Most individuals aged >60 years have progressively enlarging deposits of calcium mineral in their major arteries.1 This vascular calcification reduces aortic and arterial elastance, which impairs cardiovascular hemodynamics, resulting in substantial morbidity and mortality2-4 in the form of hypertension, aortic stenosis, cardiac hypertrophy, myocardial and lower-limb ischemia, congestive heart failure, and compromised structural integrity.5-7 The severity and extent of mineralization reflect atherosclerotic plaque burden8 and strongly and independently predict cardiovascular morbidity and mortality.9

Previously considered passive and degenerative, vascular calcification is now recognized as a pathobiological process sharing many features with embryonic bone formation. As evidence of this change in paradigm, research on vascular calcification has accelerated dramatically in the past decade. A search of PubMed (www.ncbi.nlm.nih.gov; US National Library of Medicine) under the key words vascular calcification returned ~16 articles in 1982, 100 in 1994, and 250 in 2004. This year, 400 new publications are expected, for a total >3500.

A breakthrough in this field was the recognition of its similarity to bone development and metabolism, in which endothelial, mesenchymal, and hematopoietic cells interact and respond to mechanical, inflammatory, metabolic, and morphogenetic signals governing skeletal mineralization; their counterparts in the artery wall govern arterial mineralization. With increasing age and dysmetabolic conditions in our population, the clinical burden of vascular calcification will continue to increase.

Clinical Impact of Arterial Calcification

Aortic calcification promotes congestive heart failure by eroding compliance and elastance. The hemodynamic demands of the cardiovascular system require that the aorta store energy in its elastance during systole and release it during diastole, which minimizes cardiac work and is the basis for balloon counterpulsation. This function, known as Windkessel physiology, is reflected in the high density of elastin in the arch, where mechanical energy is highest. Its loss is detectable as increased arterial pulse wave velocity in calcified arteries, resulting in thoracic summation of reflected and orthograde pressure waves, thereby increasing systolic and pulse pressures. It also increases cardiac work, promoting heart failure, left ventricular hypertrophy, and diastolic dysfunction, independently of atherosclerosis, aging, or diabetes. The link between aortic rigidity and heart failure is most evident in the hypertensive cardiomyopathy observed in patients with idiopathic infantile arterial calcification and in animal models with aortic banding.

In the aortic valve, calcification produces life-threatening aortic stenosis. Although previously considered a passive, degenerative, untreated disorder of “wear and tear” unrelated to atherosclerosis, recent findings now show that valvular calcification is regulated in a manner similar to that of atherosclerotic calcification and promoted by the systemic, inflammatory milieu characteristic of metabolic syndrome and type 2 diabetes; the molecular “fingerprints” of activated Wnt signaling identified in diabetic medial artery calcification can also be detected in calcifying aortic valves.

In coronary arteries, calcium deposits weaken vasomotor responses10 and alter atherosclerotic plaque stability, depending on the size and distribution of deposits. Lesions associated with unstable angina or infarction tend to have multiple, small calcium deposits, in “spotty” or “speckled” patterns, whereas those in stable angina are associated with few, large calcium deposits.11,12 These clinical findings are in excellent agreement with finite element analyses showing that large deposits reduce circumferential stress in adjacent plaque13 and that small deposits increase stress at their edges.14 These findings are in further agreement with the frequent arterial dissection and rupture in mouse models with aortic vascular calcification.15,16 Thus, vascular calcification introduces compliance mismatch that can promote mechanical failure due to stress concentration at the interfaces of calcium deposits with softer plaque components.

Vascular Calcification Compared With Skeletal Calcification

Eukaryotic life forms evolved in calcium-rich seas, requiring them to evolve mechanisms to prevent widespread calcium crystallization in tissues. In mollusks, mucoproteins control deposition of the calcium carbonate shell. In vertebrates, lipid vesicles and regulatory proteins control crystal formation in skeletal bone, a biocomposite of structured cellular tissue impregnated with calcium phosphate mineral that aligns
with the matrix and with the periodicity of negative charges on collagen. Crystals initially form octacalcium phosphate \([\text{Ca}_8\text{H}_2(\text{PO}_4)_6 \cdot 5 \text{H}_2\text{O}]\), which reorganizes and seeds epitaxial growth of hydroxyapatite \([\text{Ca}_{10}(\text{OH})_2(\text{PO}_4)_6]\), the characteristic mineral of bone. As in bone, vascular apatitic mineral contains carbonate and magnesium impurities. In both bone and arteries, amorphous mineralization precedes mineralized tissue biogenesis, which follows vascular ingrowth and remodeling.

The vertebrate extracellular milieu, an internal sea, has calcium concentrations approaching the solubility product for several salts. Thus, mechanisms evolved to limit nucleation and propagation of calcium deposits in vertebrate soft tissues. Perhaps, then, it is not surprising that actively regulated osteogenic processes concomitantly evolved to locally and judiciously compromise these inhibitory mechanisms. Chief among these is the induction of bone alkaline phosphatase, an ectoenzyme required for vertebrate tissue biomineralization (vide infra).

In bone hydroxyapatite mineralization, crystals nucleate and propagate as they do in inorganic crystal formation from supersaturated solutions. In biomineralization, however, organic constituents finely tune the rate of progression (Figure 1), limiting nucleation to sites on collagen fibers and matrix vesicles. These matrix vesicles are phospholipid-bound nanoparticles in which crystals are organized by phosphatidyl serine, annexins, bone sialoproteins, and crucial ectoenzymes. These ectoenzymes act on inorganic pyrophosphate, which inhibits crystal propagation and stimulates production of osteopontin, an inhibitor of nucleation.\(^{17,18}\) These osteogenic activities distinguish matrix vesicles from the apoptotic bodies that nucleate dystrophic calcification during tissue necrosis. Alkaline phosphatase, a prominent constituent of matrix vesicles, locally degrades inorganic pyrophosphate as a necessary step to permit vertebrate biomineralization. Thus, alkaline phosphatase activity is a key factor and useful marker for active osteochondrogenesis.

In arterial calcification, these same processes are called to action; matrix vesicles are found in both medial and intimal calcium deposits.\(^{19}\) Cultured vascular smooth muscle cells elaborate matrix vesicles, which can promote or inhibit mineralization depending on their content of inhibitory factors, matrix \(\gamma\)-carboxyglutamic acid protein (MGP), and fetuin.\(^{20}\) Calcified arteries and cultured vascular cells also express bone matrix proteins and regulatory factors including bone morphogenetic protein-2 (BMP-2), osteopontin, MGP, bone sialoprotein, osteonec in, collagen I, and osteocalcin.\(^{21–25}\)

Cells that spontaneously produce mineralized matrix and undergo osteoblastic differentiation have been isolated from vascular tissue and identified as (1) pericytes in microvessels, (2) pericyte-like, calcifying vascular cells in the aortic intima, (3) smooth muscle cells in the media, and (4) myofibroblasts in the adventitia.\(^{21,26,27}\) All of these cell types are closely related and may be variant phenotypes of one another.\(^{28}\) Three have multilineage potential including osteogenic, chondrogenic, adipogenic, leiomyogenic, and marrow stromogenic potential.\(^{29–32}\) It is not known whether these cells originate in the artery wall or immigrate from near (adventitial) or remote (marrow) sites or whether they result from osteogenic transdifferentiation of mature vascular smooth muscle cells, osteoblastic redifferentiation of dedifferentiated smooth muscle cells, or primary osteochondrogenic differentiation of vascular

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**Figure 1.** Schematic representation of selected regulatory factors and their possible roles in vascular biomineralization. Inorganic phosphate (P) translocates via Pit-1 to the cytoplasm. Cytoplasmic Ca\(^{2+}\) and P incorporate with alkaline phosphatase (ALP) into matrix vesicles, which bud off the plasma membrane and associate with extracellular proteins, such as collagen. NPP1 generates the mineralization inhibitor pyrophosphate (PPi), which is inhibited by alkaline phosphatase. Some important factors have not been shown for clarity. Dashed arrows indicate translocation; solid-line arrows, induction; and solid-line bars, inhibition. BSP indicates bone sialoprotein; OPG, osteoprotegerin; OPN, osteopontin; OCN, osteocalcin; PKA, protein kinase A; and ROS, reactive oxygen species.
mesenchymal stem cells or vascular pericytes. In vivo, tissues corresponding to these lineages form in the artery wall: bone, cartilage, fat, and even marrow. The predominant form of metaplasia in human vasculature is bone, which is found in 5% to 15% of specimens. Transitional stages between amorphous calcification and mature bone tissue are also seen, the latter apparently requiring microvascular invasion as in skeletal bone. Although less common than bone, ectopic cartilage also occurs in human vascular calcification, and it is the predominant form of ectopic mineralized tissue in mice, especially in the brachiocephalic arteries of apolipoprotein E (apoE)-null mice. Thus, by structural, molecular, and cellular criteria, vascular calcification proceeds via active osteochondrogenic processes. This applies regardless of whether mature bone tissue is detectable, even when the deposits are amorphous, such as in medallial calcification of type 2 diabetes mellitus. This nonatherosclerotic process also follows osteochondrogenic mechanisms but advances to ossification less often, possibly because of slower angiogenic invasion or the greater abundance of elastin, which maintains smooth muscle cell phenotype. Thus, both intimal and medial calcifications appear to be driven by osteochondrogenic molecular programs.

As in skeletal tissue, remodeling and regression may also occur in vascular calcium deposits, although not as rapidly as for other plaque components. By radiographic criteria, lipid-lowering treatment reduces progression of coronary and valvular calcification. In 2 rat models of medial artery calcification, one elicited by vitamin D (calcitriol) intoxication and the other by warfarin administration, regression has been observed. In the former, it was associated with monocytes/macrophages, which are closely related to bone-resorbing osteoclasts. This is in excellent agreement with an elegant study performed by Giachelli and colleagues showing mineral deposition and matrix acidification in ectopic valve leaflet allografts in mice. In the warfarin model, high-dose vitamin K supplementation induced regression and restored vascular compliance. Whether ossified lesions regress is unclear, such a process may require osteoclast-like cells, the monocyte-derived cells responsible for skeletal bone resorption. Such cells have been identified in arterial ossification. The potential for cell-mediated regression therapy based on osteoclast induction targeted to the vasculature remains to be explored fully.

Major Categories of Arterial Calcification
Arterial calcification has been usefully categorized by histologic and etiologic criteria (Table). Histologically, the deposits may have osteomorphic, chondromorphic, or amorphous structure. Etiologically, they may be categorized as metastatic, in which diffuse tissue calcification arises from systemically high calcium/phosphate products, or dystrophic, which is pathological but not metastatic. Anatomically, it may be intimal atherosclerotic calcification, which occurs in a patchy pattern, or it may be arterial medial calcification, which is more diffuse and independent of atherosclerosis; in the arteriolar vessels, it is known as calcific uremic arteriolopathy or, previously, calciphylaxis. Because medial and intimal layers are in close proximity, noninvasive measures of vascular calcification generally do not distinguish them.

### Intimal Atherosclerotic Calcification
Atherosclerotic calcification, the most common form of calcific vasculopathy, appears to result from induction of osteogenic differentiation in subpopulations of vascular cells by inflammatory factors, such as modified lipoproteins and cytokines, that are found in atheromatous components of plaque. Most, but not all, clinical studies link dyslipidemia with the presence, severity, and progression of vascular calcification, particularly when the duration of exposure to cholesterol is taken into account as “cholesterol-years.” Given the relevance of exposure duration, the association may be masked in cross-sectional studies that include patients on lipid-lowering agents whose current lipid level may not reflect level of exposure prior decades. Hyperlipidemia is known to promote calcification in mice. In vitro, 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors reduce vascular cell calcification via Gas-6/Axl signaling, and, in some clinical studies, they reduced progression of atherosclerotic calcification. However, in recent randomized trials, statins did not affect severity or progression.

The findings of BMP-2 and osteopontin expression in human atherosclerotic plaque provided the first molecular evidence for an osteogenic signaling mechanism. Atherogenic stimuli, such as inflammatory cytokines, oxidized lipids, and monocyte-macrophage products, promote osteogenesis and matrix calcification in vascular cell culture. High glucose levels also activate osteogenic programs, reflected in Runx2/Cbfa1-dependent alkaline phosphatase expression. Both spontaneous and induced osteoblastic differentiation of vascular cells are regulated by the cAMP pathway, Msx-2, and the Wnt signaling pathway. Oxidant stress promotes in vitro vascular cell calcification and antioxidant factors, such as α-lipoic acid and high-density lipoprotein, inhibit in vitro vascular cell calcification. In an important recent study, Aikawa and colleagues used near-infrared fluorescence imaging to explore the role of osteoclasts in calcification.
imaging to show that atherosclerotic mineralization is linked with inflammation at its earliest stages.

Given the central role of inflammation in atherogenesis, an exciting possibility is that vascular mineral itself may initiate, promote, or perpetuate atherosclerosis by inducing inflammatory cytokines in monocytes that encounter and ingest hydroxyapatite crystals.69

Murine models of atherosclerotic calcification have been developed. Several genetically distinct inbred mouse strains, including C57BL/6, Balb/C, C3H/HeJ, DBA/2J, SM/J, and MRL-lpr/lpr mice, develop spontaneous vascular calcification that increases with a high-fat/high-cholesterol diet.52,70 The occurrence of artery wall calcification differed among the strains, indicating for the first time a genetic component in the phenotype.

Vascular calcification occurs spontaneously in genetically modified mice such as apoE-deficient mice, which were recently shown to have marked cartilaginous metaplasia in the brachiocephalic artery.38,71 In one of the first models, Towler and colleagues72 demonstrated that vascular calcification occurs in response to a high-fat, diabetogenic diet in low-density lipoprotein (LDL) receptor (Ldlr)–deficient mice. In the apoE-deficient model, the calcification is accentuated by osteopontin deficiency.73,74 The Klotho mouse also develops atherosclerosis and calcification,75 but the calcification appears to be medial and is driven by hyperphosphatemia (vide infra).76

Calcifying vascular cells have served as a robust in vitro model developed on the basis of the work of Canfield and colleagues.26 Calcifying vascular cells consist of bovine aortic smooth muscle cells purified by dilutional cloning and selected for their capacity for forming nodules. These cells have many features of microvascular pericytes and are distinguished from conventional smooth muscle cells by a surface ganglioside found on pericytes. Unlike cultures of skeletal osteoblasts, which remain in monolayer, mineralize more diffusely, and require ascorbate and phosphate supplements, calcifying vascular cells spontaneously produce mineral, often in multicellular nodules that grossly and histologically resemble atherosclerotic plaque and aortic valve nodules (Figure 2). The patterns formed by these nodules in cultures of calcifying vascular cells are determined by a specific molecular mechanism. Several days after uniform plating, calcified nodules arise in a pattern of spots or ridges ~500 μm in diameter. The spatial frequency increases with transforming growth factor-β1 or 25-hydroxycholesterol treatments.77 The nodular pattern is abolished in the absence of apolipoprotein J (clusterin)78 or in the presence of forskolin.59 Importantly, the type of pattern formed by the nodules—diffuse, spotty, striped, or even labyrinthine—is determined by a well-defined "reaction-diffusion" mechanism governed by interaction between the known morphogen BMP-2 and its inhibitor, MGP. A mathematical model of this mechanism successfully predicts that warfarin, an MGP inhibitor, would double the spatial frequency of the calcium deposits.79 Given the widespread use of warfarin in patients with atrial fibrillation and the critical importance of interface area of "spotty" versus diffuse calcification in determining risk of unstable angina and myocardial infarction, this regulatory mechanism controlling the calcification pattern may have substantial clinical importance.

**Valvular Calcific Aortic Stenosis**

Calcification of the aortic valve (recently reviewed by O’Brien80) is increasingly common and carries a high mortality in the setting of advanced age, congestive heart failure, and end-stage renal disease, in which mechanical stress interacts with metabolic (mineral, diabetic, dyslipemic) and inflammatory disturbances. In seminal studies of human sclerotic aortic valves, Otto et al81 demonstrated elastin displacement, lipid accumulation, chronic inflammation, stippled calcification, and high levels of osteopontin. The cellular basis for calcific aortic stenosis was clarified by Mohler et al82 by isolating calcifying valvular cells from aortic valve tissue and, in a survey of human valve specimens, demonstrating inflammation, expression of BMP, and, importantly, mature bone tissue in >10%.47

Active osteochondrogenic differentiation and signaling were demonstrated by Rajamannan and colleagues83 in human valves, in cultured valvular cells, and in hypercholesterolemic rabbits. Using the rabbit model to test the role of inflammatory lipids, her group found that lipid-lowering agents reduced the valvular calcification.84 As evidence for osteochondrogenic signaling, they identified expression of Wnt-3a and LDLR-related protein (LRP5)–dependent activation of the canonical Wnt signaling cascade, including β-catenin accumulation in the nucleus, which serves a critical coactivator role for LEF1/TCF7 and Smad transcription.85 Indeed, β-catenin is indispensable for osteoblast development, genetically epistatic to the better-appreciated osteoblast transcriptional regulators Runx2 and Osx.
The first robust murine models of calcific aortic stenosis are making their debut. In apoe\(^{-/-}\) and ldlr\(^{-/-}\) mice, as well as mice on high-fat/high-carbohydrate diets, aortic valve leaflets have osteogenic activity.\(^{86,87}\) In an important development, ldlr\(^{-/-}\) mice engineered to express only apoB100 were shown to develop hemodynamically significant transvalvular flow gradients and thus true aortic stenosis.\(^{88}\)

Calcium deposits are also associated with the mitral valve but primarily as cartilaginous metaplasia in the annulus. This mitral anular calcification is often detected echocardiographically, and it correlates inversely with fetuin A levels\(^{89}\) and positively with atherosclerosis and cardiovascular mortality, independently of ejection fraction and coronary artery disease severity.\(^{90}\) It may have value as a clinical marker of disease. The reason one finds osteogenesis in the aortic valve leaflets, chondrogenesis in the mitral annulus, and neither in the mitral leaflets is unknown but is possibly due to differences in mechanical stress or in embryonic derivation. The aortic valve derives from embryonic neural crest cells, which are regulated by Wnt signaling. This ontogenetic history of aortic valve cells may influence their responses to mechanical and metabolic stress and account for differences between the 2 types of valves.

**Arterial Medial Calcification**

A comprehensive and insightful review of this topic has been provided by Towler.\(^{91}\) The most extensive vascular calcification is found in patients with arterial medial calcification, a highly characteristic feature of type 2 diabetes mellitus\(^{92}\) and chronic kidney disease.\(^{93}\) Arterial medial calcification was once considered benign because it was neither stenotic nor thrombogenic. It is now recognized that arterial medial calcification is associated with higher cardiovascular mortality and risk of amputation in type 2 diabetes mellitus\(^{94,95}\) and in end-stage kidney disease.\(^{96}\) There is growing evidence for heterogeneous mechanisms within the category of medial calcification. For example, hydroxyapatite is the predominant feature of aortic calcification in Marfan syndrome.\(^{117}\) Be-nign osteosarcoma, is a severely morbid and life-threatening form of chronic renal failure.\(^{97}\)

In the setting of renal insufficiency (reviewed recently by El-Abbadi and Giachelli\(^{98}\), Runx2/Cbfa1-dependent osteochondrogenic processes figure prominently, enhanced by episodic excesses in serum phosphate and calcium. Recognizing that cultured osteoblasts mineralize in response to phosphate supplements and recognizing the association of hyperphosphatemia and vascular calcification in patients with chronic renal failure, Giachelli and colleagues showed that inorganic phosphate promotes osteogenic differentiation in vascular smooth muscle cells through induction of a sodium-dependent phosphate transporter (Pit-1) and subsequent induction of Runx2/Cbfa1 and downstream osteogenic programs.\(^{99}\) Pit-1 is induced by BMP-2\(^{100}\) and suppressed by phosphonofumaric acid, which also significantly reduces smooth muscle cell calcification.\(^{101}\)

In rat models of renal failure, mature cartilage tissue and major chondrogenic factors were found in the vessels.\(^{102}\) In such models, phosphate sequestration can ameliorate vascular calcification without suppressing parathyroid hormone (PTH) levels and bone formation.\(^{103}\) Thus, interventions reducing phosphate and Pit-1 phosphate transport may help to retard the progressive arterial calcium burden in patients with chronic renal failure.

In seminal work addressing the context of type 2 diabetes, the Towler group has established that BMP-2/Mxs2/Wnt signaling, which is entrained to inflammatory redox status, figures prominently in the early stages of medial calcification.\(^{104}\) This was recently shown to occur independently of Runx2/Cbfa1.\(^{105}\) They showed that male ldlr\(^{-/-}\) mice on a Western diet develop hypercholesterolemia, type 2 diabetes, and hypertriglyceride-mia, features characteristic of the metabolic syndrome–diabetes continuum, high circulating markers of inflammation such as tumor necrosis factor-\(\alpha\), haptoglobin, and hemopexin, and concomitant valvular and medial calcification.\(^{72}\) Calcification was also demonstrated in ldlr\(^{-/-}\)-apoB100/100 mice rendered diabetic by overexpression of insulin-like growth factor-2.\(^{106}\) Procalcific BMP-2/Mxs2/Wnt signaling processes characteristic of intramembranous bone formation are upregulated in response to diet-induced obesity, inflammation, and a dysmetabolic milieu.\(^{105}\) In concert with signals arising from the macrophage, tumor necrosis factor-\(\alpha\) has emerged as a key stimulus for osteogenic differentiation in vascular cells.\(^{62}\) As further evidence of biological heterogeneity in medial calcification, patients with diabetes exhibit more severe vascular calcification at every stage of declining renal function,\(^{107}\) and the association with hyperphosphatemia is not significant in diabetic patients,\(^{108}\) suggesting the presence of nonredundant mechanisms. Altogether, it appears that different but overlapping mechanisms guide medial calcification in vitamin D toxicity, chronic kidney disease, and diabetes.

Elastin degradation appears early in many forms of medial calcification. Several investigators\(^{109–114}\) have shown that elastin metabolites can activate, and even nucleate, cell-dependent calcium deposition.\(^{115}\) Matrix metalloproteinase-9, an elastase expressed by the injured vessel wall, appears to promote arterial calcium deposition in warfarin/vitamin K models of medial calcification.\(^{116}\) Elastin degradation is a prominent feature of aortic calcification in Marfan syndrome.\(^{117}\) Because an intact elastin matrix stabilizes the vascular smooth muscle cell phenotype in vivo, changes in osteopontin expression and matrix metalloproteinase-9–dependent elastin degradation may contribute significantly to medial calcification in diabetes.

In aging, medial calcification may develop by a distinct process of unknown etiology or result from a confluence of specific processes. Aging is associated with mild degrees of several processes believed to affect vascular calcification, including chronic renal insufficiency, insulin resistance, atherosclerosis, hormonal depletion, elastolysis, and slowly manifesting genetic vulnerabilities. Any 1 or a combination of these may contribute to medial calcification in aging.

**Calcific Uremic Arteriolopathy**

Calcific uremic arteriolopathy (CUA), formerly known as calciphylaxis, is a severely morbid and life-threatening form of medial vascular calcification that leads to cutaneous necrosis and panniculitis. CUA afflicts patients with advanced chronic kidney disease, especially those receiving warfarin. Skin nodules and painful ulcers progress to black eschar and
demarcating cutaneous necrosis. CUA is an active vasculopathy characterized by patchy medial calcification of arterioles (≤0.6 mm in diameter), with intimal proliferation, thrombotic occlusion, fibrosis, and adipose inflammation and necrosis. In the dermis, adjacent subcutaneous fat necrosis also seeds calcium deposition. Mesenteric and pulmonary tissues may be involved, and mortality approaches 100% within 2 years of disease initiation. Immunohistochemistry has revealed the expression of BMP-4 in these lesions. Serum markers of systemic inflammation, including erythrocyte sedimentation rate and C-reactive protein, are markedly elevated.

An important consideration for clinical cardiology and nephrology is the possible link between warfarin and vascular calcification. In patients, warfarin is associated with vascular calcification and calcific aortic stenosis. Warfarin may affect calcification by blocking MGP and other γ-carboxylated proteins that regulate mineralization, such as osteocalcin and Gas-6. MGP is believed to inhibit mineralization by 2 mechanisms: directly as part of a complex with fetuin and indirectly by inhibiting BMP-2–induced osteogenic differentiation. To be fully functional, MGP requires posttranslational modification by γ-carboxylation, a vitamin K–dependent process that is inhibited by warfarin. Thus, a high dose of vitamin K reverses warfarin-induced vascular calcium deposition in animal models. The contributions of warfarin and Gla protein deficiency to the pathogenesis of CUA have yet to be determined.

For this devastating, but fortunately uncommon, disease, only anecdotal reports are available to guide therapy. Parathyroidectomy has been used on the basis of the potentially false impression that CUA pathobiology follows that of medial artery calcification of uremia. Traditional strategies for nonhealing ulcers, including hyperbaric oxygen, have had only modest success. Sodium thiosulfate infusion, a reducing agent that restores cellular glutathione and mobilizes amorphous calcium phosphate and brushite, has shown promise. Further research is needed to devise strategies to address this tragic disorder.

The Bone-Vascular Axis and Mineral Regulators

Epidemiological and preclinical studies have shown both parallel and reciprocal changes in arterial versus skeletal mineralization. Whereas inflammatory lipids and cytokines appear to promote vascular calcification but inhibit bone mineralization, some osteoanabolic agents, such as PTH and BMP-7, promote mineralization in the skeleton but suppress it in arteries.

The clinical association of aortic calcification with osteoporosis, often age independent, suggests a link between vascular and bone metabolism. Three causality vectors may apply: (1) vascular calcification promoting bone loss, (2) bone loss promoting vascular calcification, or (3) a common etiology. The first possibility is largely unexplored, although bone loss may be promoted by stenoses of bone supply arteries or by systemic inflammation associated with atherosclerosis. The second possibility has more supportive evidence. Bone mass is rich in regulatory factors that are also active in the vasculature, such as osteopontin, MGP, and products of the transforming growth factor-β gene superfamily. During resorption, these are released into the circulation. As evidence for their role in vascular calcification, agents that block bone resorption in animal models also block vascular calcification, however, high resorative activity is not always required because vascular calcification also occurs in conditions of low bone turnover. The third potential mechanism, a causal factor shared by vascular calcification and osteoporosis, is supported by the many risk factors common to the 2 disorders, including aging, estrogen deficiency, vitamin D and K abnormalities, dyslipidemia, hyperparathyroidism, chronic inflammation, hyperhomocysteinemia, and oxidative stress.

In renal insufficiency, synergism between hyperphosphatemia, hyperparathyroidism, calcitriol, calcium carbonate, warfarin, low fetuin, hypertension, and atherosclerosis has given it recognition as the perfect storm for vascular calcification and osteopenia. Hyperphosphatemia is associated with increased aortic calcification through Pit-1 osteoduction. Secondary hyperparathyroidism often accompanies renal failure, but it can induce aortic medial calcification independently of uremia and renal function. Importantly, however, Shao et al showed that intermittent PTH treatment has the opposite effect of continuous hyperparathyroidism, and it suppresses vascular calcification without changes in serum phosphorus. Calcitriol (1,25-dihydroxyvitamin D) has historically been used to control secondary hyperparathyroidism in these patients. Calcium carbonate is used to bind enteric phosphorus and reduce hyperphosphatemia, but it also increases serum calcium and suppresses PTH levels. In the setting of chronic kidney disease, oral calcium carbonate “paradoxically” suppresses bone formation and promotes vascular calcification, and low PTH levels correspond to low-turnover osteoporosis and severe arterial calcification.

Some possible contributors to bone-vascular interactions include osteopontin, fibroblast growth factor (FGF)-23, phosphate/PTH, and vitamin D. Osteopontin release from bone may represent a major component of the bone-vascular axis. PTH-induced bone formation increases circulating levels of intact osteopontin, a potent inducible inhibitor of vascular calcification. FGF-23 was recently identified as a circulating factor released by bone in response to high phosphate levels. It acts on the kidney to reduce phosphate resorption. Inappropriately low FGF-23 levels are associated with hyperphosphatemia and, in hemodialysis patients, with vascular calcification. Interestingly, a coreceptor for FGF-23 receptor binding is Klotho. For years, the vascular calcification and osteoporosis phenotypes of the Klotho mouse were attributed to a premature aging syndrome, but the Klotho phenotype has been reproduced in the FGF-23−/− mouse, suggesting that the vascular phenotype may be mediated by hyperphosphatemia instead. Thus, perturbations of the FGF-23/Klotho/FGF receptor–signaling axis represent additional critical components of the bone-vascular axis.

Not all regulatory systems active in both bone and artery are necessarily part of the bone-vascular axis. For example, members of the osteoprotegerin/RANKL/receptor activator of nuclear factor κ-B system may operate simultaneously, but independently, in the 2 tissues. Osteoprotegerin is protective against osteoporosis because it acts as a decoy receptor for the
pro-osteoclastic factor receptor activator of nuclear factor-κB ligand (RANKL). In humans, osteoprotegerin levels are positively associated with vascular calcification. However, in direct contrast, mice with osteoprotegerin deficiency develop vascular calcification. The possibility that the clinical correlation represents an incomplete compensatory response is supported by findings that direct osteoprotegerin treatment reduced vascular calcification in a rat model of vitamin D toxicity and in a mouse model of hyperlipidemia. Given the known immunomodulatory function of RANKL, a working model emerges. In bone, osteoprotegerin may hold in check the proresorptive effects of RANKL, whereas, in the artery, osteoprotegerin may hold in check the inflammatory effects of RANKL. In support of this, osteoprotegerin-deficient mice have T-lymphocyte infiltration in their calcified arteries. T cells are associated with valvular calcification in humans, and RANKL has been found in CD3-positive and F4/80-positive cells at the adventitial-medial junction in an atherosclerotic mouse model. Furthermore, ectopic mineralization induced by BMP-2 treatment is not accelerated by the high-turnover state of osteoprotegerin deficiency. The importance of local RANKL/osteoprotegerin signaling was highlighted by the finding that postnatal treatment with osteoprotegerin failed to reverse the vascular calcification phenotype. Thus, rather than serving as the foundation of the bone-vascular axis, RANKL/osteoprotegerin interactions may reflect tissue-specific immunomodulation of osteoprotegerin expressed in response to mechanical, endocrine, and inflammatory cues. The roles of other vascular cells that produce osteoprotegerin and RANKL, such as endothelial cells, have yet to be explored.

Similarly, bisphosphonates may also act directly on arteries, independently of their effects on bone resorption. These agents, used widely for osteoporosis treatment, are analogues of pyrophosphate, the critical endogenous inhibitor of extracellular mineralization. They inhibit vascular calcification in the vitamin D model and whether this is a direct effect on the arteries or an indirect effect through blocking bone resorption is not clear. Pyrophosphate is produced from ATP by an enzyme at the cell membrane, ectonucleotide pyrophosphatephosphodiesterase 1 (NPP1), and it is transported across the cell membrane, by the protein ANK, to its site of action in the extracellular matrix. This inhibitor is broken down by tissue-nonspecific alkaline phosphatase, secreted from osteogenic cells in matrix vesicles. Conditions that disrupt extracellular pyrophosphate levels dramatically alter vascular calcification. Terkeltaub and colleagues showed that mice deficient in NPP1 have pyrophosphate deficiency and aortic calcification. A defect in the human gene encoding NPP1 is responsible for the often-fatal disorder idiopathic infantile arterial calcification, which is now no longer “idiopathic.” Mice with mutant genes for the transport protein (ank/ank mice) are also depleted of extracellular pyrophosphate and also develop medial calcification. Millan and colleagues showed that blocking the pyrophosphatase-degrading action of alkaline phosphatase reduced the calcification in vascular smooth muscle cells from NPP1- and ANK-deficient mice, offering a new therapeutic possibility. Therefore, although extremely relevant to potential treatments, the beneficial effects of osteoprotegerin and bisphosphates in the vitamin D model may reflect, in part, direct local actions.

Thus, the bone-vascular axis may operate, directly or indirectly, through a variety of hormonal and physiological systems that could not all be covered in this limited review. Other organs and tissues besides bone and arteries also secrete hormonal factors that regulate both vascular calcification and bone including leptin, dexamethasone, aldosterone, vasopressin, adiponectin, and insulin-like growth factor-1. Bone also secretes hormonal factors independently of resorption. The diversity of molecular mechanisms is reflected in the large number of genetically modified mice with vascular calcification phenotypes, such as those deficient in fibrillin, β-glucosidase, carbonic anhydrase II, desmin, Klotho, fetuin-A, ectonucleotide pyrophosphatase, MGP, Smad6, and Abcc6.

Summary
Clinically, vascular calcification is now accepted as a valuable predictor of coronary heart disease. Achieving control over this process requires understanding mechanisms in the context of a tightly controlled regulatory network, with multiple, nested feedback loops and cross talk between organ systems, in the realm of control theory. Thus, treatments for osteoporosis such as calcitriol, estradiol, bisphosphonates, calcium supplements, and intermittent PTH are likely to affect vascular calcification, and, conversely, many treatments for cardiovascular disease such as statins, antioxidants, hormone replacement therapy, angiotensin-converting enzyme inhibitors, fish oils, and calcium channel blockers may affect bone health. As we develop and use treatments for cardiovascular and skeletal diseases, we must give serious consideration to the implications for the organ at the other end of the bone-vascular axis.

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