Vascular Calcification
Mechanisms and Clinical Ramifications
Moeen Abedin, Yin Tintut, Linda L. Demer

Abstract—Vascular calcification, long thought to result from passive degeneration, involves a complex, regulated process of biomineralization resembling osteogenesis. Evidence indicates that proteins controlling bone mineralization are also involved in the regulation of vascular calcification. Artery wall cells grown in culture are induced to become osteogenic by inflammatory and atherogenic stimuli. Furthermore, osteoclast-like cells are found in calcified atherosclerotic plaques, and active resorption of ectopic vascular calcification has been demonstrated. In general, soft tissue calcification arises in areas of chronic inflammation, possibly functioning as a barrier limiting the spread of the inflammatory stimulus. Atherosclerotic calcification may be one example of this process, in which oxidized lipids are the inflammatory stimulus. Calcification is widely used as a clinical indicator of atherosclerosis. It progresses nonlinearly with time, following a sigmoid-shaped curve. The relationship between calcification and clinical events likely relates to mechanical instability introduced by calcified plaque at its interface with softer, noncalcified plaque. In general, as calcification proceeds, interface surface area increases initially, but eventually decreases as plaques coalesce. This phenomenon may account for reports of less calcification in unstable plaque. Vascular calcification is exacerbated in certain clinical entities, including diabetes, menopause, and osteoporosis. Mechanisms linking them must be considered in clinical decisions. For example, treatments for osteoporosis may have unanticipated effects on vascular calcification; the converse also applies. Further understanding of processes governing vascular calcification may yield new therapeutic options for vascular disease. (Arterioscler Thromb Vasc Biol. 2004;24:1-10.)

Key Words: calcification • atherosclerosis • inflammation • bone • vascular

Vascular calcification is an important manifestation of atherosclerosis. For more than half a century, it has been associated with a poor prognosis attributable to vascular disease. Its presence is a strong indicator of chronic inflammatory disease, usually atherosclerosis, and its extent directly relates to the overall burden of atherosclerotic disease. Despite its clinical relevance, research on the mechanism of mineral deposition in arteries has been limited and remains at an early scientific stage relative to research on other aspects of atherosclerosis, such as lipoprotein biochemistry and inflammation.

Mechanisms of Vascular Calcification
Vascular calcification recapitulates embryonic osteogenesis. Pathologists in the 19th century recognized the presence of bone-like tissue within atherosclerotic arteries, with lamellar structure, osteoblast-like cells, and hematopoietic elements. Yet, for most of the 20th century, vascular calcification has been regarded as a passive, unregulated, degenerative process occurring within advanced atherosclerotic plaques. The concept of regulated ossification as the mechanism behind vascular calcification has re-emerged only in the past decade. Ossification has been identified histologically in 60% of restenotic aortic valves after balloon valvuloplasty. Approximately 15% of carotid atherosclerotic plaque specimens and calcified cardiac valve tissue have ossification. Vascular calcification may include both osteogenic and chondrogenic differentiation. In humans, it is primarily osteogenic with bone tissue formation, whereas in mice, it is primarily chondrogenic with cartilage formation. Although osteoblasts and chondroblasts are distinct cell types, they have substantial overlap in mineralization mechanism and gene expression, including alkaline phosphatase, Cbfa-1, and osteopontin. Many key regulators of bone formation and bone structural proteins are expressed in atherosclerotic plaques, including bone morphogenetic protein-2 (BMP-2), osteopontin, matrix γ-carboxyglutamic acid protein (MGP), and osteo-protegerin (OPG). These factors are discussed in this review. Many other bone-related factors are likely to have important roles but are beyond the scope of this review.

Development of Mineral Formation
Some of the earliest evidence that vascular calcification is regulated came from Anderson, who found matrix vesicles
Hyperparathyroidism when treated with smooth muscle cells express bone proteins and mineralize demonstrated by several groups. Primary cultures of vascular cells.16 Subsequently, we and others found expression of osteogenic factors, such as bone morphogenetic protein, osteopontin, and matrix GLA protein in cultured vascular cells.17 In vitro vascular cell calcification has been demonstrated by several groups. Primary cultures of vascular smooth muscle cells express bone proteins and mineralize when treated with β-glycerophosphate,17 which serves as an inorganic phosphate donor in the presence of alkaline phosphatase. This enzyme is expressed by these cells and is used as a marker for osteogenic and chondrogenic differentiation. Primary vascular smooth muscle cells (VSMCs) include subpopulations of cells with different phenotypes. As VSMC cultures undergo osteogenic differentiation, they also lose expression of smooth muscle-specific markers,18 indicating de-differentiation. A subpopulation of VSMC, isolated by dilutional cloning, spontaneously expresses bone proteins and produces a mineralized matrix in culture, similar to that of osteoblasts.9,19 This subpopulation, “calcifying vascular cells,” retains the ability to differentiate into other mesenchymal lineages besides osteoblasts,20 similar to cells of the clonal line C3H10T1/2. Towler et al have shown that developmental homeobox-related transcription factors regulate lineage determination in mesenchymal progenitor cells in vitro, and vascular calcification in vivo.21,22 In stem cell lines in general, cultures become a mix of differentiated and undifferentiated cells, because the parent cells gives rise to daughter cells, some of which are stem cells and some of which are differentiated. Overall, the evidence suggests that mineralized matrix appears to be produced by the osteoblastic daughter cells of a multipotent vascular cell. These early observations establishing in vitro models of vascular calcification have permitted a wide array of investigation into its cellular and molecular mechanisms.

Mineral Resorption
In most regulated biological systems, anabolic processes are accompanied by counteracting catabolic processes. In the case of vascular calcification, the catabolic process would be mineral resorption. Histologically, the bone-like structures within atherosclerotic lesions have sculpted features similar to resorptive and remodeling sites in trabecular bone. In addition, recent molecular and cellular evidence point to an active resorption process and regression in ectopic valvular calcification.23 In bone, mineral is resorbed by osteoclasts, which are large, multinucleated cells with a characteristic ruffled border. They form an actin ring, where the cell tightly apposes to mineralized matrix, allowing a protease-rich, acidic microenvironment necessary for controlled resorption.24 Functional osteoclasts express tartrate-resistant acid phosphatase, cathepsin K, calciitonin receptors, H+ATPase, and carbonic anhydrase II; however, none of these proteins is specific to osteoclasts. The definitive identifying characteristic is formation of resorption pits on mineralized surfaces. In atherosclerotic lesions, multinucleated tartrate-resistant acid phosphatase-positive cells have been observed histologically at the edges of bone-like structures.25 Osteoclasts derive from the monocytic line of hematopoietic cells, and macrophages exposed to particulate calcium mineral have been reported to undergo osteoclastic differentiation.26 Thus, atherosclerotic lesions, being rich in monocytes and macrophages,27 have an abundant source of preosteoclasts. Maturation of preosteoclasts (such as peripheral blood mononuclear cells) to osteoclasts requires 2 cytokines: monocyte colony-stimulating factor and the ligand for receptor activator of NF-κB (RANKL).28 Both are also present in atherosclerotic lesions: monocyte colony-stimulating factor is a well-established component of atherosclerotic plaque,29,30 and RANKL was recently demonstrated in the vasculature.14 At the same time that they participate in resorption, macrophages may contribute to mineral formation indirectly by producing inflammatory cytokines and producing lipid oxidation products that promote vascular cell mineralization. The presence of osteoclasts in the artery wall is controversial. Although cells with osteoclastic features have been demonstrated, functional activity of these cells is difficult to demonstrate or exclude (Table).

Nonatherosclerotic Vascular Calcification
Not all vascular calcification occurs in the presence of atherosclerosis. Metabolic disorders, such as uremia, hyperparathyroidism, and diabetes are associated with development of medial calcification that occurs even in areas without adjacent atherosclerosis. The distinction is based on the fact that medial calcification appears to be amorphous rather than

---

**TABLE. Summary of Apparent Effects of Clinical Factors on Bone and Artery Mineralization**

<table>
<thead>
<tr>
<th>Factor</th>
<th>Bone</th>
<th>Artery</th>
<th>Bone</th>
<th>Artery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estrogen</td>
<td>↑</td>
<td>↑</td>
<td>↓</td>
<td>?</td>
</tr>
<tr>
<td>Bisphosphonates</td>
<td></td>
<td></td>
<td>↓</td>
<td>?</td>
</tr>
<tr>
<td>Diabetes</td>
<td>↓</td>
<td>↑</td>
<td>↑</td>
<td>?</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>↓</td>
<td>↑</td>
<td>↑</td>
<td>?</td>
</tr>
<tr>
<td>Hyperparathyroidism</td>
<td>↓</td>
<td>↑</td>
<td>↑</td>
<td>?</td>
</tr>
<tr>
<td>Uremia</td>
<td>↓</td>
<td>↑</td>
<td>↑</td>
<td>?</td>
</tr>
<tr>
<td>Warfarin*</td>
<td>↓</td>
<td>↑</td>
<td>↑</td>
<td>?</td>
</tr>
</tbody>
</table>

*Includes vitamin K deficiency.
organized bone tissue. However, areas of atherosclerosis-associated calcification can appear amorphous, particularly in early stages. The same conditions associated with medial calcification are also associated with accelerated atherosclerosis, so that many patients with medial calcification also have atherosclerosis. Just as they overlap clinically, they may also overlap in some aspects of mechanism.

**Molecular Determinants**

MGP, first identified as a bone matrix protein, is unusual because of its small size and the post-translational modification of its glutamic acid residues by γ-carboxylation. This modification appears to be important in its function. The role of MGP in calcified atherosclerotic plaque is unclear. MGP is expressed in bone at relatively constant levels through early development and adulthood. It is also found in MGP is expressed in bone at relatively constant levels through early development and adulthood. It is also found in aortic calcification in which the wall of the aorta is replaced by ossification, but it is ultimately overwhelmed.

Homeostatic regulatory mechanism to control and limit mineralization appears to be important in its function. Lack of function (from insufficient modification of its glutamic acid residues by γ-carboxylation) leads to a decrease in cytosolic calcium, a change associated with osteopenia and cartilage metaplasia of the aorta in the MGP null mice. A more satisfactory explanation would be that MGP participates in a homeostatic regulatory mechanism to control and limit mineralization, but it is ultimately overwhelmed.

Interestingly, the MGP knockout mouse has extensive aortic calcification in which the wall of the aorta is replaced with calcified cartilage. A simple conclusion from this model is that MGP directly inhibits calcium mineralization. However, these mice also have a bone phenotype manifest as osteopenia, fractures, short stature, and inappropriate calcification of the growth plate. The simple model would not explain osteopenia and cartilaginous metaplasia of the aorta in the MGP null mice. A more satisfactory explanation would be that MGP regulates mineralization indirectly through effects on differentiation. Recent evidence indicates that MGP inhibits mesenchymal cell differentiation to the osteogenic lineage by sequestering the potent osteogenic and chondrogenic differentiation factor, BMP-2, thus preventing its interaction with receptors. Adding further complexity, the effect of MGP on BMP-2 depends on the degree of MGP γ-carboxylation and the ratio of concentrations of the 2 molecules. Lack of function (from insufficient γ-carboxylation), rather than the amount of MGP may be the factor that increases risk of calcification. Indeed, MGP isolated from calcified atherosclerotic plaque from rats has incomplete γ-carboxylation.

MGP γ-carboxylation is performed by the vitamin K-dependent enzyme, γ-carboxylase. Conditions causing a relative deficiency of functional vitamin K may increase vascular calcification because of incomplete γ-carboxylation and reduced function of MGP. In postmenopausal women, the presence of aortic calcification is associated with lower overall vitamin K intake. Because the medication warfarin interferes with the availability of bioactive vitamin K, it may also interfere with MGP function. Important questions have been raised about its use in hemodialysis patients who are subject to extensive vascular calcification and cardiovascular risk.

**Osteopontin**

Osteopontin is a matrix protein containing an RGD motif that binds to cells via the αv, β3 integrin, which functions as an important calcification inhibitor. Giachelli et al. showed that OPN binds to aortic smooth muscle cells and inhibits aortic valve calcification in vivo, most likely through self-aggregation and adhesion to apatite crystals through specific amino acid moieties. This ability of osteopontin to block growth of crystals may have important physiological and pathological significance.

OPN may also affect calcification by stimulating resorption. OPN binding to αv, β3 integrin on osteoclasts leads to a decrease in cytosolic calcium, a change associated with osteoclast activation to a resorptive phenotype. Exciting new evidence from Steitz et al. suggest that this binding also promotes resorption of ectopic calcification by inducing expression of carbonic anhydrase II, which is key in creating the acidic environment required for resorption. In wild-type mice, there is regression of calcification, concurrent with accumulation of OPN and osteoclast-like cells within transplanted cardiovascular tissue. Recombinant OPN induces resorption of ectopic bone implanted in muscle.

Mice deficient in both OPN and MGP have accelerated aortic calcification compared with mice deficient only in MGP, consistent with the concept that OPN inhibits mineralization. The phenotype of double knockout, OPN−/−, apoE−/− mice varies with gender. The male mice develop increased vascular calcification compared with wild-type and with apoE−/− mice, but no difference in overall aortic atherosclerosis. In contrast, the female double knockout mice have decreased overall aortic atherosclerosis. These findings provide in vivo evidence that atherosclerosis and vascular calcification may have at least some independent regulatory mechanisms.

**Osteoprotegerin**

The recent discovery of OPG, a member of the tumor necrosis factor-α receptor superfamily, introduced another link between bone and vascular metabolism. OPG is an indirect inhibitor of osteoclastogenesis. It functions as a soluble "decoy" receptor that binds and inhibits RANKL. RANKL, activation of its receptor, RANK, is essential for maturation of osteoclast progenitors. OPG is expressed in cultured coronary artery smooth muscle cells, and OPG and RANKL are found in normal arteries. In the rat model, OPG treatment prevents warfarin-induced vascular calcification. OPG transgenic mice lack functional osteoclasts, leading to inadequately trabecularized (osteopetrotic) bone. In contrast, OPG−/− mice have profound osteoporosis with multiple fractures. Interestingly, these mice also have vascular calcification in some arteries. The physiological role of OPG may be dependent on its levels relative to RANKL. RANKL, a tumor necrosis factor superfamily member, has now been shown to promote calcification of cardiac valve myofibro-
blasts. Unopposed RANKL activity may in part explain the medial calcification found in OPG null mice.

Recently, Pritzker et al showed a novel role of OPG in microvascular endothelial cell survival by binding TRAIL and preventing its interaction with death-inducing TRAIL receptors. Recent studies have shown an association between OPG levels and CAD in humans. In patients with cerebrovascular disease, OPG levels are independently associated with cardiovascular mortality but not with bone mineral density. In patients with stable angina, OPG levels are associated with significant coronary artery narrowing. Interestingly, estrogen therapy is associated with increased OPG levels.

Inorganic Phosphate

Phosphate levels were long thought to influence mineralization only through physico-chemical means. However, new evidence indicates that phosphate regulates and coordinates cell signaling and gene expression by dynamic transport processes. Treatment of VSMCs with supplemental inorganic phosphate (Pi) increases expression of bone regulatory proteins including Cbfa-1 and osteocalcin and, simultaneously, downregulates smooth muscle lineage markers. Extracellular phosphate is taken up by VSMC via a sodium-dependent phosphate transporter, Pit-1, and increased intracellular phosphate in VSMC induces mineralization-related genes.

Exogenous addition of proteins to cells in culture, in general, may nonspecifically affect mineralization. By virtue of the charge they carry, added protein may change the free concentration of inorganic phosphate in the media. This, in turn, can alter intracellular phosphate transport, resulting in a change in expression of mineralization-related genes.

The positive effects of inorganic phosphate on calcification are potently inhibited by inorganic pyrophosphate. Extracellular inorganic pyrophosphate, which inhibits hydroxyapatite crystal deposition, is generated by nucleotide pyrophosphate/phosphodiesterase (NPP). NPP1 knockout mice or mice expressing a truncated, inactive isozyme spontaneously develop calcification. Familial idiopathic infantile arterial calcification, a clinical syndrome associated with recessive mutations in the NPP1 gene, is characterized by extensive fibrointimal hyperplasia and premature calcification of the aorta, valvular dysfunction, and cardiomyopathy. This syndrome also includes periarticular calcification similar to that seen in NPP1-deficient mice.

Telediogy of Vascular Osteogenesis

The concept that vascular calcification is related to osteogenesis is not unexpected, given the interaction of vascular and bone cells in normal embryonic skeletogenesis. Bone formation relies on an underlying vascular architecture and scaffold. In embryonal development, endochondral ossification follows invasion of osteoanagenic vessels into calcified cartilage matrix. Once the vasculature is established, preosteoblasts, originating from the angiogenic pericytes surrounding the vasculature or from blood-borne mesenchymal cells, differentiate and initiate mineralization. The requirement for angiogenesis holds true for bone formation in fracture healing in adults as well as in skeletogenesis of the embryo.

Just as vascular calcification is closely associated with chronic inflammatory atherosclerosis, soft tissue calcification is also associated with sites of chronic inflammation. Examples include tuberculosis, hepatic echinococcal infection, foreign body retention, scleroderma, sarcoidosis, pancreatitis, and cancer. The mechanism underlying this association between ectopic ossification and chronic inflammation is not clear. One possibility is that activated immune cells recruited to sites of chronic inflammation typically release cytokines and free radicals, which oxidize lipids present in these sites. These factors may in turn module osteogenic regulatory genes, affecting differentiation of putative mesenchymal progenitor cells. For example, inflammatory cytokines as well as modified lipids and lipoproteins induce osteoblastic differentiation and mineralization of calcifying vascular cells, which were recently shown to be multipotential. In this way, soft tissue and atherosclerotic calcification may be driven by cytokines and modified lipids resulting from chronic inflammation (Figure 1). Genetic models that exhibit vascular calcification in the absence of atherosclerosis remain relevant because they may be caused by direct changes in osteogenic regulatory gene function downstream of the primary affect of atherosclerosis itself.

Clinical Significance

Histopathologically, more extensive calcification is associated with more significant coronary stenosis. Radiographically detected vascular calcification is generally accepted as a sensitive marker for atherosclerosis. The association with clinical outcome was first made with aortic calcification detected on plain radiographs, a relatively insensitive technique. Coronary artery calcification (CAC) can be detected in a more sensitive manner using electron beam and multidetector computed tomography. Current technology allows completion of a high-resolution scan in a few seconds during a single breath-hold. ECG gating can provide similar scans from conventional computed tomography imaging. These commercially available scans provide a total score indicating the extent of calcium detected, measured as “mass” or a calcium “score.” The presence of CAC is sensitive and specific for detection of clinically significant CAD and for identifying patients at risk for adverse cardiac events. The scores correlate with number of artery segments having angiographically significant disease, although not with angiographic stenosis severity. In general, they correlate well with overall atherosclerotic plaque burden.

The American Heart Association and ACC recently published guidelines for the use of coronary artery calcium score as a diagnostic tool for clinical decision-making. The guidelines affirm the sensitivity of coronary calcium for the presence of coronary atherosclerosis. Patients with no detectable coronary calcium have a relatively low risk for subsequent cardiovascular events. Therefore, it has been recommended that CAC be used as a tool to identify asymptomatic patients at low to intermediate risk, particularly the elderly, who may benefit from more aggressive risk factor modification. The guidelines emphasize that CAC should not be used.

The guidelines emphasize that CAC should not be used.
for the diagnosis of obstructive CAD given the poor specificity of CAC for significant flow-limiting coronary plaques.77

CAC may have a different significance than other types of vascular calcification (eg, valve calcification, medial artery calcification). For example, aortic valve calcification, another type of vascular calcification, is a strong independent determinant of clinical progression of aortic stenosis,78 a relationship that holds even in the setting of end-stage renal disease79 in which vascular calcification is often severe. Although not widely appreciated, CAC does not uniformly correlate with mitral valve and aortic valve calcification.80 Thus, clinical significance of vascular calcification depends in part on location.

Controversy exists over the prognostic significance of CAC. In patients with multiple cardiovascular risk factors, CAC adds modest predictive value over traditional risk factors for predicting acute coronary events.81 A consensus has developed that coronary calcification is associated with chronic symptomatic CAD rather than with acute coronary events, which are attributed to plaque rupture and coronary thrombosis. This notion is based on intravascular ultrasound studies, which have shown less extensive coronary calcification in patients with acute coronary syndromes compared with patients with chronic stable angina.82,83 In patients presenting with acute coronary syndromes, intravascular ultrasound studies show less calcification in the event-related coronary lesion than in stable plaques in other vessels.84 These findings have been interpreted as evidence that vascular calcification is protective against acute events. However, its effects on plaque stability may not depend exclusively on the amount of calcium within the plaque.

**Plaque Stability**

It is more likely that risk of plaque rupture caused by calcification is biphasic and dose-dependent, based on mechanical stress considerations. In addition to fluid stresses, the artery wall is subject to pulsatile and steady solid mechanical stresses. Stresses generated by blood pressure are pulsatile. Steady solid stresses are generated by intrinsic and extrinsic tension; arteries are usually stretched beyond rest length in their normal anatomic configuration, generating longitudinal tension even in the absence of blood pressure. Both kinds of stress can be transmitted in all 3 axes, longitudinal, circumferential, and radial. In a theoretical analysis based on anatomic configuration, plaques that had ruptured were predicted to have higher circumferential stress than normal artery segments.85 However, rupture is best predicted by failure analysis that incorporates all 3 components, circumferential, longitudinal, and radial stresses. Failure stress, known as von Mises or maximal principal stress, tends to occur at interfaces between materials of different stiffness. Because calcified atherosclerotic plaque is at least 4- to 5-times more stiff than cellular plaque,86 failure stress would be expected to concentrate at interfaces between calcified and noncalcified regions within an artery.87

As the degree of calcification increases, the number of interfaces between rigid and distensible plaque initially would increase until the point at which the rigid plaques coalesce. Further calcification would result in decreased interface area. Because failure stress concentrates at interface
areas, it follows that risk of rupture increases with the degree of calcification to the point when plaques coalesce (Figure 2). Calcification beyond this point may be associated with decreasing risk of plaque rupture. Ultimately, the most valuable prognostic parameter may be total surface area of mineral deposits rather than calcium score or mass. This hypothesis may be difficult to test epidemiologically because interface area is difficult to measure, and, like the calcium score itself, may not be independent of atherosclerosis severity.

Assessing CAC Progression as a Clinical Endpoint
The dynamics of progression of arterial calcification should be considered in clinical trials in which coronary artery calcium score progression is regarded as a surrogate end point for overall atherosclerotic disease progression. The problem lies in annualization of the CAC progression rate from studies with a short or variable time period between scans (eg, 2 months). Such annualization requires the assumption that calcium mass increases linearly with time. Evidence from Yoon et al suggests the process is nonlinear, and probably sigmoidal (Figure 3). In their study of 217 subjects who had at least 2 electron beam computed tomography scans separated by a mean interval of 25 months, the rate of progression of calcium volume score (calcification pixel area × slice thickness) increased with increasing baseline calcium score.

Studies using coronary calcium measurements must also address interscan reproducibility. Depending on whether the technique includes interslice gaps, difficulty with reproducibility of positioning can introduce variability as high as 18%, even when scans are repeated immediately. Error caused by interscan variability essentially introduces noise into the data, making studies prone to beta error. By using comparable interscan time periods and comparable baseline scores between treated and control groups, trials can prevent bias introduced by nonlinear progression. The recent ACC/American Heart Association guidelines recommend against use of serial coronary calcium measurements to assess progression or regression of coronary atherosclerosis for these reasons.

Links With Other Clinical Factors
Many clinical entities influence biomineralization. Because vascular calcification can include osteogenesis, effects on bone and vessel mineralization must be taken into account when considering treatment that affects mineralization.

Diabetes
Diabetes is clinically associated with vascular calcification and osteopenia. The presence of radiologically detectable arterial calcification is a strong marker of future cardiovascular events in diabetic patients. Although this was initially attributed to medial (rather than intimal) calcification, the distinction was based on continuous versus interrupted x-ray density pattern, which may not be sensitive enough to distinguish the 2 histological types in cases of extensive vascular calcification. At the cellular level, advanced glycation end products associated with diabetes promote mineralization in cultures of microvascular pericytes. As noted earlier, Towler et al showed that in genetically modified mice, induction of type II diabetes activates expression of developmental osteogenic transcription factors and proteins in the ascending aorta. Bidder et al showed that glucose also regulates osteopontin gene expression at the transcriptional level. Although these mice were hyperlipidemic, diabetes in

Figure 2. Relationship between plaque interface area versus total calcium mass. Biomechanical principles suggest the risk of plaque rupture should correlate with interface area, which eventually decreases as calcified plaques begin to coalesce. The corresponding figure insets illustrate how the interface (the circumference around the black area) eventually decreases as calcified areas continue to form and grow.

Figure 3. Coronary artery calcium progression. CAC progression rate increases as a function of baseline CAC volume score. The curve is extrapolated with permission from data published by Yoon et al. The dotted portion of the curve was added to illustrate that the curve is probably sigmoid-shaped, because there is a theoretical upper limit for CAC accumulation.
the absence of hyperlipidemia does induce vascular calcification in a hamster model. CAC progression appears to correlate with glycemic control in type I diabetes, and peripheral vascular calcification relates to the degree of glycemic control in type II diabetes. Interestingly, however, the degree of hyperglycemia does not clearly correlate with the degree of coronary calcification in patients with familial diabetes type II, corresponding with the limited relationship between glycemic control and cardiovascular outcomes.

**Menopause**

Controversy surrounds estrogen and hormone replacement therapy and their effects on cardiovascular disease and vascular calcification. Observational studies suggested estrogen use reduced cardiovascular disease in general and coronary calcification in particular. However, in vitro studies using cultured vascular smooth muscle cells showed that estradiol promoted vascular calcification. This in vitro finding was challenged initially based on the prevailing view that estrogen conferred cardiovascular protection. However, this view was reversed once randomized controlled trials were performed in large numbers, pointing to the hazard of reliance on observational studies, and opening the possibility that vascular calcification contributes to adverse effects. Some clinical studies now confirm higher calcium content in coronary plaques of women using hormone replacement therapy (HRT), whereas other observational studies report reduction in coronary calcification with HRT. Conclusions about the relationship between estrogen and coronary calcification cannot be reliably determined from observational studies. Unfortunately, the only data available now regarding estrogen and CAC are observational. Clinical decision-making with respect to HRT should continue to be guided by results of randomized, prospective clinical trials.

**Osteoporosis**

Osteoporosis, like atherosclerosis, is associated with aging, diabetes, smoking, inactivity, and elevated cholesterol. Osteoporosis and atherosclerosis tend to occur in the same patients and correlate in severity, even after adjustment for age. In a 10-year longitudinal study of 236 women who experienced menopause during follow-up, those with progression of aortic calcification also had significantly greater decreases in bone mineral density compared with those without progression of vascular calcification. The question has been raised whether calcium is "transferred" from bone to artery. However, calcium and phosphate levels in the serum are normally under such tight regulatory control that such a mechanism is unlikely. If transient hypercalcemia were to occur, one would expect diffuse calcium deposition in other tissues, not just the vasculature, as in hypercalcemic metastatic calcification. Moreover, passive deposition alone could not explain the presence of complete bone tissue found in vascular calcification.

Interestingly, bisphosphonate therapy for osteoporosis may affect vascular calcification. These compounds accumulate in the aorta, especially in animals with diet-induced atherosclerosis, reflecting their lipophilic nature and/or their affinity for hydroxyapatite mineral. While enhancing mineral density in skeletal bone, bisphosphonates reduce atherosclerosis in animal models. Bisphosphonates also reduce rat vascular smooth muscle cell proliferation and reduce neointimal formation after carotid balloon injury in rabbits. In a rat model using vitamin D and warfarin treatment to induce vascular calcification, bisphosphonates also inhibit mineralization. It has also been reported that patients receiving bisphosphonate therapy have no significant difference in progression of coronary calcification based on electron beam tomography imaging.

**Summary**

Vascular calcification is a clinical marker for atherosclerosis and may represent a special example of the general phenomenon of soft tissue calcification surrounding chronic inflammatory foci. Subclinical lipid deposition induces a type of "atherosclerosis," a chronic inflammatory response that contributes to the development of mature atheroma. The chronic inflammatory foci, atherosclerotic plaques, contain modified lipids, lipoproteins, and inflammatory cytokines that can regulate osteogenic differentiation of vascular cells. Evidence from clinical observations, animal models, and molecular studies suggest factors that regulate bone cell differentiation and mineralization, including BMP-2, MGP, OPN, OPG, and inorganic pyrophosphate also regulate vascular calcification.

Teleologically, ossification that encompasses inflammatory sites, such as atherosclerotic plaque and tuberculomata, may serve to limit the spread of an infectious or noxious process and physically protect neighboring tissues from injury. However, the process reduces the vascular resilience required for hemodynamic function and introduces high mechanical failure stress in the artery wall. Findings must be interpreted cautiously in light of the complex nature of biomineralization, influenced by multiple interacting factors, many of which remain to be identified. Improved understanding of the mechanism behind vascular calcification will ultimately advance therapeutics that are likely to benefit cardiovascular health.

**References**


Vascular Calcification. Mechanisms and Clinical Ramifications
Moeen Abedin, Yin Tintut and Linda L. Demer

Arterioscler Thromb Vasc Biol. published online May 20, 2004;
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2004 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/early/2004/05/20/01.ATV.0000133194.94939.42.citation

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org/subscriptions/