Abstract—This review centers on updating the active research area of vascular calcification. This pathology underlies substantial cardiovascular morbidity and mortality, through adverse mechanical effects on vascular compliance, vasomotion, and, most likely, plaque stability. Biomineralization is a complex, regulated process occurring widely throughout nature. Decades ago, its presence in the vasculature was considered a mere curiosity and an unregulated, dystrophic process that does not involve biological mechanisms. Although it remains controversial whether the process has any adaptive value or past evolutionary advantage, substantial advances have been made in understanding the biological mechanisms driving the process. Different types of calcific vasculopathy, such as inflammatory versus metabolic, have parallel mechanisms in skeletal bone calcification, such as intramembranous and endochondral ossification. Recent work has identified important regulatory roles for inflammation, oxidized lipids, elastin, alkaline phosphatase, osteoprogenitor cells, matrix γ-carboxyglutamic acid protein, transglutaminase, osteoclastic regulatory factors, phosphate regulatory hormones and receptors, apoptosis, prelamin A, autophagy, and microvesicles or microparticles similar to the matrix vesicles of skeletal bone. Recent work has uncovered fascinating interactions between matrix γ-carboxyglutamic acid protein, vitamin K, warfarin, and transport proteins. And, lastly, recent breakthroughs in inherited forms of calcific vasculopathy have identified the genes responsible as well as an unexpected overlap of phenotypes. Until recently, vascular calcification was considered a purely degenerative, unregulated process. Since then, investigative groups around the world have identified a wide range of causative mechanisms and regulatory pathways, and some of the recent developments are highlighted in this review. (Arterioscler Thromb Vasc Biol. 2014;34:715-723.)

Key Words: aortic valve ▪ arteries ▪ heterotopic ossification ▪ vascular calcification

Clinical Importance

The vasculature requires distensibility and elasticity to maintain normal hemodynamics, autoregulatory function, and vasomotion. Thus, rigid deposits of calcium mineral in the artery wall have important biomechanical consequences on circulatory function. Direct, in vivo stress-strain measurements in hyperlipidemic rabbits suggest that calcification increases stiffness and fragility,1 as expected from theoretical mechanical considerations. Increased stiffness also impairs vasomotion. Plaque rupture is a serious and devastating complication of atherosclerosis. Biomechanical analyses indicate that rigid inclusions in distensible materials, such as calcium deposits in arteries, introduce compliance mismatch and failure stress at the surfaces facing the principal direction of stress and thereby increase the risk of rupture.2 Others have also used finite element analysis to show that the risk of rupture is further increased when 2 such deposits are in close proximity.1 When cardiac valves calcify, hemodynamic effects may be severe, as in calcific aortic stenosis, which blocks outflow from the left ventricle. Overall, vascular calcification promotes significant adverse clinical effects including systolic hypertension, left ventricular hypertrophy, coronary ischemia, congestive heart failure, and possibly plaque rupture, thrombosis, and myocardial infarction.

Proposed Classification Scheme

A variety of mechanisms have been proposed for vascular calcification. Rather than being mutually exclusive, they may correctly reflect multiple causes. At risk of gross oversimplification, artery wall calcification may be loosely categorized into 3 broad types as shown in Table 1. For simplicity, certain known forms, such as those associated with irradiation, pharmacological agents, toxins, and hypercalcemia of cancer, are not included here. The 3 different types listed may coexist or overlap in the same subject at the same time and even at the same arterial site. Many patients and experimental models with diabetes mellitus have both hyperlipidemia and chronic kidney disease (CKD), resulting in both intimal atherosclerotic calcification and medial calcification in the same arterial segment. In addition, even though osteochondrogenic
differentiation factors and ossification are primarily associated with intimal atherosclerotic calcification, evidence shows that medial calcification is also associated with osteoblastic differentiation markers and, occasionally, even ossification. Many of the genetic disorders also have their effects through downstream metabolic mediators. Although osteogenic and chondrogenic differentiation are assumed to precede mineralization in the vasculature, as in bone, the reverse order may also occur, as described later.

**Comparable Processes in Skeletal Bone Formation**

Although the majority of vascular calcium deposits seem to be amorphous calcification, ≈15% have advanced to fully formed, structured bone tissue—true ossification—including osteoid, trabeculae, osteocytes, osteoclast-like cells, and marrow. The use of more than 1 route to mineralization is also a feature of skeletal bone formation (Table 2). These include the following: intramembranous bone formation, by which the skull and clavicles arise; endochondral bone formation, by which long bones form; and callus formation, by which fractures repair. Intramembranous ossification occurs by direct mineralization of matrix produced by mesenchymal cells that undergo osteogenic differentiation. Endochondral ossification, on the contrary, occurs with cartilage as an intermediate step. For example, in the growth plate of long bone, chondrocytes occupy sites of future bone, creating a gradient of chondrocytes in stages of maturation: proliferation, prehypertrophy, hypertrophy, and apoptosis. The apoptotic bodies serve as nucleation sites for calcium–phosphate crystals, producing calcified cartilage. This calcified cartilage matrix serves as the scaffold, into which microvessels invade, bringing endothelial cells and pericytes. Monocyte-derived chondroclasts dissolve the calcified cartilage and that microvascular pericytes, on exposure to this calcified matrix, and possibly transglutaminase (TG), undergo osteogenic differentiation and produce bone matrix, osteon, matrix vesicles, and new hydroxyapatite mineral to replace the cartilage.

As cells mature into osteoblasts or chondrocytes, they express osteochondrogenic genes and release a type of membrane-invested microparticle, known as matrix vesicles into the extracellular milieu. These matrix vesicles contain a variety of enzymes and factors, including alkaline phosphatase and annexins. They concentrate calcium and initiate hydroxyapatite mineral crystallization. Over time, it appears that hydroxyapatite crystals nucleate within these matrix vesicles and propagate until they breach the vesicle membrane and, in some manner, merge with existing bone mineral. Matrix vesicles, themselves, arise from cells by more than 1 mechanism. A pioneer in this field, H. Clarke Anderson, noted that matrix vesicles may form by budding from the plasma membrane and from cell degeneration; more than 1 mechanism may function in the same tissue. The term matrix vesicle primarily refers to the microparticle found in the matrix of skeletal bone and cultured bone cells. Similar structures are found in other tissues, such as vascular tissue, and other cultured cells, including endothelial, dendritic, and smooth muscle cells (SMCs). These have been termed extracellular vesicles, apoptotic bodies, and microvesicles.

**Parallels Between Vascular and Skeletal Mineralization**

As hypothesized by Anderson, the matrix vesicles in the vasculature appear to be analogous to the matrix vesicles of skeletal tissues. For example, Kapustin et al and Chen et al showed that vascular SMCs (VSMCs) produce matrix vesicles that, like bone matrix vesicles, contain alkaline phosphatase as well as annexins and that nucleate calcium mineral. Both groups further showed that the alkaline phosphatase activity in the VSMC matrix vesicles is regulated by exogenous calcium and β-glycerophosphate. VSMCs may also, like osteoblasts, produce nucleating vesicles as a result of primary osteogenic differentiation or as a result of degenerative processes. In novel work from the Aikawa group, evidence suggests that microvesicles released by inflammatory cells, such as macrophages, are capable of mineralization. And, as it does for
pericytes, exposure to hydroxyapatite mineral may induce osteoblastic differentiation of VSMCs.11

The concentrations of calcium and phosphate in extracellular fluid are near those required for spontaneous precipitation of calcium–phosphate crystals. This is held in check at baseline by inhibitory factors. Extrasosseous biomineralization may be driven not only by upregulation of activating factors but also by downregulation of such inhibiting factors. It is likely that some forms of vascular calcification are driven solely by gain of activators, others, solely by loss of inhibitors, and some, by a combination.

**Teleology**

Vascular calcification may often be a result of an adaptive mechanism gone awry. Evolutionary pressure from numerous parasitic and bacterial infections may have driven an emphasis on immunologic and inflammatory responses. Soft tissue abscesses, ulcers, and other lesions of chronic inflammation often produce ectopic ossification, which may form a wall of bone entrapping the noxious site, an ultimate immune defense against objects resistant to ordinary immune defenses.

**Relationship to Osteoporosis**

Some epidemiologic studies have found that the correlation between vascular calcification and osteoporosis is independent of age.13,14 The mechanisms for such an age-independent association are not clear. One possibility is that the same underlying factor driving mineralization of vascular tissue also underlies demineralization of bone tissue, even though the outcomes are opposite in the 2 tissues. For example, it is well known that chronic inflammation has opposite effects on soft versus hard tissue, promoting mineralization in the former and demineralization in the latter (Figure). Clinical examples are calcific tendonitis and osteomyelitis.5 Thus, because hyperlipidemia and diabetes mellitus promote systemic and local inflammation by glycoxidative modification of lipids in bone and artery wall tissue, simultaneous vascular calcification and osteoporosis may result. Lipid deposition and oxidation have been shown in skeletal bone.15-17 With respect to metabolic vascular calcification, the associated CKD promotes hyperparathyroidism, which promotes bone loss, now known as the CKD-mineral and bone disorder.

A common question is whether the calcium lost from bone in osteoporosis is transferred to the vascular tree. In normal individuals, this would be unlikely given that circulating levels of calcium are tightly regulated by parathyroid hormone and renal excretion and also because of abundant mineralization inhibitors in the circulation, such as fetuin.18 In contrast, patients with CKD often have dysregulated calcium–phosphate metabolism, attributable to hyperparathyroidism or treatment regimens, possibly resulting in hyperphosphatemic calcification. Another potential mechanism linking osteoporosis and vascular calcification is release into the circulation, during resorption of skeletal bone, of unidentified regulatory factors that trigger mineralization in the arteries.

**Inflammation, Oxidized Lipids, Hyperlipidemia, and Fish Oil**

Chronic inflammation seems to be a central factor in aberrant soft tissue calcification in general, and sites of chronic inflammation in the vasculature have been shown to become sites of atherosclerotic calcification in mice.19 This phenomenon has now been confirmed at the level of human imaging studies. Abdelbaky et al20 evaluated imaging data from 137 patients aged >1 to 5 years and showed that sites of focal aortic inflammation, detected by 18F-Deoxyglucose positron emission tomographic scanning, are significantly associated with subsequent calcification detected by computed tomographic scanning. These findings support the view that atherosclerotic calcification in humans arises from chronic inflammation. The most common source of chronic vascular inflammation is atherosclerosis, and its underlying contributing factor is accumulation of oxidized lipids. Earlier studies showed that fish oils, such as eicosapentaenoic acid, which inhibit lipid oxidation, also inhibit vascular calcification.21 Addressing the mechanism of this effect, Kageyama et al22 recently showed that osteoblastic differentiation and mineralization in VSMCs induced by the free fatty acid, palmitic acid, is blocked by eicosapentaenoic acid through a mechanism requiring long-chain acyl–coenzyme A synthetase-3 and nuclear factor-κB. These findings suggest that pro-oxidant lipids positively regulate, and antioxidant lipids negatively regulate, vascular calcification.

The importance of osteogenic differentiation in hyperlipidemic vascular calcification was shown in vivo by Sun et al,23 who generated mice with vascular-specific deficiency of Runx2, the master regulatory factor for osteogenic differentiation. This defect markedly inhibited vascular calcification induced by a high-fat diet. Interestingly, this group at University of Alabama also observed a reduction in infiltration of macrophages and their differentiation into osteoclastic cells,23 which may have important implications as we develop a better understanding of the coupling between osteoblastic

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<tr>
<th>Skeletal site</th>
<th>Cranial and clavicular</th>
<th>Long bones</th>
<th>Fracture sites</th>
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<tr>
<td>Primary events</td>
<td>Primary osteogenic differentiation of resident osteoprogenitors</td>
<td>Chondrocyte hypertrophy, release of transglutaminase, autophagy, apoptosis, release of nucleating microvesicles, amorphous calcification of cartilage matrix</td>
<td>Hematoma containing marrow mesenchymal stem cells, chondrogenic differentiation of marrow stem cells into soft callus</td>
</tr>
<tr>
<td>Secondary events</td>
<td>Production of osten, release of nucleating microvesicles, bone formation</td>
<td>Microvascular invasion, resorption of calcified matrix, osteoblastic differentiation of pericytes, production of osten, release of nucleating microvesicles, bone formation</td>
<td>Microvascular invasion, resorption, osteoblastic differentiation of pericytes, osten formation, release of nucleating microvesicles, formation of bone as hard callus</td>
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and osteoclastic cells in the artery wall, similar to the functionally important coupling between these cell types in bone.

**Alkaline Phosphatase and Phosphatase, Orphan 1**

Tissue nonspecific alkaline phosphatase is a phosphatase that breaks down the mineralization inhibitor, pyrophosphate. Recently, Millán’s group identified an additional phosphatase, phosphatase, orphan 1 (PHOSPHO1), in matrix vesicles. They demonstrated that mice deficient in PHOSPHO1 have abnormalities in skeleton, including osteomalacia and reduced plasma levels of tissue nonspecific alkaline phosphatase and pyrophosphate. Interestingly, mutant mice deficient in PHOSPHO1 but overexpressing tissue nonspecific alkaline phosphatase do not have corrected mineralization phenotype in skeleton, despite corrected pyrophosphate levels, whereas mutant mice deficient in both PHOSPHO1 and tissue nonspecific alkaline phosphatase have complete absence of skeletal mineralization. These findings led the authors to conclude that PHOSPHO1 has a nonredundant functional role as an initiator of mineralization. Most recently, Kiffer-Moreira et al showed that inhibition of PHOSPHO1 in VSMCs also inhibits matrix calcification in vitro.

**Osteoprogenitor Cells**

The osteoprogenitor cells in the artery wall may arise from several potential sources, and there is evidence for most of them. These include, among others, the following: resident progenitor cells; mesenchymal stem cells from the marrow stroma via the circulation; subendothelial pericyte-like cells; calcifying vascular cells, also known as vascular mesenchymal cells; adventitial myofibroblasts; transdifferentiated SMCs; previously mature SMCs that have dedifferentiated and redifferentiated; and endothelial cells that have undergone epithelial–mesenchymal transition/transformation. Whether mature SMCs have the plasticity to de- and redifferentiate is an area of controversy. Many of these cells undergo mineralization in vitro. In pericytes and calcifying vascular cells, the mineral forms within 3-dimensional cellular aggregates known as nodules, which self-organize in periodic patterns through a mechanism involving reaction–diffusion, polarized cell division, and left–right chirality.

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**Figure.** Schematic diagram depicting relationships between soft tissue mineralization and bone mineralization in chronic infection vs chronic inflammation. Soft tissue calcification is associated with bacterial infection and abscess in liver, vascular calcification with chronic inflammation, bone tissue decalcification with bacterial infection in osteomyelitis, and bone tissue calcification with chronic inflammation in osteoporosis.
In an exciting new development from Towler’s group, Cheng et al. showed that vascular endothelial cells, previously considered relatively innocent bystanders in vascular calcification, underwent endothelial–mesenchymal transition and osteogenic differentiation in response to overexpression of the Wnt7 inhibitor, Dickkopf1 (Dkk1). Furthermore, Yao et al. showed that cells with endothelial markers colocalize with osteogenic markers in Mgp−/− mice and Ins2Akita−/−, a diabetes mellitus mouse model, and that overexpression of matrix γ-carboxyglutamic acid protein (MGP) in human umbilical vein endothelial cells attenuates osteogenic differentiation. An adventitial source of multipotent vascular cells is evidenced by the findings from Yang et al. Altogether, such findings begin to paint an image similar to the transitional stages of differentiation and renewal found in intestinal epithelium and skin.

**MGP and Transglutaminase 2**

This small protein, MGP, is well established as a key inhibitor of vascular calcification, and its function is affected by inflammatory state. MGP-deficient mice are known to develop rapid and extensive vascular calcification. Recent studies suggest that MGP can bind and inhibit bone morphogenetic protein as well as calcium mineral itself, suggesting that the aortic calcification in the deficient mice may be attributable to unopposed bone morphogenetic protein activity or unopposed mineral growth. Both mechanisms may have a role, given that the mice develop aortic calcification within 9 days of age, and express osteo-chondrogenic genes 5 days later. Thus, osteoblastic differentiation either may result from unopposed bone morphogenetic protein activity or it may proceed as a consequence of mineral formation perhaps attributable to endocytosed calcium nanocrystal effects on gene induction.

An interesting feature of MGP is that it requires post-translational modification, vitamin K–dependent γ-glutamyl carboxylatation. Some epidemiological evidence supports a role for vitamin K in vascular calcification. Rats with vitamin K deficiency and CKD have increased vascular calcification, which is attenuated by vitamin K repletion. Warfarin, which blocks vitamin K action by blocking the reductase step of vitamin K cycling, interferes with MGP γ-glutamyl carboxylatation and induces vascular calcification in rats and in dilute brown non-agouti mice, which is reversed by vitamin K treatment. In catalyzing γ-carboxylation, vitamin K is converted to vitamin K oxide and then recycled to active vitamin K through a reductase. Recently, Schurgers et al. extended this experimental model and superimposed it on the hyperlipidemic model of atherosclerotic calcification by showing that warfarin significantly increases vascular calcification in hyperlipidemic mice. Clinical studies are underway to assess the role of warfarin in human vascular and valvular calcification. Together, these findings suggest that vitamin K deficiency promotes vascular calcification through insufficient post-translational modification of MGP. This may have important clinical applications.

The mechanism of warfarin-induced vascular calcification may also involve TG2, which is known to cross-link matrix proteins. It is key in intermediate stages of endochondral ossification in the appendicular bones, where it is released by hypertrophic chondrocytes during production of calcified cartilage matrix. Cross-linking seems to render the matrix more vulnerable to mineral deposition, possibly by increasing or enhancing nucleation sites. This is consistent with the increase in calcification of bioprosthetic valves treated with another cross-linker, glutaraldehyde.

Using cultured VSMCs and organ culture of aortic rings from Tg2−/− deficient mice, Johnson et al. showed a substantial inhibition of phosphate-induced calcium deposition, indicating an essential role for TG2 in this form of vascular calcification. Beazley et al. went on to show that TG2 inhibitors block vascular calcification in vitro and in vivo in warfarin-treated rats, possibly independently of effects on MGP. Chen et al demonstrated elevated tissue levels of TG2 in rats with CKD and inhibition of ex vivo calcification in rat aortic rings by TG2 inhibition. Together, these findings support a role for TG2 in 2 metabolic forms of vascular calcification driven by warfarin and CKD.

**Elastin, Pseudoxanthoma Elasticum, Generalized Arterial Calcification of Infancy, ABC6, and ENPP1**

Pathologists have long observed that early stages of human vascular calcification occur along the edges and frayed ends of elastin fibers. As noted earlier, elastin haploinsufficiency reduces the vascular calcification associated with MGP deficiency in mice. The authors interpreted this finding to mean that MGP either protects mineralization initiation sites on elastin or alters extracellular matrix. These findings suggest that, in the context of MGP insufficiency, elastin calcification is metabolic in origin and that metabolic mineralization may lead to osteo-chondrogenic differentiation. Results showed a reduction in calcification and an increase in life span. The potential importance of elastin in other forms of vascular calcification is supported by its role as the target of calcification in the hereditary human disease, pseudoxanthoma elasticum (PXE), described below.

Clues about the role of elastin in metabolic vascular calcification come from the hereditary human disorder, PXE, which features fragmentation and calcification of elastin and extracellular matrix, as well as changes in the skin. Its autosomal recessive inheritance was traced to mutations in the gene ABC6, resulting in absence or nonfunction of the multidrug resistance protein, MRP6, a transmembrane organic anion transporter found primarily in kidney and liver. However, it is not clear whether ABC6 is located on the basal membrane of hepatocytes or on the mitochondrial membrane, and more importantly, the organic anion whose transport is disrupted in this disease remains to be identified.

Several lines of evidence suggest that PXE is a systemic, rather than local, disease. ABC6 is not found at the sites of disease, and disease expression requires absence of ABC6 in the liver, suggesting that the mechanism is related to transport of some unknown factor from the liver to the periphery. In recent work, making use of the observation that mice with PXE develop whisker calcification, Jiang et al. transplanted snout tissue, including whiskers, between Abcc6 null and...
wild-type mice. The whiskers from null mice calcified when grafted to Abcc6 null mice, but did not when grafted to wild-type mice. Conversely, whiskers from wild-type mice, grafted to null mice, did calcify, indicating that the calcification depended on the recipient (systemic) condition rather than donor (local) condition.

This, together with the evidence for vitamin K effects described above, led investigators in Amsterdam to the exciting theory that vascular calcification in PXE is attributable to inadequate delivery of vitamin K to the periphery, by absence of a factor needed to deliver it.65 This raised the possibility of vitamin K treatment for PXE. Interestingly, vitamin K, being a fat-soluble vitamin, must be carried to the periphery on lipoproteins, which are produced by the liver, and levels of lipoproteins are influenced by ABCC6 deficiency.57 Other investigators in Amsterdam proceeded to test the possibility of vitamin K therapy and found benefit in some types of vascular calcification, but, surprisingly, not that in PXE mice.58,59

In an unexpected twist, mutations of Abcc6 were recently found to underlie some cases of a different, equally mysterious, inherited disorder of vascular calcification, now known as generalized arterial calcification of infancy,52 which was previously believed to arise from mutations of the gene, Enpp1, which encodes a pyrophosphate-generating enzyme. Apparently, the 2 disorders, generalized arterial calcification of infancy and PXE, overlap substantially in phenotype, having a final common pathway of diffuse vascular calcification, with variable degree of severity. Even more remarkably, the converse was shown that full features of each disease may arise from mutations in either gene: some cases of generalized arterial calcification of infancy are attributable to Abcc6 mutations, and some cases of PXE are attributable to Enpp1 mutations.60

Receptor Activator of Nuclear Factor-κB Ligand and Osteoprotegerin

Oxidized lipids and oxidant stress also act on the key driver of osteoclastic bone resorptive activity, receptor activator of nuclear factor-κB ligand (RANKL). Byon et al61 demonstrated that oxidant stress induces RANKL in mouse VSMCs via the osteogenic differentiation factor, Runx2. Similarly, Mazière et al showed that oxidized lipids upregulate RANKL in a dose-dependent manner in human vascular cells, possibly through the associated increase in reactive oxygen species.62 Earlier work showed that mice deficient in a decoy receptor for RANKL, osteoprotegrerin (OPG), develop extensive vascular calcification63 and that OPG treatment reduced vascular calcification in hyperlipidemic mice.64 This effect of OPG treatment may occur through downregulation of Notch1–recombination signal binding protein for immunoglobulin kappa J region pathway and downstream mediators, MxS2 and alkaline phosphatase.65 To determine whether the vascular calcification in Opg−/− mice results from leaking of mineral from skeletal bone where excessive osteoclastic differentiation promotes resorption, Callegari et al66 showed that vascular calcification in Opg−/− mice was reduced by transplantation of Opg+/+ marrow. Interestingly, angiotensin II infusion increases both RANKL expression and calcification in hyperlipidemic mice.67

CKD, Fibroblast Growth Factor 23, and Klotho

Hyperphosphatemia, a metabolic disorder that is almost universal in CKD, stimulates release of the hormone fibroblast growth factor 23 (FGF23) from skeletal osteocytes as part of a negative feedback loop. It seems that FGF23 and Klotho have a role in vascular calcification; however, whether it is direct or indirect remains under investigation. Together with a coreceptor, Klotho, FGF23 acts on FGF receptors in the kidney to decrease renal phosphate resorption and downregulate vitamin D activation, both effects serving to reduce serum phosphate. Klotho deficiency is associated with premature aging syndromes.68 As evidence for a role for Klotho in CKD-related vascular calcification, Hu et al69 showed that mice overexpressing Klotho have reduced vulnerability to CKD-induced vascular calcification and, conversely, that mice with Klotho insufficiency have greater vulnerability to CKD-induced vascular calcification. It has been suggested that CKD-associated vascular calcification represents a state of klotho deficiency. Lim et al70 showed that Klotho knockdown in VSMCs potentiated development of cell calcification via Runx2 and myocardin serum response factor–dependent pathways. Some investigators have shown endogenous Klotho expression in human VSMCs and arteries as well as responsiveness to FGF23,71 whereas others have found neither expression of Klotho nor FGF23 responsiveness in human or mouse VSMCs or arteries.72

In clinical studies, a cross-sectional analysis of >2000 patients with atherosclerosis showed a positive and independent association between FGF23 and coronary calcification.72 However, in a study that analyzed >1500 patients with CKD, the independent correlation between FGF23 levels and coronary calcification severity lost significance after adjustment for cardiovascular risk factors.73 These divergent findings may be explained, in part, by the different patient populations, atherosclerosis versus CKD. Another consideration is that the statistical adjustment included several factors that may lie on a causal pathway between FGF23 and calcification, such as prior cardiovascular disease, diabetes mellitus, hypertension, and smoking history. In this manner, a causal relationship between FGF23 and coronary calcification, if present, could be masked by the statistical adjustment.

Diabetes Mellitus

Although not as dramatic as in patients with CKD, there is a strong predilection for vascular calcification in subjects with diabetes mellitus,73 resulting in a major clinical problem, given the large and growing numbers of such patients. Several mechanisms may be involved. One is the formation of advanced glycation end products. The receptor for these advanced glycation end products (RAGE) has a role in atherosclerosis, and in calcific human carotid artery specimens, RAGE colocalizes with inflammatory cells in unstable regions with microcalcifications.74 In mice with diet-induced diabetes mellitus, RAGE is upregulated and colocalizes with VSMCs undergoing osteochondrogenic differentiation.75 Ligands for RAGE are quenched by the soluble form of the receptor, and serum levels of this decoy receptor in hemodialysis patients are inversely associated with vascular calcification.76


suggesting a role for RAGE. Moreover, hyperlipidemic mice overexpressing extracellular RAGE-binding protein, S100A12, in VSMCs have accelerated vascular calcification, and this is mediated, in part, by oxidative stress.77 Another mechanism by which diabetes mellitus may affect vascular calcification is through promoting release of osteoprogenitor cells from the marrow into the circulation. Two recent studies found that the proportion of circulating progenitor cells with osteogenic markers is significantly increased in patients with diabetes mellitus.78,79 An additional mechanistic consideration, based on a streptozotocin-treated rat model, is that diabetes mellitus may promote vascular calcification by reducing the vitamin K–dependent activation of the inhibitor of calcification, MGP.80 A combination of diabetic factors may synergize to promote vascular calcification. For example, the combination of hyperglycemia and elastin degradation products, coupled with transforming growth factor-β1 (commonly elevated in diabetes mellitus), increases osteogenic markers, such as alkaline phosphatase, osteocalcin, and Runx2 in vascular cells.81 These and other mechanisms may explain a predilection for initiation of vascular calcification in diabetic subjects. There may also be features of diabetes mellitus that facilitate progression of existing vascular calcification. One study of ≈200 veterans with diabetes mellitus suggests that coronary vascular calcification progresses more rapidly with the use of cholesterol-lowering statins.82 Although statins are expected to reduce inflammation, because they also promote bone growth,83 the counterintuitive findings from the study of veterans raises the interesting possibility that once vascular calcium deposits achieve a certain level of maturity in osteogenic differentiation, then statins may enhance progression.

Apoptosis, DNA Damage, Prelamin A, Autophagy, and Matrix Vesicles

The chronic inflammatory milieu generally includes cell injury and death, including DNA damage, autophagy, and apoptosis. During apoptosis, VSMCs release both matrix vesicles and other microparticles, such as apoptotic bodies, which are larger than matrix vesicles, but share many functions, including the ability to concentrate calcium and nucleate calcium–phosphate crystals.6 In further studies related to cell injury, Liu et al84 have shown that in vitro prelamin A overexpression, which blocks DNA damage repair, induces vascular cell osteoblastic differentiation and mineralization. This finding suggests that DNA damage signaling induces pro-osteogenic expression. Another recent important finding is that an autophagic response, transiently observed in chondrocyte maturation, which reduces matrix vesicle release from cells exposed to excess phosphate, prevents in vitro vascular calcification, supporting the concept that matrix vesicle release is critical to the process of hyperphosphatemic vascular calcification.85

Conclusion

As with many clinical disorders, there are multiple causes for calcific vasculopathy, including inflammatory, metabolic, genetic, and epigenetic mechanisms, and these have substantial mechanistic overlap, suggesting that soft tissue calcification may be viewed as a spectrum. Lessons learned from this research in vascular calcification may also contribute to the understanding of a broad range of inflammatory diseases that result in soft tissue calcification.

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Disclosures

None.

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