Vitamin D and the Cardiovascular System

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Several epidemiologic and clinical studies have suggested that there is a strong association between hypovitaminosis D and cardiovascular disease (CVD). Hypovitaminosis D was reported as a risk factor for increased cardiovascular events among 1739 adult participants in the Framingham Offspring Study. Analysis of more than 13,000 adults in the Third National Health and Nutrition Examination Survey (NHANES III) showed that even though hypovitaminosis D is associated with an increased prevalence of CVD risk factors, its association with all-cause mortality is independent of these risk factors. Importantly, epidemiologic studies suggested that patients who had chronic kidney disease and were treated with activated vitamin D had a survival advantage when compared with those who did not receive treatment with these agents. Mechanistically, emerging data have linked vitamin D administration with improved cardiac function and reduced proteinuria, and hypovitaminosis D is associated with obesity, insulin resistance, and systemic inflammation. Preliminary studies suggested that activated vitamin D inhibits the proliferation of cardiomyoblasts by promoting cell-cycle arrest and enhances the formation of cardiomyotubes without inducing apoptosis. Activated vitamin D has also been shown to attenuate left ventricular dysfunction in animal models and humans. In summary, emerging studies suggest that hypovitaminosis D has emerged as an independent risk factor for all-cause and cardiovascular mortality, reinforcing its importance as a public health problem. There is a need to advance our understanding of the biologic pathways through which vitamin D affects cardiovascular health and to conduct prospective clinical interventions to define precisely the cardioprotective effects of nutritional vitamin D repletion.

A growing body of evidence suggests that people who are deficient in 25-hydroxyvitamin D [25(OH)D] have higher risks for numerous chronic medical conditions (1–3). In this review, we present the emerging data that support this link. Vitamin D is either produced in the skin *via* the effects of ultraviolet light or ingested with food and/or supplements. This vitamin D undergoes hydroxylation in the liver to produce 25(OH)D. It is widely believed that the renal enzyme 25(OH)D-1α-hydroxylase is primarily responsible for producing the circulating levels of the activated form of vitamin D, the hormone 1,25(OH)D. 25(OH)D-1α-hydroxylase is located in the inner mitochondrial membrane and is a cytochrome P-450 monooxygenase. Most of the biologic activities of 1,25(OH)D are mediated by its binding to a high-affinity receptor that acts as a ligand-activated transcription factor. The major steps involved in the control of gene transcription by the vitamin D receptor (VDR) include ligand binding, heterodimerization with retinoid X receptor, binding of the heterodimer to vitamin D response elements, and recruitment of other nuclear proteins into the transcriptional preinitiation complex. 1,25(OH)D may also act through non–VDR- or nongenomic VDR-mediated pathways (4–6).

There is increasing evidence that the kidney is not unique in its ability to convert 25(OH)D to 1,25(OH)D, It was recently demonstrated that many tissues not only express the VDR but also possess 25(OH)D-1α-hydroxylase activity. The role of this paracrine production of 1,25(OH)D in these tissues is not well understood, but a variety of *in vitro* studies indicated that this process may be involved in a wide range of physiologic functions, including regulation of cytokines, inflammatory and/or fibrotic pathways, the renin-angiotensin system, vascular and cardiac cell function, immune response modulation, cell growth and differentiation, and others (7–15). Several of the biologic pathways through which the effects of 1,25(OH)D are mediated remain poorly understood but may account for its role in cardiovascular health (Figure 1).

Epidemiology of Hypovitaminosis D and Cardiovascular Disease in the General Population

Several epidemiologic and clinical studies have suggested that there is a strong association between hypovitaminosis D and cardiovascular disease (CVD). Scragg *et al.* (16) reported an inverse relationship between serum 25(OH)D levels and myocardial infarction in a community setting. This affect may be
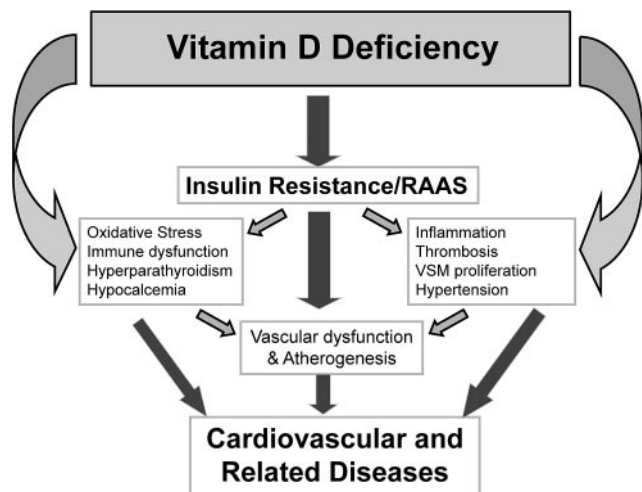
Figure 1. Conceptual model of major pathways through which vitamin D deficiency may lead to CVD.

mediated in part through increased physical activity (17). Martins *et al.* (18) analyzed data from more than 13,000 adults in the Third National Health and Nutrition Examination Survey (NHANES III) and demonstrated that there is a strong association between hypovitaminosis D and key CVD risk factors (BP, diabetes, overweight, hypertriglyceridemia, but not hypercholesterolemia) after adjustment for multiple variables. Furthermore, several studies have now shown that hypovitamin-

osis D is a predictor of all-cause and cardiovascular mortality (Table 1), independent of the traditional risk factors (19–21). Thus, these data have consistently demonstrated that there is a graded, inverse relationship between serum 25(OH)D levels and CVD that seems to be most evident as levels fall below 20 to 25 ng/ml. In addition, Pfeifer *et al.* (22) reported a fall in BP, heart rate, and parathyroid hormone levels after the short-term administration of 800 IU of 25(OH)D plus 1200 mg of calcium *versus* 1200 mg/d calcium alone to elderly women (mean age 74 yr; mean 25(OH)D level <20 ng/ml), suggesting that inadequate intake of 25(OH)D and calcium could play a contributory role in the pathogenesis and progression of hypertension and CVD in this cohort.

Epidemiology of Hypovitaminosis D and CVD in Patients with Chronic Kidney Disease

Chronic kidney disease (CKD) is now recognized as a powerful cardiovascular risk factor (23). Agarwal *et al.* (24) found that the administration of an activated vitamin D analog (paricalcitol) reduced proteinuria in 51% of the 57 patients with CKD in comparison with only 25% of the 61 study participants who received placebo ($P = 0.004$), suggesting a direct vascular effect of vitamin D. At least three observational studies of patients with CKD—one in non–dialysis-dependent and two in dialysis-dependent patients—have reported an increased risk for death (both non–dialysis-dependent and dialysis-dependent patients) or composite

Table 1. Summary of epidemiologic studies that have shown an association between hypovitaminosis D and mortality/cardiovascular events in the general population

Reference	N	Population	Follow-up	Key Findings
Dobnig *et al.*, (21) 2008	3258	Single center, referred for coronary angiography	7.7 yr	Lower two quartiles of serum 25(OH)D with higher all-cause and cardiovascular mortality
Giovannucci *et al.*, (56), 2008	18,255	Health Professionals Follow-up Study, men 40 to 70 yr	Up to 10 yr	Adjusted hazards for myocardial infarction compared with 25(OH)D levels ≥30 ng/ml: ≤15, 2.09; 15 to 22.5, 1.43; and 22.6 to 29.9, 1.60
Pilz *et al.*, (57), 2008	3316	Single center, referred for coronary angiography	7.7 yr	Low vitamin D levels associated with increased fatal/nonfatal strokes
Wang *et al.*, (19), 2008	1739	Framingham Offspring Study	5.4 yr	Low serum 25(OH)D levels (<15 ng/ml *versus* ≥15 ng/ml) associated with increased cardiovascular events
Melamed *et al.*, (20) 2008	13,311	Third National Health and Nutrition Examination Survey	8.7 yr	Lowest quartile of serum 25(OH)D (<17.8 ng/ml) with higher all-cause mortality than highest quartile

risk for death and ESRD (nondialysis patients) in patients with low serum 25(OH)D levels (Table 2).

Equally as important, observational studies have shown an association between treatment with activated vitamin D (presumably for secondary hyperparathyroidism) and lower all-cause and cardiovascular mortality in patients with CKD, both in the early stages (25–27) and in those who receive renal replacement therapy (28–30). Unfortunately, data on serum 25(OH)D were not available in any of these studies.

Vitamin D and Cardiac Hypertrophy

Left ventricular hypertrophy (LVH) is recognized as one of the strongest risk factors for cardiovascular mortality, especially in patients with CKD (31). The increased rates of pressure and volume overload in patients with CKD can accelerate cardiac myocyte apoptosis and fibrosis. At the same time, activation of neurohormonal systems, growth factors, and cytokines as well as associated downstream intracellular signals can affect cardiac hypertrophy, apoptosis, and fibrosis, leading to LVH and/or cardiac dysfunction (32–34); however, the large variability in LV mass in the CKD population suggests that other risk factors may also play a role.

Not only are low levels of 25(OH)D pervasive in patients with CKD (35), but also two small clinical trials of hemodialysis patients have shown that treatment with activated vitamin D [1,25(OH)D or related analogs] may lead to regression of LVH, suggesting a cardioprotective action (36,37). Indeed, activated vitamin D may affect the myocardium directly and play a role in the regulation of myocyte hypertrophy, because cardiac hypertrophy has been observed in the hearts of VDR knockout mice (38). Activated vitamin D has been shown to downregulate proliferation and hypertrophy in cultured cardiomyocytes (39,40). Moreover, Bodyak *et al.* (41) showed that activated vitamin D, paricalcitol, attenuated sodium-induced LV abnormalities in Dahl salt-sensitive hypertensive rats and when administered to dialysis patients improved diastolic function and reduced LV thickness when compared with patients who did not receive activated vitamin D. In preliminary studies, we found that in comparison with cardiomyocyte cells cultured in the absence of activated vitamin D, the addition of 1,25(OH)D

(1) inhibited cell proliferation; (2) enhanced cardiomyocyte formation; (3) exerted a protective effect against apoptosis; and (4) promoted a generalized decreased in the expression of genes related to the regulation of the cell cycle, such as cyclins A1, C, and E and cyclin-dependent kinases Cdk2 and Cdk4 (42). In summary, these findings suggest that it is potentially possible that correcting vitamin D deficiency could contribute to improve cardiac structure and function, thereby ameliorating cardiovascular risk.

Vitamin D and Vascular Health

The ubiquity of the VDR and tissue 25(OH)D-1α-hydroxylase provides insights into several pathobiologic pathways through which hypovitaminosis D may mediate vascular health in addition to those outlined already (Figure 2). The VDR knockout mouse, an animal model that emulates vitamin D deficiency, displays increased BP, serum angiotensin, and tissue renin (14). *In vitro* studies using a juxtaglomerular cell model have shown that 1,25(OH)D as well as other vitamin D analogs directly suppress renin gene expression *via* a vitamin D response element that is present in the renin gene (14). Vitamin D analogs have been shown to inhibit the production of several proinflammatory Th-1 cytokines, such as IL-2 and IFN-γ, while stimulating the effects of Th-2 lymphocytes, leading to a reduction in matrix metalloproteinase (MMP) and reducing plaque production and/or instability (43); it also favors the induction of T regulatory cells partly through the induction of tolerance-inducing dendritic cells. Furthermore, it has been shown to have immunosuppressive effects whereby it reduces the proliferation of lymphocytes and the production of cytokines, which have been identified as having an important role in atherogenesis (43) (Figure 3).

VDR agonists have also been shown to downregulate plasminogen activator inhibitor 1 (PAI-1) in human aortic smooth muscle cells but not endothelial cells. This is likely due to the lack of VDR expression in the endothelial cells studied (8). Studies with mouse embryonic fibroblasts from VDR knockout mice showed an increase in profibrotic factors (NF-κB transcriptional activity, IL-6, and TNF-α) in comparison with cells cultured from wild-type mice (44). Although provocative, the

Table 2. Summary of epidemiologic studies that have shown an association between hypovitaminosis D and mortality/cardiovascular events in individuals with CKD

| Reference	N	Population	Follow-up	Key Findings
Wolf *et al.*, (58), 2007	825	Patients with ESRD, undergoing hemodialysis	90 d	<10 ng/ml with higher all-cause mortality than with >30 ng/ml
Wang *et al.*, (59), 2008	230	Patients with ESRD, undergoing peritoneal dialysis	Up to 3 yr	Significant increase in risk for fatal/nonfatal cardiovascular events with levels <18 ng/ml
Ravani *et al.*, (60), 2009	168	Stages 2 to 5 CKD, CKD clinic	4 yr	25(OH)D is an independent inverse predictor of ESRD and mortality
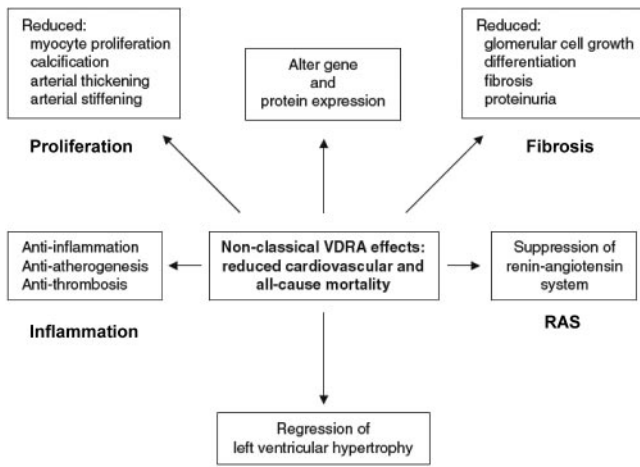
Figure 2. Nonclassical aspects of differential VDR activation: Implications for survival in patients with CKD. Adapted from reference 54.

knockout mouse does not allow direct inference to deficient levels of vitamin D and is confounded by a myriad of a counterregulatory set of gene activities in the setting of an absent VDR. To examine more closely the VDR-mediated, parathyroid hormone/calcium-independent effects of vitamin D on profibrotic pathways, we performed a series of experiments on a mesenchymal multipotent cell line with potential to differentiate along endothelial lines and to be used for applications such as neovascularization and tissue engineering (45). We found that incubating this multipotent cell line with vitamin D led to

increased expression and nuclear translocation of the VDR; decreased expression of TGF-β1 and plasminogen activator inhibitor 1; decreased expression of collagens I and III and other collagens isoforms; and increased expression of several antifibrotic factors, such as bone morphogenic protein 2 and 7, (TGFβ antagonist), MMP8 (a collagen breakdown inducer), and follistatin (an inhibitor of the profibrotic factor myostatin) (15) (Figure 4).

Vitamin D is associated not only with multiple pathways that regulate the early stages of atherogenesis, *per se*, but also with vascular calcification. The role of activated vitamin D administration on vascular calcifications has remained a paradox, but recent findings using a mouse model suggested that the administration of calcitriol and paricalcitol, at dosages sufficient to correct secondary hyperparathyroidism, were protective against aortic calcification, whereas higher dosages stimulated aortic calcification (46). Protective dosages of 1,25(OH)D suppressed aortic osteoblastic gene expression, which is normally increased in CKD, suggesting a mechanism for this inhibition of aortic calcification. In addition, evidence suggested that Wnt signaling may modulate vascular calcification *via* osteoblastic differentiation of vascular smooth muscle cells, reinforcing the role of osteoblast regulation in vascular calcification (47,48). The relationship between vitamin D and vascular health is reinforced at a clinical level by Melamed *et al.*, who reported an 80% increase in the adjusted prevalence of peripheral artery disease among NHANES participants with the lowest quartile of 25(OH)D ($<$17.8 ng/ml) in comparison with those in the highest quartile ($\geq$29.2 ng/ml) (49). Similar associations of
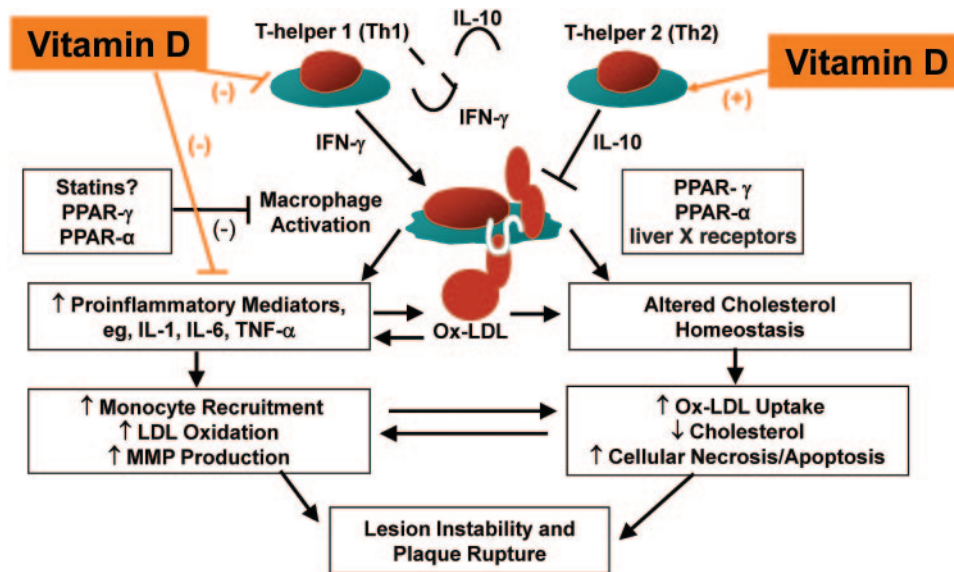

Figure 3. Vitamin D, lymphocyte, and macrophage involvement in atherosclerosis. Recent studies suggest that lymphocytes and macrophages play the initial role in the generation of atheromas. It is hypothesized that Th1 cells start producing excess IFN-γ, which is a potent stimulator of macrophage activity. Activated macrophages secrete IL-1β, IL-6, and TNF-α. These cytokines recruit additional monocytes, increase LDL oxidation, and generate production of MMPs that can destabilize the plaque to cause rupture and thrombosis. In contrast, the TH2 lymphocyte subset is called the antiatherogenic phenotype, because these cells produce IL-10, a good cytokine that suppresses macrophage activation and Th1 proliferation. PPAR, peroxisome proliferator–activated receptor. Adapted from references 43 and 55.
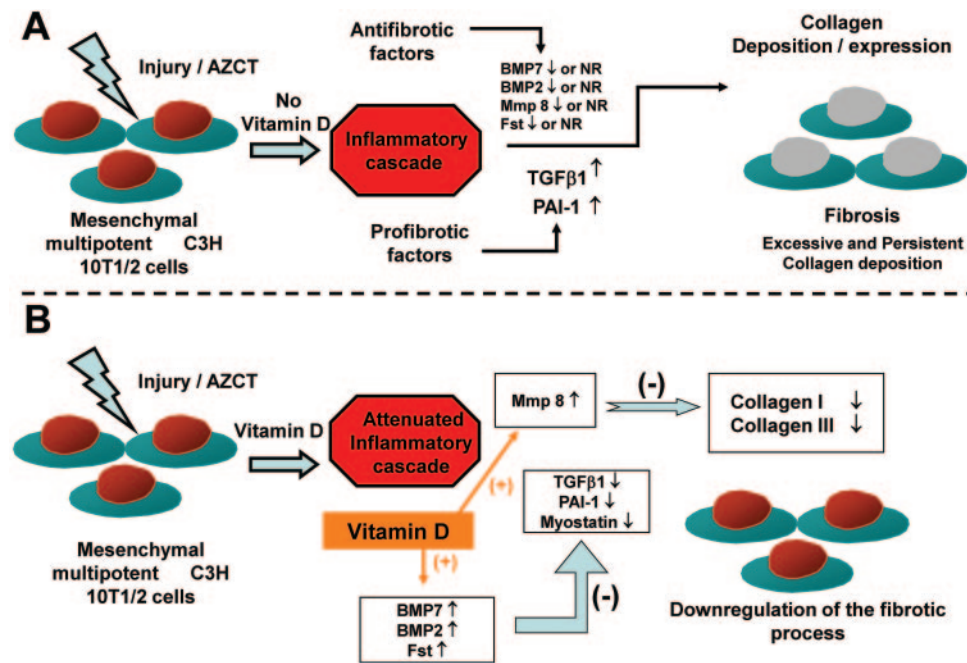
Figure 4. Vitamin D reverts the fibrotic process induced by 5′-azacytidine (AZCT) in mesenchymal multipotent cells. (A) In the absence of vitamin D, an injury triggers the inflammatory cascade, which can lead to a fibrotic process (progressive scarring). (B) 1,25(OH)D (the active form of vitamin D) induces a VDR-mediated antifibrotic signaling phenotype in multipotent mesenchymal cells characterized by a decreased expression of profibrotic markers (TGF-β1, plasminogen activator inhibitor 1 [PAI-1], myostatin) and an increased expression of antifibrotic markers (bone morphogenic protein 2 [BMP2] and 7 [BMP7], follistatin, MMP8), leading to an effective reduction in collagen expression, the ultimate marker of a fibrotic condition. Adapted from reference 15.

peripheral artery disease and low serum levels of 25(OH)D were reported by Fahrleitner and colleagues (50,51).

The aforementioned data suggest that vitamin D has multiple immunomodulatory effects and regulates cytokines, profibrotic, and proinflammatory pathways and the renin-angiotensin system (9–14) through the VDR. Some effects, such as downregulation of TGF-β1, may occur through vitamin D–mediated nongenomic pathways (52). The preponderance of data link vitamin D deficiency or lack of activation of the VDR with the modulation of gene transcription and associated signaling pathways that may contribute to the acceleration of cardiovascular pathology.

Future Directions

Prospective studies are needed to assess the efficacy of 25(OH)D repletion in patients with CKD/ESRD, in both the presence and absence of activated 1,25(OH)D (or analog) repletion. Studies are needed to evaluate the existence of potential "nongenomic" or other direct cellular effects of 25(OH)D and how these may be linked to 1,25(OH)D through modulation of the VDR or 25(OH)D-1α-hydroxylase expression. Studies are needed to understand better the role of 25(OH)D and local 25(OH)D-1α-hydroxylase on vascular and cardiac function as well as the role of 24,25(OH)D in select organs. Studies are needed to characterize the gene–environment interactions that influence 25(OH)D and 1,25(OH)D, as well as to define the interdependence of 25(OH)D, 1,25(OH)D, and novel vitamin D analogs for vascular health. As we extend our understanding of

25(OH)D, 1,25(OH)D, and novel vitamin D analogs on the renin-angiotensin system, endothelial function, cardiac and renal fibrosis, immune regulation, insulin resistance, adipokines, and related vascular mediators, we will be better positioned as a renal community to advance the emerging recognition of 25(OH)D deficiency as an underestimated public health problem and 25(OH)D repletion as an important public health solution.

Conclusions

Vitamin D seems to play an important role in cardiovascular health. Emerging studies suggest that vitamin D has important indirect effects *via* traditional and possibly nontraditional CVD risk factors as well as direct effects on cardiac and vascular cells. This confluence of emerging reports on the ubiquitous role of vitamin D in cardiovascular health and the graded relationship between vitamin D levels and clinical outcomes supports the need to reassess the current position on which levels of vitamin D constitute vitamin D insufficiency and necessitate vitamin D repletion. The current recommended levels of serum 25(OH)D (30 to 32 ng/ml) and vitamin D supplementation guidelines (see next paragraph) are primarily based on predicted needs to maintain optimum bone health and to prevent rickets but do not address the vitamin D requirements that may be necessary to minimize the initiation and/or progression of CVD and CVD risk factors.

Given our evolving understanding of vitamin D and cardiovascular health, more effective strategies are needed to address

the unattended epidemic of hypovitaminosis D with nearly 50% of Americans estimated to have serum 25(OH)D levels <30 ng/ml. Presently, the recommended daily vitamin D intake is 200 IU/d for people up to 50 yr of age, 400 IU/d for people between 51 and 70 yr of age, and 600 IU/d for people who are older than 70 yr; however, given the daily metabolic use of vitamin D being approximately 4000 IU many authorities now believe vitamin D supplementation in the range of 1000 to 4000 IU/d may be required to maintain adequate levels in the general population; studies suggest that dosages as high as 10,000 IU/d can be given safely (53). These findings reinforce the importance of firmly establishing the evidence base required to address hypovitaminosis D as an important public health problem and to inform more precisely future clinical guidelines as to what is likely required to achieve adequate vitamin D levels in people who are at risk for CVD, in both the absence and presence of CKD. There still remains a great need to advance our understanding of the biologic pathways through which vitamin D mediates cardiovascular function to improve our ability to target therapies, as well as prospective clinical interventions to advance our evidence of the efficacy of vitamin D repletion on cardiovascular health.

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