought to induce systolic and/or diastolic heart failure as well as various arrhythmias all of which likely predispose CKD patients to sudden cardiac death.

In daily practice, assessment of cardiovascular calcifications in CKD patients cannot be recommended as routine diagnostics on a regular basis and should be reserved for particular situations (e.g. ultrasound of the iliac arteries for transplant listing, echocardiography to detect calcific aortic valve stenosis or when patients want to know their individual cardiovascular risk). In addition, we feel that in many patients a diagnostic workup for calcifications has little clinical consequence. Thus, in old dialysis patients with a relatively short life expectancy, the detection of cardiovascular calcifications usually does not alter their treatment. Vice versa in younger dialysis patients, in particular those waiting for a kidney transplant, every measure should be undertaken to prevent the development of calcification rather than wait for them to become detectable and to act only then.

### Pathomechanisms

#### Calcification promoters

An imbalance of calcification promoters and inhibitors (Table 1) in CKD paves the way for the development of extrasosseous calcifications [10]. Of note, these factors may act differently on different parts of the arterial tree [11, 12].

**Altered mineral homeostasis.** The main constituents of vascular calcifications are calcium and phosphate mainly in the form of hydroxylapatite [13]. Elevated serum levels of both calcium and phosphate are risk factors for increased mortality in CKD patients [14] and promote mineralization of vascular smooth muscle cells (VSMCs) (Figures 1 and 2). Experimental studies have shown that increasing phosphate concentrations (entering the cell via the sodium-dependent phosphate cotransporter PiT-1) can induce human arterial VSMCs to transdifferentiate towards an osteoblastic phenotype (see below). In contrast to bone morphogenetic protein (BMP)-7, other BMPs such as BMP-2 appear to promote calcification [15]. As ageing is an important confounder when investigating human arteries and pathomechanisms of calcification, arteries of uraemic children are important models to gain insight into the process of calcification. Indeed, in studies on arteries of children with CKD, phosphate could induce progression of calcification and osteogenic transdifferentiation, and calcium could potentiate these effects [16].

Disturbed calcium and phosphate metabolism in uraemia is often accompanied by dysregulation of PTH and FGF23. High PTH levels implicate rapid bone turnover with associated calcium and phosphorus release into the circulation (and likely the vasculature) whereas suppressed PTH can produce adynamic/low-

### Table 1. Promoters and inhibitors of cardiovascular calcifications

<table>
<thead>
<tr>
<th>Promoters</th>
<th>Inhibitors</th>
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<tr>
<td>BMP-2, 4 and 6</td>
<td>Matrix Gla protein</td>
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<tr>
<td>Osteocalcin</td>
<td>Osteopontin</td>
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<tr>
<td>Bone sialoprotein</td>
<td>Osteoprotegerin</td>
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<tr>
<td>Alkaline phosphatase</td>
<td>Fetuin-A</td>
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<tr>
<td>Calcium and phosphate ions</td>
<td>Klotho</td>
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<td>Oxidative stress</td>
<td>Pyrophosphate</td>
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<tr>
<td>Inflammatory cytokines (IL-6, IL1, TNF)</td>
<td>Carbonic anhydrase</td>
</tr>
<tr>
<td>Diabetes (high glucose, AGE)</td>
<td>BMP-7</td>
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<tr>
<td>Modified lipids, cholesterol</td>
<td>Vitamin K</td>
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<tr>
<td>Coumarin derivatives</td>
<td>Magnesium</td>
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<tr>
<td>Matrix vesicles/exosomes</td>
<td>Sodium thiosulfate</td>
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<tr>
<td>Apoptosis/apoptotic bodies</td>
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<tr>
<td>MMP2, 3 and 7</td>
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<tr>
<td>Runx2</td>
<td></td>
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<tr>
<td>Sox9</td>
<td></td>
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<tr>
<td>Osterix/Sp7</td>
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</table>

**Figure 1:** Microcalcifications of a uraemic artery visualized by TEM. Microcalcifications show various morphologies with a lamellar core-shell structure in many particles which suggest that apoptotic bodies or matrix vesicles may serve as calcification nidus.

G. Schlieper et al.
Altered vascular extracellular matrix. Extracellular matrix molecules such as collagen type 1, bone sialoprotein, fibronectin and decorin have been shown to be involved in the process of biomineralization in the vasculature. Genetic diseases such as pseudoxanthoma elasticum (PXE) or Marfan’s syndrome are characterized by a clinical phenotype that is similar to that of arterial remodelling which is linked to calcification [22, 23]. Arterial remodelling is defined as structural and functional changes of the vascular wall that occur in response to disease, injury or ageing. Vascular remodelling results in VSMC phenotypic switching (see below), thereby promoting calcification. Matrix metalloproteinases (MMP) that degrade components of the ECM can modulate artery calcification [24]. Cathepsin S deficiency, which normally exhibits elastolytic activity, abolishes vascular calcification in renal disease [25]. Indeed, calcification often occurs along elastin fibres, and elastin degradation can enhance calcification [26]. Recently, elastin haploinsufficiency has been shown to impede the progression of arterial calcification in matrix Gla protein (MGP)-deficient mice [27]. Elastin degradation occurs in experimental CKD [28]; however, elastin degradation could not be detected in human uremic arteries [13].

Altered vascular enzyme activity. Alkaline phosphatase indirectly facilitates hydroxyapatite formation by reducing levels of pyrophosphate, a calcification inhibitor (see below) [29]. Within this context, loss of CD73 activity, an enzyme that generates adenosine and anorganic phosphate from AMP, resulted in an increase in alkaline phosphatase activity and thereby seems to induce calcification [30]. Recently, another phosphatase called PHOSPHO1 has been shown to be involved in vascular calcification as inhibition of PHOSPHO1-inhibited calcification [31].

Other calcification promoters. An involvement of lipids and/or oxidation in vascular calcification has been shown in experimental studies. In VSMCs, lipids such as palmitic acid can induce mineralization through Acyl-CoA synthetases and NFKB [32], and oxidized low-density lipoprotein promoted osteoblast differentiation by up-regulating osterix expression dependent on Msx2 [33]. Several other factors such as microRNAs may also promote vascular calcification [34]. Overexpression of the adipokine c1q/tumour necrosis factor-related protein-3 promoted phosphate-induced VSMC calcification [35].

Drugs and diseases such as diabetes are known to be associated with cardiovascular calcifications. For example, warfarin is known to induce cardiovascular calcifications by inhibiting the vitamin K cycle thereby leading to less active MGP, and inflammation leads to reduced fetuin-A levels (see below). Recently, endothelial microparticles have been shown to mediate inflammation-induced vascular calcification [36] pointing to an interaction between different vascular cells and layers, thereby opening a more complex and fascinating research field.

Calcification inhibitors

Even in healthy subjects, body fluids are supersaturated with regard to calcium and phosphate. Spontaneous pathological precipitation of calcium–phosphate crystals, however, does not occur. This is due to the tight control of calcium precipitation by multiple calcification inhibitors, which either work alone or in concert. Below, we will address the major actors involved in calcification inhibition.

Fetuin-A. Fetuin-A (AHSG; 2-Heremans-Schmid glycoprotein) is a hepatic plasma protein belonging to subgroup 3 of the cystatin superfamily, which also includes fetuin-B, histidine-rich glycoprotein, and kininogen [37]. Fetuin-A circulates in plasma at high concentrations, ranging from 0.5 to 1.0 g/L in humans [38]. Fetuin-A-deficient mice on a DBA/2 background clearly established the role of fetuin-A as a systemic inhibitor of ectopic calcification [39]. Lack of fetuin-A turnover bone disease [17], which is often associated with the presence of cardiovascular calcifications. Klotho serves as a co-receptor for FGF23, and Klotho deficiency has been implicated in the development of arterial calcification and the resistance to protective vascular effects of FGF23 [18]. FGF23 itself does not seem to induce vascular calcification [19]. VSMCs express lower levels of the calcium-sensing receptor in calcified arteries, and such reduced levels thereby could contribute to calcification [20]. Of interest, calcimimetics prevented experimental medial calcification [21].

Mechanisms of cardiovascular calcification in CKD
contributes to vascular damage in the case of preexisting damage [40]. In haemodialysis patients, low fetuin-A levels were shown to be associated with inflammation and connected vascular calcification to mortality in patients [38]. Calciprotein particles (CPP) were identified as a trigger for ectopic calcification with a major role for fetuin-A in the formation and removal of these particles [41]. Locally, in the vasculature, small extracellular vesicular structures derived from VSMCs accumulate fetuin-A to prevent calcification [42]. Recently, fetuin-A was shown to alter the cytotoxicity of calcifying particles on late fetuin-A to prevent calcification [41]. Locally, in the vasculature, small extracellular vesicular structures derived from VSMCs accumulate fetuin-A to prevent calcification [41].

Matrix Gla protein. MGP is a vitamin K-dependent protein. It is widely expressed and accumulates in cartilage and calcified tissues. The function of MGP became clear in MGP-deficient mice, which die within 2 months after birth as a consequence of massive haemorrhages due to blood vessel rupture caused by arterial calcification [45]. The necessity for vitamin K as a cofactor to activate MGP was shown by chemical inhibition of MGP function [46] and by mutagenesis of the glutamate residues [47]. The precise function of MGP has not been unravelled yet, but likely includes inhibition of calcium crystal growth, blocking BMP-2 and BMP-4 function and shielding the nidus for calcification [48]. Like fetuin-A, MGP accumulates in extracellular vesicles thereby forming a break on unwanted calcium–phosphate precipitation [49].

Osteoprotegerin. OPG acts as a soluble (decoy)-receptor for RANKL. OPG is widely expressed, but particularly highly expressed in VSMCs and endothelial cells of arteries. Its function is to prevent binding of RANKL to RANK [50], thereby inhibiting osteoclast function and subsequent bone resorption. Mice with OPG deficiency have both increased osteoporosis and vascular calcification [51]. Clinically, high circulating levels of OPG are associated with atherosclerosis or risk factors of atherosclerotic disease indicating a compensatory increase [52].

Pyrophosphate, ectonucleotide pyrophosphatase phosphodiesterase. ENPP1 is a gene, which encodes a pyrophosphate-generating enzyme that regulates extracellular phosphate. The ENPP1 gene has been linked to ectopic calcification through a mouse model known as the ‘tiptoe walking mouse’ [53]. Normal levels of extracellular pyrophosphate are sufficient to prevent vascular calcification [54]. In haemodialysis patients, plasma levels of pyrophosphate are decreased and correlate inversely with vascular calcification.

Osteopontin. OPN is an extracellular phosphoprotein that has many phosphoserines that are negatively charged [55]. This negative charge gives OPN its strong affinity for hydroxypatite. OPN is present in mineralized tissues such as bones and teeth. OPN-deficient mice suffer from accelerated vascular calcification [56]. OPN regulates mineralization by inhibiting calcium crystal growth and by promoting osteoclast function. In healthy arteries, OPN is not present, however, in calcified plaques it is highly upregulated [57].

Altered phenotype and responses of vascular smooth muscle cells

Osteochondrogenesis. VSMCs are key in suppressing mineralization of vascular tissue. They are armed with an array of proteins that inhibit calcification such as BMP-7 [58], osteopontin [57] and MGP [48]. In addition, they can take up from the circulation the potent inhibitor fetuin-A [42]. Paradoxically, VSMCs also have the ability to promote mineralization for example by producing extracellular vesicles (EVs) that act as nucleation sites for calcification in a manner similar to matrix vesicles in bone formation [16]. Why pro-calcification mechanisms are executed by VSMCs is still poorly understood. It may simply be an undesired bystander effect of the innate capacity of VSMCs to adapt to a changing environment by switching phenotype in combination with a pathological environment, such as that present in CKD.

VSMCs can reversely switch phenotype from a contractile to a synthetic (migratory/proliferative) [59] and osteochondrogenic phenotype [60]. Inflammation [61], oxidative stress [62] and ageing (see below) can cause upregulation of osteochondrogenic transcription factors in VSMCs including Msx2, Runx2, Sox9 and osterix. These transcription factors mediate switching into osteochondrogenic phenotypes with upregulated expression of bone and chondrocyte proteins such as osteopontin, osteocalcin and alkaline phosphatase and downregulated expression of VSMC contractile proteins such as SM22-α and SM α-actin [63]. Uraemia can aggravate this process, e.g. with de novo expression of osteocalcin and downregulation of alpha smooth muscle actin [64]. Mice deficient for smooth muscle cell-specific Runx2 are largely protected from vascular calcification [65].

The contractile phenotype is a predominantly quiescent and anticalcifying phenotype whereas synthetic and osteochondrogenic phenotypes are associated with an increased propensity to promote calcification [66] (L. Schurgers, unpublished observations). Osteochondrogenic markers have been observed during vascular calcification in animal models [60] and in calcifying arteries of CKD patients [67] underscoring the theory that VSMCs switching into the osteochondrogenic phenotype precedes vascular calcification.

Vascular ageing and senescence. Phenotypic switching of VSMCs has also been observed during normal vascular ageing with associated cellular senescence [68]. Investigating the VSMC phenotype associated with diseases and cellular senescence at the molecular level led to a hypothesis that vascular calcification in diseases such as CKD can be regarded as the result of premature ageing of VSMCs [69]. Prelamin A, an unprocessed product of the LMNA gene, is considered a driving factor in ageing of VSMCs. In vitro, prelamin A expression by VSMCs caused DNA damage, upregulation of Runx2, osteochondrogenic differentiation and calcification [70]. In vivo prelamin A has been detected in VSMC nuclei in calcified arteries of aged individuals [71] and young CKD patients [70] indicating accelerated ageing in the latter. This interesting perspective will offer novel potential strategies and targets to prevent and combat vascular calcification.

Extracellular vesicles and mineralization. Extracellular vesicles are considered major players in promoting VSMC-mediated
calcification *in vitro* [49] and *in vivo* [72]. Extracellular vesicles are membrane-encapsulated vesicles that provide nucleation sites for formation of seed calcium crystals that, fuelled by various mechanisms [73], can grow further [42, 49]. They can be produced by living cells (micro- and matrix vesicles, exosomes) and dying cells (apoptotic bodies) [74] and are present in calcified arteries of CKD patients [13].

VSMCs can generate extracellular vesicles *in vitro* that differ in composition and calcifying properties depending on VSMC environmental factors such as Ca\(^{2+}\) and phosphate, and phenotype [49, 75]. VSMCs normally produce extracellular vesicles that contain inhibitors of calcification such as carboxylated MGP and fetuin-A. Elevated Ca\(^{2+}\) and phosphate, inflammatory cytokines and reactive oxygen species can cause phenotypic switching and a shift of production towards extracellular vesicles lacking those inhibitors and exposing pro-calciifying phosphatidylserine and annexins [49].

In addition to actively participating in vascular calcification while alive, VSMCs can produce pro-calciifying extracellular vesicles during their demise through apoptosis and secondary necrosis. Apoptotic bodies, which are membrane-encapsulated cell fragments resulting from apoptosis, resemble matrix vesicles and show pro-calciifying properties [76]. Chronic VSMC apoptosis in an animal model causes calcification of atherosclerotic plaques of young animals [77]. ZVAD, an inhibitor of apoptosis, strongly inhibited calcification of CKD vessels in an *ex vivo* model [16].

### CLINICAL THERAPEUTIC CONSEQUENCES

#### Calcium and phosphate balance

Advanced CKD predisposes to calcium retention if dietary sources exceed 800 mg/day [78]. Dialysate composition is another important consideration in the calcium mass balance. Dialysate calcium concentrations of 1.25 mmol/L are usually associated with a near neutral balance, whereas higher concentrations promote calcium loading during haemodialysis [79]. Particularly in patients on calcium-containing phosphate binders, the consensus is reached to go even lower with dialysate calcium [80]. Another important consideration is serum phosphate, since cardiovascular calcification is markedly accelerated in patients with a positive intradialytic calcium mass balance and insufficient control of phosphate (E. Ok, personal communication). Fine-tuning of the dialysate calcium should also include circulating iPTH. Thus, dialysate calcium concentrations of 1.25 mmol/L or lower stimulate PTH release, whereas 1.375 mmol/L led to stable PTH levels [81]. The former would be desirable in cases of low baseline PTH, i.e. below the 2-fold upper normal range of the assay, which is usually associated with adynamic bone disease and thus relative inability of the bone to incorporate calcium and phosphate. Importantly, however, there are presently no large outcome studies demonstrating that a very low dialysate calcium is safe and improves clinical outcome. Most centres therefore do not decrease dialysate calcium below 1.25 mmol/L unless patients are overtly hypercalcaemic. Currently, there is a discussion on the optimal dialysate calcium [82, 83] with recent calculations favouring a calcium of 1.25 mmol/L or lower [80] or using an individualized approach [84]. However, the hard evidence for a lower calcium dialysate is questioned [85].

Phosphate retention is another consequence of advanced CKD, in particular if dietary intake is high. Measurements of urinary phosphate excretion in particular fractional excretion may provide information on the dietary intake, at least in stable patients, and may be used to guide dietary counselling. In addition to diet, phosphate binders are usually required in advanced CKD. Several studies have investigated whether the choice of phosphate binders, in particular calcium-containing or calcium-free, affects the progression of cardiovascular calcification in dialysis patients (Table 2). Collectively, these studies show consistently a slower progress if calcium-containing phosphate binders are avoided [86–90]. Smaller pilot studies described similar observations for the comparison of lanthanum carbonate with calcium carbonate [91, 92]. In non-dialysis-dependent CKD patients (CKD stages 3–4), an Italian study noted the slowest progression of cardiovascular calcifications in patients given a phosphate-restricted diet plus sevelamer when compared with diet alone or diet and calcium acetate [93]. This notion was challenged by another study in 148 CKD stage 3–4 patients, which concluded that phosphate...

#### Table 2. Randomized clinical trials in dialysis patients assessing the effect of different phosphate binders on the progress of cardiovascular calcification

<table>
<thead>
<tr>
<th>Reference</th>
<th>Population</th>
<th>Comparators</th>
<th>Study duration (months)</th>
<th>Key finding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chertow et al.</td>
<td>200 prevalent HD patients</td>
<td>(A) Calcium acetate or carbonate (B) Sevelamer chloride</td>
<td>12</td>
<td>Faster progress of CAC and aorta calcium score with calcium compared with sevelamer</td>
</tr>
<tr>
<td>Block et al.</td>
<td>129 incident HD patients</td>
<td>(A) Calcium acetate or carbonate (B) Sevelamer chloride</td>
<td>18</td>
<td>Faster progress of CAC scores with calcium-containing phosphate binders only with preexisting calcification at baseline</td>
</tr>
<tr>
<td>Gallasi et al.</td>
<td>109 incident HD patients</td>
<td>(A) Calcium acetate or carbonate (B) Sevelamer chloride</td>
<td>18</td>
<td>Faster progress of CAC scores with calcium-containing phosphate binders in a diabetic subgroup (n=64)</td>
</tr>
<tr>
<td>Qinibi et al.</td>
<td>203 prevalent HD patients</td>
<td>(A) Calcium acetate plus statin (B) Sevelamer plus statin</td>
<td>12</td>
<td>No significant difference in calcification progression between the groups</td>
</tr>
<tr>
<td>Kakuta et al.</td>
<td>183 prevalent HD patients</td>
<td>(A) Calcium carbonate</td>
<td>12</td>
<td>Faster progress of CAC scores with calcium-containing phosphate binders</td>
</tr>
<tr>
<td>Toussaint et al.</td>
<td>45 prevalent HD patients</td>
<td>(A) Calcium carbonate (B) Sevelamer chloride</td>
<td>18</td>
<td>Less aortic calcification progression with lanthanum carbonate</td>
</tr>
<tr>
<td>Ohtake et al.</td>
<td>52 prevalent HD patients</td>
<td>(A) Calcium carbonate (B) Lanthanum carbonate</td>
<td>6</td>
<td>Faster progress of CAC scores with calcium carbonate versus lanthanum carbonate</td>
</tr>
</tbody>
</table>

CAC, coronary artery calcification; HD, haemodialysis.
binders in such patients actually promote rather than retard the progression of vascular calcification compared to placebo [94]. The authors speculated that subsequent to binding phosphate in the intestinal lumen, luminal wall phosphate transport is up-regulated and thus more rather than less phosphate was absorbed. In our view, it is more likely, however, that the authors created an artefact by pooling patients randomized to calcium acetate, lanthanum carbonate or sevelamer carbonate into one ‘phosphate binder’ group. Indeed, inspection of the supplemental data, with all the limitations of small group sizes, suggests that only the group receiving calcium acetate but not the other groups exhibited progressive cardiovascular calcification. Thus, in our view, there is a very consistent data base that in patients with advanced CKD or on dialysis and with a reasonable life expectancy, calcium-containing phosphate binders should be avoided. Concerning outcome data, most studies failed to detect a survival advantage for calcium-free phosphate binders (most likely due to insufficient power of the single studies); however, a meta-analysis reported a 22% risk reduction with calcium-free phosphate binders [95].

**Vitamin D derivatives**

We are not aware of large randomized controlled trials in advanced CKD patients that have investigated the effects of active vitamin D derivatives on calcification progress. Clearly, historical experience suggests that large doses of calcitriol or other active vitamin D compounds can accelerate vascular calcification. Another area of uncertainty is native vitamin D. Here, a recent small randomized trial in haemodialysis patients compared placebo or cholecalciferol (25 000 IU) therapy every 2 weeks [96]. After a 12-month period, calcification scores had progressed similarly in both study arms.

**Calcimimetics**

The only large randomized study to assess the effects of cinacalcet on cardiovascular calcification progress was the ADVANCE trial [97]. In this trial, 360 haemodialysis patients were randomized to cinacalcet plus low-dose calcitriol or vitamin D analogue, or flexible vitamin D therapy. The primary end point, i.e. the percentage change in coronary artery calcification scores from baseline to Week 52 did not differ significantly between the two arms but the study noted a consistent trend in favour of the combined regimen at all sites investigated. Furthermore, the study may have been confounded by the excessive use of vitamin D analogue in the combination arm [98]. Case reports additionally describe resolution of extraosseous calcifications following the institution of cinacalcet in patients with extremely high PTH levels [99].

**Vitamin K and vitamin K antagonists**

So far, the effects of vitamin K supplementation on cardiovascular calcification has not been tested in CKD patients. However, we have reported that uncarboxylated MGP is markedly reduced in dialysis patients upon administration of up to 360 μg vitamin K2 per day [100]. These data lay the foundation for the randomized VitaVasK trial in which haemodialysis patients are given 5 mg of vitamin K1 thrice weekly while calcification progress is monitored over 18 months [101]. Vice versa, based on the experimental observations with MGP (see above), the approximately 10-fold increased risk of calciphylaxis and the uncertain cost-benefit ratio, we have advocated against a liberal use of vitamin-K antagonists in dialysis patients [102].

**Other approaches**

Other than a very small pilot study, which noted no progression of calcification with a magnesium-containing phosphate binder [103], no data are available to substantiate the hypothesis that magnesium interferes with calcification in CKD. Similarly, pilot data suggest an arrest of calcification in dialysis patients given intravenous sodium thiosulfate after each dialysis for 5 months, but the long-term effects of this approach on bone remain uncertain [104]. Finally, in 50 patients with CKD stages 3–4, alendronate did not decrease the progression of vascular calcification compared with placebo over 18 months [105]. A number of small studies suggested a halt or slow down of calcification progression (e.g. [106]), but a larger study still reported progression of vascular calcification in transplant patients [107]. PTH was not an independent predictor for progression in that study [107].

In our view, a combination of the above-mentioned therapies (individualized to each patient) and not a single option might be the best therapeutic approach to prevent the development and progression of cardiovascular calcifications.

The treatment of calcific uraemic arteriolopathy remains a challenge, and so far treatment consists of a largely empiric, multimodal approach (including optimization of mineral metabolism, administration of sodium thiosulfate, gentle debridement, etc.) [108]. The CKD-MBD working group of the ERA-EDTA has initiated a call for action by defining calcific uraemic arteriolopathy as one of the outstanding research targets for the upcoming years.

**CONCLUSIONS**

Taken together, a plethora of different factors contribute to the development of cardiovascular calcifications in CKD. Different pathologies (atherosclerotic calcification, media sclerosis and valvular calcification) may have overlapping yet distinct mechanisms. VSMCs are actively involved in the process of media calcification. Therapy aims at correcting the imbalance of promoters and inducers to prevent the initiation and progression of cardiovascular calcifications.

**ACKNOWLEDGEMENTS**

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**CONFLICT OF INTEREST STATEMENT**

G.S. has received speaker and consultancy honoraria from Amgen and Sanofi and J.F. has received speaker and consultancy honoraria from Amgen, Fresenius, Sanofi, Shire and Vifor.
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FULL REVIEW

Mechanisms of Cardiovascular Calcification in CKD
Salt and nephrolithiasis

Andrea Ticinesi1,2, Antonio Nouvenne2, Naim M. Maalouf3, Loris Borghi1,2 and Tiziana Meschi1,2

INTRODUCTION

Excessive salt intake has been focused on in the last few years as one of the main elements that influence health status. In particular, salt is linked to hypertension and cardiovascular disease, and therefore many professional organizations have

ABSTRACT

Dietary sodium chloride intake is nowadays globally known as one of the major threats for cardiovascular health. However, there is also important evidence that it may influence idiopathic calcium nephrolithiasis onset and recurrence. Higher salt intake has been associated with hypercalciuria and hypocitraturia, which are major risk factors for calcium stone formation. Dietary salt restriction can be an effective means for secondary prevention of nephrolithiasis as well. Thus in this paper, we review the complex relationship between salt and nephrolithiasis, pointing out the difference between dietary sodium and salt intake and the best methods to assess them, highlighting the main findings of epidemiologic, laboratory and intervention studies and focusing on open issues such as the role of dietary salt in secondary causes of nephrolithiasis.

Keywords: hypercalciuria, nephrolithiasis, salt, urinary calcium, urinary sodium

Salt and nephrolithiasis

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