Arteriosclerosis, vascular calcifications and cardiovascular disease in uremia

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Purpose of review

Arterial calcification in chronic kidney disease (CKD) is associated with increased cardiovascular risk. The mechanisms responsible for arterial calcification include alterations of mineral metabolism and expression of mineral-regulating proteins.

Recent findings

Arterial calcification is similar to bone formation, involving differentiation of vascular smooth muscle cells (VSMCs) into phenotypically distinct osteoblast-like cells. Elevated phosphate and/or calcium trigger a concentration-dependent increase of calcium precipitates in VSMC *in vitro*. The calcification is initiated by VSMC release of membrane-bound matrix vesicles and formation of apoptotic bodies. The presence of serum prevents these changes, indicating the presence of calcification inhibitors. Arterial calcification occurs in two sites: the tunica intima and tunica media. Intimal calcification is a marker of atherosclerotic disease and is associated with arterial stenotic lesions. Medial calcification influences outcome by promoting arterial stiffening whose principal consequences are left-ventricular hypertrophy and altered coronary perfusion. Aortic stiffness is an independent predictor of all-cause and cardiovascular mortality in CKD patients. Age, duration of dialysis, smoking and diabetes are risk factors for the development of arterial calcification in end-stage renal disease. Oversuppression of parathyroid hormone and low bone turnover potentiate the development of arterial calcification.

Summary

Arterial disease in CKD patients is characterized by extensive calcification. Evidence has accumulated pointing to the active and regulated nature of the calcification process. Elevated phosphate and calcium may stimulate sodium-dependent phosphate cotransport involving osteoblast-like changes in cellular gene expression. Arterial calcification is responsible for stiffening of the arteries with increased left-ventricular afterload and abnormal coronary perfusion as the principal clinical consequences.

Keywords

arterial stiffness, chronic kidney disease, hypercalcemia, hyperphosphatemia, vascular calcification

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Abbreviations

BMP	bone morphogenic protein
CKD	chronic kidney disease
EBCT	electron-beam computed tomography
ESRD	end-stage renal disease
MGP	matrix Gla protein
VSMC	vascular smooth muscle cell

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Introduction

Cardiovascular complications are the leading cause of death of patients with end-stage renal disease (ESRD) [1], and epidemiological and clinical studies have shown that damage of large arteries is a major contributory factor to mortality of these patients [2–4]. The frequency of traditional risk factors does not fully explain this high rate of cardiovascular disease, and other factors associated with chronic kidney disease (CKD) and ESRD must contribute [5,6]. Arterial calcification is a common complication in CKD and ESRD [7–10], and the extents of arterial calcification were predictive of subsequent cardiovascular mortality beyond established conventional risk factors [11–15]. The precise pathophysiology of arterial calcification in ESRD is unknown, but calcium and phosphate metabolism abnormalities are thought to be particularly important determinants. For many years, arterial calcification was considered to be the result of passive mechanisms due to elevated phosphate levels and high calcium phosphate ion products, resulting in super saturated plasma [16–18]. However, recent studies have shown that arterial calcification is a regulated process with plasma constituents maintaining minerals in solution and inhibiting their deposition in tissues, and evidence indicates that many proteins involved in bone metabolism can be expressed in arterial tissues, reflecting changes of the phenotype of vascular smooth muscle cells (VSMCs) [19–26]. Disturbances of calcium and phosphate metabolism in ESRD are associated with uremic bone disease, and an inverse relationship among arterial calcification and bone density and bone turnover has been documented in uremic patients [7,27•]. In the general population, arterial calcification and osteoporosis are also associated, and a relationship exists between the clinical courses of the two processes [28]. In this article, we discuss the mechanisms and clinical significance of arterial calcification in CKD and ESRD.

Methods for assessing vascular calcification

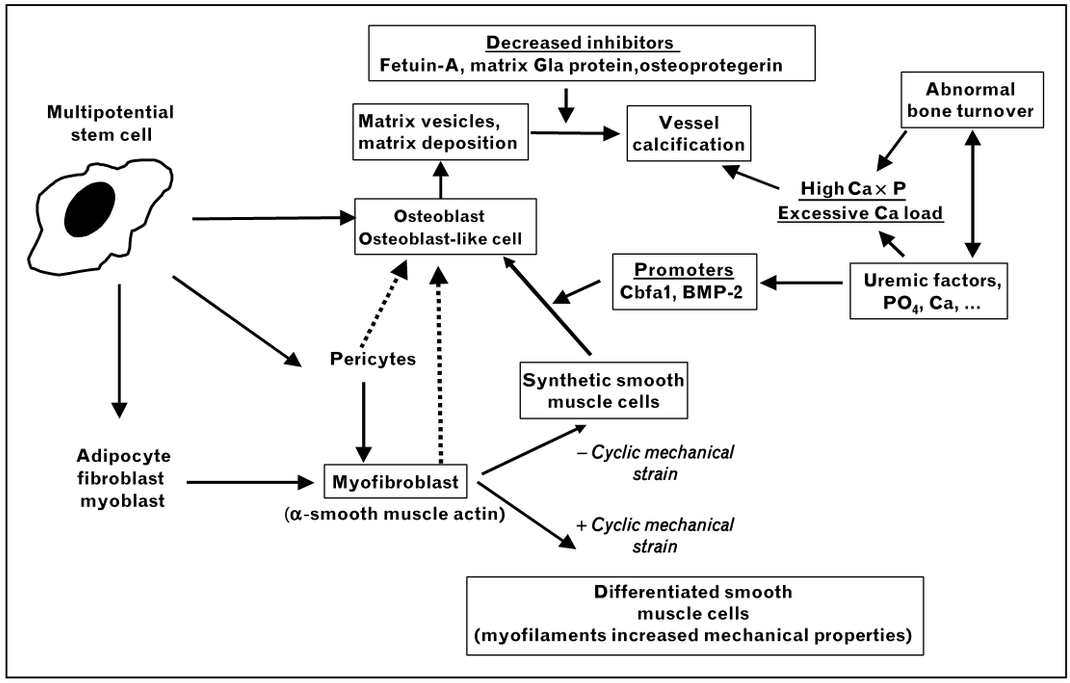
There are several non-invasive methods that enable arterial calcification to be detected and quantified. The most widely used methods that allow quantitative analyses are electron-beam computed tomography (EBCT) and multi-slice (spiral) computed tomography [29,30]. The quantity and progression of arterial calcification can be assessed with both of these techniques. The results are typically reported using the Agatson score, which is based on the product of the calcified plaque area and density coefficient [29]. A limitation of these two imaging techniques is their inability to distinguish between the two predominant arterial calcification sites, i.e. intimal and medial calcification. Ultrasonography and plain radiographs are semi-quantitative techniques that can be used to detect calcification. They are good initial screening tools for detecting the presence of calcification, but they have relatively low sensitivity and are less useful for the quantification of calcification progression over time. With plain radiographs, it is sometimes possible to discriminate between intimal and medial calcification in peripheral arteries [14,31,32].

Mechanisms of vascular calcification

Recent studies have shown that arterial calcification is an active process that is regulated by a variety of genes and proteins [19–26]. Arterial calcification appears to be a process similar to bone formation implicating a variety of proteins involved in bone and mineral metabolism detected in atherosclerotic plaques and/or medial calcifications [33,34]. It has been demonstrated in animal models that arterial calcification can be induced by selective deletion of various genes, such as those encoding matrix Gla protein (MGP), fetuin-A (α_2-HS glycoprotein or AHSG) or osteoprotegerin [19, 35,36].

The calcification process involves differentiation of VSMCs into phenotypically distinct cells that have been shown to generate calcification *in vitro* [33]. *In vitro*, VSMC transformation into osteoblast-like cells, with subsequent mineralization, is induced or regulated by a variety of factors, including calcium and phosphate [24,37–39,40••,41••]. Exposing human VSMCs to high inorganic phosphate levels induced a concentration-dependent rise of calcium phosphate precipitates in VSMCs in association with extracellular matrix synthesis *in vitro*. Increased calcium concentrations had a similar effect. Indeed, recent in-vitro studies showed that calcium and phosphate act synergistically and independently on VSMC calcification [40••,41••]. This process was inhibited by phosphonophormic acid, an antagonist of the sodium–phosphate cotransporter Pit-1, indicating that calcification induction is an active cellular mechanism [40••]. Phosphate may initiate calcification by enhancing the activation of Cbfa-1, a factor that stimulates the differentiation of mesenchymal cells into osteoblasts [37,39]. Elevated intracellular phosphate levels induce osteoblast-like changes in VSMCs, including the formation of matrix vesicles and nodules [40••]. Calcification was initiated by VSMC release of membrane-bound matrix vesicles and the formation of apoptotic bodies, thereby suggesting that physiological or pathological cell death may also be a primary initiating event in arterial calcification formation [40••]. Release of matrix vesicles and apoptotic body formation serve as initiation sites for apatite crystallization. Moreover, VSMCs synthesize bone-associated proteins, including alkaline phosphatase, osteocalcin and osteopontin, and a coat of collagen-rich extracellular matrix [38,42,43]. However, in the presence of serum, matrix vesicles did not contain elemental calcium and phosphate and VSMCs do not become calcified and are able to inhibit spontaneous calcium and phosphate precipitation in solution [40••]. These observations indicate that systemic calcification inhibitors are present in serum and VSMCs. The VSMCs constitutively express potent local inhibitors of calcification such as MGP [44]. MGP-depleted knockout mice develop overwhelming calcification throughout the arterial tree, leading to arterial rupture and hemorrhage, which cause death [36]. Inactivation of the osteopontin gene enhances arterial calcification of MGP-deficient mice, indicating that osteopontin is an inducible inhibitor of arterial calcification *in vivo* [45,46]. MGP may limit arterial calcification by binding to bone morphogenic protein (BMP)-2, a potent osteogenic differentiation factor. BMP-2 has been detected in human calcified arteries and its concentration is elevated in human uremic serum [47]. Because MGP activity is vitamin K-dependent, warfarin markedly increases the calcifying potential [48], perhaps by affecting the availability of vitamin K, which is a coenzyme necessary for the γ-carboxylation of MGP. In warfarin-treated rats, pathological changes consistent with defective tissue-specific MGP expression were observed [49]. Warfarin use has been associated with some cases of calciphylaxis, almost exclusively in some ESRD patients [50]. Osteoprotegerin is another local factor that might act as an inhibitor of arterial calcification and osteoprotegerin-deficient animals develop severe arterial calcification of the media of aorta and renal arteries, as well as osteoporosis [35]. Pertinently, osteoprotegerin inhibited in-vivo warfarin and vitamin D-induced arterial calcification in rats [51]. Fetuin-A is a potential circulating arterial calcification inhibitor that is abundant in plasma [52]. *In vitro*, fetuin-A inhibits the formation and precipitation of calcium phosphate, thereby blocking hydroxyapatite crystallization [53]. Fetuin-A-knockout mice exhibit more ectopic calcifications [19]. *In vitro*, the phosphate-stimulated apatite production can be inhibited completely by adding pyrophosphates that antagonize the cellular sodium–phosphate cotransport system [37,38,54]. Mutations in

Figure 1. Schematic and hypothetical pathogenesis of vascular calcifications



Dotted lines indicate more hypothetical relationships. BMP, bone morphogenic protein.

the cell-surface enzyme ecto-nucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1), which generates pyrophosphate, cause infantile arterial calcification of the internal elastic lamina of muscular arteries [55] (Fig. 1).

Arteries are under the influence of cyclic mechanical stresses, such as tensile and shear stress. These stresses and strains are important stimuli that regulate the VSMC phenotype and maintain the contractile phenotype [56]. Using the three-dimensional engineered smooth muscle model, Nikolovski *et al.* [57] demonstrated that expression of bone-associated genes was down-regulated in tissue exposed to cyclic strain, and that cyclic strain inhibited the switching of VSMCs to an osteoblast-like phenotype. Furthermore, long-term strain played a protective role in terms of calcification, because unstrained tissues exhibited more calcium deposits (Fig. 1).

Factors associated with arterial calcifications in chronic kidney disease and end-stage renal disease

In-vitro studies have shown that in comparison with pooled control human serum, pooled uremic serum induced the expression of Cbfa-1 in bovine VSMCs, supporting the view that this key regulatory factor is upregulated in response to 'uremic toxins' [58]. A number of factors have been associated with increased severity or extent of arterial calcification in uremic patients [59,60]. Age, duration of dialysis and diabetes are clearly risk factors for arterial calcification development [8–14]. Arterial calcification in ESRD may reflect the interplay between factors that either promote or inhibit calcium deposition in arterial tissues. Whereas some investigators have found a link between hyperphosphatemia and hypercalcemia and arterial calcification frequency and extent [4,8,14], others have not [7,9]. The total dose of calcium-based phosphate binder administered to ESRD patients has also been identified as a factor associated with arterial calcification [8,9,14]. The role of parathyroid hormone as a risk factor for calcification is not clear. The authors of a few studies have found an association between elevated intact parathyroid hormone and the higher rate of vascular and/or valvular calcification [4,15]. Others were unable to establish a relationship between arterial calcification and parathyroid hormone levels [7–9,14]. Low parathyroid hormone levels have also been associated with more arterial calcification [61]. Oversuppression of parathyroid hormone by excessive calcium balance or vitamin D_3 potentiates cardiovascular complications [62]. In-vitro studies showed that 1,25-dihydroxyvitamin D_3 increased arterial calcification by modulating the effects of parathyroid hormone-related peptide, which is an endogenous inhibitor of VSMC calcification [63,64]. According to a recent study, arterial calcification was more extensive in ESRD patients with biopsy-proven low bone activity and adynamic bone, and the association between hyperparathyroidism and arterial calcification is more likely related to the

high-bone-turnover-associated release of calcium and phosphate from bone than the direct action of parathyroid hormone [27]. The results of an EBCT study on patients with coronary artery disease showed that serum concentrations of calcium, 1,25-dihydroxyvitamin D_3 and parathyroid hormone were not correlated with coronary calcifications [65]. Recent investigation of diabetic low-density lipoprotein receptor-deficient mice showed that human parathyroid hormone 1-34 (Teriparatide) inhibits arterial calcification and aortic osteogenic differentiation via a direct action and circulating osteopontin, and exerts beneficial effects on macrovascular disease [66]. Moreover, treatment with parathyroid hormone increases bone strength and bone mineral density in women with postmenopausal osteoporosis [67,68]. Epidemiological studies on the general population have shown an inverse relationship between bone mineralization and arterial calcification. In the Framingham Heart Study, the progression of abdominal aortic calcification was associated with the magnitude of bone loss [69]. A similar relationship has been found in dialysis patients [7,70].

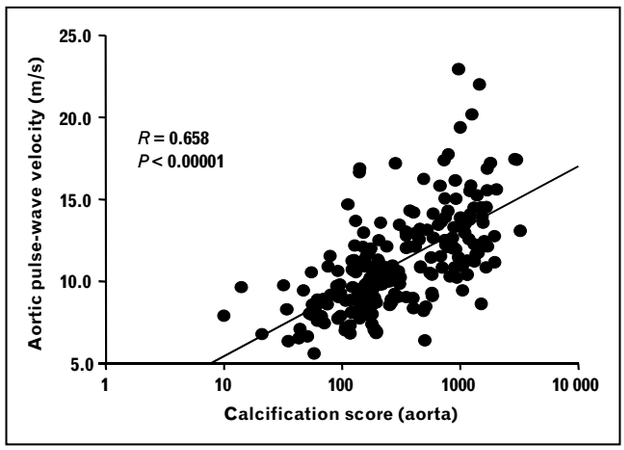
In a recent evaluation of dialysis patients, serum fetuin-A concentrations were significantly lower than in the healthy controls, and low fetuin-A levels were associated with higher levels of the inflammatory marker hs-CRP and mortality [71]. Experimental studies have shown that inflammatory cytokines and inflammatory lipids can promote VSMC calcifications [72–76]. The association between inflammatory markers, and arterial and cardiac valve calcifications has also been documented in the general population [77] and ESRD patients [9,14,78]. The results of epidemiological studies on the general population indicated a relationship between coronary and cardiac valve calcification, and serum low-density lipoprotein, and an inverse relationship with high-density lipoprotein hyperlipidemia and arterial calcification [79,80]. The association of dyslipidemia and arterial calcification in CKD patients is less evident, and was negative [8,9] or positive [81,82]. Several studies in humans have shown that genetic factors play a significant role in the development of arterial calcification in different populations [83–85]. The genetic influence was also observed in ESRD patients with a higher arterial calcification frequency in Caucasians [10,14].

Clinical impact of arterial calcifications

Arterial calcification occurs in two distinct sites: the intimal and medial layers of the arterial wall. Intimal calcification occurs when minerals are deposited within atherosclerotic plaque in the intima of the arterial wall. This process is a progressive feature of common atherosclerosis found in the general population and is not specific to CKD, except for its higher frequency in ESRD patients. Calcified intimal lesions are irregular, patchy and are restricted around areas of atherosclerotic plaque leaving the surrounding intima unaffected. Intimal calcifications are a sensitive marker of underlying atherosclerotic disease [86], even though, in ESRD patients, no relationship has been established between coronary calcification scores determined by EBCT and the degree of stenosis evaluated by coronary angiography [87]. Coronary and large artery calcifications have been associated clearly with increased cardiovascular morbidity and mortality in the general population and ESRD patients [10,13,14]. In patients with known coronary artery disease, coronary calcium scores were shown to be a strong predictor of cardiovascular events, independent of the degree of coronary luminal obstruction [88].

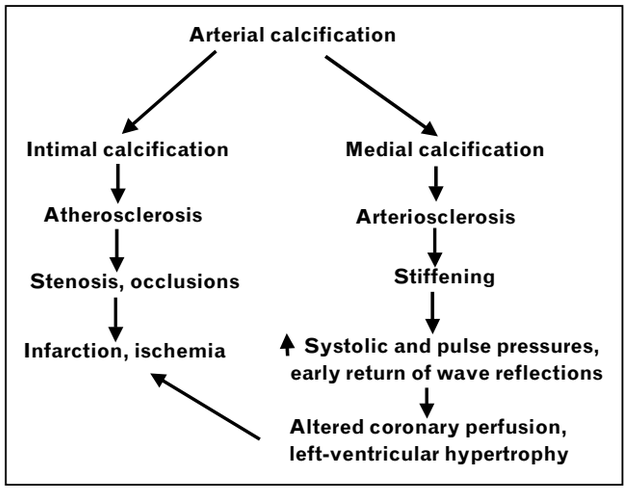
Medial calcification (Mönckeberg's sclerosis or medial calcinosis) is characterized by diffuse mineral deposits within the arterial tunica media. While medial calcification is frequently observed with aging in the general population, it is significantly more pronounced in patients with metabolic disorders, such as metabolic syndrome, diabetes or CKD. Diffuse medial calcification may influence morbidity and mortality by promoting arterial stiffness and a progressive loss of the cushioning function of blood vessels (arteriosclerosis, arterial hardening) [9,13,14,89]. The degree of arterial stiffening can be inferred directly by increases of pulse-wave velocity [90] (Fig. 2), and indirectly by elevated pulse pressure. Pulse-wave velocity is a reproducible, non-invasive measure of the speed with which the arterial-pulse pressure wave (ventricular-ejection pressure wave) moves away from the heart [89]. The consequence of increased pulse-wave velocity is the early return of wave reflections from peripheral reflective sites to central arteries and the aorta. The principal consequences of this early return are abnormally increased aortic and left-ventricular systolic pressures with increased cardiac workload, increased

Figure 2. Correlation between aortic calcification score and aortic pulse-wave velocity



Taken from personal data (G.M. London, unpublished observations).

Figure 3. Schematic representation of the clinical effects of arterial intimal and arterial medial calcifications



oxygen consumption and left-ventricular hypertrophy. Additional effects include decreased diastolic pressure and diastolic pressure-time integral, and altered coronary perfusion [89,91] (Fig. 3). Aortic pulse-wave velocity is an independent predictor of all-cause and cardiovascular mortality in the general population and ESRD patients [92–95].

Conclusion

Once present, arterial calcification rarely regresses and therefore the primary goals are prevention and stabilization of existing calcifications. Preventive measures include controlling serum phosphate levels, avoiding oversuppression of parathyroid activity and adynamic bone, controlling diabetes, cessation of smoking and early transplantation [8,9,27]. Because elevated serum phosphate and calcium are associated with arterial calcification and cardiovascular mortality in CKD and ESRD, their control should be a priority [96]. However, some evidence suggests that arterial calcification could be, at least in part, an iatrogenic phenomenon resulting from a positive calcium balance associated with the overuse of excessively high doses of calcium-based phosphate binders, pharmacological doses of vitamin D and high calcium concentration in the dialysate. These findings served as the stimulus for the development of new phosphate binders that do not contain calcium, newer vitamin D analogues that may be less hypercalcemic, and calcimimetic compounds for treatment of secondary hyperparathyroidism. Use of non-calcium-containing phosphate binders, such as sevelamer or lanthanum chloride, can reduce the total calcium load, while still effectively controlling serum phosphate [97,98]. Using sevelamer, in conjunction with effective phosphate and calcium control, may slow the progression on arterial calcification in ESRD patients [99–101]. It should be noted that patients treated with sevelamer also have lower low-density lipoprotein-cholesterol levels which could also account for the beneficial effects on arterial calcification progression. Several endogenous calcification inhibitors, such as BMP-7 [102,103], osteopontin [104], fetuin-A [71] and parathyroid hormone 1-34 (Teriparatide) [66], are in early developmental stages and may be of clinical benefit in treating ectopic calcification.

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- •• of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 605).

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