Vascular Calcification
Pathobiological Mechanisms and Clinical Implications

Rebecca C. Johnson, Jane A. Leopold, Joseph Loscalzo

Abstract—Once thought to result from passive precipitation of calcium and phosphate, it now appears that vascular calcification is a consequence of tightly regulated processes that culminate in organized extracellular matrix deposition by osteoblast-like cells. These cells may be derived from stem cells (circulating or within the vessel wall) or differentiation of existing cells, such as smooth muscle cells (SMCs) or pericytes. Several factors induce this transition, including bone morphogenetic proteins, oxidant stress, high phosphate levels, parathyroid hormone fragments, and vitamin D. Once the osteogenic phenotype is induced, cells gain a distinctive molecular fingerprint, marked by the transcription factor core binding factor α1. Alternatively, loss of inhibitors of mineralization, such as matrix γ-carboxyglutamic acid Gla protein, fetuin, and osteopontin, also contribute to vascular calcification. The normal balance between promotion and inhibition of calcification becomes dysregulated in chronic kidney disease, diabetes mellitus, atherosclerosis, and as a consequence of aging. Once the physiological determinants of calcification are perturbed, calcification may occur at several sites in the cardiovascular system, including the intima and media of vessels and cardiac valves. Here, calcification may occur through overlapping yet distinct molecular mechanisms, each with different clinical ramifications. A variety of imaging techniques are available to visualize vascular calcification, including fluoroscopy, echocardiography, intravascular ultrasound, and electron beam computed tomography. These imaging modalities vary in sensitivity and specificity, as well as clinical application. Through greater understanding of both the mechanism and clinical consequences of vascular calcification, future therapeutic strategies may be more effectively designed and applied. (Circ Res. 2006;99:1044-1059.)

Key Words: vascular calcification ■ cardiac valve calcification ■ vascular smooth muscle cells ■ atherosclerosis ■ imaging

Ectopic calcification has been noted in the vasculature for many decades. Until recently, however, this phenomenon was simply viewed as a passive consequence of aging. Accumulating evidence now points toward a tightly regulated process, with competition between factors promoting calcification and inhibitors of mineralization, but the precise molecular and cellular mechanisms facilitating ectopic mineral deposition are unclear. Additionally, the specific clinical ramifications of vascular calcification remain controversial. In this review, we attempt to explain the current theories of the clinical consequences of cardiovascular calcification, as well as the potential mechanisms underlying its pathobiology.

Mechanisms of Vascular Calcification
In the past 15 years, the prevailing perspective on vascular calcification has evolved. Although it is recognized that ectopic vascular calcification is a consequence of a dysregulated process, the specific molecular etiology remains unclear. It has been shown that cardiovascular calcification recapitulates processes inherent to orthotropic, or skeletal, bone formation. Here, mineralization may proceed by endochondral or intramembranous ossification. Endochondral calcification is characterized by osteoblast-mediated calcification of a cartilage skeleton, whereas intramembranous ossification results from osteoblast-induced calcification of collagen extracellular matrix (ECM) in the absence of a cartilage template.1,2 Herein, we attempt to outline the predominant proposed mechanisms for vascular calcification; these pathobiological mechanisms broadly fall into 2 categories: induction of osteogenesis and loss of inhibitors of mineralization.

Induction of Osteogenesis

Origin of Osteoblast-Type Cells
One major hypothesis of vascular calcification, notably advanced by Demer and colleagues,3,4 is that it is a consequence of active bone formation in situ by osteoblast-type cells. Different hypotheses abound as to the origin of these cells; some studies indicate that the presence of osteoblastic cells in the vascular wall is the end result of phenotypic change of vascular smooth muscle cells (VSMCs), although other ob-
Pericytes have long been suspected to be a subpopulation of smooth muscle cells (SMCs) in the vascular wall, termed “calcifying vascular cells” (CVCs), that spontaneously form nodules and calcify when maintained in long-term culture. These nodules share many properties with bone, including increased alkaline phosphatase (ALP) activity and osteocalcin, osteonectin, and osteopontin (OPN) expression. Other evidence suggests that activation of resident pericytes or circulating stem cells (Figure 1).

The ability to undergo reversible differentiation is characteristic of the VSMC phenotype; these cells are in their differentiated, contractile form at baseline but respond to various stimuli by entering a proliferative, synthetic state to produce ECM. Several stimuli induce VSMCs to undergo osteogenic differentiation, including oxidative stress, bone morphogenetic proteins (BMPs), or changes in pyrophosphate levels, among others to be discussed in more detail below. As the atherosclerotic lesion progresses to a fibrocalcific plaque, certain markers of osteogenic differentiation begin to be expressed, most notably BMP2 and the transcription factors core binding factor-α1 (Cbfa1) and osterix.

Demer and others have established the existence of a subpopulation of smooth muscle cells (SMCs) in the vascular wall, termed “calcifying vascular cells” (CVCs), that spontaneously form nodules and calcify when maintained in long-term culture. These nodules share many properties with bone, including increased alkaline phosphatase (ALP) activity and osteocalcin, osteonectin, and osteopontin (OPN) expression. These cells also have the potential for multiple mesenchymal lineages, including osteoblasts, and represent (by some estimates) 20% to 30% of the total VSMC population.

Other evidence suggests that activation of resident pericytes in the vessel wall contributes to vascular calcification. Pericytes are intimately associated with the endothelium in the microvasculature; their migration represents a crucial step in the later stages of angiogenesis. Pericytes share several phenotypic markers with CVCs, including α-actin, β-actin, and the 3G5 epitope of monoclonal antibody-defined ganglioside antigen. Pericytes have long been suspected to be mesenchymal progenitors (as reviewed by Tilton) and have the potential to develop into osteoblasts and chondrocytes. This potential was first demonstrated by Sato and Urist, who showed the ability of pericytes to differentiate into osteoprogenitor cells in a BMP-stimulated model of skull wound repair. Furthermore, in athymic mice, pericytes implanted in diffusion chambers developed into a variety of skeletal tissues, including bone, cartilage, mineralized cartilage, fibrocartilage, and nonmineralized cartilage-like regions. Like CVCs, pericytes produce large nodules of both cells and ECM when in long-term culture; these nodules contain type I collagen, OPN, matrix Gla protein (MGP), and osteocalcin. Activation of this cell type in the atherosclerotic lesion may, therefore, provide a source of osteoprogenitors in the artery wall.

Role of Angiogenesis

In addition, there are several lines of evidence that implicate neoangiogenesis as a necessary requirement for vascular and valvular calcification. Neovascularization is abundant in areas of lesional calcification. In one study, silicone polymers were injected into fixed human hearts, demonstrating abundant new vessel formation in both the media and neointima of lesions. The degree of angiogenesis observed correlated with the severity of the lesion, and it was found that newly formed vessels proliferated around calcified deposits. With respect to valvular calcification, Mohler et al found neovascularization to be present in all pathological specimens, and, furthermore, the newly formed vessels in these valves were juxtaposed to regions of woven bone.

The mechanism that accounts for the colocalization of angiogenesis and calcification is likely multifactorial as these processes involve the coordinated interplay of vascular endothelial and SMCs, pericytes, circulating and resident osteoprogenitors, as well as osteoblastic cells. As reviewed by Collett and Canfield, cytokines, such as BMP-2 and -4, as well as the angiogenic factor vascular endothelial growth factor (VEGF) stimulate migration and differentiation of osteoblasts. Notably, the role of VEGF is of particular interest. VEGF has been shown to be elaborated by osteoblasts and chondrocytes and VEGF receptors have been localized on these cell types as well as chondrocytes. In this manner, VEGF modulates the function and phenotype of these cells and stimulates chondrocytes activity. Other
mediators of angiogenesis, including bone sialoprotein and OPN, have been shown to be present in fibrocalcific plaques.

Alternatively, it has been suggested that newly formed vessels may simply serve as a conduit to deliver stem and other progenitor cells to new areas of the lesion: circulating mesenchymal precursors may eventually differentiate into osteoblasts in the vessel wall; however, this is likely to represent only a small portion of the contribution of neangiogenesis to vascular and valvular calcification.

Inducers of Osteochondrogenic Phenotype

BMP and Bone Formation

Long known to induce ectopic bone formation, BMPs are members of the largest subclass of the transforming growth factor-β (TGF-β) superfamily and are increasingly being recognized as mediators of vascular calcification; BMP2 and BMP4 have been implicated in both mineralization and local induction of inflammation, whereas BMP7 has been shown to retard vascular calcification. BMP2 is synthesized as a 60-kDa precursor that is processed in the secretory pathway to a small 18-kDa monomer; 2 monomers then associate to form the active homodimer, which binds to its receptor. The BMP receptor is a heterodimer consisting of 2 serine/threonine kinases, types 1 and 2. The heterogeneity of type 1 and type 2 BMP receptors allows for multiple possible heterodimer combinations. Once the BMPR2 and BMPR1 receptors interact through ligand binding, BMPR2 phosphorylates the regulatory Smads. These Smads then modulate target gene expression. However, some effects of BMP2 also appear to be non–receptor mediated. Importantly, the BMP-signaling pathways at work in vascular calcification have yet to be fully elucidated.

In contrast to the actions of BMP2, BMP7 promotes a VSMC phenotype o wing to the induction of p21 and upregulation of Smad 6 and 7. BMP7 has been shown to upregulate expression of α-smooth muscle actin (αSMA), a VSMC phenotypic marker, as well as prevent and reverse transition to an osteoblastic phenotype. In murine models of atherosclerosis-mediated vascular calcification and renal failure, BMP7 treatment abrogated the progression of vascular calcification observed in non–BMP7-treated animals. This finding was associated with a decrease in osteocalcin expression in atheromatous plaques and the vessel wall. It has been proposed that the differential effects of BMP2 and BMP7 reflect diversity in receptor-specific binding properties or activation of downstream signaling pathways.

In fact, BMPR2 mutations have been implicated in familial pulmonary hypertension. Of note, approximately half of the patients with chronic pulmonary hypertension have dystrophic calcification in the pulmonary vasculature on histological examination, even though the pulmonary vessels are not normally subject to calcification. Interestingly, the rare condition of idiopathic infantile arterial calcification and persistent pulmonary hypertension combines these 2 phenotypes. This condition has an etiology of autosomal recessive inheritance, and there is some suggestion that disordered calcium homeostasis is involved. Although further study is needed to define the metabolic error(s) involved, the combination of phenotypes supports the view that dysregulated BMP signaling and vascular calcification may be linked.

BMP2 can drive both osteogenic and chondrogenic differentiation of multipotent mesenchymal progenitors. As atherosclerotic lesions evolve, bone matrix proteins are increasingly expressed; several investigators have found BMP to be expressed by a variety of cells in atherosclerotic lesions, including endothelial cells, foam cells, and SMCs. Additionally, a number of studies have associated some of the common mediators of endothelial dysfunction with increased BMP expression and calcification, including oxidative stress, turbulent blood flow, and hypoxia. Incubation of VSMCs with tumor necrosis factor-α (TNF-α) or oxidized-low density lipoprotein (LDL) significantly increases BMP2 expression.

Cbfal and Osterix

Although BMP2 is clearly a crucial mediator of vascular calcification, its downstream effects are achieved through the upregulation of key osteogenic transcription factors, including Msx2, Cbfal, and osterix. Cbfal, also known as runt-related transcription factor 2 (Runx2) or polonya-enhancer binding protein 2α (Pebp2αA), is a key regulator of osteoblastic differentiation, although for full effect, it is believed to require activation of osterix. Cbfal-null mice lack functional osteoblasts and are unable to produce hypertrophic cartilage or mineralized bone. In humans, mutations in Cbfal cause the autosomal dominant condition of cleidocranial dysplasia, with a phenotype marked by open fontanelles, supernumerary teeth, absent clavicles, and short stature. Just as it is crucial in bone formation, Cbfal expression in VSMCs serves as an early, definitive marker of osteoblastic differentiation, the initial step in vascular calcification. In tissue samples from patients with chronic kidney disease (CKD), Cbfal is selectively expressed in calcified arterial tissue.

Several factors that contribute to vascular calcification have been shown to induce Cbfal expression, including increased inorganic phosphate concentration. Cbfal controls the expression of a number of proteins of osteoblastic differentiation, including osteocalcin, OPN, and type I collagen. There is, however, a poor correlation between Cbfal mRNA or protein levels and the expression of osteoblast-related genes, with Cbfal expression preceding osteocalcin expression and osteoblast differentiation by several days. In several osteoblast cell culture systems, Cbfal protein levels do not correlate well with expression of target genes.

Cbfal-dependent transcription is not simply regulated by the levels of the Cbfal protein but is also regulated by ECM/cell interactions (mediated by integrins). This signal transduction pathway is mediated by the MEK/ERK branch of the mitogen-activated protein kinase (MAPK) pathway. U0126, an inhibitor of extracellular signal-regulated kinase 1/2 (ERK1/2) phosphorylation, rapidly and specifically inhibits ERK phosphorylation and ECM-dependent induction of the osteocalcin gene. BMP signal transduction in osteoblasts also requires ECM interactions, which are blocked by...
U0126.54 Thus, although both BMP2 and Cbfa1 are crucial mediators of osteoblastic differentiation, alone they are insufficient to induce the phenotype.

Instead, it is believed that Cbfa1 requires activation of a downstream transcription factor, osterix, to induce the calcifying phenotype. Osterix is a zinc finger–containing transcription factor that has high sequence homology with members of the SP family of transcription factors.55 In osterix-null mice, bone formation is absent, owing to a lack of bone matrix deposition; cells from these animals do, however, express Cbfa1. In contrast, cells isolated from Cbfa1-null mice do not express osterix, suggesting that this transcription factor resides downstream of Cbfa1.56 This finding was confirmed recently in mesenchymal cells, where it was shown that Cbfa1 binds to and activates the osterix promoter to modulate transcription.57 In this manner, osterix transcripts are direct targets for Cbfa1 and thereby establishes the link between these 2 master genes for calcification.

**Phosphate, PTH, and Vitamin D**

More recently, some of the pathological mechanisms underlying the epidemiological associations with vascular calcification have begun to be defined. Patients with CKD have a disproportionate burden of vascular calcification. Ultrasonographic studies have shown a much higher prevalence of calcified plaques in CKD patients than in age-matched controls.58 One hypothesis accounting for the disproportionate calcification burden in these patients is that high serum phosphate levels contribute to vascular calcification; patients with CKD have elevated serum phosphate as a consequence of both reduced phosphate filtration and secondary hyperparathyroidism. Although it was initially believed that high phosphate concentrations trigger vascular calcification simply by exceeding the calcium-phosphate solubility product, causing precipitation, studies have now shown that high phosphate levels induce VSMCs to differentiate into an osteoblastic phenotype. In cell culture, the addition of high levels of inorganic phosphate to media have been shown to induce mineralization in VSMCs51,59 and increase expression of OPN and ALP.60

The contribution of high phosphate levels to VSMC phenotypic change and increased calcification appears to be dependent on Pit-1, a type III sodium-dependent phosphate cotransporter. Pit-1 may be upregulated in renal failure; rats with induced renal failure that were fed a high-phosphorus, low-calcium diet had a 2.63-fold increase in Pit-1 mRNA levels in aortic VSMCs.61 These transporters modulate osteoblastic differentiation of VSMCs brought on by high phosphate content in serum,59 and Pit-1 directly induces Cbfa1 expression.62 Furthermore, inhibition of Pit-1 expression and activity with small interfering RNA (siRNA) prevents the induction of Cbfa1 and osteocalcin expression that is otherwise noted under high-phosphate growth conditions.63

Phosphate levels alone, however, do not explain the increased incidence of vascular calcification in CKD patients; serum from these patients—dependent of phosphate concentration—induces OPN and ALP expression and calcification.60 Furthermore, Cbfa1 expression can also be induced by serum from uremic patients, again, regardless of the phosphate content.49 The factor(s) promoting vascular calcification in “uremic serum” remains an area of active investigation.

Parathyroid hormone (PTH) plays a crucial role in calcium homeostasis, and, as such, PTH and PTH-related peptide (PTHrP) may function as mediators of pathological calcification, as well. Both PTH and PTHrP prevent VSMC calcification in a dose-dependent manner by inhibiting ALP activity.54 Additionally, PTHrP is secreted from VSMCs, an action that is decreased by calcitriol.65 The development of secondary hyperparathyroidism is a common clinical sequela of CKD. In contrast to its inhibitory role in the vasculature, PTH actively promotes osteoblastic gene expression in bone, and there is some evidence to suggest that protein kinase A, which is activated by PTH-receptor-mediated signaling, can phosphorylate and activate Cbfa1 in osteoblasts.53,66 There are, however, no data as yet linking PTH to Cbfa1 expression in VSMCs. PTH levels are frequently elevated in the secondary hyperparathyroidism that accompanies CKD, and there are also elevated levels of PTH fragments, many of which are nonfunctional competitive inhibitors for the PTH receptor. The coexistence of both ectopic vascular calcification and renal osteodystrophy observed in patients with CKD and their link to the disruption of normal PTH regulation provides a promising avenue for continued study.

Further complicating the clinical management of patients with CKD, both phosphate binders and vitamin D analogs, which are used to prevent renal osteodystrophy (a consequence of secondary hyperparathyroidism), aggravate vascular calcification. In some cases, these therapeutics trigger the highly morbid condition of vascular calciphylaxis. For both main groups of therapeutics, alternative strategies may provide efficacious prevention of osteitis fibrosa without increasing ectopic calcification. Sevelamer is a non–calcium-containing phosphate binder that may offer some therapeutic advantages over traditional calcium-containing phosphate binders. In animal studies, the addition of sevelamer reduced serum phosphate and PTH levels in uremic rats, which was associated with a reduction in aortic calcification and renal osteodystrophy.67 Although sevelamer and calcium carbonate are similarly efficacious at reducing phosphate levels in rats with induced renal failure, sevelamer-treated animals had a decreased calcium-phosphate product and PTH levels, attenuated rates of progression of vascular calcification, and improved renal function compared with the calcium carbonate–treated group.68

In clinical trials, patients with preexisting evidence of coronary artery disease who were initiating hemodialysis and were randomized to treatment with sevelamer exhibited delayed progression of vascular calcification compared with patients randomized to calcium-phosphate binders.69 After 1 year of maintenance hemodialysis, patients treated with sevelamer also demonstrated a significant reduction in total and LDL cholesterol, as well as markers of inflammation.70 In a randomized clinical trial in 200 dialysis patients comparing sevelamer to calcium-based phosphate binders, patients treated with sevelamer were found to have attenuated coronary and aortic calcification by electron beam computed tomography (EBCT). In those patients in whom increased vascular calcification was observed, it was found that they
were more likely to have frequent episodes of hypocalcemia.\textsuperscript{71} The findings of this study have been under debate as patients were treated with 2 types of calcium-based phosphate binders (calcium phosphate and calcium acetate) that are not equivalent in efficacy. In addition, it was noted that the study did not control for other factors such as dialysate calcium levels, vitamin D levels, and lipid profiles, all of which may influence vascular calcification.\textsuperscript{72} Interestingly, a follow-up study found that patients treated with sevelamer were less likely to experience hypercalcemia than calcium salt-treated patients, and there was no progression of vascular calcification on EBCT despite a greater degree of calcification at baseline. In contrast, calcium salt–treated patients demonstrated significant progression of vascular calcification.\textsuperscript{73} Furthermore, treatment with sevelamer was associated with a reduction in skeletal bone loss compared with calcium salt–treated patients.\textsuperscript{74}

Another commonly used treatment for secondary hyperparathyroidism, calcitriol (1,25-dihydroxyvitamin D, the active form of vitamin D) may also exacerbate dystrophic calcification. Vitamin D toxicity is a common animal model used to study vascular calcification.\textsuperscript{75} Calcitriol dose-dependently increases both calcification and ALP activity in VSMCs.\textsuperscript{65} Furthermore, in response to interferon-\gamma, macrophages express 25-hydroxyvitamin D 1\alpha-hydroxylase, the enzyme required to convert 25-hydroxyvitamin D into calcitriol.\textsuperscript{65} Once calcitriol binds to its receptor, signaling through this pathway has pleiotropic effects; the vitamin D receptor influences many genes in the vessel wall, including VEGF, matrix metalloproteinase 9, myosin, and structural proteins, including elastin and type I collagen.\textsuperscript{76–79} These effects on structural protein expression may help to explain some of the effects of calcitriol on vascular calcification. In CKD patients, treatment with calcitriol often results in hypercalcemia and hyperphosphatemia, owing to increased intestinal absorption of calcium and phosphate, which then results in an overall increased risk of soft tissue calcification. In cell culture, VSMCs treated with calcitriol calcify and have increased ALP activity.\textsuperscript{64} Despite these observations, the effects of vitamin D analog therapy on vascular calcification remain controversial. In fact, 2 clinical studies of patients at moderate to high risk for cardiovascular disease found that therapy with active vitamin D analogs was associated with decreased indices of vascular calcification.\textsuperscript{80,81}

Newer treatment modalities include the use analogues of vitamin D that suppress PTH synthesis without increasing serum calcium or phosphate as well as calcimimetic agents that increase the sensitivity of calcium-sensing receptors in the parathyroid glands.\textsuperscript{82,83} A recent retrospective study showed a 16\% survival advantage in CKD patients treated with one of these novel vitamin D analogs, paracalcitriol, over patients treated with calcitriol.\textsuperscript{84} Patients with CKD and secondary hyperparathyroidism treated with the calcimimetic agent AMG 073 achieved up to a 10\% decrease in serum calcium levels and a concomitant decrease in PTH levels, serum phosphorous levels, and the calcium-phosphate product.\textsuperscript{85} These studies suggest that therapy with agents such as sevelamer, paracalcitriol, and calcimimetic agents may help to stave off ectopic calcification in CKD patients, as well as improve survival.

**Warfarin**

Warfarin, a vitamin K antagonist used clinically as an oral anticoagulant for the management of atherothrombotic cardiovascular disorders, promotes calcification by preventing \gamma-carboxylation of MGP, an inhibitor of calcification. As such, warfarin therapy has been implicated in vascular and valvular calcification in experimental animal models, as well as in patients. Rats treated with warfarin developed focal calcification of the elastic lamellae of the aorta and aortic valve after only 2 weeks; after 5 weeks, calcification was evident on radiographs.\textsuperscript{86} In patients treated with warfarin (mean duration 88±113 months), multislice spiral computed tomography (SCT) revealed increased coronary and valvular calcium scores as compared with patients who were not on warfarin therapy.\textsuperscript{87}

**Glucocorticoids**

Glucocorticoids, a class of steroid hormones with antiinflammatory properties, have also been shown to mediate osteoblastic differentiation and, thereby, promote ectopic calcification. Interestingly, long-term glucocorticoid use has associated with osteoporosis; however, in vascular cells, these compounds have been shown to initiate differentiation to an osteochondrogenic phenotype.\textsuperscript{88} For example, VSMCs treated with dexamethasone express a gene program and phenotypic markers consistent with osteoblastic differentiation.\textsuperscript{89} Similarly, pericytes, which reside in the vascular wall, that are exposed to dexamethasone exhibit decreased expression of MGP, OPN, and vascular calcification–associated factor mRNA that results in increased ALP activity and calcium deposition. These studies, therefore, suggest that prolonged glucocorticoid therapy is linked to aberrant vascular calcification.\textsuperscript{88}

**Other Factors Inducing Bone Formation**

**Reactive Oxygen Species**

As described, oxidative stress can stimulate BMP2 and Cbfa1 expression in the vessel; additionally, reactive oxygen species (ROS) signaling can induce other markers of osteoblastic differentiation. In the subpopulation of CVC, the induction of oxidative stress can recapitulate osteogenesis in these cells from their undifferentiated state.\textsuperscript{89} CVCs treated with minimally oxidized LDL,\textsuperscript{40} oxidized 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphorylcholine (ox-PAPC), and the isoprostane 8-isoprostane E\textsubscript{2} all showed a dose-dependent increase in ALP activity. Interestingly, there is some indication that BMP2 signaling may be mediated through ROS formation and signaling. Endothelial cells, when exposed to oxidized LDL, express BMP2.\textsuperscript{44} BMP2 induces cyclooxygenase-2 mRNA expression and prostaglandin production in cultured osteoblasts.\textsuperscript{90} In addition, in endothelial cells, BMP2 increases expression of the noxl subunit of NADPH oxidase, thereby increasing superoxide formation; the proinflammatory downstream gene effects of BMP2 in these cells, including increased intracellular adhesion molecule-1 expression, appear to depend on increased noxl expression and
activity, as confirmed by siRNA inhibition of Nox1 expression. Unpublished data (authors and B. Maron, 2006) from our laboratory indicate that BMP2 induces both oxidant stress and osteochondrogenic differentiation in VSMCs and that the induced phenotypic change is conditional on ROS signaling. The role of ROS formation and signaling in vascular calcification may provide a link between inflammation and vascular calcification, which often colocalize in the atherosclerotic lesion. Indeed, calcific vasculopathy may be a compensatory response to the chronic inflammation of atherosclerosis, akin to the calcification surrounding tuberculoma, carcinomas, helminthic infections, or foreign body inclusions.

**Alkaline Phosphatase**

As mentioned, ALP is a functional phenotypic marker of osteoblasts, and ALP activity is often used as a molecular marker for vascular calcification, as it is an early indicator of ECM deposition. ALP activity is thought to be essential to bone mineralization because a missense mutation in the human tissue nonspecific ALP gene was identified in hypophosphatasia, a rare heritable form of rickets. ALP activity is crucial to hydroxyapatite formation during enchondral ossification; once expressed in the vasculature, ALP is thought to act similarly. Accordingly, medial SMCs in medial calcification express higher levels of ALP, and the classic stimuli of atherosclerotic vascular calcification, including BMP2 and oxidized LDL, increase ALP activity in cultured VSMCs. The mechanism by which ALP modulates vascular calcification is by decreasing levels of inorganic pyrophosphate; pyrophosphate is a substrate for ALP and a recognized potent inhibitor of vascular calcification (discussed below).  

**Leptin**

Additionally, there is evidence to suggest that increased leptin levels may play a role in vascular calcification. Serum leptin levels are elevated both with progressive obesity and in renal failure (as a consequence of reduced leptin filtration). Leptin mediates ectopic calcification by binding to, and activating, its receptors on ventromedial hypothalamic neurons as well as β-adrenergic receptors on osteoblasts. In murine models, modulation of leptin levels influenced bone mass; increasing leptin levels were associated with decreased bone mass, whereas lowering leptin levels had the converse effect. Although these studies revealed the mechanism by which leptin influences orthotopic bone formation, the role of leptin in heterotopic bone formation has not yet been evaluated in these models; however, a number of in vitro studies suggest that leptin, via the mechanisms outlined above, mediates vascular calcification. For example, leptin increases marrow stem cell differentiation into an osteoprogenitor phenotype (as defined by ALP mRNA and protein levels). Furthermore, leptin can also induce osteoblastic differentiation and calcification of CVCs in vitro. Leptin may selectively act on certain VSMCs, perhaps those that are most prone to osteoblastic differentiation; the leptin receptor is expressed by CVC and by certain subpopulations of cells in the mouse artery wall. In aortic endothelial cells, leptin increases oxidative stress, which has been shown to induce BMP2 production by these cells.

**Apoptosis**

Yet another proposed mechanism for vascular calcification is the increased rate of apoptosis of VSMCs in a number of pathological conditions and as a normal consequence of aging. BMPs have been shown to stimulate apoptosis in pulmonary artery SMCs by downregulating expression of Bcl2, an antiapoptotic mediator. Moreover, in vitro inhibition of apoptosis has been shown to inhibit calcification, and, conversely, stimulation of apoptosis increases the rate of calcification 10-fold. Apoptotic bodies of dead foam cell and VSMC debris, in addition to matrix vesicles derived from viable VSMCs and CVCs, may serve to concentrate locally calcium and phosphate, thereby providing a suitable micro-environment for nucleation. Interestingly, not all matrix vesicles are subject to mineralization. Under normal physiological conditions, vesicles released by VSMCs failed to calcify; this occurred only after prolonged exposure to calcium and phosphate. Although the data relating apoptosis and calcification highlight the central role that apoptosis plays in medial calcification, which occurs with high prevalence in the elderly, concomitant phenotypic change of VSMC or stem cell differentiation contributes significantly to the central mechanism underlying atherosclerotic calcification.

**Loss of Inhibition**

The other predominant mechanism by which vascular calcification occurs is through the loss of physiological inhibitors of vascular calcification or their inadequacy. A number of structural and circulating proteins exist that normally inhibit vascular calcification; physiological levels of calcium and phosphate are precariously close to the calcium-phosphate solubility constant in serum, and it is hypothesized that these inhibitors are necessary to prevent soft tissue calcification under basal conditions.

**Inorganic Pyrophosphate**

Inorganic pyrophosphate is a potent inhibitor of vascular calcification and increasingly recognized as a vascular paracrine factor that mediates this process. In addition to serving as a substrate for ALP, pyrophosphate levels are maintained by the activity of ectonucleotide pyrophosphatase/phosphodiesterase I (NPP1) and the cellular pyrophosphate exporter ankyrin (ANK). Deficiency in NPP1 is associated with prevalent vascular calcification in infantile idiopathic arterial calcification, whereas decreased ANK potentiates enchondral vascular calcification. The mechanism by which pyrophosphate modulates vascular calcification is through direct physiochemical inhibition of hydroxyapatite formation and preventing propagation of tissue calcium deposition. More recently, it was found that pyrophosphate also prevents VSMC transition to an osteochondrogenic phenotype. This finding was not associated with an increase in BMP2; however, OPN levels were decreased in NPP1null cultures. Furthermore, in NPP1-null mice, arterial and ligamentous calcification were prevalent. Studies such as these suggest
that restoring pyrophosphate levels by inhibiting ALP, up-regulating vascular NPP1 or ANK-mediated secretion of intracellular pyrophosphate may serve as strategies to limit vascular calcification. In fact, early studies with the bisphosphonate etidronate, a pyrophosphate analog, have shown that this agent decreases the progression of vascular calcification in hemodialysis patients.

MGP, Osteopontin, and Osteoprotegerin
The role of MGP in calcification is complex; MGP has been shown to modulate both cell differentiation and calcification through mechanisms that have not been fully elucidated. MGP functions as a noggin-like protein by inhibiting BMP/BMPR2 interactions as well as by binding BMP2 directly. In this manner, it is the relative ratio of MGP to BMP2 that regulates mineral deposition and osteogenic differentiation; low levels (<1-fold) or high levels (>15-fold) favor calcification, whereas intermediate levels (1 to 15-fold) inhibit this process. MGP requires vitamin K–dependent γ-carboxylation (in the endoplasmic reticulum) to be fully functional. The γ-carboxylated MGP can then undergo a conformational change upon calcium binding that is necessary for the MGP/BMP2 complex to form, which prevents BMP2 interactions. The γ-carboxylated, but not the non–γ-carboxylated, form of MGP is carried in plasma by fetuin, another circulating inhibitor of mineralization. The non–γ-carboxylated form is associated with vascular calcification, whereas the γ-carboxylated form is present in normal vasculature. There are abundant animal data to highlight the role of MGP in normal vascular function. MGP-null mice develop massive arterial calcification, even in the absence of atherosclerosis. In data from humans, the extent of coronary calcification, as determined by EBCT, is correlated with serum MGP levels. MGP is not present in normal blood vessels; however, it is expressed at loci of arterial calcification. These findings probably reflect an attempt by the body to maintain homeostasis. Eventually, this attempt at regulation fails, and the expression of MGP is decreased globally before atherosclerotic or medial calcification occurs. Finally, a weak genetic association between MGP polymorphisms and risk of plaque calcification and myocardial infarction has been demonstrated. In addition to MGP, OPN and osteoprotegerin (OPG) are crucial inhibitors of vascular calcification. For an extended discussion of these inhibitors, see the online data supplement, available at http://circres.ahajournals.org.

Fetuin
In contrast to OPN, OPG, and MGP, which function in the vessel wall, fetuin, also known as α2-Heremans-Schmid glycoprotein (Ahsbg), is a circulating inhibitor of calcification. In vitro, fetuin acts to inhibit de novo formation of hydroxyapatite crystals, but does not affect the crystals once they have formed. Animal data also support the importance of fetuin as mice deficient in this protein develop extensive soft tissue calcifications in the myocardium, kidney, tongue, and skin. Fetuin levels may also have clinical relevance; Ketteler et al showed that lower serum fetuin levels in hemodialysis patients correlate with increased mortality. Furthermore, circulating levels of fetuin-a are increased in patients with calciphylaxis, perhaps indicating a failed attempt to maintain calcium homeostasis.

Smad 6
Finally, other inhibitors specifically influence BMP signaling. Smad 6 is an inhibitor of intracellular BMPR2 signaling. Smad 6-null mice have extensive ectopic calcification of all layers of the vessel wall, and cartilaginous metaplasia in the aortic outflow tract.

The cellular mechanisms underlying vascular calcification continue to be defined. Although competing theories abound, most likely, there is a complex interplay between inhibition and promotion of calcification (Figure 2). It may be that active processes, such as phenotypic change of VSMCs, are aggravated by more passive processes, such as calcium-phosphate precipitation in apoptotic bodies. Ongoing investigation will more clearly define these pathobiological mechanisms, and may eventually yield novel treatment modalities to prevent the progression and adverse clinical sequelae associated with vascular calcification.

Inverse Relationship Between Orthotopic and Heterotopic Bone Formation
It is also worthwhile to note that the skeleton itself plays an active, yet inverse, role in ectopic calcification. For an extended discussion of this topic, see the online data supplement.

Types of Cardiovascular Calcification and Cardiovascular Risk
Clinically, 4 different types of cardiovascular calcification exist: atherosclerotic calcification, medial artery calcification, cardiac valve calcification, and vascular calciphylaxis. These 4 types are the consequence of distinct yet overlapping pathological mechanisms, and they are by no means mutually exclusive of one another. In particular, medial and atherosclerotic calcification occur frequently in concert and contribute synergistically to disease.

Atherosclerotic Calcification
Atherosclerotic calcification occurs at sites of atherosclerotic plaques, where there is a combination of cellular necrosis, inflammation, and cholesterol deposition (Figure 3A). LDL, when oxidized, recruits T cells and macrophages to the lesion. Atherosclerotic calcification forms via a process similar to endochondral ossification; chondrogenesis (in this case, cartilaginous metaplasia) precedes osteoblast induction and lamellar bone formation. As the lesion progresses, osteogenesis is evident and is occasionally quite advanced, with marrow formation visible in some pathological specimens. The expression of bone-related proteins is present as early as the intimal xanthoma stage of lesion progression, and histological evidence of calcification is detectable once a lipid core has formed.

Medial Artery Calcification
In contrast to atherosclerotic calcification, medial artery calcification (MEC) (also known as Mönckeberg’s sclerosis) proceeds through a process similar to matrix vesicle–medi-
ated intramembranous bone formation, with no cartilage intermediate required (Figure 3B). This condition is common in diabetes, CKD, and aging; in elderly patients, the calcification in distal arteries is so pronounced as to be evident on radiographic images, appearing in a characteristic “railroad track” pattern. In fact, this phenomenon is even more evident in patients with diabetes and CKD. Vattikuti and Towler, in their review of vascular calcification in diabetes, argue that MEC is a consequence of stimulation of a migratory population of adventitial myofibroblasts that acquire an osteoblastic phenotype by VSMC elaboration of OPN.

Cardiac Valve Calcification
Cardiac valve calcification is a more amorphous, disorganized process with aspects similar to both medial and atherosclerotic calcification (Figure 3C). The primary stimulus for valvular calcification appears to be the combination of mechanical stress and inflammation in the valve. These calcifying valves initially have macrophage and T-cell infiltrates in response to endothelial injury; BMP2 and BMP4 are then expressed by myofibroblasts and preosteoblasts adjacent to these lymphocytic infiltrates. Furthermore, cardiac valves express markers of osteoblastic differentiation, including Cbfa1 and osteocalcin. These valves also calcify in a manner similar to osteogenesis, with lamellar bone evident in the majority of pathological specimens examined. Congenitally bicuspid aortic valves uniformly show signs of calcification by the time individuals reach the age of 30 years, which may, in part, be attributable to the particular mechanical stressors to which these valves are subjected. Recently, the molecular mechanism underlying...
bicuspid aortic valve calcification was solved. Mutations in the transcriptional regulator NOTCH1 resulted in aortic valve anomalies and severe calcification, owing to impaired repression of the osteoblast stimulator Runx2.137

Calciphylaxis
In contrast to the other types of vascular calcification, vascular calciphylaxis or calcific uremic arteriolopathy (Figure 3D) is a more systemic process characterized by diffuse calcification of the media of small to medium sized arteries and arterioles with intimal proliferation resulting in tissue necrosis. This phenomenon results in widespread subcutaneous soft tissue calcification that occurs when the physiological calcium phosphate solubility threshold is exceeded (>60 mg/dL) and is not dependent on active osteogenic processes. This rare complication of chronic renal failure and secondary hyperparathyroidism is often preceded by either warfarin therapy or significant weight loss; it has also been associated with immunosuppressive drugs, diabetes, obesity, and hypercoagulable states.138,139

Clinical Relevance of Vascular Calcification
Just as there are conflicting theories as to the mechanisms underlying cardiovascular calcification, the pathological and prognostic importance of vascular calcification is also a matter of debate.

Atherosclerotic Calcification
The contribution of atherosclerotic calcification to plaque rupture is undefined. Virmani et al describe a specific type of lesion with a calcified nodule that is prone to rupture; these lesions consist of a fibrous cap disrupted by thrombus and dense, calcific nodules. Virmani et al hypothesize that these plaques may rupture owing to physical stress exerted by the nodules.140 Abedin et al argue that plaque calcification initially destabilizes a plaque; plaques are most prone to rupture at areas of interface between high and low density tissue. Thus, as the plaque begins to calcify, the number of these junctions increases up to a point, after which calcifications begin to coalesce, and the number of interfaces decreases. Abedin et al argue that this process suggests a biphasic risk of plaque rupture as calcification in any particular lesion evolves.2 Yet, other evidence indicates that calcified plaque may be more stable and, thus, less prone to rupture than noncalcified lesions. An intravascular ultrasound study of previously ruptured plaques demonstrated that they contain less-deep calcification than of nonruptured plaques; however, there was no difference in superficial calcification between the 2 groups.141 In addition, focal calcification is a significant contributor to dissection after balloon angioplasty, with a potential relationship between the degree of calcification and the extent of balloon-induced dissection.142 The contribution of focal calcification to plaque vulnerability remains unclear; to stratify risk appropriately, further investigation is required. In future studies, it will be particularly important to distinguish between superficial, focal atherosclerotic calcification and deep, concentric medial calcification.

Medial Artery Calcification
MEC contributes to vascular stiffness, which, in turn, increases pulse-wave velocity to decrease diastolic blood pressure and increase systolic blood pressure. These changes lead to both increased pulse pressure143 and left ventricular hypertrophy,144 factors that are both highly associated with mortality in patients with cardiovascular disease. This potential pathological mechanism is supported by the epidemiological data: medial calcification is strongly correlated with coronary artery disease and future cardiovascular events in patients with CKD,145 and in diabetic subjects,146–148 where higher levels of MEC are a risk factor for amputation.146

Cardiac Valve Calcification
Although the mechanisms are distinct, there are most likely overlapping causes of calcification in both the valve and the vessel; mitral and aortic annual calcification are significantly associated with calcification in the vascular bed, even after adjusting for traditional cardiovascular risk factors,149 possibly reflecting a predilection in certain subpopulations for ectopic calcification. Clinically, cardiac valve calcification is a major mechanism of valve failure (both native and bioprosthetic).150 In addition, patients with mitral annular calcification (MAC) have a higher incidence of stroke, atrial fibrillation, and cardiac death than those without annular calcification. Data from the Framingham Heart Study indicate that MAC carries a relative risk of stroke of 2.10, with risk increasing as calcification worsens151; moreover, Framingham data also associate MAC with increased cardiovascular disease, cardiac death, and all-cause mortality.152

In light of the increased risk of mortality and morbidity associated with valvular calcification, therapeutic strategies to prevent and even reverse this process are particularly appealing. 3-Hydroxy-3-methylglutaryl–coenzyme A (HMG-CoA) reductase inhibitors (statins) are already widely prescribed for their lipid-lowering benefit. A growing body of research, however, indicates that statins may have additional therapeutic advantages in calcific aortic stenosis. Statins have antiinflammatory effects beyond those of lipid-lowering; for example, statins lower serum levels of C-reactive protein independent of LDL reduction.153 Furthermore, in vitro studies demonstrate that statins reduce the secretion of proinflammatory cytokines from endothelial cells154 and macrophages.155 Histological examinations of stenotic heart valves demonstrate T-lymphocyte accumulation, suggesting that, like atherosclerosis, calcific aortic stenosis may be the result of a chronic inflammatory process. In a hypercholesteremic animal model, Rajamannan et al were able to induce Cbfα1, ALP, and OPN expression in cardiac valves; this increase in bone matrix protein expression was attenuated by atorvastatin treatment.156 In human studies, the data have been conflicting. Retrospective studies show a decrease in progression of aortic stenosis in patients receiving statin therapy for hyperlipidemia.157–159 The only prospective, randomized clinical trial to date, however, showed no beneficial effect of high-dose statin therapy on aortic stenosis progression.160 Although this study diminishes the promise of statins as a treatment for aortic stenosis, important distinctions between this negative study and the positive retrospective studies exist; patients were excluded from the prospective study if they required statin therapy for hyper-
cholesterolemia, and patients in the retrospective studies generally received statin treatment for a longer duration. Importantly, these conflicting results question whether the observed therapeutic benefit of statins on aortic stenosis is attributable to “pure” antiinflammatory effects or antiinflammatory effects secondary to LDL reduction. In fact, recent studies have suggested an alternative mechanism; statins limit calcification by inhibiting apoptosis and restoring the prosurvival growth arrest–specific gene 6 (Gas6)/Axl pathway, a member of the vitamin K–dependent protein family. Gas6 activity is dependent on Gla residues, similar to MGP, and γ-carboxylation is necessary for its antiapoptotic actions.161 In human VSMCs, inorganic phosphate-mediated calcification and associated cellular apoptosis was abrogated by treatment with atorvastatin; atorvastatin stabilized Gas6 mRNA and, thereby, restored Gas6/Axl signaling.162 Further investigation is needed to ascertain clearly whether this mechanism is operative in valvular calcification processes.

Calciphylaxis
Although the incidence of calciphylaxis is approximately 1% of patients on hemodialysis, the onset of calciphylaxis represents an extremely ominous development. Patients with calciphylaxis have ischemic necrosis of several tissues, including skin, muscle, and fat. As discussed, newer treatment modalities may help to prevent this extreme form of vascular calcification. Calciphylaxis has a mortality rate ranging from 60% to 80%, with mortality being significantly higher in patients with proximal necrotic skin lesions.163

Imaging Modalities to Detect Vascular Calcification

Chest Roentgenography, Fluoroscopy, and Echocardiography
Only extensive vascular calcification may be detected by radiography (Figure 4A).164 Although fluoroscopy can be used to detect moderate to large foci of calcification, it is unable to detect smaller calcifications. The sensitivity of fluoroscopy, when compared with EBCT, is 52%,165 although, unlike EBCT, specificity is uniformly high.166 Trans-thoracic echocardiography is an excellent tool to detect valvular calcification (Figure 4B).167 Unfortunately, this technique can rarely be used to visualize the coronary arteries and does not allow for objective quantification of calcium.

Intravascular Ultrasound
Intravascular ultrasound (IVUS) detects calcification as intensely echogenic areas within vessels, including within plaques, the intima, media, and adventitia (Figure 4C), although highly fibrotic plaques may occasionally be similarly hyperechoic.168 When used to identify coronary calcium, IVUS has excellent sensitivity and specificity, 90% and 100%, respectively, in one study.169 Although IVUS is highly sensitive and specific for vascular calcification, it is invasive, is semiquantitative, and visualizes only a limited portion of the coronary tree. IVUS is an excellent tool in the armamentarium of the interventional cardiologist168; especially given the increased risk of dissection in plaques with focal calcification, its application in the identification of areas of extensive calcification has particular utility. Its invasive nature, however, precludes its use as a screening test in the general population.

Electron Beam Computed Tomography
EBCT has emerged as a noninvasive, sensitive method to quantify coronary calcium (Figure 4D and 4E).170 Unique among vascular calcification imaging methodologies, EBCT images of the coronary arteries are quantifiable, using the standardized Agatston scoring system to produce coronary artery calcium (CAC) scores.165 Studies have consistently shown increased CAC scores to be positively correlated with atherosclerotic plaque burden.170,171 CAC scores may be an
incremental risk factor in addition to traditional cardiovascular prognostic factors. The Rotterdam Coronary Calcium Score Study, a large population-based study, showed a graded association between CAC score and stroke. The American College of Cardiology (ACC) and American Heart Association (AHA) guidelines acknowledge the sensitivity of CAC to aid diagnosis of coronary atherosclerosis, with a similar predictive value to cardiac stress tests. Although EBCT may have specific utility in identifying asymptomatic patients at low to moderate risk, the ACC/AHA guidelines currently do not recommend EBCT screening in patients owing to the paucity of data relating CAC and cardiovascular risk in the asymptomatic population.

EBCT is, however, limited in that CAC scores may have little to do with luminal obstruction. Indeed, the precise relationship between CAC and cardiovascular disease risk remains unclear; moderate to severe CAC are consistently associated with abnormal stress-testing results, but minimal or no CAC does not preclude abnormal testing. Patients with little or no CAC can and do experience cardiac events. Furthermore, the reliability of EBCT is questionable; relative risks vary widely, and specificity has been disappointingly low.

Another frequent criticism of EBCT as a measurement of CAC and progression of risk is the inherent subjectivity of the scan, even with use of the standardized Agatston score system. One evaluation of EBCT quantification of aortic valve calcification showed a low intra- and interobserver variability of approximately 4%, with reliable interobserver variability. However, reproducibility between successive scans has been poor, ranging from 14% to 51% variability. Furthermore, EBCT is not superior to other noninvasive diagnostic procedures in diagnosing CAD, including exercise stress tests, exercise echocardiography, or exercise perfusion imaging. Physicians should proceed with caution, especially when using this test to screen asymptomatic patients. The clinical significance of positive, and especially negative, results is unfortunately nebulous. At present, a better understanding of the relationship between EBCT and prognosis is needed before its widespread application is advisable.

Spiral Computed Tomography
In contrast to EBCT, spiral, or helical, computed tomography (SCT) with gating has demonstrated value in detecting CAC in patients with CKD. In patients that received a renal transplant or in those undergoing dialysis, SCT offered lower intrareader variability compared with EBCT. Using this technique, investigators have shown that in patients with CAC demonstrated at baseline by SCT, the incidence of cardiovascular events is 3.7 times greater than among those without CAC. Despite these advantages, SCT is associated with increased radiation exposure and decreased temporal resolution that limit its widespread use.

Conclusion
The role of vascular calcification within the greater context of cardiovascular pathology is beginning to be defined. At the molecular level, new insights into the factors regulating mineralization provide greater appreciation of its complexity. Far from a passive process, calcification in the vasculature proceeds similarly to orthotopic bone formation, sharing common regulators, including BMP2, Cbfa1, vitamin D, pyrophosphate, OPN, OPG, and MGP. The seminal event in vascular calcification appears to be the introduction of cells with an osteochondrogenic phenotype. These cells may be derived from a dedicated population of CVC within the vessel wall, or may represent maladaptive changes in the normal, contractile VSMC phenotype. Another attractive hypothesis holds that pericytes, which are associated with angiogenesis, have a propensity toward an osteogenic phenotype. Alternatively, circulating mesenchymal precursors may provide the source of osteoblastic cells. Once osteochondrogenic cells are established, mineralization proceeds within the ECM.

The precise clinical consequences of vascular mineralization remain under investigation. Further, calcification has differing clinical ramifications depending on its cardiovascular location. Atherosclerotic calcification may contribute to balloon dissection and may cause plaque rupture in some cases, whereas medial calcification increases pulse pressure by contributing to total vascular stiffness. Additionally, calcification of cardiac valves is the major cause of valve failure. A number of imaging modalities are available to visualize vascular calcification. In particular, EBCT has gained attention, owing to its relative sensitivity and noninvasive nature. Caution is warranted in the widespread application of EBCT, however, until the proper clinical interpretation of both positive and negative results is defined. Through continued advances in understanding both the molecular mechanisms and clinical consequences of vascular calcification, new therapeutic strategies and screening techniques may eventually be added to our armamentarium against cardiovascular disease.

Acknowledgments
We thank Stephanie Tribuna for expert assistance with manuscript preparation.

Sources of Funding
This work was supported in part by a Stanley J. Sarnoff fellowship grant (to R.C.J.); NIH grants HL081110 and HL04399 and an American Heart Association Grant-in-Aid (to J.A.L.); and NIH grants HL58976, HL61795, PO1 HL55993, and NO1 HV28178 (to J.L.).

Disclosures
None.

References


1056 Circulation Research November 10, 2006


1057

Johnson et al Vascular Calcification


181. Klatt EC. Monckeberg’s medial calcific sclerosis. The Internet Pathology Laboratory for Medical Education. Tallahassee: Florida State University College of Medicine. Available at: http://www.medlib.med.utah.edu/WebPath.


Vascular Calcification: Pathobiological Mechanisms and Clinical Implications: Correction

In the article that appears on page 1044 of the November 10, 2006, issue, an incorrect grant number printed in the Acknowledgments section on page 1054. Grant number P01 HL 55993 is incorrect. The correct grant number is P01 HL 81587. The authors regret this error.

This error has been noted in the online version of the article, which is available at http://circres.ahajournals.org/cgi/content/full/99/10/1044

Reference


DOI: 10.1161/RES.0b013e3181bc3a11