VITAMIN D AND THE HEART

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Summary

Vitamin D receptors (VDR) are found in cells throughout the cardiovascular system. A variety of experimental studies indicate that the liganded VDR may play an important role in controlling cardiac hypertrophy and fibrosis, regulating blood pressure and suppressing the development of atherosclerosis. Some, but not all, observational studies in humans provide support for these experimental findings, raising the possibility that vitamin D or its analogues might prove useful therapeutically in the prevention or treatment of cardiovascular disease.
INTRODUCTION

Vitamin D or, more specifically, its bioactive metabolite, 1,25 (OH)$_2$ vitamin D$_3$ [1,25 (OH)$_2$ D$_3$], is a nuclear hormone receptor ligand which is known to have profound effects on calcium and phosphate homeostasis. From the standpoint of mineral homeostasis, vitamin D’s primary targets are located in the small bowel, bone and, to a lesser extent, kidney. However, a growing literature indicates that vitamin D receptors (VDRs) are found in a variety of cells and tissues that are either loosely related or totally unrelated to mineral homeostasis. These include malignant breast, colon and prostate cells and normal cells of the immune system, kidney, heart and vasculature. The heart is particularly noteworthy in that 1) plasma 25 (OH) vitamin D$_3$ [25(OH)D$_3$] levels have been shown to correlate inversely with the incidence of a variety of cardiac disorders, including ischemic heart disease and heart failure and 2) interventional studies in a variety of cell culture systems and animal models suggest that the liganded VDR can exert anti-hypertrophic activity in cardiac myocytes in vitro and in vivo. The present work will review the recent literature supporting a role for the liganded VDR and, inferentially, vitamin D in the regulation of cardiac structure and function.
Experimental studies

Expression of VDR and 1-hydroxylase genes in heart

Early studies showed that the VDR gene is expressed in the heart (15, 48, 50, 63, 73, 78) where it localizes to both the cardiac myocyte (19, 58) and fibroblast (15). VDR expression in both cell types increases following exposure to hypertrophic stimuli in vitro, as well as in hypertrophied hearts in vivo (15). Cardiac ventricular tissue also expresses the 1-hydroxylase (Cyp27B1) implying that it has the capacity to produce 1,25(OH)₂D₃, the cognate ligand of the VDR (15), from circulating 25(OH)D₃. Ventricular tissue also expresses the 25 hydroxyvitamin D-24 hydroxylase (Cyp24A1) which is responsible for producing inactive vitamin D metabolites (15). Data gathered in our laboratory suggest that the liganded VDR possesses anti-hypertrophic activity in the heart (see below), implying that this hormone-ligand system may operate in a negative feedback loop that regulates the magnitude and duration of the hypertrophic response.

Role of the VDR in cardiac development

Experimental studies have shown that the VDR is linked to quail cardiac atrial development (79, 80). The slow myosin heavy chain 3 gene (MyHC3), an atrial chamber-specific gene, has been used as a marker to investigate atrial and ventricular lineage specification (21, 80, 81, 97). Slow MyHC3 is initially expressed throughout the early tubular heart. While atrial chamber-restricted expression is maintained at a constant level, down-regulation of ventricular slow
MyHC3 gene expression during chamber formation (80) leads to atrial specification. Expression of the slow MyHC3 gene is achieved through the positive effects of a GATA factor-binding element in the cardiac atria and the negative effects of a vitamin D response element (VDRE) in the cardiac ventricle (80). The liganded VDR inhibits slow MyHC3 expression through a process that involves an Iroquois family homeobox gene, Irx4. Expression of Irx4 is restricted to the ventricular myocyte throughout heart development (79). Downregulation of the slow MyHC3 gene by Irx4 requires the VDRE element. Of note, Irx4 does not bind directly to the VDRE but forms an inhibitory complex through interaction with RXR, in the heterodimeric VDR-RXR, complex.

Despite VDR’s well defined role in avian heart development, the role of the VDR in mammalian heart development remains undefined; however, atrial and ventricular specification appears to be normal in the VDR−/− mouse (37) implying that if it does play a role, it may be redundant with other developmental regulatory systems in the heart.

Relationship of the liganded VDR to hypertrophy of the rodent heart

Using the neonatal rat cardiac ventricular myocyte system, we have documented that the liganded VDR suppresses myocyte hypertrophy in vitro. We have shown that 1,25(OH)2D3, as well as a number of the less calcemic analogues, act in both atrial (92) and ventricular (93) myocytes to inhibit the activation of phenotypic markers associated with hypertrophy. The vasoactive
peptide endothelin (ET) promotes changes in fetal gene expression and promoter activity, cell size and protein synthesis (93) that parallel changes that occur with hypertrophy \textit{in vivo}. These changes are at least partially reversed by 1,25(OH)$_2$D$_3$ or its less calcemic analogues (i.e. oxacalcitriol). Similar findings have been reported by others using cultured cardiac HL-1 myocytes (48) where 1,25(OH)$_2$D$_3$ was found to reduce cell proliferation and atrial natriuretic peptide (ANP) gene expression, a marker of the hypertrophy-sensitive fetal gene program. We have characterized the structural requirements for VDR’s anti-hypertrophic activity \textit{in vitro} using human (h) ANP gene promoter activity as a surrogate marker of hypertrophy. Inhibition of this promoter requires an intact DNA-binding and ligand-binding domain of the VDR (17). It also requires the capacity of the liganded VDR to heterodimerize with the RXR (12) and preservation of the activation domain of the receptor, particularly in the area surrounding the co-activator binding pocket (13). Intriguingly, the same residues that are critical for association with co-activator proteins and triggering an increase in target gene transcription, also play a role in mediating the inhibitory effect on hANP promoter activity (13). Simpson’s group using a vitamin D-deficient rat model was among the first to report a connection between vitamin D status and cardiovascular function (86). They found that when they provided Sprague-Dawley rats a low vitamin D diet for more than 2 weeks, the rats developed transient hypertension and significant hypocalcemia. Despite the fact that the rats were maintained on the same vitamin D deficient diet, by 8 weeks (and extending to 18 weeks) there was no...
difference in blood pressure between the vitamin D deficient group and control animals (86). Weishaar et al. (85) went on to find a significant increase in heart weight to body weight ratio, a reliable index of cardiac hypertrophy, in rats after 9 or 18 weeks of vitamin D deficiency. They found that the hypertrophy was not accompanied by loss of soluble cardiac enzymes (e.g., creatine phosphokinase) or myocardial edema (85). It was not reversed by restoration of serum calcium to normal, nor was it prevented by normalization of serum calcium levels during the period of vitamin D deficiency. Microscopic analysis of the ventricular sections demonstrated that myofibrils from the vitamin D deficient rats were smaller than those in vitamin D sufficient rats, and there was a significant increase in the amount of extracellular matrix protein (85). Independent studies demonstrated that vitamin D-deficient cardiac hypertrophy is associated with myocyte hyperplasia and increased expression of the proto-oncogene c-myc (49), but the precise mechanism underlying cardiac hypertrophy in these vitamin D deficient rats remains unknown. Extending this anti-hypertrophic activity to a developmental paradigm, Gezmish et al. have reported that maternal vitamin D deficiency in Sprague-Dawley rats led to cardiac hypertrophy in 4 week old offspring. This was accompanied by an increase in cardiomyocyte number and size (24).

Both the VDR and the 1-OHase gene knockout mice are hypertensive and both display elevations in renin production in the kidneys (37, 100) and in the case of the VDR knockout, the heart (94). Both models also display increased plasma angiotensin II levels. Of note, the elevations in plasma renin levels are
independent of hypocalcemia and hyperparathyroidism in these mice (45, 112).

Yuan et al. have shown that CREB (Cyclic AMP Response Element Binding Protein), a transcription factor which binds to a cAMP response element (CRE) in the mouse renin gene promoter, is linked to the observed increase in renin gene transcription. The liganded VDR directly interacts with CREB, thereby preventing its association with the CRE in the mouse renin gene promoter and directly suppressing renin gene transcription (96). In addition to the observed elevations of blood pressure, both of these mouse models display significant cardiac hypertrophy with enlargement of individual myocytes. The VDR knockout mouse also displays elevations in plasma ANP levels and ventricular ANP transcript levels (94), presumably a consequence of the hypertrophy. Since renin activity is increased in both groups of knockout mice, one might predict that interference with the renin-angiotensin system might revert the phenotype. In fact, treatment with angiotensin converting enzyme inhibitors reverses both hypertension and cardiac hypertrophy in these mice (37, 100). Renin expression is increased in the hypertrophied heart of the VDR gene knockout mouse; however, the role of this cardiac vs. systemic renin activity in contributing to hypertrophy of the myocardium remains undefined. Simpson et al. confirmed that VDR knockout mice develop cardiac hypertrophy (62) with significant hypertrophy of individual myocytes, as well as an increase in cardiac interstitial fibrosis. However, they found no difference in blood pressure between the wild type and VDR knockout mice. They also found no evidence for elevated plasma renin activity, plasma angiotensin II or plasma aldosterone levels in the VDR knockout mice. The
reasons behind these seemingly discrepant results (37, 62) remain undefined.

Chen and co-workers have recently documented that isolated deletion of the VDR within the cardiac myocyte leads to cardiac hypertrophy and activation of the fetal gene program without increased cardiac renin gene expression (16).

While all studies with the 1-(OH)ase or VDR gene knockout mice consistently demonstrate cardiac hypertrophy, there is relatively little information dealing with cardiac function in these models. Zhou et al. (100) have shown impaired systolic function in 1-(OH)ase gene knockout mice that normalized following 1,25(OH)_{2}D_{3} administration. Paradoxically, cardiac myocytes isolated from 6-month-old VDR knockout mice have shown accelerated rates of contraction and relaxation as compared to age-matched wild type controls (73). The same study showed that acute treatment of wild type cardiac myocytes with vitamin D accelerates myocyte relaxation. Subsequent studies suggested that the vitamin D effect may be dependent upon an interaction between the VDR and caveolin-3 in the T-tubules and sarcolemmal membrane, operating through a rapid, non-genomic pathway (99).

**Relationship of liganded VDR to pro-fibrotic activity in the rodent heart.**

Vitamin D regulates myocardial extracellular matrix integrity through its effects on the expression of matrix metalloproteinases (MMPs), as well as tissue inhibitors of metalloproteinases (TIMPs) (84). Imbalance in the expression of MMPs and TIMPs in the myocardium is associated with the initiation and progression of both diastolic and systolic dysfunction in the heart (6). Neonatal
rat cardiac fibroblasts express the VDR gene (15) and this expression is increased following exposure to the pro-hypertrophic/pro-fibrotic agonist ET (15). Interestingly, pretreatment with 1,25(OH)_2D_3 leads to a reduction in pre-proendothelin gene expression (~20% decrease in pre-proendothelin mRNA levels relative to vehicle-treated controls; Gardner, DG, unpublished data) in cultured cardiac fibroblasts. This suggests a negative feedback loop in which activation of pathological hypertrophy/fibrosis, accompanied by an increase in pre-proendothelin gene expression, leads to an increase in VDR expression. VDR, in turn, feeds back to suppress ET gene expression and, by inference, the pro-hypertrophic and pro-fibrotic activities that it promotes. As noted above, rats with vitamin D deficiency develop cardiac hypertrophy in association with interstitial fibrosis (85). VDR gene knockout mice display an increase in myocardial interstitial fibrosis (57) with increased expression of cardiac MMP-2 and MMP-9 and reduced expression of TIMP-1 and TIMP-3 suggesting that vitamin D plays an important role in suppressing remodeling activity in the myocardium. This is consistent with a study in humans, which showed an inverse correlation between circulating MMP-9 levels and 25(OH)D_3 concentrations (71).

**Relationship of the liganded VDR to atherosclerosis**

Atherosclerotic involvement of the coronary arteries (CAD) represents the leading cause of cardiovascular disease worldwide. Recent experimental studies suggest that vitamin D may impact the development of atherosclerotic disease.
Oh et al. (51), using macrophages from obese, diabetic, hypertensive patients with vitamin D deficiency, showed that 1,25(OH)\(_2\)D\(_3\) suppressed foam cell formation by reducing acetylated or oxidized low density lipoprotein (LDL) cholesterol uptake in diabetic but not in non-diabetic subjects. This resulted from downregulation of JNK activity, improved insulin signaling, reduced oxidized LDL-derived cholesterol uptake, as well as suppression of macrophage endoplasmic reticulum (ER) stress and promotion of an anti-atherogenic monocyte/macrophage phenotype (51, 59). More recently, Szeto et al. (66) showed that VDR deficiency (i.e., VDR\(^{-/-}\) genetic background) accelerated the atherosclerotic process in the low density lipoprotein receptor deficient (LDLR\(^{-/-}\)) mouse. This effect appeared to operate through a mechanism involving suppression of the renin-angiotensin system in the macrophage cell.

Weng et al. (87) have shown that vitamin D deficient mice fed a high fat diet had elevated plasma renin levels, increased systolic and diastolic blood pressure and a 2-8 fold greater incidence of atherosclerosis in the aortic arch, thoracic and abdominal aorta compared to vitamin D sufficient mice. This was accompanied by increased macrophage infiltration into atherosclerotic plaques. Of note treatment of vitamin D-deficient mice on the high fat diet with the ER stress suppressor 4-phenyl butyric acid (PBA) reduced atherosclerosis, and peritoneal macrophage foam cell formation as it reduced levels of ER stress proteins, but without altering blood pressure.
Takeda et al. (67) showed that oral 1,25(OH)\(_2\)D\(_3\) reduced atherosclerotic lesions, macrophage accumulation and CD4(+) T cell infiltration in the aortic sinus of Apo E\(^{-/-}\) mice. This was accompanied by an increase in Foxp3(+) regulatory T cells and a decrease in CD80(+)CD86(+) dendritic cells in both immune tissues and in atherosclerotic lesions, suggesting that the hormone’s immunosuppressive properties may be responsible for its anti-atherosclerotic activity. Collectively, the data, though limited, appear to support the hypothesis that VDR ligands oppose the development and progression of the atherosclerotic phenotype.

**Treatment with 1,25(OH)\(_2\)D\(_3\) or its analogues suppresses cardiac hypertrophy**

Severe vitamin D deficiency in humans is associated with cardiomyopathy and congestive heart failure (75). These disorders have been shown to be responsive to vitamin D repletion. As noted above, mice with targeted deletion of the 25(OH)D 1-(OH)ase, which cannot produce endogenous 1,25 (OH)\(_2\)D\(_3\), develop myocardial hypertrophy and cardiac dysfunction. The administration of 1,25(OH)\(_2\)D\(_3\) normalizes serum calcium and phosphorus levels as well as cardiac structure and function (100). Chronic infusion with angiotensin II (800 ng/kg/min over 14 days) in mice leads to moderate hypertension, cardiac hypertrophy and interstitial fibrosis, all of which are partially reversed by the synthetic vitamin D analogue paricalcitol (14).
Dahl Salt Sensitive (DSS) rats display salt sensitive hypertension and cardiac hypertrophy. These rats have been shown to have low levels of plasma 25(OH)D$_3$ (70) and they respond to treatment with pharmacological doses of 1,25(OH)$_2$D$_3$ analogues, paricalcitol (7) or doxercalciferol(18), with inhibition of hypertrophy and improvement in cardiac function. Paricalcitol also has been shown to prevent progression of hypertrophy and development of heart failure in this same rat strain (5). Spontaneously hypertensive rats (SHR) display cardiac hypertrophy and low levels of vitamin D (56). One month old SHR rats treated with either paricalcitol or doxercalciferol for 2 months displayed reduced natriuretic peptide gene expression and a 65-80% reduction in LV wall thickness (34). When either of these vitamin D analogues was paired with losartan, reversal of hypertrophy was virtually complete. 1,25(OH)$_2$D$_3$ treatment of spontaneously hypertensive – heart failure prone (SHR-HFP) rats, fed a high salt diet, resulted in reduced myocyte hypertrophy, left ventricular diameter, and improved stroke volume (40).

Mice subjected to transverse aortic constriction develop significant cardiac hypertrophy and fibrosis. Noteworthy, treatment with paricalcitol failed to reverse hypertrophy. However, it did reduce cardiac ANP gene expression, as well as expression of a number of extracellular matrix proteins (i.e., fibronectin, collagen III and TIMP-1), and it was successful in reducing fibrosis and improving indices of LV contraction and relaxation (44). An intriguing study from Gupta et al. (27) showed that vitamin D deficiency induces cardiac hypertrophy, as well as inflammation in epicardial fat, in hypercholesterolemic swine. Noteworthy, the
epicardial inflammation was associated with diminished suppressor of cytokine signaling (SOCS) 3 gene expression.

In uremic rats, which express low levels of the 1- (OH)ase in the kidney, treatment with paricalcitol prevented left ventricular hypertrophy (47).

Collectively, these findings are consistent with the notion that 1,25(OH)₂D₃, as well as its less hypercalcemic analogues, can reduce cardiac hypertrophy in these well defined experimental models. Table 1 summarizes the studies supporting the anti-hypertrophic activity of the VDR ligands.

1,25(OH)₂D₃ and its analogues suppress cardiac fibrosis

Cardiac fibrosis is frequently seen together with cardiac hypertrophy, and it plays a major role in contributing to the dysfunctional state seen in advanced cardiomyopathy. 1,25(OH)₂D₃ has been shown to possess both anti-inflammatory (20, 95) and anti-fibrotic (98) activity in different experimental models. However, 1,25(OH)₂D₃ and its analogues have demonstrated inconsistent results in the suppression of cardiac fibrosis. Koleganova et al. (33) showed that treatment with sub-hypercalcemic doses of 1,25(OH)₂D₃ reduced interstitial fibrosis of the heart in subtotally nephrectomized rats. A second group showed that paricalcitol prevented left ventricular hypertrophy as well as myocardial and perivascular fibrosis in uremic rats (47). Interestingly, these findings have been linked to upregulation of VDR gene expression, reduced myocardial proliferating cell nuclear antigen (PCNA) labeling (an index of cellular proliferative activity) and...
reduced myocardial oxidative stress (57). In an acute myocarditis-induced, extensive fibrosis model, treatment of infected, susceptible mice with the vitamin D analog ZK 191784 resulted in a reduction in myocardial expression of osteopontin, metalloproteinase-3, TIMP-1, urinary plasminogen activator and procollagen-1 (65), all of which have been associated with fibrosis. An independent study showed that paricalcitol reversed interstitial fibrosis and extracellular matrix protein gene expression in an aortic constriction model of cardiac hypertrophy(44). By way of contrast, Repo et al. (58) reported that paricalcitol aggravated cardiac perivascular fibrosis in rats with renal insufficiency, a model that is typically associated with low circulating 1,25(OH)2D3 levels. In another study, while treatment with 1,25(OH)2D3 led to decreased cardiac hypertrophy in the SHR-HF rat, there was no significant reduction in myocardial collagen content (40). Collectively, the experimental data have provided a mixed result (Table 1). While these studies have raised the possibility that vitamin D possesses anti-fibrotic activity in the heart, definitive proof of this hypothesis has proven difficult to demonstrate.

Clinical Studies

Cardiovascular Disease (CVD)

A growing literature suggests an association between low 25 (OH) D3 levels and the risk of CVD and, more specifically, ischemic cardiac disease. However, this area
remains highly controversial with a number of studies supporting (4, 9, 25, 30, 35, 43, 46, 64, 82, 83, 101) and questioning this association (24, 38, 54, 64, 74, 75, 98).

Analysis of a cross section of the NHANES III population revealed an association between vitamin D deficiency (25(OH)D₃ < 20ng/mL) and coronary artery disease (CAD), defined as self reported angina, myocardial infarction or stroke with an odds ratio of 1.20 (95% CI: 1.01–1.36) (45). Similar findings were reported in the Health Professionals Follow Up Study. In that study the relative risk (RR) for nonfatal MI or fatal CVD was 2.42 (95% CI 1.53-3.84) in those with vitamin D deficiency(25). Analysis of more than 100,000 participants from the Nurses’ Health Study and the Health Professionals Follow-Up Study showed that a higher total vitamin D intake (from foods and supplements) was associated with a decreased risk of CVD, including CAD and stroke, although the effect appeared to be confined to men(64). In the Framingham Offspring Study the rate of cardiovascular events was also higher in those patients with 25(OH)D₃ levels < 15 ng/mL, although the association was confined to patients with hypertension, RR 2.13, (95% CI: 1.30 - 3.48). A study of more than 10,000 men and women from the Danish general population compared cardiovascular risk in individuals with plasma 25 (OH)D₃ levels at 1-4th percentile with those at the 50-100th percentile (9). The multivariable adjusted risk was increased by 40% for ischemic heart disease, 64% for myocardial infarction, 57% for sudden death and 81% for fatal ischemic heart disease/myocardial infarction. A meta analysis of 17 studies reported in the literature, including several of those described above, showed that the risk of ischemic heart disease and early death were increased by 39% and 46% for the lowest versus highest quartile of 25 (OH)D₃ levels(12). In aggregate, these studies would appear to support
the link between low 25(OH)D₃ levels and CVD. However, a prospective cohort study (10) of almost 1500 postmenopausal women followed over a 5 year period failed to establish a link between vitamin D levels and cardiovascular events. Noteworthy, those with the lowest 25 (OH)D₃ levels at baseline tended to have more risk factors for CVD – a confounding element, which the authors speculated, may have contributed to the associations identified in previous studies.

To date there have been no randomized clinical trials designed to assess CVD as a primary outcome of vitamin D therapy; however, several studies have examined this relationship in secondary analyses. Overall, results have failed to demonstrate a significant effect of vitamin D supplementation with or without calcium (54), but there was a trend towards benefit in several studies. In a study of vitamin D supplementation over 4 months, a small and insignificant trend towards a reduction in cardiovascular death was observed (74). Similar results were seen in a study designed to assess vitamin D status and fracture risk, in which a greater percentage of women receiving placebo (vs. vitamin D) had a cardiovascular event (2.0 vs. 1.3%), although this did not reach statistical significance (55). Trivedi et al. found no significant effect of vitamin D supplementation on cardiovascular mortality (or total mortality) after 5 years(74). A prospective study of post menopausal women that assessed dietary vitamin D intake and vitamin D supplement use failed to demonstrate a reduction in CVD with vitamin D supplementation (8). In a second study of 114 post menopausal women with serum 25(OH)D₃ levels >10 and < 60 ng/ml who received 2500 IU of vitamin D₃ vs. placebo daily for 4 months, there was no improvement in endothelial function, arterial stiffness or inflammation markers (23) thus failing to identify a potential mechanism for the putative
reduction in CVD risk. Analysis of the results from the Women’s Health Initiative also showed no significant difference in the rate of MI, angina or stroke (29) among groups with varying degrees of vitamin D deficiency and no effect of vitamin D/calcium supplementation on coronary or cerebrovascular risk after 7 years of follow up. At this point in time, there is very little support for the hypothesis that vitamin D supplementation will reduce the incidence of CVD; however, most of these interventional studies have been small, short term, inadequately powered or otherwise flawed. Hopefully this debate will be resolved with the ongoing Vitamin D and Omega-3 Trial (VITAL; www.vitalstudy.org). This is a randomized, placebo-controlled trial that will attempt to determine whether daily vitamin D supplementation (2000 IU) reduces incident heart disease, stroke and cancer (41).

Cardiac hypertrophy, cardiomyopathy and heart failure

Given the anti-hypertrophic activity that VDR ligands have demonstrated in the preclinical studies, it was an obvious move to determine whether the same anti-hypertrophic activity was operative in human subjects (i.e., a more selective segment of CVD). Even here, results have been mixed. In a longitudinal study of 256 subjects of the Hoorn Study, a population-based cohort in the Netherlands, low serum 25(OH)D$_3$ levels were associated with higher left ventricular mass index (LVMI) after 8 years of follow up, but only in subjects without prior CVD and in subjects with low kidney function (76). The effect in the latter group was attenuated after adjustment for parathyroid hormone levels leading the authors to conclude that no strong association exists between 25(OH)D$_3$
levels and myocardial structure and function. In the PIVUS study, another community-based study, 870 participants without prior CVD, impaired left ventricular end systolic dimension, fractional shortening and ejection fraction were all associated with low 25(OH)D₃ levels at baseline, but the latter were not associated with changes in LV geometry or function over the ensuing 5 year period (22). A third population-based study from the Baltimore Longitudinal Study of Aging found that in a group of largely vitamin D-sufficient subject without CVD, left ventricular geometry was optimal at intermediate 25(OH)D₃ levels and deteriorated at the extremes of plasma 25(OH)D₃ levels (2). In contrast, in a more recent study of an older Icelandic population, 25(OH)VD₃ was not associated with LV mass or thickness as measured by MRI (77). Interestingly, serum PTH was associated with both greater LV mass and decreased function in this study.

CVD is the leading cause of death in patients with chronic kidney disease (27, 28) and some element of cardiac hypertrophy is present in most patients with advanced kidney disease. Park and coworkers (52) were the first to demonstrate that administration of 1,25(OH)₂D₃ to hemodialysis patients with secondary hyperparathyroidism resulted in a dramatic reduction of left ventricular hypertrophy as well as reduction in plasma renin, angiotensin II and ANP levels. These changes correlated strongly with reductions in plasma parathyroid hormone (PTH) levels. Similar findings of anti-hypertrophic effects of vitamin D in renal failure patients have been reported by two other groups (36, 42). It is notable that in the study from Lemilla et al. (36), the reductions in ventricular hypertrophy were confined to those individuals with elevated PTH levels (36). However, Bucharles et al. (10) showed that hemodialysis
patients with low vitamin D levels, displayed findings of concentric left ventricular hypertrophy and a high level of inflammation (high sensitivity CRP, IL-6 and serum albumin were used as markers), even in the absence of high immunoreactive PTH levels (< 300pg/ml). Cholecalciferol supplementation in a subset (30 subjects) of these patients suppressed levels of the inflammatory markers and left ventricular hypertrophy (14). Thadhani and the PRIMO investigators (69) carried out a multinational, double-blind, randomized, placebo-controlled trial among 227 patients with chronic kidney disease, mild-to-moderate left ventricular hypertrophy and preserved left ventricular ejection fraction. Study participants received paricalcitol or placebo over a 48 week period. They found that treatment with paricalcitol reduced PTH levels effectively but there was no effect on left ventricular mass index, a metric of cardiac hypertrophy, or diastolic function assessed by echocardiography. Noteworthy, hypercalcemia was more common in the paricalcitol vs. placebo treated group. Followup analysis of 196 patients from this same group showed that paricalcitol did promote a reduction in left atrial volume index and attenuated the rise in B-type natriuretic peptide (BNP) levels (68). A summary of these studies is presented in Table 2.

Vitamin D deficiency has been strongly linked to heart failure in a variety of observational studies (38), although its causal relationship to heart failure remains highly controversial (1). Heart failure has been reported in infants with rickets (39) (3) (28, 31) (11), although, in this setting, profound hypocalcemia may contribute to the observed cardiac dysfunction. In one study vitamin D supplementation (vs. placebo) in 80 infants with congestive heart failure (71), all of whom had baseline 25 (OH)D₃ levels that were below the lower end of the
reference range, led to a significant improvement in heart failure score, several
echocardiographic indices of myocardial function, including left ventricular
ejection fraction, and the serum cytokine profile (61). It is noteworthy that patients
with hereditary vitamin D resistant rickets, who harbor mutations of the VDR
gene, showed no abnormalities in circulating plasma renin activity, angiotensin
converting enzyme activity, angiotensin II or aldosterone levels; nor was there
evidence of hypertension or echocardiographic pathology (72).

In cross sectional studies, vitamin D deficiency has been linked to the
presence of heart failure (19) (53). In a study drawn from the NHANES III
database, vitamin D insufficiency, defined as 25(OH)D₃ < 30 ng/mL was
associated with an OR of 1.7 (95% CI:0.87-3.32) for heart failure and 3.52 (95%
CI: 1.58-7.84) for CVD and heart failure combined (32). Similar results have been
reported in a study of patients referred for coronary angiography. Vitamin D
levels were negatively correlated with N-terminal pro-BNP, a marker of cardiac
dysfunction and failure, and negatively correlated with NYHA classification and
impaired LV function. After correction for cardiovascular risk factors, the hazard
ratio for death due to heart failure was 2.84 (95% CI: 1.2-6.74) and 5.05 for
sudden cardiac death (95% CI: 2.13-11.97) when vitamin D deficient patients
with 25(OH)D₃ levels ≤10 ng/ml were compared with replete patients with levels
≥ 30 ng/ml (53). Interestingly, a recent report linked a functional polymorphism in
the 1-(OH)ase gene, the rate limiting step in the synthesis of active
1,25(OH)₂D₃, with increased risk for heart failure (88).
The effect of vitamin D supplementation, in combination with other micronutrients, has been shown to improve left ventricular function (91), although the intervention also included several compounds thought to be beneficial in heart failure. Recently, a small, randomized, 20 week study compared the effect of vitamin D vs. placebo in patients with heart failure. Vitamin D levels improved and BNP levels fell in the vitamin D-treated group. However, there was no benefit in functional studies and, surprisingly, perceived quality of life was reduced in those receiving vitamin D. (89). A short term randomized, controlled trial examining the effects of vitamin D supplementation (2500 IU/d) on endothelial function, arterial stiffness or inflammation – indirect measures of cardiovascular health – showed no effect of the supplementation (23). A similar small randomized study comparing calcium and vitamin D supplementation showed statistically significant reductions in TNF and increases in the anti-inflammatory cytokine IL-10 in the vitamin D treatment group; however, no change in LV function was observed (60). In a study from Zia et al. (113), a small group of 14 African American subjects with vitamin D deficiency (25(OH)D levels (14.4 +/- 1.3 ng/ml at entry) and dilated cardiomyopathy with reduced ejection fraction (EF) (<35%) were treated with oral ergocalciferol for 8 weeks followed by a maintenance phase of cholecalciferol plus calcium carbonate supplementation for 6 weeks. Treated patients demonstrated increased 25(OH)D levels, reduced PTH and plasma 8-isoprostane (a biomarker of lipid peroxidation) levels and improvement of EF (from 24.3 +/- 1.7% to 31.3 +/- 4.3%). Gotsman et al. (26) studied more than 3000 heart failure patients in an Israeli HMO and established that vitamin D deficiency was an independent predictor of mortality in patients with heart failure (HR 1.52; CI 1.21-1.92; p<.001) and in the control
group (HR 1.91; CI 1.48-2.46; p<.00001). Intriguingly, vitamin D supplementation in
1783 patients was independently associated with reduced mortality in the heart failure
patients (HR 0.68; CI 0.54-0.85; p<.0001). Heart failure is also associated with skeletal
myopathy that can lead to poor exercise tolerance. Witham and coworkers(90) showed
that treatment of elderly vitamin D-deficient, heart failure patients with vitamin D2 for 10
weeks failed to improve functional capacity or quality of life in this patient population.
Quality of life was, in fact, slightly, but significantly, worse in the vitamin D
supplemented group. In aggregate, data gathered to date indicate that there is an
inverse relationship between 25(OH) D levels and the presence of heart failure;
however, studies carried out to date have not been able to demonstrate a palliative
effect of vitamin D supplementation on the various clinical parameters associated with
heart failure.

CONCLUSION

A growing body of data suggests that vitamin D and VDR, its cognate receptor,
play an important role in the regulation of cardiovascular homeostasis. The ability of this
hormonal system to inhibit the renin-angiotensin system, control blood pressure, inhibit
cellular proliferation and hypertrophy, reduce fibrosis and suppress immune function
suggests a variety of plausible mechanisms that could contribute to these palliative
effects (Fig. 1). However, the cardioprotective “hypothesis” has not been without
controversy and results to date have been inconclusive. While the preponderance of
evidence gathered from the experimental studies have supported the hypothesis, and
the clinical epidemiological studies have established a clear link between low vitamin D levels and CVD, it remains to be determined whether the latter are mechanistically linked and, inferentially, whether CVD is treatable with supplemental vitamin D. We anxiously await the results of clinical trials, currently underway, to help define the role of vitamin D and its analogues in the prevention and treatment of CVD.

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91. Witte KK, Nikitin NP, Parker AC, von Haehling S, Volk HD, Anker SD, Clark AL, and Cleland JG. The effect of micronutrient supplementation on quality-of-life and...


Fig. 1. (A) The VDR binds RXR at the vitamin D receptor element (VDRE) to regulate gene expression. (B) Vitamin D deficiency and/or VDR gene deletion affects the heart, vasculature and immune system which may in turn, contribute to the development of heart failure, hypertension and increased atherosclerosis.
**Table 1. Relationship of the liganded VDR to cardiac hypertrophy and/or fibrosis in animal models**

<table>
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<tr>
<th>Animal Model</th>
<th>Treatment</th>
<th>Outcome</th>
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</tr>
</thead>
<tbody>
<tr>
<td>VDR knockout mice</td>
<td>N/A</td>
<td>Cardiac hypertrophy, increased myocyte size, cardiac fibrosis</td>
<td>16, 94</td>
</tr>
<tr>
<td></td>
<td>Captopril</td>
<td>Reduced cardiac hypertrophy and myocyte size</td>
<td></td>
</tr>
<tr>
<td>1- 25-hydroxylase knockout mice</td>
<td>N/A</td>
<td>Cardiac hypertrophy, increased myocyte size, impaired systolic function</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>1,25(OH)₂D₃ or Captopril or</td>
<td>Normalized cardiac structure and function</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Losartan</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mice with transverse aortic constriction</td>
<td>N/A</td>
<td>Cardiac hypertrophy and fibrosis</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>Paricalcitot</td>
<td>No effect on hypertrophy, but reduced fibrosis and improved cardiac function</td>
<td></td>
</tr>
<tr>
<td>Ang II-treated mice</td>
<td>N/A</td>
<td>Cardiac hypertrophy and fibrosis</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>ZK191784</td>
<td>Reduced fibrosis and expression of fibrotic markers</td>
<td></td>
</tr>
<tr>
<td>Vitamin D deficient hypercholesterolemic</td>
<td>N/A</td>
<td>Cardiac hypertrophy and epicardial inflammation</td>
<td>27</td>
</tr>
<tr>
<td>swine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin D deficiency rats</td>
<td>N/A</td>
<td>Cardiac hypertrophy, increased myofibril size and matrix protein production</td>
<td>85</td>
</tr>
<tr>
<td>Maternal vitamin D deficiency in rats</td>
<td>N/A</td>
<td>Cardiac hypertrophy, increased cardiomyocyte number and size</td>
<td>24</td>
</tr>
<tr>
<td>SHR/SHR-HFP</td>
<td>N/A</td>
<td>Cardiac hypertrophy and fibrosis, increased LV diameter and stroke volume</td>
<td>34, 40, 56</td>
</tr>
<tr>
<td></td>
<td>Paricalcitol or Doxercalciferol or 1,25(OH)₂D₃</td>
<td>Reduced hypertrophy and improved cardiac function, but no change in collagen content</td>
<td></td>
</tr>
<tr>
<td>Uremic rats</td>
<td>N/A</td>
<td>Cardiac hypertrophy and fibrosis</td>
<td>33, 47, 58</td>
</tr>
<tr>
<td></td>
<td>1,25(OH)₂D₃ or Paricalcitot</td>
<td>Reduced hypertrophy; prevented fibrosis. In one case (ref 58) fibrosis increased</td>
<td></td>
</tr>
<tr>
<td>Dahl-salt sensitive rats</td>
<td>Paricalcitot</td>
<td>Partially reversed cardiac hypertrophy and fibrosis</td>
<td>5, 7, 18</td>
</tr>
<tr>
<td></td>
<td>N/A</td>
<td>Cardiac hypertrophy, heart failure</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Paricalcitot or Doxercalciferol</td>
<td>Inhibited hypertrophy and improved cardiac function</td>
<td></td>
</tr>
</tbody>
</table>
Table 2. Clinical studies of vitamin D effects on cardiac hypertrophy

<table>
<thead>
<tr>
<th>Study</th>
<th>Population</th>
<th>N</th>
<th>Primary Measure</th>
<th>Outcomes</th>
<th>Ref</th>
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</thead>
<tbody>
<tr>
<td>Cohort</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Fall T, et al.</td>
<td>Older age</td>
<td>870</td>
<td>25(OH)D$_3$</td>
<td>Increased LVESD, FS, EF</td>
<td>22</td>
</tr>
<tr>
<td>Ameri P, et al.</td>
<td>Subjects without CVD</td>
<td>711</td>
<td>25(OH)D$_3$</td>
<td>Increased LVMI, LV thickness</td>
<td>2</td>
</tr>
<tr>
<td>van Ballegooijen AJ, et al.</td>
<td>Older age</td>
<td>969</td>
<td>25(OH)D$_3$</td>
<td>No association detected with LV structure</td>
<td>77</td>
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<tr>
<td>Interventional</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Park CW, et al.</td>
<td>ESRD with secondary HPT</td>
<td>Treatment (15) Controls (10)</td>
<td>Calcitriol treatment</td>
<td>Decreased IVW, LVPW, LV thickness, and LVMI</td>
<td>52</td>
</tr>
<tr>
<td>Lemmila S, et al.</td>
<td>ESRD</td>
<td>10</td>
<td>Calcitriol treatment</td>
<td>Decreased IVW, LVPW, LV thickness, and LV dimension</td>
<td>36</td>
</tr>
<tr>
<td>Bucharles, S, et al.</td>
<td>ESRD</td>
<td>30</td>
<td>Cholecalciferol treatment</td>
<td>Decreased LVMI</td>
<td>10</td>
</tr>
<tr>
<td>Matias, PJ, et al.</td>
<td>ESRD</td>
<td>158</td>
<td>Paricalcitol treatment</td>
<td>Decreased LVMI</td>
<td>42</td>
</tr>
<tr>
<td>Randomized Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thadhani, R, et al.</td>
<td>CKD</td>
<td>Paricalcitol (115) Placebo (112)</td>
<td>Paricalcitol or placebo treatment</td>
<td>No change in LVMI or diastolic function</td>
<td>69</td>
</tr>
</tbody>
</table>

Left Ventricular Mass Index (LVMI), Left Ventricular End Systolic Volume (LVESD), Left Ventricular Ejection Fraction (LVEF), Intraventricular Wall (IVW), Left Ventricular Posterior Wall (LVPW), Hyperparathyroidism (HPT), End Stage Renal Disease (ESRD), Chronic Kidney Disease (CKD), 25-hydroxyvitamin D3 (25(OH)D$_3$), Cardiovascular Disease (CVD).
A Gene Regulation

1,25 (OH)2VD3

B Vitamin D Deficiency and Cardiovascular Disease

Cardiac Hypertrophy
Cardiac Fibrosis
Impaired Contractility

Increased Renin Angiotensin System
Endothelial Dysfunction
Increased Reactive Oxygen Species

Impaired Innate Immunity
Impaired Macrophage Function
Altered Cytokine Production

Congestive Heart Failure
Hypertension
Atherosclerosis