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Cardiovascular Calcifications in Old Age: Mechanisms and Clinical Implications

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Abstract Vascular calcification, a hallmark of aging, is accelerated in patients with hypertension, diabetes, and chronic kidney disease. It may be viewed as the result of disturbances of the complex and subtle balance between inhibitors and promoters, acting at both the systemic and local levels. Ethnic differences in certain components of the atherosclerosis process were identified previously; however, recent evidence suggests that atherosclerosis is not a modern disease and may be viewed as an inherent component of human aging, unrelated to any specific diet or lifestyle. In this review, we highlight the mechanisms governing vascular calcification and its association with aging. By understanding the pathways involved in these processes, novel drug targets may be proposed in an effort to reduce the effects of vascular calcification as a risk factor.

Keywords Aging · Medial artery calcification · Arteriosclerosis · Vascular calcification · Calcium deposit · Arterial stiffness · Pulse wave velocity

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Introduction

Vascular calcification is a hallmark of aging [1, 2] and is accelerated in patients with hypertension [3], diabetes [4], and chronic kidney disease (CKD) [5]. Although aging is well recognized as a potent cardiovascular (CV) risk factor, the mechanisms underlying the association between aging and vascular calcification remain unknown. Although ethnic differences previously were identified in certain components of the atherosclerosis process [6], recent evidence suggests that atherosclerosis is not a disease of the modern era and may be viewed as an inherent component of human aging not related to any specific diet or lifestyle [7••].

Although the mechanisms responsible for arterial stiffening have not been elucidated completely, they are thought primarily to involve structural changes within the media, particularly fatigue fracturing of elastin and collagen deposition. An additional mechanism is arterial calcification, which may occur in the intima, in conjunction with atherosclerotic plaques, or in the media as arteriosclerosis [8].

Medial arteriosclerosis may be defined as a process characterized by lumen enlargement with wall thickening (remodeling) and a reduction of elastic properties (stiffening) at the level of large elastic arteries [9]. Although aging is the main determinant of arterial stiffening (medial arteriosclerosis) [1, 10], this process is not uniform along the arterial tree, as distal muscular arteries do not present the same age-dependent stiffening [10]. Development of intimal atheromatous plaques (atherosclerosis) has a different pathophysiologic evolution. Although the two clinical conditions (medial vs. intimal disease) certainly may coexist and share some pathways, they also may be observed separately, both spatially and temporally [11].

Atherosclerotic calcification is intimally oriented and eccentric, initiating at the base of necrotic fibrofatty plaques via apoptotic vesicles arising from dead and dying vascular

smooth muscle cells (VSMCs) [12]. Major features of atherosclerotic calcification that differ from medial artery calcification (MAC) include abundant fibrosis, extensive cellular necrosis, apoptotic body formation, and cholesterol crystal accumulation [12].

MAC differs greatly from the eccentric, calcified atherosclerotic plaque [12] of atherosclerosis. MAC is a feature of diabetes and CKD [4] and entails matrix vesicle-nucleated mineralization with apatitic calcium phosphate deposition in the tunica media in the absence of atheroma and neointima. The tunica media of large arteries consists of a structured assembly of VSMCs, elastic lamellae, and collagen fibrils into functional muscular–elastic sheets. Mechanical properties are determined by cross-links between the extracellular matrix components and cell matrix [13–15]. Smooth muscle cells account for 30% to 50% of the volume and are the least rigid component in the arterial wall. The elastic lamellae, representing 25% of the volume, largely determine the elasticity of the arterial wall in the normal physiologic range of pressures [16]. Collagen fibers, which represent 35% of the volume, are stiffer than elastin and VSMCs, thus conferring vascular integrity to tensile strength [17]. Collagen is recruited mainly at higher pressures, at which the arteries are significantly distended [17]. Thus, the elasticity of large arteries decreases with pressure loading, because the stiffer components of the arterial wall are recruited sequentially. This efficient organization is modified during aging, determining the progressive stiffening of large arteries [1, 2].

Age-associated changes in the arterial properties may contribute to significant increases in vascular disease in older adults [1]. Mounting evidence suggests that large artery calcification and remodeling contribute directly to arterial stiffening [1]. Although the number of cells decreases over time, cells are replaced by fibrotic tissue and the residual cells also become hypertrophic. The decay of the elastic network gradually transfers the wall tension to the collagen fibers, which normally are recruited as the vessel pressure and diameter increase [2]. Because the collagen fibers are stiffer than the elastic network, resistance to additional dilation often is tempered by the dilatation that already has occurred [2]. In addition, cross-linking by advance glycation end products (AGEs) and elastocalcinosis further increases vascular stiffness. Calcium deposition also may promote the destruction of elastic fibers, thereby exacerbating the aging process [2, 18]. Thus, in arteriosclerosis, large artery calcification and remodeling may be viewed as two parallel consequences of the degradation of the elastic network.

Pulse wave velocity (PWV), a noninvasive index of vascular stiffening, increases with age in both men and women. PWV is determined in part by the intrinsic stress/strain relationship (stiffness) of the vascular wall and by the mean arterial pressure. Increased PWV traditionally has been linked to structural changes in the vascular media, including increased collagen, reduced elastin content, elastin breakdown, and calcification.

Because age-associated increases in PWV have been demonstrated in healthy subjects with little or no atherosclerosis, arterial stiffening may occur independently of atherosclerosis [10, 14, 15]. However, several studies indicate that increased arterial stiffness also is associated with atherosclerosis, diabetes, and CKD [3–5]. The role of the structural changes within the matrix and endothelial dysregulation of vascular smooth muscle tone and other aspects of vascular wall structure/function overlap and interrelate with one another. Endothelial dysfunction occurs at an early stage in the pathophysiology of atherosclerosis, diabetes, and hypertension [19]. Therefore, changes in mechanical properties of the artery wall influence the development of atherosclerosis, and the latter, via endothelial dysfunction and other mechanisms, influences arterial stiffness.

Cellular Aspects at a Glance

Once considered a passive process, vascular calcification has emerged as an actively regulated form of calcified tissue metabolism (Fig. 1).

Recent evidence suggests a pathophysiologic link between vascular (intimal and medial) calcification and bone metabolism because of the presence of bone-related proteins and cells at the site of calcification [20, 21]. The mechanism of vascular calcification is complex, but the dedifferentiation or transformation of VSMCs into an osteoblastic/chondrocytic phenotype is thought to be the initiating process.

Most studies describe an age-related decline of VSMCs [22], which has been attributed to a generalized reduction of cellular activity that cannot counterbalance the cellular apoptosis of the arterial wall. VSMCs originate from a similar mesenchymal stem cell as osteoblasts, the latter occurring with up-regulation of the Runt-related transcription factor 2 (RUNX2), msh homeobox 2 (MSX-2), and SRY (sex-determining region Y)-box 9 (SOX9) [23]. MSX-2 is required for membranous bone formation, whereas RUNX2 is necessary for osteoblastic transformation, neovascularization, and endochondral ossification [24]. SOX9 may determine whether ensuing ossification is endochondral or membranous in type [25, 26]. The actions of bone morphogenetic proteins (BMPs) on target cells such as VSMCs are conflicting to some extent, suggesting the existence of intrinsic autoregulatory and overlapping mechanisms. In fact, because BMP2 signaling up-regulates RUNX2 as well as MSX-2 and can drive both chondrogenic and osteogenic differentiation of pluripotent mesenchymal progenitors, BMP2-regulated processes are implicated in all histoanatomic variants of vascular calcification. However, depending on the underlying disease process and the primary mechanisms driving vascular calcification, the relative contributions of BMP2–MSX-2/RUNX2 signaling

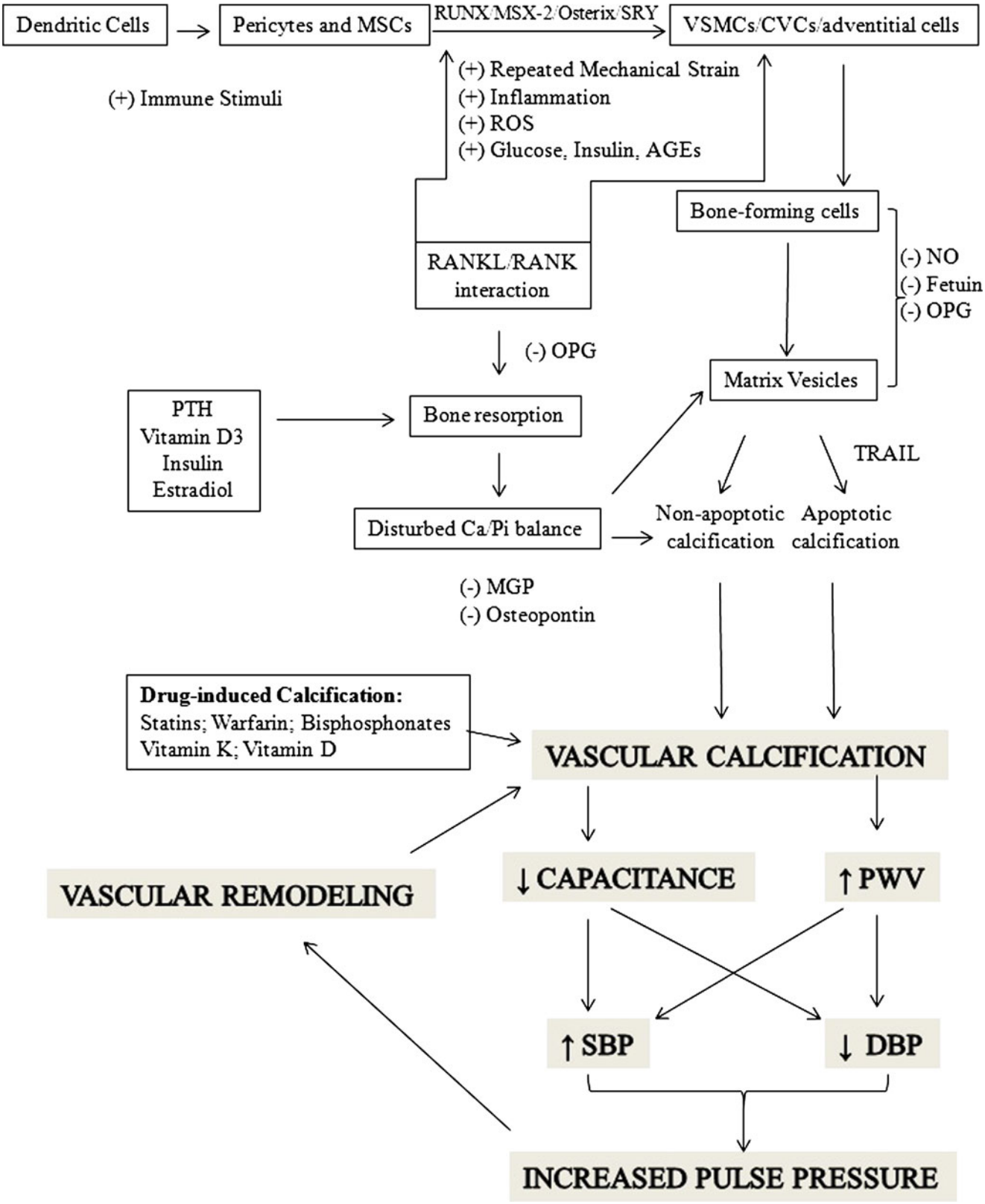

Figure 1 Proposed diagram depicting the alterations of large arteries during aging, leading to their stiffening. The consequences of the increased stiffness on central hemodynamics also are represented. *AGEs* advanced glycation end products; *CVCs* calcifying vascular cells; *DBP* diastolic blood pressure; *MGP* matrix Gla protein; *MSCs* mesenchymal stem cells; *MSX-2* Msh homeobox 2; *NO* nitric oxide; *OPG* osteoprotegerin; *PTH* parathyroid hormone; *PWV* pulse wave velocity; *RANK* receptor activator of nuclear factor-κB (NF-κB); *RANKL* RANK ligand; *ROS* reactive oxygen species; *RUNX* Runt-related transcription factor; *SBP* systolic blood pressure; *SRY* sex-determining region Y-box 9; *TRAIL* TNF-related apoptosis-inducing ligand; *VSMCs* vascular smooth muscle cells.

likely will differ [27, 28]. The signals that initiate regional osteogenic and chondrogenic differentiation arise in part from lipid and glucose metabolism and inflammatory cytokines [29, 30]. In relation to different stimuli, VSMCs transdifferentiate into noncontractile cells with bone-forming capacity and acquire properties of osteoblasts, that is, the ability to synthesize alkaline phosphatase (ALP), bone sialoprotein (osteopontin), osteocalcin, and type I collagen [31, 32].

VSMCs also secrete matrix proteins, and once the matrix is prepared, these cells mineralize the matrix through the secretion of matrix vesicles [29] or through apoptosis/fibrosis [32]. Matrix vesicle formation is a feature of normal osteogenesis [33]. Matrix vesicles are rich in alkaline phosphatase, which catalyzes the breakdown of pyrophosphate, itself an inhibitor of calcification. Phosphate is actively transported into the vesicle by a sodium-dependent phosphate pump, itself enhanced by increased phosphate concentration in the extracellular fluid. Matrix vesicles also contain calcium-binding proteins; thus, phosphorus and calcium increase the mineralizing potential of matrix vesicles [34••]. The vesicle membrane later disintegrates, and calcium phosphate reacts with extracellular matrix constituents, forming mature crystals of hydroxyapatite [35]. The extrusion of matrix vesicles by VSMCs and related osteogenic cells may be stimulated partly when these cells undergo apoptosis [35]. Apoptosis itself may result from a variety of factors, including the action of tumor necrosis factor-α (TNF-α), receptor activator of nuclear factor-κB (NF-κB [RANK]) ligand (RANKL), and TNF-related apoptosis-inducing ligand (TRAIL). Their actions are inhibited by the glycoprotein osteoprotegerin (OPG). RANK is expressed primarily on cells of the macrophage/monocyte lineage, including preosteoclastic cells, T and B cells, dendritic cells, and fibroblasts [36]. RANKL is highly expressed in bone, bone marrow, and lymphoid tissues, stimulating osteoclast differentiation and activity and inhibiting osteoclast apoptosis [37]. Besides RANK, RANKL also binds to OPG. The major biologic action of OPG has been its binding to RANKL and consequent inhibition of RANK stimulation, then its decreasing of osteoclast differentiation and activity in bone.

Recent studies also defined a role for prelamin A accumulation in the vascular degeneration observed in aged and atherosclerotic patients within the general population [38, 39]. Increased prelamin A accumulation was observed in atherosclerotic arteries and medial VSMCs of aged patients with MAC. In human and animal studies, prelamin A accumulated selectively in the vasculature [40]. Prelamin A has been shown to modulate DNA damage repair signaling, leading to premature cell senescence. It also has been shown to induce gene expression changes that modulate both Wnt and Notch signaling, as well as extracellular matrix production, to affect VSMC phenotype. Finally, it has been reported that prelamin A promotes VSMC calcification and aging by inducing persistent DNA damage signaling, which acts upstream of VSMC osteogenic differentiation and the senescence-associated secretory phenotype [41•].

Calcification is a balance between promineralizing factors that stimulate VSMC dedifferentiation and inhibitors of calcification. In both bone and arteries, there are inhibitors of calcification, including matrix Gla protein (MGP), pyrophosphate, and osteopontin, and circulating inhibitors such as fetuin-A [28]. MGP is present in VSMCs and, like osteocalcin, belongs to a family of mineral-binding proteins that contain γ-carboxyglutamic acid, which has a high affinity for hydroxyapatite. MGP inhibits induction of ALP by BMPs [42]. MGP-deficient mice demonstrate extensive endochondral bone formation in the arterial wall, as well as generalized calcification of cartilage. There is mounting evidence of complex coordination of the actions of fetuin-A, MGP, and other factors in the release of calcium and phosphate accompanying bone lysis [43]. Osteopontin is a phosphoprotein secreted by different cell types, including preosteoblasts, osteoblasts, and osteoclasts; consequently, it is involved in several biologic functions. Osteopontin is a major constituent of bone matrix and has been shown to inhibit calcification by adhering to calcium apatite crystals [44]. Fetuin-A is an acute-phase glycoprotein synthesized in the liver [43]. It inhibits the actions of BMP2 and limits matrix vesicle formation by inhibiting hydroxyapatite formation. In addition, fetuin-A reduces apoptosis and enhances phagocytosis of matrix vesicles by VSMCs. Reduced synthesis of fetuin-A is a major determinant of increased arterial calcification in CKD [43]. Polymorphisms in the gene encoding fetuin-A are associated with poor CV outcome; indeed, an inverse correlation has been demonstrated between circulating levels of fetuin-A and CV and all-cause mortality in humans [45]. Finally, other factors that might influence the development of osteogenic cells and vascular calcification are nitric oxide (NO) and high-density lipoprotein (HDL). NO inhibits calcification and osteoblastic transformation of VSMCs in vitro, an effect shown to be mediated through inhibition of the actions of transforming growth factor-β (TGF-β) and its downstream effects on the phosphorylation of SMAD proteins and of plasminogen activator inhibitor-1 [46]. The administration of HDL to calcifying vascular cells (CVCs) in vitro reduces ALP activity (a marker of osteogenic transformation of CVCs induced by BMPs), including that stimulated by proinflammatory cytokines interleukin (IL)-1β and IL-6 [47].

Vessel structure also may be regulated by alterations in matrix cross-linking. Endothelial NO synthase (NOS)-dependent nitric oxide also regulates tissue transglutaminase 2 (TG2) cross-linking activity and location in endothelial cells. Decreased endothelium-dependent NO synthesis in the aging vasculature leads to reduced TG2 *S*-nitrosylation and, thus, enhanced transamidation activity. This, in turn, results in increased cross-linking of matrix proteins and, consequently,

to decreased compliance and increased stiffness of aging conduit blood vessels [48].

It is known that angiotensin II induces both matrix metalloproteinase 2 (MMP2) and calpain-1 expression and activity in the arterial wall. Overexpression of calpain-1 induces MMP2 transcripts, protein levels, and activity, in part, by increasing the ratio of membrane type 1 MMPs to tissue inhibitor of MMP2. These effects of calpain-1 overexpression-induced MMP2 activation are linked to increased collagen I and III production and vascular calcification. Overexpression of calpain-1 also induces TGF-β1/SMAD signaling, elastin degradation, alkaline phosphatase activation, and total calcium content, but reduces the expression of calcification inhibitors, osteopontin, and osteonectin, in cultured VSMCs in vitro and in carotid artery rings ex vivo [49•].

Aging and Structural Changes in the Artery Wall: Collagen and Elastin Fibers

Collagen isoforms found in the aorta are predominantly types I and III (about 80%–90%) with some type IV, and their concentration gradually increases after the age of 50. A hallmark of arterial aging is the progressive thinning, splitting, and fragmentation of elastic lamellae [1, 2]. Elastin is the most abundant protein of the vascular wall of large arteries and represents 90% of elastic fiber content, which also is composed of glycoproteins. Elastic fibers consist of soluble tropoelastin monomers assembled and cross-linked on several residues. The metabolism of elastin seems age dependent, being synthesized mainly during early development, with a subsequent slow turnover. Several elastin cross-links decrease with age, thus contributing to the reduction in the rubber-like properties of the polymer. The gradual disruption of the elastic lamellae is a slow mechanical senescence of the network by repetitive influences of systolic stretching [1, 2]. Once elastin is degraded, elastin peptides are susceptible to calcification and calcium binding; thus, they may serve as initiation sites for calcification [50]. MMPs may serve to degrade medial elastin, resulting in the liberation of soluble elastin peptides. These peptides can bind the elastin–laminin receptor (ELR), which is present on most cells, to stimulate production of MMPs and other serine elastases. This represents a positive feedback mechanism triggering a cycle of MMP-mediated elastin degradation, inflammatory cell recruitment, and more MMP secretion. Additionally, soluble elastin peptides may interact with the ELR to induce osteogenic changes in VSMCs [50].

AGEs

Increased oxidative stress with aging leads to greater generation of AGEs. AGEs have been found in arterial and cardiac tissue as well as atherosclerotic lesions in dialysis patients [51]. Because only protein catabolism removes AGEs, collagen and elastin are highly susceptible to AGE accumulation because of their slow turnover. Increased cross-linking confers a resistance to enzymatic degradation of collagen that promotes its accumulation in the arterial wall [52]. Other relevant effects of AGEs include binding to their receptor (RAGE) to promote the release of fibrotic cytokines and inhibition of cell adhesion that may enhance apoptosis [53]. AGE accumulation on collagen and elastin and age-related aortic stiffness were correlated [54]. AGE-modified elastin and calcification have been found in the aortic media of dialysis patients, and calcium binding to elastin plays a key role in the pathogenesis of medial calcification [54]. In cultured VSMCs, AGEs may accelerate calcification of microvascular pericytes. AGEs induce the expression of RUNX2 mRNA and ALP activity and calcification [31]. RAGE is expressed in a variety of cells, including VSMCs, and these AGE-mediated changes in VSMCs are partially attenuated by a neutralizing antibody to RAGE [55]. Moreover, experiments using aminoguanidine and pyridoxamine, inhibitors of AGE cross-linking formation, reported prevention of arterial stiffening [56]. Furthermore, AGE cross-link breakers restored large artery properties in aging rodents, dogs, nonhuman primates, and humans, providing a scientific rationale for their development [57].

Genetic Pathways

In complex disorders such as vascular calcification, multiple contributing genes, as well as environmental exposures, mediate outcome [58, 59]. For vascular calcification, mechanisms affecting smooth muscle proliferation, endothelial function, response to reactive oxygen species, vitamin K metabolism, and osteochondral differentiation, together with a growing list of physiologic modulator molecules and environmental factors, likely contribute to both intimal calcification in atherosclerosis and calcification of the tunica media of the arteries.

Vascular calcification has a high heritable component [58, 59]. Genome-wide association studies have identified several loci linked to coronary artery calcification (CAC), and some of these loci are the same as those for coronary atherosclerosis. The 9p21 locus, which previously was linked to vascular disease, also is associated with subclinical coronary atherosclerosis and calcification [60]. Recently, a meta-analysis of CAC and myocardial infarction involving nearly 10,000 subjects from five cohorts identified 48 single-nucleotide polymorphisms (SNPs) at 9p21 near the cyclin genes (*CDKN2B* and *CDKN2A*) significantly linked to CAC [61]. These genes encode cyclins that may be linked broadly to cellular senescence and inflammation. This meta-analysis also found a single SNP at 6p24 in the *PHACTR1* gene [61], and this same locus has been linked to myocardial infarction [62].

Gene identification in rare diseases associated with arterial calcification, including generalized arterial calcification of infancy, pseudoxanthoma elasticum, calcification of joints and arteries, and familial idiopathic basal ganglia calcification, has helped researchers gain insight into the molecular pathophysiology of arterial calcification [63]. Mutations in the underlying disease genes *ENPP1*, *ABCC6*, *NT5E*, and *SLC20A2*, respectively, lead to arterial media calcification [64–67]. The encoded proteins nucleotide pyrophosphatase/phosphodiesterase-1 (NPP1), adenosine triphosphate (ATP)-binding cassette subfamily C member 6 (ABCC6), glycosylphosphatidylinositol-linked plasma membrane CD73 ectoenzyme (CD73), and type III sodium-dependent phosphate transporter 2 (PiT2), respectively, appear to be responsible for preventing spontaneous calcification by modulating ATP metabolism, adenosine, and inorganic phosphate. However, the role of ABCC6 in this pathway remains to be defined [68]. NPP1 also seems to play a role in intima calcification and atherosclerotic plaque development [69]. Whether the other proteins, including ABCC6, CD73, and PiT2, also are involved in atherosclerotic plaque calcification remains to be elucidated.

Another interesting genetic pathway for vascular calcification is represented by the klotho gene. This gene, identified as an "aging suppressor" gene in mice, encodes a single-pass transmembrane protein expressed predominantly in the distal tubular epithelial cells of the kidneys, parathyroid glands, and choroid plexus of the brain [70, 71]. Klotho originally was identified in a mutant mouse strain that could not express the gene; these mice developed multiple disorders resembling human aging and had a shortened life span [72, 73•]. The aging phenotypes include atherosclerosis and endothelial dysfunction, osteopenia, sarcopenia, skin atrophy, and impaired cognitive function. In an atherosclerotic mouse model, the in vivo gene delivery of klotho protects against endothelial dysfunction. HMG-CoA reductase inhibition enhances klotho protein expression in the kidneys and inhibits atherosclerosis in rats with chronic blockade of NOS [74]. Mounting evidence also suggests that klotho deficiency is a marker for CKD progression and acute kidney injury [75•]. There are two forms of klotho: a membrane form and a secreted form, each with distinct functions. Membrane klotho acts as an obligate coreceptor for fibroblast growth factor 23, a bone-derived hormone that induces phosphate excretion into the urine [76]. Secreted klotho is involved in the regulation of NO production in the endothelium, maintenance of endothelial integrity and permeability, calcium homeostasis in the kidneys, and inhibition of intracellular insulin and insulin-like growth factor-1 signaling [77]. Recent data show that low serum klotho levels are associated with poor skeletal muscle strength and the prevalence of CV disease and all-cause mortality in community-dwelling adults [78–80]. Low serum klotho levels also have been reported in patients with metabolic disorders such as obesity and diabetes mellitus [81]. The expression of local vascular klotho has been observed to decrease in human arteries in patients with CKD compared with healthy individuals [82]. An association between klotho deficiency and vascular calcification also has been reported in aging mice and in a mouse model of CKD [72, 73•]. Interestingly, recent data show that a decrease in the serum soluble klotho level is an independent biomarker of arterial stiffness in patients with CKD [83].

Clinical Implications

Arterial stiffness is emerging as an important risk factor in hypertension [84]. PWV predicts future changes in systolic blood pressure (SBP) and future development of hypertension in healthy individuals [84]. Furthermore, increased arterial stiffness has been associated with increased morbidity and both all-cause and CV mortality in hypertensive patients [85, 86]. A recent meta-analysis confirmed that arterial stiffness, as measured by carotid–femoral PWV, is an independent predictor of adverse CV events and all-cause mortality: for each 1-m/s increase in aortic PWV, CV risk rises by more than 10% [87].

A large body of evidence supports the concept of increased arterial stiffness in type 1 diabetes [88, 89]. This is an early phenomenon that occurs before the onset of clinically overt micro- or macrovascular disease, and arterial stiffness is enhanced further in the presence of microvascular complications. Similar findings have been reported with regard to pulse pressure: subjects with type 1 diabetes show an increase in pulse pressure around the third/fourth decade of life, suggesting accelerated arterial aging, and the age–pulse pressure relationship is even steeper in the presence of microvascular complications [88, 90]. Whether increased arterial stiffness is a cause (because greater arterial stiffness is associated with higher pressures in small arteries and capillaries) or a consequence (because microvascular damage will increase wave reflection and thus increase pulse pressure) of microangiopathy—or, alternatively, whether both phenomena are the result of other damage pathways, such as endothelial dysfunction or inflammation—remains to be elucidated. Taken together, these data highlight the accelerated arterial aging in type 1 diabetes and may explain, at least in part, the increased CV risk in these patients. A large body of evidence supports the concept of increased arterial stiffness in type 2 diabetes [91, 92]. Similar to that observed in type 1 diabetes, this again is an early phenomenon because much already occurs in the impaired glucose metabolism state (i.e., impaired fasting glucose and/or impaired glucose tolerance). In addition, the presence of micro- and macrovascular complications in type 2 diabetes is associated with a further increase in arterial stiffness [93]. Furthermore, as in patients with type 1 diabetes, those with type 2 diabetes show a rapid age-related increase in arterial stiffness compared with their nondiabetic counterparts, and

these increases are amplified further by micro- and macrovascular complications [94].

Several studies in the general population have shown that aortic calcification increases overall and CV mortality [95]. London et al. [96] studied the effect of arterial intimal and medial calcification on mortality in 202 hemodialysis patients. Compared with patients who had intimal calcification, those with medial calcification had longer survival; however, patients with medial calcification showed significantly shorter survival than patients without calcifications. These findings suggest that arterial medial calcification is a strong prognostic marker of all-cause and CV mortality in hemodialysis patients, independent of classic atherogenic factors, acting mainly through increased arterial stiffness. In a previous study in 110 hemodialysis patients, the same group of investigators showed that the presence and extent of vascular calcifications were strong predictors of CV and all-cause mortality [97]. Vascular calcification is an important determinant of CV outcomes after kidney transplantation. In a clinical trial with 112 kidney transplant recipients, aortic calcification, diagnosed by electron beam CT, was prevalent and found to be a strong predictor of CV events [98]. Arterial stiffness has been independently associated with reduced creatinine clearance in patients with mild to severe renal insufficiency [5], and it is independently associated with all-cause mortality and CV events in CKD [99]. Data show that mild CKD (glomerular filtration rate [GFR] of 60–89 mL/min per 1.73 m^2 body surface area) is a risk factor for CV disease [100]. Several studies have demonstrated the existence of a relationship between the degree of GFR loss and arterial stiffness, even in individuals with GFR values in the "normal to mildly impaired renal function" range (GFR ≥60 mL/min per 1.73 m^2 body surface area) [101], suggesting a cause–effect relationship and/or common underlying mechanisms. In hemodialysis patients, aortic stiffness is a powerful independent predictor of CV and all-cause mortality [102]. Notably, the lack of an aortic PWV decrease in response to a drug-induced decrease in blood pressure was a significant predictor of CV death in patients with end-stage renal disease [103].

Several lines of evidence suggest that age-associated bone demineralization and arterial calcification are highly regulated processes and share common mechanisms and signaling pathways [104]. Previous studies measuring bone mineral density (BMD) with dual-energy x-ray absorptiometry (DEXA) in postmenopausal women showed an inverse relationship between PWV and bone demineralization [105, 106]. In a recent study conducted in community-dwelling men and women with a wide range of BMDs, we measured BMD with CT, which unlike DEXA can distinguish between cortical and trabecular bone. We found that a decrease in cortical cross-sectional area is associated with an increase in PWV in women but not in men, suggesting that mediators of this association probably are differentially regulated between men and women [107].

The correlation of osteoporosis with calcium deposits suggests a significant role of calciotropic hormones in the pathogenesis of vascular calcification. Both endothelial cells and VSMCs have vitamin D receptors. Vitamin D might influence vascular homeostasis through a direct effect on endothelial cells and VSMCs, but also from indirect interaction with other calciotropic hormones and immunomodulation. Activation of vitamin D nuclear receptor (VDR) induces the change in expression of almost 200 genes influencing the cell cycle, reducing proliferation, differentiation, and apoptosis of VSMCs [108]. VDR activation also modulates cardiac calcium flux and thereby induces an accelerated relaxation of cardiomyocytes, which may improve diastolic function of the heart. Vitamin D–mediated regulation of cardiac extracellular matrix turnover also may be important in maintaining CV health [109]. Vitamin D also may protect against atherosclerosis, vascular calcification, and endothelial dysfunction [110]. Studies showed that poor vitamin D status, as well as vitamin D intoxication, may contribute to vascular calcification. Vitamin D levels that are too high or too low intensify the activity of metalloproteinases, key enzymes for vascular remodeling. Excessive vitamin D supplementation may result in intense calcium deposit accumulation in the tunica intima and media, elastin degradation, increased arterial stiffness, and left ventricular hypertrophy [111]. Recent studies reported an association between vitamin D insufficiency and increased arterial stiffness [112, 113] and endothelial dysfunction [114]. Vitamin D may reduce vascular calcification by inhibiting BMPs, but data on this topic are somewhat controversial [110], and both observational and interventional studies showed inconsistent results regarding the association of vitamin D with subclinical atherosclerosis [110]. Several, but not all, interventional studies showed that vitamin D supplementation improves endothelial function [114]. Further research is needed to identify the mechanisms by which vitamin D affects arterial stiffness and to explore whether vitamin D supplementation may prevent CV disease.

Management of Arterial Aging

Treatment of Vascular Calcification

Treatment strategies for vascular calcification represent a daily challenge for the medical community dealing with CKD patients. Although current treatment strategies focus on correcting abnormal calcium, phosphorus, parathyroid hormone, or vitamin D levels in CKD, a better understanding of the mechanisms of abnormal tissue calcification may lead to the development of new therapeutic agents that can reduce vascular calcification and improve the CV outcome of CKD patients.

Hyperphosphatemia contributes to secondary hyperparathyroidism, CV mortality, and all-cause mortality. The phosphorus binders currently used to manage hyperphosphatemia include sevelamer, lanthanum, and the calcium-based phosphate binders (CBPBs) calcium carbonate and calcium acetate.

Sevelamer is an aluminum- and calcium-free phosphorus binder that does not promote hypercalcemia, allows better serum phosphorus control compared with CBPBs, suppresses the progression of aortic calcification in hemodialysis patients, and has a favorable effect on the lipid profile [115]. In 200 hemodialysis patients, sevelamer attenuated the progression of coronary and aortic calcification better than CBPBs after 1 year of treatment [116]. Another study confirmed these findings, showing that treatment with sevelamer, rather than calcium carbonate, was associated with less vascular calcification within the myocardium, aorta, and kidney [117]. More recently, a randomized trial conducted in hemodialysis patients (n = 91 treated with sevelamer; n = 92 treated with calcium carbonate) showed that sevelamer treatment slowed the increase in CAC and suppressed AGE accumulation [118]. The possible mechanism consists of a strong phosphorus-binding capacity of sevelamer at the intestinal level without excessive calcium loading. However, the Renagel in New Dialysis study in patients with baseline CAC scores of 30 or higher showed no significant difference in the rate of progression of calcification at any point up to 18 months of follow-up between a group of patients treated with sevelamer and a group treated with CBPBs [119]. Moreover, in a study with more than 1,000 hemodialysis patients followed up to 45 months, the overall mortality was not reduced significantly by sevelamer compared with CBPBs, except in patients older than 65 years, in whom sevelamer reduced the risk of death [120]. Finally, a systematic review of the clinical efficacy and safety of sevelamer in dialysis patients failed to show any evidence that sevelamer reduced all-cause mortality, CV mortality, the frequency of symptomatic bone disease, or health-related quality of life [121].

In vitro studies have shown that acetylated low-density lipoprotein (LDL) promotes VSMC calcification, whereas HDL inhibits it [122]. In human studies, sevelamer has been shown consistently to reduce LDL and often to increase HDL levels. The improved lipid profile may play a role in the lower degree of vascular calcification observed after sevelamer therapy. Interestingly, in the Calcium Acetate Renagel Evaluation-2 study, intensive LDL cholesterol–lowering therapy with atorvastatin disclosed similar progression of CAC in the group of hemodialysis patients treated with calcium acetate and those treated with sevelamer [123].

The calcium-sensing receptor (CaR) is a G-protein–coupled cell surface receptor that senses extracellular calcium ions and enables cells to respond to small changes in the extracellular calcium ion concentration [124]. Data suggest that a close relationship between CaR and vascular calcification may exist locally in the vessel wall. In fact, low levels of CaR immunoreactivity were found in atherosclerotic, calcified human arteries compared with noncalcified arteries [125].

Because bisphosphonates have been shown to reduce vascular calcification in experimental models, a future role in the management of vascular calcification has been evoked. In hemodialysis patients, etidronate has been found to reduce and even reverse the progression of CAC in some patients [126, 127]. In a more recent trial, atorvastatin plus etidronate combination therapy for 12 months significantly reduced both thoracic and abdominal aortic plaques, whereas atorvastatin monotherapy reduced only thoracic aortic plaques and etidronate monotherapy reduced only abdominal aortic plaques [128]. Although the underlying mechanisms still are uncertain, it may be speculated that bisphosphonates inhibit bone resorption, with reduced efflux of calcium and phosphate, limiting their availability for deposition in the vessels, or may influence the activity of the sodium/phosphate cotransporter in VSMCs [129].

Treatment of Arterial Stiffness

Mounting evidence suggests that changes in arterial stiffness might be induced by either nonpharmacologic or pharmacologic interventions. Nonpharmacologic interventions that can reduce arterial stiffness include exercise training [130, 131], weight loss and various dietary modifications [132], and continuous positive airway pressure [133].

Pharmacologic treatments include (1) antihypertensive treatments such as angiotensin-converting enzyme (ACE) inhibitors, angiotensin receptor blockers (ARBs), β-blockers, calcium-channel antagonists, and diuretics; (2) lipid-lowering agents such as statins; and (3) AGE breakers.

Antihypertensives

Renin–angiotensin–aldosterone system inhibitors, such as the ACE inhibitors and ARBs, have been widely suggested to have a blood pressure–independent effect on arterial stiffness [134]. The mechanism by which antihypertensives improve arterial stiffness seems to be related to the reduction of the wave reflection and augmentation index [135], with subsequent lowering of SBP and less adverse left ventricle remodeling. The effect on central blood pressure reduction mediated by the ARB olmesartan in combination with either a calcium-channel blocker or a diuretic was investigated in hypertensive patients [136]. Interestingly, despite a similar reduction in brachial SBP observed in the two groups, the decrease in central SBP in the olmesartan/calcium-channel blocker group was significantly greater than in the olmesartan/diuretic group. In addition, PWV was significantly more reduced in the

olmesartan/calcium-channel blocker group, suggesting that the regulating capacity of arterial stiffness and wave reflections might differ among antihypertensive patients. It has been suggested that β-blockers are inferior to other classes of drugs in reducing vascular stiffness, because they are less effective than other antihypertensive drugs in reducing the central pulse pressure and augmentation index. REASON (Regression of Arterial Stiffness in a Controlled Double-Blind Study) compared perindopril (2 mg/d) plus indapamide (0.625 mg/d) versus atenolol (50 mg/d) alone for 12 months in hypertensive patients. Interestingly, at 1-year follow-up, the brachial and central SBP reduction achieved with combination therapy (ACE inhibitor/diuretic) was greater than that with a β-blocker or an ACE inhibitor alone. This might be ascribed to the greater structural changes of arterial stiffness that are more pronounced in central than in peripheral arteries [137]. Nebivolol, a selective β-blocker with NO-mediated vasodilatory effects, has been shown to decrease the augmentation index slightly compared with atenolol [138]. In the Anglo-Scandinavian Cardiac Outcomes Trial (ASCOT), amlodipine proved to be more effective than atenolol for reducing CV events [139]. The Conduit Artery Functional Endpoint (CAFE) study showed that the reduction in central SBP and pulse pressure was greater in the amlodipine than the atenolol treatment group, despite similar reductions in blood pressure at the brachial level [140]. This might be explained predominantly by the β-blockers' effects on heart rate and stroke volume. Whether this would result in favorable outcomes needs further investigation.

Statins

The role of statins in arterial stiffening remains controversial [141–143]. Some studies reported that statins produced significant reductions in PWV levels in different segments of the arterial tree [144, 145], whereas others showed no change [146] or even an increase [147] in arterial stiffness. The inconsistency of findings might be related to methodologic issues (i.e., small sample sizes and short study duration) as well as other aspects, such as the statin dosage, the baseline cholesterol levels, and the methodology used to assess arterial stiffness (many studies used suboptimal indices of arterial stiffness or included measurements of PWV only in peripheral muscular–type arterial segments).

AGE Breakers

AGEs have been implicated in increased myocardial and vascular stiffness, and AGE cross-linked breakers have emerged as a potential therapeutic target [148, 149]. Kass et al. [57] demonstrated that in elderly patients with arterial stiffening at baseline, alagebrium, a novel drug showing a good safety and tolerability profile in phase I and II studies, significantly improved arterial compliance, carotid–femoral PWV, and pulse pressure after 8 weeks of treatment. The potential clinical value of these interventions, however, remains to be established [149].

Conclusions

Extensive calcification of the vascular system is a key characteristic of aging. Although arterial calcification may be viewed as a uniform response to vascular injury, it is a heterogeneous disorder with overlapping and distinct mechanisms of initiation, progression, and clinical consequences. Through understanding of the pathways involved in these processes, novel drug targets may be proposed in an effort to reduce the effects of vascular calcification as a risk factor.

Compliance with Ethics Guidelines

Conflict of Interest Francesco Giallauria, Carlo Vigorito, Nicola Ferrara, and Luigi Ferrucci declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- •• Of major importance

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