Vitamin D and Osteogenic Differentiation in the Artery Wall

Jeffrey J. Hsu,* Yin Tintut,* and Linda L. Demer*†
Departments of *Medicine and †Physiology/Biomedical Engineering, David Geffen School of Medicine at University of California, Los Angeles, Los Angeles, California

Vascular calcification is widespread, particularly in patients with chronic kidney disease, who receive, among other treatments, active vitamin D supplements. Emerging evidence indicates that vascular calcification is a regulated process that resembles embryonic endochondral osteogenesis, involving osteoblastic differentiation of vascular smooth muscle cells. In experimental animal models, high dosages of vitamin D consistently promote vascular calcification. In particular, the vitamin D–fed rat is frequently used as a model to assess putative regulators of calcific vasculopathy. The artery wall calcification in these animals most likely results from multiple mechanisms involving systems physiology of the complex, bone-vascular-renal-endocrine axis. Genetically engineered mice with upregulated vitamin D signaling pathways have also shed light on the molecular intermediaries, including fibroblast growth factor-23 and transcriptional intermediary factor 1-α. In contrast to the studies of animals, studies of humans show that vitamin D has an inverse relationship or little effect. This difference between in vitro and in vivo findings is most likely, again, due to the complex, systemic feedback regulatory mechanisms that control calcium-phosphate metabolism. Recent epidemiologic evidence suggests that there is a narrow range of vitamin D levels in which vascular function is optimized. Levels above or below this range seem to confer a significant increase in risk for cardiovascular disease. There is some evidence to suggest that dietary vitamin D may be carried by lipoprotein particles into cells of the artery wall and atherosclerotic plaque, where it may be converted to active form by monocyte-macrophages. These findings raise interesting questions regarding the effects of vitamin D intake on atherosclerotic calcification and cardiovascular risk.

Cardiovascular disease is the leading cause of death among adults with chronic kidney disease (1), with 10- to 20-fold higher mortality rate (2). It seems that a key contributor to the cardiovascular complications seen in these patients is vascular calcification (calcific arteriolopathy), a pathological process that is widely known to increase the risk for cardiac and vascular events (e.g., myocardial infarction, stroke) (3–5). Vascular calcification is particularly prevalent in patients with chronic kidney disease (CKD), (6) even among pediatric patients (7). Growing evidence indicates that multiple factors contribute to vascular calcification in CKD (8). Hyperphosphatemia is a common clinical finding in patients with CKD, and Wada et al. (9) showed that increased serum levels of inorganic phosphate induce matrix mineralization in smooth muscle cells in vitro. In addition, calcitriol has been used widely in patients with CKD to prevent development of secondary hyperparathyroidism as a result of the impaired ability of the diseased kidney to convert vitamin D into its active form, 1α,25(OH)2D3. Calcitriol increases serum calcium levels and suppresses parathyroid hormone (PTH) release; although this treatment addresses the secondary hyperparathyroidism, the resulting increase in serum calcium levels, together with increased serum phosphate (10), may have the adverse effect of promoting mineralization in the vasculature. The greater prevalence of vascular calcification in patients with CKD and the increased use of vitamin D metabolites in the treatment of these patients together beg the question of whether vitamin D intake contributes to vascular calcification. An in-depth review on this question has been provided by Norman and Powell (11).

The cellular and molecular mechanisms of vascular and cardiac valvular calcification have been studied in great detail (see reviews by Shao et al. [12] and Abedin et al. [13]). In seminal studies, Towler and colleagues (14) demonstrated a key role of molecular Wnt signaling cascades in osteoblastic differentiation of vascular cells. Calcium deposits may be found at many sites in the cardiovascular tree, including the medial layer of the large arteries (medial arterial calcification); within atherosclerotic plaque (intimal calcification); on cardiac valves (15), particularly mitral and aortic; and in the microvessels (calcific uremic arteriolopathy). Calcium deposition at these different sites follows different clinical courses, and they seem to have at least some distinct pathophysiologic features. In approximately 15% of human atherosclerotic plaque, the calcium deposits develop complete bone architecture, histologically indistinguishable from trabecular bone, even including marrow and...
cartilage (16). Transitional stages corresponding with the stages of embryonic endochondral ossification may be seen in human plaque (17). This seeming contrast between the clinical and laboratory views of the relationship of vitamin D to the osteogenic differentiation of vascular cells is the focus of this brief survey highlighting some of the current literature in this complex field.

Cell and Organ Culture Studies

Vascular cell calcification has been shown to occur in vitro in bovine and human cells (18–20), following the same molecular events and a similar time course as seen in osteoblast differentiation (21). The in vitro model has been used extensively to identify a wide range of activators and inhibitors that control osteogenic differentiation of vascular cells (22). In both bovine and human vascular smooth muscle cell cultures, active vitamin D treatment induces osteoblastic differentiation as evidenced by induction of alkaline phosphatase activity, an established marker of osteogenesis in cultured cells, as well as its gene expression (23). This effect is mediated, at least in part, through increased secretion of PTH-related peptide (23). In human vascular smooth muscle cells, 1α,25(OH)₂D₃ has been shown to induce matrix calcium incorporation in the presence of TNF-α, oncostatin M, and IFN-γ (24). Cellular uptake of calcium is also induced by 1α,25(OH)₂D₃ in rat aortic smooth muscle cells (25). In contrast, in organ culture of rat aortic segments in serum-free medium, calcitriol treatment did not promote calcium incorporation (26).

Other aspects of vascular function besides mineralization are also affected by vitamin D. Canfield and colleagues (27) showed that vitamin D inhibits vascular endothelial growth factor–induced endothelial cell sprouting, as well as the formation of endothelial cell networks within three-dimensional collagen gels. They further showed that 1α,25(OH)₂D₃ promoted cellular regression as a result of apoptosis, specifically within the sprouting cell population, a phenomenon confirmed in vivo. Even artery wall contractility is affected by vitamin D, as shown in ex vivo arterial segments (28).

It is interesting that 1α,25(OH)₂D₃ is carried by low density lipoprotein particles and internalized by cells via the LDL receptor (29); whereas vitamin D generated by ultraviolet exposure is primarily carried in the bloodstream on vitamin D binding protein. As a fat-soluble vitamin, dietary calciferol may be carried into the bloodstream from the intestinal villi inside chylomicrons and transferred to LDL particles in the liver (30). Thus, LDL particles accumulating in artery walls to produce atherosclerotic plaque may bring vitamin D with them. Approximately 16% of 1α,25(OH)₂D₃ and approximately 3% of 25(OH)D are carried in lipoprotein particles (29). Once in the subendothelial space or atherosclerotic plaque, 25(OH)D may be converted to active form by 1α-hydroxylase expressed in the abundant monocyte-derived cells (31). Vitamin D is known to induce a variety of factors that contribute to atherogenesis or vascular calcification (e.g., type I collagen, vascular endothelial growth factor, matrix metalloproteinases, elastin) and to induce apoptosis, which may promote vascular calcification in vitro (32). Thus, it is possible that dietary vitamin D may have a direct role in vascular pathology (see the comprehensive review by Norman and Powell [11]).

Animal Models of Vascular Calcification

Hypervitaminosis D Induces Vascular Calcification

It has been known for decades that high-dosage vitamin D reliably produces vascular calcification in animal models. In the 1960s, vitamin D excess was shown to induce vascular calcification and thrombosis in rats. Hypervitaminosis D also produces atherosclerosis and vascular calcification in arteries of rabbits with moderate hyperlipidemia (33). Vitamin D was also shown to accelerate vascular calcification in Rhesus monkeys with experimental arteriosclerosis generated by nicotinic acid and high dietary cholesterol (34). This in vivo effect of vitamin D on vascular calcification has been reproduced consistently in studies from several investigative groups using cholecalciferol and/or calcitriol in the rat model (35–38).

Rodent Models of Hypervitaminosis D

Price et al. (39) developed a rat model of vascular calcification using warfarin, which inhibits vitamin K–dependent γ-carboxylation of matrix GLA protein, a known inhibitor of vascular calcification in vivo (40, 41). In this warfarin model, which, as expected, resembles the matrix GLA protein–deficient mouse phenotype, vascular calcification occurred much more rapidly with high-dosage cholecalciferol treatment (37). In rats with surgically induced renal insufficiency, vitamin D treatment leads to aortic calcification, with consequent hypertension, left ventricular hypertrophy, and aortic aneurysms (42).

Genetically Modified Mice

A link between vitamin D signaling and vascular calcification is suggested by the finding of vascular calcification in mice deficient in transcriptional intermediary factor 1-α (TIF1α), a tumor suppressor in hepatocytes. TIF1α represses the vitamin D receptor pathway, and, as a result, the TIF1α-deficient mice have enhanced vitamin D receptor signaling, suggesting a link between increased vitamin D receptor signaling and calcific vasculopathy.

Another link was initially suggested by the finding of calcific arteriolopathy (medial calcification) in mice deficient in fibroblast growth factor-23, which negatively regulates 1α-hydroxylase. These mice develop vascular calcification (43, 44); however, fibroblast growth factor-23 also inhibits renal phosphate resorption in response to high phosphate levels, and it was subsequently shown that a low-phosphate diet reversed the phenotype, indicating that hyperphosphatemia accounted for the vascular calcification in this model (45).

Modifiers of Vitamin D–Induced Vascular Calcification

A ubiquitous serum protein, fetuin-A, also known as α2-Heremans-Schmid glycoprotein, is considered by some to be a systemic inhibitor of vascular calcification, and loss of serum fetuin has been observed with excess vitamin D (46, 47). Thus, in some cases, vitamin D may promote calcification by reducing circulating levels of fetuin-A. Several investigative groups have shown that osteoprotegerin (OPG), a soluble decoy receptor in
the TNF receptor family, inhibits vitamin D–induced calcification (35,47–49). The findings suggest that vitamin D may affect atherosclerotic calcification via effects on OPG or its target, the ligand for receptor activator of NF-κB, a critical osteoclast differentiation factor. It is interesting that OPG also reduces hyperlipidemia-induced calcification, independent of vitamin D levels (50). Osteoclast-like cells have been found in the artery wall, and their resorptive activity is driven by carbonic anhydrase, which has a vitamin D response element in its gene promoter (51). One positive modifier of vitamin D–induced vascular calcification is magnesium deficiency. Low dietary magnesium has been shown to enhance coronary arterial calcification and ultrastructural changes of endothelial cells in vitamin D–fed swine (52).

**Clinical Studies of Vitamin D and Coronary and Aortic Calcification**

**Inverse Relationship between Endogenous Vitamin D Levels and Vascular Disease**

In contrast to the findings in animals, many clinical studies show an inverse relationship between vitamin D levels and vascular calcification. Calcification of the coronary arteries, measured as a “calcium score” by electron beam computed tomography (EBCT), correlates inversely with 1α,25(OH)2D3 in patients with familial hypercholesterolemia and coronary artery disease, as shown by Watson et al. (53). Consistent with this, Doherty et al. (54) showed an inverse relationship between EBCT calcium score and endogenous 1α,25(OH)2D3 in patients with high scores on the Framingham coronary risk scale. This relationship was independent of racial/ethnic origin. More recently, London et al. examined patients with CKD for a relationship of serum vitamin D parameters with aortic calcification, on the basis of roentgenographic and echocardiographic images, as well as with aortic stiffness measured by pulse-wave velocity. Results showed no relationship with relatively weak measures of aortic calcification, yet a significant inverse relation between vascular stiffness and both 25(OH)D3 and 1α,25(OH)2D3 was seen (55). Because aortic stiffness often reflects calcification, one might expect that quantitative measures of aortic calcification, such as calibrated computed tomographic analysis, may reveal a relationship; however, in patients who underwent coronary angiography and calibrated EBCT scanning, serum concentrations of 1α,25(OH)2D3 had no significant correlation with levels of coronary calcification (56). The more physiologically relevant comparison between quantitative aortic calcification and levels of 25(OH)D3 remains to be determined.

**Supplemental Vitamin D and Vascular Disease**

It is notable that in animals, vitamin D treatment has a positive relationship with vascular calcification, whereas in humans, endogenous vitamin D levels have a negative relationship with coronary calcification. One consideration is that vitamin D may have a biphasic relation with risk, promoting vascular calcification in both excess and deficiency. If so, then treatment studies may reflect the effects of high vitamin D levels for which the relationship is positive, whereas observational studies of endogenous levels may reflect the lower range for which the relationship is inverse. Another consideration is that, although dietary intake of vitamin D may correlate with levels of 25(OH)D (57), serum levels of 1α,25(OH)2D3, which is not considered a marker of hormonal status, may vary independently of dietary intake and inversely with 25(OH)D (58). Most clinical studies involve either no vitamin D supplementation or retrospective, nonrandomized vitamin D supplementation. Fewer studies involve prospective, randomized supplementation. In nonrandomized studies, calcitriol intake was not related to the presence of coronary artery calcification, although coronary calcification did correlate with higher serum phosphorus, calcium-phosphorus product (Ca × P), and daily intake of calcium in pediatric patients with CKD (6). In another study of pediatric patients with CKD, in which coronary calcification incidence was 15%, calcitriol intake was a minor contributor to coronary calcification in a multivariable regression model. The major predictors were serum phosphorus and cumulative exposure to calcium-containing oral phosphate-binding agents (7). In another observational treatment study of humans, vitamin D supplementation had a positive independent association with microvascular calcification (calcific uremic arteriolopathy) (59); however, in the National Institutes of Health Women’s Health Initiative studies, investigators found no significant effect of calcium/vitamin D supplementation on cardiovascular disease in >36,000 healthy postmenopausal women (60). Recent evidence supports the existence of a biphasic relationship of serum 25(OH)D with cardiovascular disease. In the Framingham Offspring Study, involving >1700 participants without symptoms and previous cardiovascular disease and a mean follow-up of 5.4 yr, the relationship between serum vitamin D [25(OH)D] and cardiovascular disease was found to be biphasic with risk increasing at levels <15 and >30 ng/ml. Thus, both vitamin D deficiency and vitamin D excess increase the risk for cardiovascular disease. This relation could be mediated in part by secondary hyperparathyroidism (61).

**Calcific Uremic Arteriolopathy**

Calcific uremic arteriolopathy (CUA), formerly known as calciphylaxis, is a life-threatening form of medial arterial calcification that leads to necrotic skin lesions and gangrene. Microvessels and surrounding soft tissue may also mineralize, the latter of which occurs in a process referred to as calcinosis. This process leaves painful nodules and necrosis, sometimes gangrenous, in affected skin. CUA is an active vasculopathy characterized by patchy medial calcification of arterioles (≤0.6 mm diameter), and it afflicts patients with advanced CKD, especially those who receive warfarin. Clinical studies have demonstrated an association between calcitriol intake and CUA (62).

**Vitamin D Analogs and Calcimimetics**

Vitamin D receptor activators, such as paricalcitol and doxercalcerol, have been proposed as alternative therapeutic measures for secondary hyperparathyroidism in CKD to prevent the increased serum Ca × P that may contribute to increased vascular calcification. In studies of rats, Mizobuchi et al. (36)
showed that, although calcitriol significantly increased the Ca × P and aortic calcium content, paricalcitol did not. Calcium-mimetics may have similar effects: By simulating serum calcium, they reduce PTH release from the parathyroid gland, which reduces the Ca × P product and vascular calcification (63,64). In a rat model of secondary hyperparathyroidism, cinacalcet did not produce vascular calcification (65).

**Conclusions**

The relationships of vitamin D with atherosclerotic calcification and with aortic medial calcification are strong and most likely involve multiple mechanisms within the complex, bone-vascular-renal endocrine axis. In cell culture and in animal models, vitamin D treatment is clearly associated with increased vascular calcification; however, clinical studies show either no effect or an inverse relationship between vitamin D levels and vascular calcification, probably as a result of the complex, bone-vascular-renal-endocrine axis. Nevertheless, clinical studies also indicate that there is a narrow range of vitamin D levels within which vascular function is optimized, and levels above or below this range seem to confer increased risk for cardiovascular disease.

**Disclosures**

None.

**References**


