Vitamin D and Cardiovascular Disease

P.E. Norman, J.T. Powell

Abstract: Vitamin D plays a classical hormonal role in skeletal health by regulating calcium and phosphorus metabolism. Vitamin D metabolites also have physiological functions in nonskeletal tissues, where local synthesis influences regulatory pathways via paracrine and autocrine mechanisms. The active metabolite of vitamin D, 1α,25-dihydroxyvitamin D, binds to the vitamin D receptor that regulates numerous genes involved in fundamental processes of potential relevance to cardiovascular disease, including cell proliferation and differentiation, apoptosis, oxidative stress, membrane transport, matrix homeostasis, and cell adhesion. Vitamin D receptors have been found in all the major cardiovascular cell types including cardiomyocytes, arterial wall cells, and immune cells. Experimental studies have established a role for vitamin D metabolites in pathways that are integral to cardiovascular function and disease, including inflammation, thrombosis, and the renin–angiotensin system. Clinical studies have generally demonstrated an independent association between vitamin D deficiency and various manifestations of degenerative cardiovascular disease including vascular calcification. However, the role of vitamin D supplementation in the management of cardiovascular disease remains to be established. This review summarizes the clinical studies showing associations between vitamin D status and cardiovascular disease and the experimental studies that explore the mechanistic basis for these associations. (Circ Res. 2014;114:379-393.)

Key Words: cardiovascular disease ■ vitamin D ■ 1,25(OH)₂D

Vitamin D Physiology and Metabolism

Vitamin D (nutritional term for compounds with biological activity of 1α,25-dihydroxyvitamin D; also used to indicate summation of Vitamin D₃ and D₇) is a fat-soluble vitamin that functions as a steroid hormone. It plays a crucial role in mineral homeostasis and skeletal health and its deficiency classically leads to rickets in children and osteomalacia in adults. Its primary action on the skeletal system is to regulate calcium and phosphorus metabolism by influencing intestinal absorption, bone resorption, and renal retention (Figure 1). Vitamin D metabolites also play an integral physiological role in non-skeletal tissues and have been implicated in a wide range of chronic pathology, including skin and autoimmune disease, cancer, diabetes mellitus, hypertension, and cardiovascular disease (CVD). Interest in the role of vitamin D in CVD arose from evidence of adverse cardiovascular effects of vitamin D deficiency in animal models, and epidemiological studies reporting the increase in cardiovascular events in winter and at increasing distance from the equator.

Vitamin D₃ (cholecalciferol) is mainly produced nonenzymatically from precursors when skin is exposed to ultraviolet light. Skin synthesis of vitamin D₃ is much more important than any dietary sources such as fatty fish. Vitamin D₂ (ergocalciferol) is a plant-derived form of vitamin D manufactured through exposure of yeast to ultraviolet light. The inactive precursors (or prohormones) from either skin production or diet undergo 25-hydroxylation by 1 of 4 cytochrome P450 enzymes (most importantly CYP2R1) in the liver producing 25-hydroxyvitamin D [25(OH)D], the form usually considered as a circulating biomarker of vitamin D status. Subsequent conversion by 1α-hydroxylase (another cytochrome P450 enzyme; CYP27B1) into the active form, 1α,25-dihydroxyvitamin D [1,25(OH)₂D] or calcitriol, occurs primarily in the kidney (Figure 1). Strict transcriptional feedback mechanisms involving parathyroid hormone and fibroblast growth factor 23 control renal production of 1,25(OH)₂D, and all target cells express 24-hydroxylase that converts 1,25(OH)₂D into an inactive form [1,24(OH)₂D]. Various extrarenal tissues, including many cell types involved in CVD, also express CYP27B1 and are therefore able to produce 1,25(OH)₂D. This local production and breakdown is not subject to the same feedback controls as renal production.

Vitamin D Deficiency and Excess

There is controversy about the definition of vitamin D deficiency (or hypovitaminosis D), the optimum serum level of 25(OH)D, and the dietary requirements of vitamin D. The Endocrine Society Clinical Practice Guideline defines vitamin D deficiency as 25(OH)D level <50 nmol/L (20 ng/mL) and insufficiency as 52.5 to 72.5 nmol/L. The International Osteoporosis Foundation defines vitamin D deficiency as 25(OH)D level <25 nmol/L and insufficiency as <50 nmol/L.
and a target level of 75 nmol/L. The Institute of Medicine recommends that a target level of 50 nmol/L is sufficient for bone health. This lack of consensus, along with inherent fluctuations with season, diet, and supplements, makes the assessment of the relationship between vitamin D status and fluctuations with season, diet, and supplements, makes the recommendation that a target level of 50 nmol/L is sufficient for bone health. This lack of consensus, along with inherent fluctuations with season, diet, and supplements, makes the assessment of the relationship between vitamin D status and CVD complex.2 The variability between the different assays assessment of the relationship between vitamin D status and CVD complex.2 The variability between the different assays assessment of the relationship between vitamin D status and CVD complex.2 The variability between the different assays assessment of the relationship between vitamin D status and CVD complex.2 The variability between the different assays assessment of the relationship between vitamin D status and CVD complex.2 The variability between the different assays assessment of the relationship between vitamin D status and CVD complex.2 The variability between the different assays assessment of the relationship between vitamin D status and CVD complex.2 The variability between the different assays assessment of the relationship between vitamin D status and CVD complex.2

Vitamin D Receptor

VDR is a member of the nuclear receptor superfamily, and it regulates numerous genes whose promoters contain vitamin D response elements. These genes are involved in regulatory processes of potential relevance to CVD, including cell proliferation and differentiation, apoptosis, oxidative stress, membrane transport, matrix homeostasis, tissue mineralization, and cell adhesion.20 VDRs have been found in all the major cardiovascular cell types, including VSMC,21 EC,22 cardiomyocytes,23 most immune cells,24 and platelets.25 VDR binds 1,25(OH)2D with high affinity and specificity and then heterodimerizes predominantly with retinoid X receptor (Figure 2). Structurally, VDR consists of a short N-terminal domain before the DNA-binding domain comprising 2 zinc fingers, the first conferring vitamin D response element specificity and the second providing a site for heterodimerization.2 The rest of the molecule contains the lipophilic 1,25(OH)2D-binding domain and the C-terminal, the coactivator-binding domain. Gene expression is dependent on the involvement of tissue-specific coactivators, including steroid receptor coactivator, and the silencing of corepressors that modify chromatin structure thereby allowing gene transcription; the DNA-binding domain is small, and contact is limited to 6 nucleotides in the opened DNA groove.26 These protein complexes influence target gene specificity that may be of relevance in CVD; for example, mothers against decapentaplegic homolog 3 that is involved in transforming growth factor-β transcription also coactivates the VDR.25 VDR downregulates some genes, including those for parathyroid hormone and CYP27B1.20 There has been some interest in epigenetic effects of vitamin D metabolites with emerging evidence that VDR activation can influence post-translational events via the generation of specific subsets of microRNAs.28 As with some other nuclear receptors, VDR displays rapid (seconds to minutes) nongenomic effects involving transmembrane calcium transport and various second messengers that may in turn modulate gene expression (Figure 2).26,25 Our understanding of the importance of this signaling concerning CVD is limited.26

Mechanistic Basis of the Effect of 1,25(OH)2D on Cardiovascular Function

Several pathways and cell types that are relevant to cardiovascular physiology and pathology are influenced by vitamin D metabolites as summarized in Figure 3 and the Table. Most cardiovascular and inflammatory cells express CYP27B1, enabling local synthesis of 1,25(OH)2D.35 This target cell synthesis of 1,25(OH)2D seems to be particularly important in the nonskeletal actions of vitamin D and may explain evidence, suggesting that serum concentration of 25(OH)D correlates better with clinical outcomes than 1,25(OH)2D concentration.49

Inflammation: Endocrine, Paracrine, and Autocrine Actions of Vitamin D Metabolites

Immune and inflammatory cells play key roles in all forms of CVD including atherosclerosis.30 VDR has been identified in most immune cells, notably in macrophages, dendritic cells, and activated T cells. Overall, 1,25(OH)2D controls inflammatory and immune responses keeping them within physiological boundaries (Figure 3). Although early

Nonstandard Abbreviations and Acronyms

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innate immune responses to infection are stimulated with enhanced cytokine release, this is tempered by negative feedback loops, and adaptive immune responses to 1,25(OH)2D are generally anti-inflammatory (Figure 4). Proinflammatory cytokines such as interleukin-1 (IL-1), IL-2, IL-6, IL-23, tumor necrosis factor-α, and interferon-γ are downregulated, and anti-inflammatory ones such as IL-4 and IL-10 are upregulated.50–52 Both 1,25(OH)2D and 25(OH)D inhibit the production of tumor necrosis factor-α and IL-6 by targeting monocyte/macrophage mitogen-activated protein kinase phosphatase-1.53 There is some evidence that epigenetic mechanisms may be involved in the attenuation of Toll-like receptor-mediated inflammation by 1,25(OH)2D; the suppressor of cytokine signaling 1 is stimulated by 1,25(OH)2D via downregulation of proinflammatory microRNA-155 production in macrophages (Figure 5).49 In patients with type 2 diabetes mellitus, endoplasmic reticulum stress and the uptake of cholesterol by macrophages is suppressed by 1,25(OH)2D, with reduced foam cell formation.56,57

An important feature of the modulation of immune function by 1,25(OH)2D is suppression of the development and responses of Th1 and Th17 cells and favoring Treg and Th2 cells.59 This is highly relevant to atherosclerosis given that cytotoxic T cells promote vulnerable plaque in apoE-deficient mice.72 There is animal model evidence that 1,25(OH)2D inhibits atherosclerosis by inducing tolerogenic dendritic cells and regulatory T cells58 and, in a clinical study, higher serum 25(OH)D concentrations were associated with a smaller plasma compartment of CD8+ cells in patients with type 1 diabetes mellitus.73

Experimental evidence demonstrating modulation of immune and inflammatory cell differentiation and related cytokine release suggests important roles for vitamin D metabolites in the pathogenesis of atherosclerosis, aneurysm formation, and other inflammatory vascular disease. There is some limited clinical and epidemiological evidence to support this. A good example of stepping beyond the physiological boundaries for inflammatory and immune responses at low serum concentrations of 25(OH)D comes from patients with diabetes mellitus, where there is some

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**Figure 1. Endocrine pathways of vitamin D metabolites.**

FGF-23 indicates fibroblast growth factor 23. Adapted from Holick1 with permission of the publisher. Copyright © 2007 Massachusetts Medical Society. Authorization for this adaptation has been obtained both from the owner of the copyright in the original work and from the owner of copyright in the translation or adaptation.
intriguing information to suggest that polymorphisms in vitamin D metabolism genes may have a role in the early phases of islet autoimmunity.\(^7^4\) One randomized trial has shown that 1 year of vitamin D supplementation reduced circulating IL-6 concentrations in overweight subjects, although high sensitivity C-reactive protein concentrations increased.\(^7^5\) A much larger population study (NHANES) showed a strong inverse relationship between serum vitamin D and CRP concentrations in those with the lowest concentrations of 25(OH)D, suggesting that vitamin D supplementation for those with vitamin D deficiency might beneficially reduce inflammation.\(^7^6\) Finally, there is some evidence that vitamin D is an important influence on T cell regulation in patients with Behcet’s disease.\(^7^7\)

**EC and VSMC Function**

EC dysfunction is associated with a proinflammatory and prothrombotic state, increased arterial stiffness and ultimately atherosclerosis. Human EC express VDRs and inflammatory cytokines cause rapid induction of endothelial CYP27B1 thereby allowing local production of 1,25(OH)\(_2\)D.\(^2^2,3^5\) Exogenous vitamin D resulted in decreased proliferation of EC and increased monocyte adhesion, which was independent of intercellular adhesion molecule-1 or vascular cell adhesion molecule-1 induction. In addition, EC stress results in upregulated expression of the VDR. These effects on EC suggest an autocrine or paracrine role for 1,25(OH)\(_2\)D with the potential to influence early atherogenesis by modulation of EC adhesion adversely and hence VSMC migration and proliferation.\(^7^9\) At least one of the mechanisms by which 1,25(OH)\(_2\)D inhibits EC stimulation of VSMC proliferation in vitro involves the suppression of endothelin-induced activation of cyclin-dependent kinase 2.\(^3^4\)

Recent clinical studies also suggest a relationship between vitamin D status and endothelial function.\(^7^9–8^2\) Levels of 25(OH)D in healthy volunteers are independently associated with various measures of endothelial function, arterial stiffness, and coronary flow reserve.\(^8^0,8^3\) In a subgroup of participants with vitamin D deficiency, normalization of 25(OH)D levels at 6 months was associated with a significant increase in reactive hyperemia indices (monitoring endothelium-dependent relaxation). Similarly in other studies, treatment with vitamin D improved arterial stiffness (pulse wave velocity).\(^8^4,8^5\) Vitamin D deficiency is associated with increased EC expression of nuclear factor \(\kappa\)B (NF\(\kappa\)B) and IL-6, and inhibition of NF\(\kappa\)B (with oral salsalate) improved endothelium-dependent dilatation to a greater extent in subjects with the lowest 25(OH)D levels.\(^3^0\) Low 25(OH)D levels are associated with reduced flow–mediated dilation of the brachial artery in patients with type II diabetes mellitus.\(^8^6\) It has been suggested, on the basis of in vitro evidence, that vitamin D may attenuate the adverse effects (including increased NF\(\kappa\)B expression) of advanced glycation endproducts on EC.\(^5^7\)

In addition to any vascular effects mediated via EC, 1,25(OH)\(_2\)D influences VSMC directly via the VDR (Table).\(^8^8\) The production of vascular endothelial growth factor by VSMC is stimulated by 1,25(OH)\(_2\)D, and this may be relevant.
to endothelial repair. Vitamin D analogs have been shown to upregulate type-B endothelin receptor gene (which influences NO release) and downregulate the oxytocin receptor gene in human VSMC, a gene expression pattern that favors vessel relaxation. Exposure to 1,25(OH)₂D increases proliferation of quiescent VSMC but decreases proliferation in nonquiescent cells. Migration of VSMC is also induced by 1,25(OH)₂D via a nongenomic response. In addition, VSMC express CYP27B1 that can be upregulated by parathyroid hormone, providing a potential autocrine mechanism influencing VSMC proliferation and differentiation. These effects of 1,25(OH)₂D are consistent with a role in physiological vascular remodeling although the relevance to either pathological intimal hyperplasia or carotid intima/media thickening is yet to be established.

**The Renin–Angiotensin System**

Murine gene deletion or knockout (KO) studies provide in vivo evidence that 1,25(OH)₂D is involved in the regulation of the renin–angiotensin system (RAS). This is relevant to CVD at multiple levels. Experimental atherosclerosis is accelerated in low-density lipoprotein receptor−/−/VDR−/− mice via loss of macrophage VDR signaling and resultant upregulation of local RAS. Renin gene expression is suppressed by 1,25(OH)₂D via reduction in the activity of the cAMP response element in the renin gene promoter. In the VDR KO model, there is a marked increase in the expression of renin and consequently of angiotensin II production, resulting in hypertension and cardiac hypertrophy. Pancreatic RAS also seems to be upregulated in this model, and this is reversed by treatment with 1,25(OH)₂D resulting in increased insulin secretion. In the CYP27B1 KO model, mice have elevated levels of renin, hypertension, and cardiac hypertrophy, which are reversed by treatment with 1,25(OH)₂D. Activation of the VDR by 1,25(OH)₂D also improves EC function by reduced reactive oxygen species production via downregulation of angiotensin II type 1 receptor expression.

Both renin activity and hypertension have been found to be inversely associated with 25(OH)D levels in clinical observational studies. For example in a cross-sectional analysis of patients with low renin hypertension, renin was inversely associated with 25(OH)D levels and positively associated with salt sensitivity, supporting a role for vitamin D metabolites in hypertension. In NHANES, the odds ratio for risk of hypertension in participants with 25(OH)D levels in the first versus fourth quartile was a modest 1.3 (95% confidence interval [CI], 1.13–1.49). A subsequent meta-analysis of 4 prospective and 14 cross-sectional studies confirmed that 25(OH)D level was inversely associated...
with hypertension with an odds ratio of 0.73 (95% CI, 0.63–0.84) with a significant dose–response effect.96

The influence of vitamin D metabolites on both endothelial function and RAS suggests an important role in physiological control of vascular tone and blood pressure and hence in the pathophysiology of hypertension.

**Thrombosis**

Experimental data suggest that 1,25(OH)₂D influences thrombotic tendency. Ohsawa et al.44 demonstrated that 1,25(OH)₂D downregulated tissue factor and upregulated thrombomodulin expression in cultured monocyctic cells via a VDR-mediated mechanism. In an in vitro study of human VSMC, vitamin D
analogs (paricalcitol and calcitriol) caused downregulation of plasminogen activator inhibitor-1 and thrombospondin-1 mRNA expression and protein production and upregulation of thrombomodulin.46 Given that plasminogen activator inhibitor-1 and thrombospondin-1 both involved thrombus formation, and thrombomodulin overexpression may reduce thrombosis,97 this pattern of effects of 1,25(OH)₂D is consistent with an antithrombotic role. Results from a KO model confirmed initial in vitro studies, with VDR null mice exhibiting a prothrombotic state because of downregulation of both antithrombin and thrombomodulin and upregulation of tissue factor gene expression.98 Another antithrombotic effect of 1,25(OH)₂D is the attenuation platelet activation by EC.99 There are few clinical reports implicating vitamin D deficiency with increased thrombosis. In the 1958 British Birth Cohort, there was a significant inverse association between vitamin D status and tissue plasminogen activator antigen concentration and to a lesser extent with fibrinogen and D-dimer concentrations.100 Low levels of 25(OH)D in patients with antiphospholipid syndrome (a prothrombotic condition) have been associated with reduced expression of tissue factor.101 A recent population-based study (n=18,791) has found that there is a stepwise increase in risk of deep vein thrombosis for each lower tertile of 25(OH)D level.102 This association was independent of cardiovascular risk factors, albeit only in nonsmokers. Although there may be an association between venous thrombosis and atherosclerosis, the relevance of this observation to CVD is unknown.

Matrix Homeostasis

Several lines of evidence suggest that vitamin D metabolites play a role in matrix homeostasis. This may be significant in aneurysmal disease, which is characterized by inflammation, matrix destruction, and proteolysis.103,104 In vitro studies indicate that 1,25(OH)₂D suppresses matrix-metalloproteinase (MMP) production in various cell types and, in the VDR KO mice, the expression of tissue inhibitor of matrix metalloproteinase-1 and tissue inhibitor of matrix metalloproteinase-3 is downregulated, and that of MMP-2 and MMP-9 is upregulated in cardiac muscle.60 There is some clinical evidence that vitamin D insufficiency is associated with increased circulating levels of MMP-2 and MMP-9.105,106 In a study using cultured skin fibroblasts, VDR gene expression was decreased in individuals with Marfan syndrome.107 Given that the VDR is a negative regulator of transforming growth factor-β transcriptional activation,47 this could contribute

Figure 4. Vitamin D metabolites and immune modulation: endocrine, paracrine, and autocrine responses. IL indicates interleukin; INF-γ, interferon-γ; PGE2, prostaglandin E2; TGF-β, transforming growth factor-β; TLR, toll-like receptor; TNF-α, tumor necrosis factor-α; and VDR, vitamin D receptor. (Illustration credit: Ben Smith.)
to aneurysmal phenotype.\textsuperscript{107} However, vitamin D metabolites are also known to repress elastin gene expression via a post-transcriptional mechanism.\textsuperscript{45,108} Because most aortic elastin is synthesized during the fetal and early postnatal period,\textsuperscript{109} it has been hypothesized that exposure of excess vitamin D during fetal life could influence elastin deposition in the developing aorta, thereby predisposing an individual to aneurysm formation in later life.\textsuperscript{110} This is paradoxical given that low vitamin D status in adults may be associated with abdominal aortic aneurysm. Because exposure to excess vitamin D is now relatively rare, the significance of this hypothesis in humans is uncertain.

As with occlusive arterial disease, there is some epidemiological evidence that vitamin D deficiency is associated with an increased risk of aneurysmal disease.\textsuperscript{111} In a large population-based study of older men (n=4233), there was a significant association between low level of 25OHD and abdominal aortic aneurysms\textsuperscript{≥}35 mm in diameter, and the level of 25OHD correlated with aneurysmal diameter in a dose–response fashion.\textsuperscript{112} A similar finding was noted in a small series of patients (n=236) with abdominal and thoracic aneurysms.\textsuperscript{111}

### Cardiomyocyte Function and Cardiac Hypertrophy

Cardiomyocytes express VDR, and their physiological function is influenced by 1,25(OH)\textsubscript{2}D.\textsuperscript{23,62} There is experimental evidence that vitamin D deficiency results in maladaptive cardiac remodeling attributable to progressive myocyte hypertrophy and interstitial fibrosis. The VDR KO mice exhibit ventricular hypertrophy and increased matrix turnover (see above).\textsuperscript{50,64} Because this model is associated with hypertension and elevated parathyroid hormone levels, both of which may cause cardiac hypertrophy,\textsuperscript{10,111} the mechanism for this effect of 1,25(OH)\textsubscript{2}D was unclear. In a model using selective deletion of the VDR gene from cardiomyocytes, Chen et al\textsuperscript{66} have recently confirmed a direct in vivo antihypertrophic effect of 1,25(OH)\textsubscript{2}D on cardiomyocytes, but with little effect on fibrosis. It seems that 1,25(OH)\textsubscript{2}D suppresses expression of modulatory calcineurin inhibitory protein 1, a direct downstream target of suppression of the calcineurin/NF of activated T cells. However, in a murine model of viral myocarditis, a vitamin D analog reduced myocardial fibrosis by inhibition of osteopontin expression.\textsuperscript{114} The secretion of atrial natriuretic peptide (which influences proliferation) is also suppressed by 1,25(OH)\textsubscript{2}D, although it is unclear whether this plays a role in any antihypertrophic effect.\textsuperscript{62} In addition, the expression of type 1 natriuretic peptide receptor-A, an inhibitor of RAS and myocardial hypertrophy, is increased by 1,25(OH)\textsubscript{2}D.\textsuperscript{63}

The rate and magnitude of cardiomyocyte sarcomere contraction is modulated by 1,25(OH)\textsubscript{2}D via interaction with caveolin-3 in t-tubules.\textsuperscript{67} This rapid nongenomic process is thought to be mediated by membrane-bound VDR. Cardiomyocyte relaxation is accelerated by 1,25(OH)\textsubscript{2}D, which may improve coronary perfusion during diastole.\textsuperscript{62} Intracellular calcium influx into cardiomyocytes is also increased by 1,25(OH)\textsubscript{2}D via modulation of β-adrenergic pathways.\textsuperscript{115}

The clinical significance of these experimental observations in cardiac tissue remains to be established. There is evidence that vitamin D deficiency during pregnancy is associated with dilated cardiomyopathy in infants.\textsuperscript{116} Although epidemiological studies have often demonstrated an association between vitamin D deficiency and heart failure in adults, the association may not be significant once adjustment for other risk factors is made.\textsuperscript{19,117} Furthermore 2 clinical trials of the effects of vitamin D in heart failure did not report any significant clinical benefits.\textsuperscript{118,119} An alternative suggestion is that low 25(OH)D concentrations are associated with poor prognosis in patients with heart failure, an effect mediated by activation of RAS and inflammation.\textsuperscript{120}
Vascular Calcification

Vascular calcification is seen in the intima in atherosclerosis, the media in both atherosomal disease and diabetic arterial disease, and in valvular heart disease. This ectopic mineralization process is poorly understood and has been recently reviewed elsewhere.121 The present review will be limited to aspects of vascular calcification that are relevant to vitamin D and its metabolites. The relationship between vitamin D and vascular calcification is complex and may be partly dose dependent: excess vitamin D in the presence of renal impairment results in experimental vascular calcification, yet in contrast, 1,25(OH)2D levels are inversely associated with coronary artery calcification.122 The actions of 1,25(OH)2D on mineral metabolism (increasing phosphate and calcium concentrations) and on osteoblastic gene expression may favor vascular calcification, whereas other effects such as modulation of inflammation may reduce vascular calcification.13

High dose vitamin D metabolites and analogs induce an osteoblastic phenotype in VSMC,42,123 and this has been used in animal models to produce vascular calcification in both the presence124,125 and absence126 of uremia. Macrophyages also influence vascular calcification by enhancing osteogenic phenotype in VSMC.127 Treatment of cocultured macrophages and VSMC with VDR activators induces osteopontin and inhibits bone morphogenetic protein 2 and tumor necrosis factor-α expression, with reversal of the effect of macrophages on VSMC calcification.128 There is some evidence that 1,25(OH)2D increases in vitro VSMC calcification42,123 although this has not be confirmed in other studies.129 Wu-Wong et al41 reported that vitamin D analogs did not influence expression of alkaline phosphatase, osteopontin, or receptor activator for NFκB ligand in VSMC. However, in another study, exposure of VSMC to clinically relevant concentrations of 1,25(OH)2D and phosphate increased the expression of osteoblast-associated genes such as matrix gla protein, osteopontin, and osteonectin.42 In the VDR−/− and Low-Density Lipoprotein receptor−/− mouse model, a low vitamin D diet increased aortic calcification via a mechanism involving upregulation of osteoblast transcription factors.44 These differences in the influence of 1,25(OH)2D or its analogs on both calcification and the gene expression of calcification factors may be pharmacological rather than physiological. Because vitamin D is activated to 1,25(OH)2D in the kidney, uremia changes homeostatic mechanisms, where vitamin D signaling plays a critical role, with vitamin D agonists offering therapeutic benefits.129 In a mouse chronic kidney disease model, therapeutic dosage (sufficient to correct secondary hyperparathyroidism) of the VDR activator, paricalcitol, reduced aortic osteoblastic gene expression and calcification, but high dosage (400 ng/kg) caused a significant increase in calcification.130 This highlights the dose-dependent role of vitamin D in vascular calcification, and presumably cardiovascular health.

Klotho is a gene first described as being associated with premature aging in mice. It is a 130-kDa transmembrane protein that is mainly expressed in the distal nephron.131 The extracellular domain of Klotho is subject to proteolytic cleavage, and the soluble form of Klotho is secreted into the circulation. Klotho has been suggested as a master regulator of arterial calcification. It is a coreceptor for fibroblast growth factor 23 and together they maintain calcium and phosphate balance. Their expression is increased by activation of VDR, with promotion of phosphaturia and autoregulatory inhibition of the conversion of 25(OH)D to 1,25(OH)2D. Two recent studies, one in mice and one in humans, have indicated strongly that Klotho is protective against vascular calcification by preventing the conversion of VSMC to an osteotypic phenotype.132,133 Ectopic calcification seen in mice models with loss of function for either Klotho or fibroblast growth factor 23 is prevented by genetically reducing 1,25(OH)2D production (possibly by reversing hyperphosphatemia).13,134 Klotho also influences endothelium-dependent vasodilation and inflammatory pathways involving VSMC.13 Thus, interactions between Klotho, fibroblast growth factor 23, and vitamin D metabolites are likely of critical importance to the development of vascular calcification.

Clinical and Epidemiological Studies of Vitamin D Deficiency and CVD

Significant associations between low vitamin D status and both prevalent135 and incident136 degenerative CVD have been reported in large-scale studies using composite end points. Vitamin D deficiency has also been linked to several cardiovascular risk factors such as hypertension (see above), dyslipidemia, and diabetes mellitus.

Some clinical studies have shown that high levels of 25(OH)D are associated with favorable lipid profile.137,138 However, these observations are subject to confounding, and a recent meta-analysis of 12 clinical trials (1346 participants) of the influence of vitamin D supplementation on lipid profiles showed little evidence of a beneficial effect.139 In a large population-based study (n=107 811) using serial laboratory results, Ponda et al138 have also cast doubt on the impact of vitamin D on lipid levels. In a subgroup of 6260 subjects with vitamin D deficiency at baseline, and biochemical evidence of improved vitamin D status 4 to 26 weeks later (resulting from vitamin D supplementation), there was no improvement in lipid profile.

Several cross-sectional studies have demonstrated a consistent relationship between vitamin D deficiency and diabetes mellitus. In NHANES III, a 25(OH)D level in the first versus fourth quartile was associated with diabetes mellitus with an odds ratio of 1.73 (95% CI, 1.38–2.16).95 There is a negative correlation between insulin resistance and β cell function in individuals at risk of type 2 diabetes mellitus.140 Longitudinal studies have also shown that higher baseline vitamin D status reduces the risk of incident diabetes mellitus.5,141 Despite the experimental and human observational evidence implicating vitamin D in diabetes mellitus, interventional studies have been inconsistent or inconclusive.142,143

Coronary Heart Disease and CVD Mortality

Several large-scale prospective studies using a range of methodologies have found that baseline 25(OH)D concentration is associated with the incidence of coronary heart disease events in a dose–response fashion. In the Health Professionals Follow-up Study (n=18 225 men aged 40–75 years who were free of CVD at baseline), there was a graded independent relationship between low levels of 25(OH)D and risk of
myocardial infarction; for example, the relative risk for levels ≤37.5 nmol/L versus ≥75 nmol/L was 2.09 (95% CI, 1.24–3.53). In a meta-analysis of 18 prospective studies (n=82982 participants of mixed ethnicity), the risk of any coronary heart disease events was increased by 33% (95% CI, 28%–38%) for the lowest versus the highest quartile of 25(OH)D level. Cerebrovascular disease, although less studied than coronary heart disease, shows a similar pattern: in a meta-analysis of 10 prospective cohort studies (n=58384 participants with 2644 events), for individuals with 25OHD levels in the lowest versus highest quartile, the hazard ratio for ischemic stroke was 1.54 (95% CI, 1.43–1.65).

Observational studies have also shown a graded relationship between vitamin D deficiency and risk of CVD (Figure 6). Significant associations between vitamin D deficiency and CVD mortality have been reported in older adults, although in a report from NHANES of 13331 participants, this was not significant. A recent population-based study of 9146 younger (30–71 years) adults found significant associations with all-cause mortality but not CVD mortality. In a meta-analysis of 17 prospective studies (n=77155 participants of mixed ethnicity), the risk of any early death (as a surrogate for cardiovascular death) was increased by 35% (95% CI, 31%–42%) for the lowest versus the highest quartile of 25(OH)D level.

Peripheral Arterial Disease and Extent of Atherosclerosis

Several cross-sectional population studies have demonstrated an association between the extent of peripheral arterial disease (PAD) in subjects and vitamin D deficiency. In such studies, PAD is usually defined by measuring the ankle brachial index systolic pressure index, which is a specific marker of lower limb atherosclerosis (and an index of overall atherosclerotic burden) rather than a clinical manifestation of lower limb disease. In NHANES 2001 to 2004, the risk of having PAD (based on an ankle brachial index <0.9; n=4818) was almost twice as high for subjects with a 25(OH)D level <50 nmol/L versus those with ≥75 nmol/L; odds ratio 1.82 (95% CI, 1.26–2.61).

In addition to any relationship between vitamin D deficiency and PAD as a marker of atherosclerosis, the role of immobility in both may be important. PAD is associated with decreased mobility (which indirectly reduces sun exposure and impairs vitamin D synthesis), sarcopenia, and an increased risk of hip fracture. In this situation, vitamin D deficiency could be considered a consequence, rather than a cause, of both PAD and osteoporosis.

Therapeutic Implications

Although vitamin D deficiency is undoubtedly bad for cardiovascular health, there is also some evidence that high levels of vitamin D may also be associated with adverse arterial remodeling and poor outcomes. In NHANES III, there was a U-shaped relationship between vitamin D and mortality risk, with an apparent increase in mortality, particularly in women, with 25(OH)D levels >50 ng/L. Although 1 meta-analysis that included 8 studies that assessed relatively high (>65 nmol/L) levels of 25(OH)D found no significant change in risk of CVD, another meta-analysis reported evidence of increased mortality with 25(OH)D concentrations >97.5 nmol/L. Although part of this increased mortality at high levels of 25(OH)D seems to be attributable to cancer, a similar pattern has been reported for cardiovascular outcomes in some studies. In the Framingham Offspring Study, there was a nonlinear relationship between baseline vitamin D status and the adjusted hazard ratio for incident cardiovascular events. Carotid intima/media thickness and cardiac calcification scores were also found to have a bimodal distribution across 25(OH)D levels in a study comparing children on dialysis with controls. These studies did not contain enough individuals with high levels of 25(OH)D to establish whether there is a threshold above which the risk of adverse cardiovascular events is significant (Figure 6). As such, the importance of the various nonlinear patterns remains to be established, and further research is required. Nevertheless a recent review of the risks of vitamin D supplementation suggests that “…there should be a degree of caution about recommending high serum 25(OH)D concentrations for the entire population.”

Ethnicity is relevant when interpreting circulating levels of 25(OH)D because levels are lower in blacks than in whites. There is evidence that low levels of 25(OH)D are associated
with increased risk of coronary heart disease events in whites and Chinese but not among black or Hispanic people.\textsuperscript{160} The reason for this difference is unclear but may relate to differential CYP27B1 or 24-hydroxylase activity and hence tissue availability of 1,25(OH)\textsubscript{2}D.\textsuperscript{161} These differences need to be taken into account when considering therapeutic intervention, at least in the context of cardiovascular risk.

The major problem with epidemiological studies is that vitamin D status may just be a surrogate marker for sociodemographic risk factors and poor metabolic health, thereby confounding any observational associations with CVD.\textsuperscript{162,163} The importance of this confounding has been highlighted in gene association studies. Although a large study suggested that a variant within the VDR gene (rs7968585) increased the composite risk of outcomes, which included myocardial infarction,\textsuperscript{164} a recent Mendelian randomization study failed to demonstrate any consistent relationship between various genetic variants associated 25(OH)D metabolism and cardiovascular outcomes.\textsuperscript{165} This raises important questions concerning the role of vitamin D deficiency in the causal pathway of CVD, although the role of variants in VDR and other components of vitamin D signaling has not been extensively investigated.

Despite the wide-ranging experimental and epidemiological evidence implicating 1,25(OH)\textsubscript{2}D in many aspects of cardiovascular health, a meta-analysis of 51 trials of vitamin D in the prevention of various cardiovascular outcomes showed no overall benefit.\textsuperscript{165} At present, it is unclear whether vitamin D supplementation can reduce the risk or consequences of CVD, and it is not recommended for this indication.\textsuperscript{9,162}

**Future Directions**

Although the results of current trials assessing the role of vitamin D supplementation in the prevention of CVD are awaited with interest,\textsuperscript{166} there are important knowledge gaps which need addressing. First, there is a requirement to better define the therapeutic window for vitamin D supplementation with respect to age and ethnicity, probably in tandem with better quality control of vitamin D metabolite assays. Second, more needs to be learned about the autocrine and paracrine influence of 1,25(OH)\textsubscript{2}D on cardiovascular physiology and pathology at a cellular level, particularly with respect to control of inflammatory networks and local calcification. Areas of potential interest include tissue-specific patterns of 1,25(OH)\textsubscript{2}D–induced gene expression, the role of nongenomic actions of 1,25(OH)\textsubscript{2}D, and the relevance of 1,25(OH)\textsubscript{2}D to matrix homeostasis in aneurysmal disease, diabetic arterial disease, and calcific aortic valve disease. Also of importance is the bioactivity of 1,25(OH)\textsubscript{2}D within vascular tissue and its autoregulation, which is dependent on 25(OH)D acting, not only as a substrate for 1,25(OH)\textsubscript{2}D production, but also as an inducer of CYP27B1 and VDR expression.\textsuperscript{30} Although 1,25(OH)\textsubscript{2}D limits its own availability by induction of 24-hydroxylase in undifferentiated monocytes, this control is antagonized in activated macrophages by interferon-\(\gamma\)–induced Stat-1\(\alpha\) (signal transducers and activators of transcription-1 \(\alpha\)) production.\textsuperscript{167} Given the prominence of interferon-\(\gamma\) in atherosclerosis, the significance of this potentially unregulated local production of 1,25(OH)\textsubscript{2}D is intriguing. Insights into the importance of 1,25(OH)\textsubscript{2}D in regulating the cell cycle in cancer and other pathologies have the potential to inform cardiovascular research. The rates of proliferation, migration, and apoptosis in VSMC are normally low but are all increased in atherosclerosis and injury-induced intimal hyperplasia. Most of these cellular processes are influenced by 1,25(OH)\textsubscript{2}D in cancer, including the regulation of cyclins and their dependent kinases; the transcription of p53 and other genes affecting apoptosis; the expression of insulin-like growth receptors and binding proteins influencing mitogenic pathways; reactive oxygen species generation; and associated effects on inflammation and proliferation.\textsuperscript{168} Interactions between 1,25(OH)\textsubscript{2}D and cell adhesion molecules may also be relevant: the Wnt/\(\beta\)-catenin pathway, which is important in VSMC function and atherosclerosis,\textsuperscript{169} is regulated by 1,25(OH)\textsubscript{2}D in cancer cells; the expression of E-cadherin is increased by 1,25(OH)\textsubscript{2}D, thereby normalizing cellular shape, adhesion, and homophilic interactions.\textsuperscript{166} The influence of 1,25(OH)\textsubscript{2}D on all these pathways in models of atherosclerotic or other CVD merits further exploration.

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