

Effects of Vitamin D on Blood Pressure and Endothelial Function

Bokyung Min

College of Pharmacy, Catholic University of Daegu, Gyeongsan 712-702, Korea

Vitamin D deficiency is prevalent, primarily due to limited sun exposure, which may be observed in urban areas, or as a result of modern lifestyles. Common myths about vitamin D persist, including that it is mostly obtained from the diet and is only essential for bone and mineral homeostasis. Nonetheless, advances in biomedical science suggest that vitamin D is a hormone that is integral to numerous physiologic functions in most cells and tissues. Therefore, abnormal vitamin D levels may contribute to health disturbances. A number of recent reports on potential associations between vitamin D deficiency and cardiovascular disease have highlighted its role in this system. A focus over the previous decade has been to better understand the mechanisms behind vitamin D regulation and the pathophysiology associated with suboptimal vitamin D levels. Vitamin D deficiency is highly associated with the incidence of cardiovascular diseases, even when considering other well-known risk factors. In this process, the renin-angiotensin system is disrupted, and hypertension and endothelial dysfunction contribute to the risk of cardiovascular disease. Likewise, clinical outcomes upon the normalization of vitamin D levels have been investigated in different patient populations. It makes sense that vitamin D supplementation to improve vitamin D status among vitamin D-deficient individuals could be useful without requiring a sudden lifestyle change. This manuscript provides a brief overview of vitamin D metabolism and the vitamin D receptor. It also summarizes the current clinical research relating to vitamin D supplementation and its effects on hypertension and endothelial dysfunction in cardiovascular medicine.

Key Words: 25-hydroxyvitamin D, Blood pressure, Calcitriol, Endothelial dysfunction, Vitamin D

INTRODUCTION


Cardiovascular disease is considered a public health burden in developed countries, and over the recent decades, it has emerged as a problem in developing countries as well. Because of the high prevalence of cardiovascular disease in the general population, it is essential to develop strategic prevention and management plans that include early screening and health education. Early identification of cardiovascular disease risk factors is important, as is modifying those risk factors. To be effective, these risk factors need to be good predictors of future cardiovascular health, and the costs of screening and intervention programs should be low. To help manage the mortality and morbidity related to cardiovascular disease, ideal candidates for screening may be hypertension and endothelial dysfunction because they are common, predictive, and modifiable risk factors.

Vitamin D status can be influenced by multiple variables, including race, geographic latitude, environment, lifestyle, and genetics. It is now recognized across many scientific disciplines that vitamin D deficiency is pandemic [1-3]. Following observations that the risk of cardiovascular disease increases with increasing distance from the equator, and therefore decreasing sunlight exposure, it has been postulated that vitamin D contributes to cardiovascular health [4,5]. With advances in the understanding of cellular and molecular physiology, our knowledge about vitamin D has rapidly evolved and expanded. Vitamin D receptors (VDRs) are abundant in various tissues throughout the human body, including renal tubule cells, parathyroid tissue, bone, immune cells, coronary artery endothelial cells, endothelial cells, cardiomyocytes, and vascular smooth muscle cells. This widespread presence indicates that vitamin D might mediate biological effects and regulate physiological functions beyond just bone and mineral homeostasis [3,5-8].

The objectives of this review are as follows: 1) to provide

Received August 12, 2013, Revised August 27, 2013,
Accepted September 2, 2013

Corresponding to: Bokyung Min, College of Pharmacy, Catholic University of Daegu, 13-13 Hayang-eup, Gyeongsan 712-702, Korea. (Tel) 82-53-850-3609, (Fax) 82-53-850-3602, (E-mail) bmin0@cu.ac.kr

 This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

ABBREVIATIONS: VDR, Vitamin D receptors; DBP, vitamin D binding protein; RXR, retinoid X receptor; VDRE, vitamin D response elements; CRP, C-reactive protein; TNF- α , tumor necrosis factor- α ; IU, international unit; BMD, bone mineral density; RAAS, renin-angiotensin-aldosterone system; ANP, atrial natriuretic peptide; c-AMP, cyclic adenosine monophosphate; CREB, cyclic adenosine monophosphate (c-AMP) response element binding protein; PKA, protein kinase A; SD, standard deviation; FMD, flow-mediated vasodilation; IMT, intima medial thickness; LDL, low density lipoprotein.

an overview of the molecular mechanisms relating to pathophysiological changes with a focus on endothelial dysfunction related to vitamin D; 2) to identify the clinical implications of vitamin D status from an evidence-based perspective; and 3) to suggest future directions for research in this area.

VITAMIN D AND ITS METABOLISM

Although it is generally classified as a vitamin, the biological characteristics of vitamin D align more closely with those of hormones. Vitamin D is supplied from food sources or is synthesized following UVB irradiation, which is the major source. The term "vitamin D" is commonly used to refer to cholecalciferol (vitamin D₃), ergocalciferol (vitamin D₂), or 25-hydroxyvitamin D (25(OH)D₃). This biologically inert compound is metabolized to its active forms alpha-calcidol (1-hydroxyvitamin D₃), doxercalciferol (1-hydroxyvitamin D₂), and calcitriol (1,25-dihydroxyvitamin D₃ [1,25(OH)₂D₃]) in the kidney and peripheral tissue, where they are involved in multiple functions. The level of 25-hydroxyvitamin D is an indicator reflecting the overall body storage pool of vitamin D. Historically, a normal value for vitamin D as 25(OH)D has been defined as ≥ 30 ng/ml in all age groups [9].

The cytochrome P450 system is involved in vitamin D metabolism. The enzymes vitamin D-25-hydroxylase (cytochrome P27A1), 25-hydroxyvitamin D-1 α -hydroxylase (cytochrome P27B1), and 25-hydroxyvitamin D-24-hydroxylase (cytochrome P24) participate in each step of the metabolic pathway to activate the precursor for vitamin D and to degrade individual vitamin D [10].

Vitamin D might share common pleiotropic effects with statins in a number of cells and tissues. Investigations of possible relationships between vitamin D and statins (hydroxymethylglutaryl-CoA [HMG-CoA] reductase inhibitors) have recently emerged. Cytochrome P450-mediated pharmacokinetic interactions via common metabolic pathways followed by competitive receptor binding might result in an elevated serum statin concentration. This elevated concentration can cause troublesome muscle pain, which is known to reduce long-term compliance with statin use. Similarly, other studies have reported that some statins, namely atorvastatin and rosuvastatin, significantly elevate 25-hydroxyvitamin D levels. However, there was no evidence of risk or mortality reduction. Further research on these interactions is required to establish a potential causal relationship [11-13].

The metabolism of vitamin D depends primarily on vitamin D binding protein (DBP) [14]. In other words, the maintenance of optimal vitamin D levels is regulated by the interaction between vitamin D and DBP. Vitamin D and DBP complex are filtered by the glomerulus and then reabsorbed in the renal proximal tubule where they are activated to 1,25-dihydroxyvitamin D₃ in a rate-limiting manner.

VITAMIN D RECEPTORS

The VDR is a nuclear factor receptor with which 1,25(OH)₂D₃ interacts to form a heterodimer conjugation complex with retinoid X receptor (RXR). It is then able to function hormonally to influence gene expression. More specifically, 1,25(OH)₂D₃, which is bound to DBP in circulation, dissociates to enter the cell nucleus where it interacts with the VDR. The binding of 1,25(OH)₂D₃ to the VDR initiates

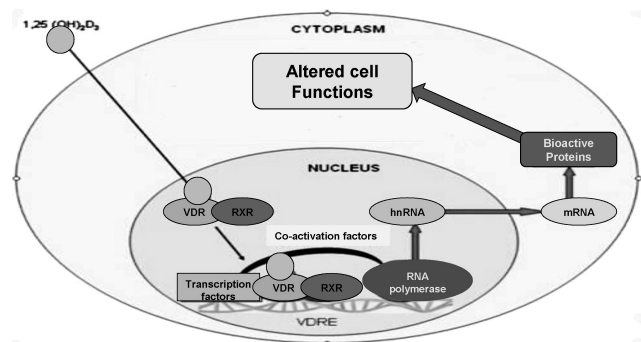


Fig. 1. Interactions between vitamin D receptor and retinoid X receptors inducing vitamin D response elements. Adapted from reference 17.

the recruitment of cofactors to ultimately form a complex with RXR (VDR-RXR) that attaches to vitamin D response elements (VDRE) in the promoter region of target genes to initiate and regulate transcription (Fig. 1). This course of highly regulated gene expression occurs in cells where VDRs are present, including myocytes, cardiomyocytes, pancreatic β -cells, vascular endothelial cells, neurons, immune cells, cells of the parathyroid gland, and osteoblasts. VDRs homodimerize or heterodimerize, depending on the receptor specificity in each cell type [14-19].

VDR gene polymorphism has been recognized, and this genetic heterogeneity might be associated with various chronic diseases such as autoimmune diseases, endocrinological abnormalities, other chronic inflammatory diseases, and some types of cancers. Likewise, the reported risk for hypertension in FF homozygotes was 2.2 times greater than in Ff heterozygotes and 2.2 times greater than in ff homozygotes in the analysis of the VDR *Fok1 I* polymorphism and genetic susceptibility to essential hypertension. However, when comparing Ff and ff genotypes, no significant difference was noted. Therefore, the FF genotype and allele F conferred a risk of developing hypertension regardless of the presence of family history and smoking status. When investigating the relationship between bone mineral density (BMD) and carotid artery intimal medial thickness (IMT) as a surrogate marker of endothelial dysfunction among Mexican women, the VDR *BsmI BB* genotype demonstrated significantly higher forearm BMD and IMT. Furthermore, the association of the VDR genotype with IMT was not necessarily dependent on the association between VDR and BMD. Interestingly, the *BsmI* polymorphism was more vulnerable to Graves' disease and atherosclerosis following long-term valproate exposure in Asians but not in Caucasians. Larger genome-wide cohort studies are warranted to establish relationships between genetic variations and subsequent functional consequences [20-24].

ENDOTHELIAL DYSFUNCTION

Endothelial dysfunction is a hallmark of the pathophysiology of arterial vasculature. Risk factors for endothelial dysfunction include hyperlipidemia, obesity, aging, and smoking. Endothelial dysfunction is an important target for the prevention and early recognition of subclinical cardiovascular diseases [25].

The vascular endothelium is a thin single layer of cells

lining all blood vessels and is responsible for regulating key functions of the vasculature such as (1) serving as a barrier for the exchange of fluid, electrolytes, macromolecules, and cells between the intravascular and extravascular space; (2) regulating smooth muscle through the synthesis of vasoactive substances like nitric oxide, PGI₂, and endothelin-1; (3) modulating platelet aggregation; and (4) modulating leukocyte adhesion and transendothelial migration and expression of adhesion molecules [26].

Results of *in vitro* studies indicate that VDR agonists reduce proinflammatory cytokine production and release. This finding was supported by reports of increased C-reactive protein (CRP) levels in patients with renal impairment due to various etiologies. In similar patient groups, the level of NF- κ B activity was suppressed and the inflammatory response was diminished after 1,25(OH)₂D₃ supplementation [27].

The conversion to the bioactive form 1,25(OH)₂D₃ occurs via the enzyme 1 α -hydroxylase in the endothelial and vascular smooth muscle cells and results in the protection of the vascular walls by vitamin D. Further, 1,25(OH)₂D₃ inhibits cytokine-mediated endothelial cell activation as well as adhesion molecule expression that involves tumor necrosis factor- α (TNF- α). A multitude of evidence exists indicating that the induction of adhesion molecules is a crucial step in the progression from endothelial damage to atherosclerosis. Adhesion molecules facilitate the recruitment of circulating monocytes, which then differentiate into macrophages. Oxidized low-density lipoprotein (LDL) cholesterol is gradually absorbed by macrophages and then incorporated into foam cells. The formation of foam cells amplifies the inflammatory response. Cytokines secreted from endothelial injury stimulate smooth muscle proliferation and migration into the intima and media. Therefore, in essence, vitamin D reduces the expression of adhesion molecules, inhibits endothelium-dependent vasoconstriction, modulates the renin-angiotensin-aldosterone system (RAAS), and hinders vascular smooth muscle cell proliferation and macrophage activation [6,19,28-31].

THE RENIN-ANGIOTENSIN-SYSTEM AND BLOOD PRESSURE

In early animal studies, VDR knockout mice displayed disrupted renin expression and angiotensin II production without any changes in the circulating angiotensinogen level. Although the development of hypertension led by the augmentation of angiotensin II is reasonable to expect, additional experiments are needed to confirm this. Simultaneously, VDR-derived activation in the RAAS was independent of Ca²⁺ homeostasis through the parathyroid hormone. Adverse consequences of RAAS activation included left ventricular hypertrophy due to enlarged cardiomyocytes. Additionally, circulating atrial natriuretic peptide (ANP) and ANP mRNA were elevated [28].

Yuan et al. helped to elucidate the mechanics of the influence of vitamin D on the renin gene transcription (Fig. 2). They identified that 1,25(OH)₂D₃ is not involved in the vitamin D response element pathway but rather binds to the cyclic adenosine monophosphate (c-AMP) response element. This occurs after VDR acts as a ligand and then blocks the c-AMP response element binding protein (CREB)-mediated activation of the rennin gene promoter. c-AMP, a primary intracellular signal in rennin expression,

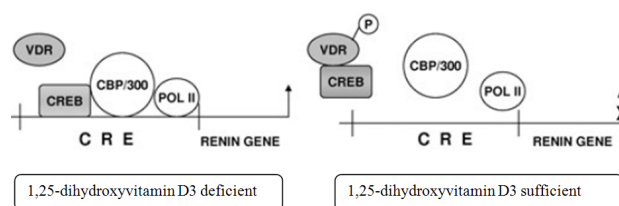


Fig. 2. A proposed mechanism of 1,25-dihydroxyvitamin D₃ on renin-expression [Adapted from reference 28].

binds to the regulatory subunit on c-AMP-dependent protein kinase A (PKA). The catalytic subunit on PKA phosphorylates and activates CREB at the nucleus. In this manner, the c-AMP response element in the promoter region of the renin is bound to CREB and triggers gene transcription [32].

CLINICAL TRIALS WITH VITAMIN D SUPPLEMENTATION TO TREAT VITAMIN D DEFICIENCY

Ecological and epidemiological studies have reported that vitamin D deficiency increases the risk of cardiovascular diseases. Nevertheless, a causal relationship is yet to be established. Some investigators might argue that vitamin D deficiency is not a cause but rather a consequence of cardiovascular disease. Patients with cardiovascular disease participate in fewer outdoor activities due to psychological or physical limitations and are therefore less likely to be physically active or exposed to sunlight. Without UVB irradiation, reliance on food sources of vitamin D increases. It is difficult to meet the daily vitamin D requirements with food alone, and the risk of vitamin D deficiency and the subsequent effects on cardiovascular health increases. Vitamin D deficiency affects cardiovascular disease through the action of several core factors including vascular endothelial dysfunction, RAAS activation, and an adaptive immunity-mediated inflammatory response. Endothelial dysfunction is an early subclinical sign of forthcoming fatal cardiovascular diseases, and it participates in the underlying etiology of premature atherosclerosis. Hormonal abnormality, including RAAS activation, is considered to be a likely contributor to the development of (essential) hypertension. Core risk factors for future life-threatening cardiovascular events include hypertension and endothelial dysfunction [33-35]. We will focus on these 2 risk factors as they relate to vitamin D deficiency, including a review of the relevant clinical research, in the rest of this review.

Hypertension

Pathophysiologic changes accompanying suboptimal levels of vitamin D act unfavorably on the cardiovascular system. Negative consequences include RAAS activation and endothelial dysfunction, both of which increase the risk of developing hypertension and contribute to the worsening of existing hypertension. Thus, it is a reasonable expectation that vitamin D supplementation would ameliorate blood pressure control. In laboratory and animal experiments, vitamin D insufficiency or deficiency contributed to high blood pressure through RAAS activation, which resulted in ventricular hypertrophy and therefore the deterior-

ration of cardiac function [29]. By regulating RAAS, calcitriol administration not only normalized blood pressure but also restored cardiac function. The activity of a renin expression gene promoter was attenuated by calcitriol replacement. Interestingly, captopril or losartan treatment has been shown to effectively control high blood pressure as well as cardiac hypertrophy without the effects on RAAS activation. The effects of vitamin D deficiency on hypertension are not affected by the underlying reason for deficiency (VDR or vitamin D synthesis abnormality) [31].

Epidemiological and observational studies prompted the first laboratory experiments to elucidate why vitamin D deficiency increases the risk of cardiovascular mortality and morbidity. Scragg et al. identified, in the third National Health and Nutrition Examination Survey, that an inverse relationship existed between 25-hydroxyvitamin D and systolic blood pressure, and this relationship remained significant even after adjustment for age, sex, ethnicity, physical activity, and body mass index. People in the highest quintile had lower systolic blood pressure than those in the lowest 25-hydroxyvitamin D quintile [36].

In a retrospective analysis of 2 large cohort studies, 25-hydroxyvitamin D levels and the risk of developing hypertension were evaluated. One cohort was followed for 4~8 years while the other cohort was followed for 16~18 years. Men whose levels of 25-hydroxyvitamin D were in the lowest (<15 ng/ml) category were at the highest risk of hypertension relative to men whose levels of 25-hydroxyvitamin D were in the highest (≥ 30 ng/ml) category (relative risk [RR], 6.13; 95% confidence interval [95% CI], 1.00 to 37.80). In the same comparison with women, the multivariate relative risk was 2.67 (95% CI, 1.05 to 6.79) [37].

Since findings from observational or epidemiologic studies consistently revealed that suboptimal vitamin D levels were linked with higher all-cause mortality rates, including those from cardiovascular disease, it could be assumed that vitamin D replacement or normalization would reduce the risk of cardiovascular disease and its effects.

The effects of 8 weeks of supplementation with vitamin

D plus calcium compared to calcium-only supplementation on the blood pressure among 148 women aged more than 70 years (mean \pm standard deviation [SD], 74 \pm 1 years) was studied. Vitamin D plus calcium significantly lowered systolic blood pressure by 9.3%, and the calcium-only supplementation lowered it by 4.1% compared to baseline. Diastolic blood pressure was not significantly different in the 2 groups. The vitamin D supplementation was more effective in restoring overall biochemical stability, including electrolyte correction, than the calcium-only treatment [38].

Cardiovascular surrogate markers were assessed following vitamin D supplementation for 16 weeks in 49 young black people with vitamin D insufficiency or deficiency. At baseline, both systolic (112.5 \pm 9.1 mmHg) and diastolic (68.8 \pm 9.4 mmHg) blood pressure measurements were similarly normotensive. Daily supplementation of 2000 international units (IU) of vitamin D was provided to one group, and it optimized vitamin D levels and significantly improved carotid-femoral pulse-wave velocity, which is considered a key test for arterial stiffness measurement. The changes in the blood pressure measurements were not related to the vitamin D supplementation status, but adiposity, measured as total body fat mass, was inversely correlated to the levels of 25-hydroxyvitamin D in the 2000 IU vitamin D treatment group [39].

Despite the large number of clinical studies that have been conducted to examine the effect of vitamin D supplementation on blood pressure, there is still no clear consensus on the potential antihypertensive effect of vitamin D. This might be due to the differences in rationale and methodology between studies. In many studies, blood pressure was not the primary endpoint. Baseline characteristics were heterogeneous, ranging from young normotensive to elderly hypertensive subjects with other comorbidities. Small sample sizes and short follow-up periods (<1 year) were shortcomings of the randomized double-blind controlled trials. There was no consistent effect on blood pressure across the studies. Meta-analyses and systematic reviews have been conducted on these studies to determine

Table 1. Summary of meta-analysis or systematic review on hypertension with vitamin D

| Clinical trial | Number of subjects/studies included | Inclusion | Outcomes | Comments |
|-------------------------|-------------------------------------|--------------------------------|---|--|
| Witham et al. 2009 [40] | 716/11 | Randomized, controlled trial | SBP: WMD -3.6 mmHg; 95% CI -8.0 to 0.7 DBP: WMD -3.1 mmHg; 95% CI -5.5 to -0.6 | Subgroup analysis between Vt D ₂ vs. calcitriol on BP: only Vt D ₂ ↓ BP, p=0.05 |
| Pittas et al. 2010 [41] | 37,162/9 | Randomized trial | SBP: WMD -1.9 mmHg; 95% CI -4.2 to 0.4 (I ² =69%) DBP: WMD -0.1 mmHg; 95% CI -0.7 to 0.5 (I ² =23%) | Observation cohort studies were separately analyzed: Lower Vt D was associated with incident hypertension |
| Wu et al. 2010 [42] | 429/4 | Randomized, double-blind trial | SBP: WMD -2.44 mmHg; 95% CI -4.86 to -0.02 DBP: WMD -0.02 mmHg; 95% CI -4.04 to 4.01 | Vt D doses and study length did not affect on a change of BP |
| Elamin et al. 2011 [43] | 1,518/14 | Randomized trial | SBP: WMD -0.06 mmHg; 95% CI -1.98 to 1.87 (I ² =61%) DBP: WMD -0.34 mmHg; 95% CI -1.03 to 0.35 (I ² =0%) | Mortality analyzed: RR, 0.96; 95% CI 0.93 to 1.00; p=0.08 No significant effect on MI or stroke |

SBP, systolic blood pressure; WMD, weight mean difference; CI, confidence interval; Vt D, vitamin D; BP, blood pressure; ↓, change; ↓, reduce; RR, relative risk; MI, myocardial infarction; I², a statistic to estimate heterogeneity across studies, used to evaluate inconsistency of results or findings.

whether vitamin D interventions are efficacious for blood pressure control. Four meta-analyses were evaluated and tabulated in this review (Table 1). Witham et al performed a systematic review and meta-analysis including 11 randomized controlled trials and found that the blood pressure lowering effect resulting from vitamin D supplementation was neutral. Systolic blood pressure decreased by an average of 3.6 mmHg (95% CI, -8.0 to 0.7 mmHg), and diastolic blood pressure significantly decreased by an average of -3.1 mmHg (95% CI, -5.5 to -0.6 mmHg). Additionally, the types of vitamin D formulations that were used produced differences in blood pressure reduction. Ergocalciferol or cholecalciferol with ultraviolet B demonstrated a greater decrease in systolic blood pressure (-6.2 mmHg; 95% CI, -12.32 to -0.04 mmHg) than calcitriol (0.7 mmHg;

95% CI, -4.8 to 6.2 mmHg) [40].

To date, the antihypertensive effect of vitamin D therapy has been inconclusive, unless it was evaluated with concurrent overt kidney disease or hyperparathyroidism. By correcting electrolyte (calcium and phosphorus) imbalances, vitamin D supplementation resulted in decreased blood pressure as well as maintenance of electrolyte homeostasis. Therefore, the effects of vitamin D supplementation on blood pressure remain inconclusive.

Endothelial dysfunction and atherosclerosis

Endothelial dysfunction is a powerful surrogate marker of cardiovascular risk. However, lifestyle changes have been shown to be the most effective intervention, but long-term

Table 2. Summary of clinical trials on endothelial dysfunction with vitamin D

| Clinical trial | Study subject | Study design | Intervention | Outcomes |
|---------------------------|---|--|--|--|
| Tarcin et al. 2009 [45] | Control n=23, normal 25(OH)D Treatment n=23, 25(OH)D < 25 nmol/l | Prospective 3 mos | Control: none (just compared with post-treatment values) Treatment: Vit D ₃ 300K IU Outcome measure: FMD | Control: FMD greater than post-treatment values visually. Treatment: ↑ 0.4 +/- 3.3% |
| Yiu et al. 2013 [46] | Type 2 DM Control n=50 Treatment n=50 | Randomized, double-blind, placebo-controlled 12 wks | Control: placebo Treatment: Vit D ₃ 5K IU Outcome measure: FMD | Treatment effect between two groups (95% CI): 0.83 (-0.41 to 2.08), p=0.19 |
| Harris et al. 2011 [47] | Overweight African American adults Control n=23 Treatment n=22 | Randomized, double-blind, placebo-controlled 16 wks | Control: placebo Treatment: Vit D ₃ 60K IU/mo Outcome measure: FMD | Control: -1.3 +/- 0.6% Treatment: 1.8 +/- 1.3%, p < 0.05 |
| Sugden et al. 2008 [44] | Type 2 DM Control n=17 Treatment n=17 | Randomized, double-blind, placebo-controlled | Control: placebo Treatment: Vit D ₂ 100K IU Outcome measure: FMD | Control: 3.55 +/- 21.55% Treatment: 1.17 +/- 26.68%, p=0.78 |
| Stricker et al. 2012 [48] | PAD, 25(OH)D < 30 ng/ml Control n=31 Treatment n=31 | Randomized, double-blind, placebo-controlled | Control: placebo Treatment: Vit D ₃ 100K IU single dose Outcome measure: ΔLDF | Control: 15.2 +/- 14.0 → 14.3 +/- 10.8, p=0.77 Treatment: 13.5 +/- 16.8 → 20.6 +/- 16.0, p=0.097 p=0.06 for between groups |
| Witham et al. 2010 [49] | Type 2 DM 25(OH)D < 100 nmol/l Control n=22 Treatment1 n=19 Treatment2 n=20 | Randomized, double-blind, placebo-controlled | Control: placebo Treatment1: Vit D ₃ 100K IU Treatment2: Vit D ₃ 200K IU single dose Outcome measure: FMD | 8 wks Control: 5.2 +/- 3.1% Treatment1: 4.3 +/- 2.3% Treatment2: 4.9 +/- 3.2% 16 wks Control: 5.1 +/- 1.8% Treatment1: 5.2 +/- 2.1% Treatment2: 6.5 +/- 2.6%, All p > 0.05 |
| Witham et al. 2010 [50] | Stroke 25(OH)D < 75 nmol/L Control n=28 Treatment n=30 | Randomized, double-blind, placebo-controlled | Control: placebo Treatment: Vit D ₂ 100K 16 wks Outcome measure: FMD | Control: 3.7 +/- 3.1% (8 wks) 5.5 +/- 4.4% (16 wks) Treatment: 6.9 +/- 3.5% (8 wks) 4.7 +/- 3.5% (16 wks) p=0.007 (8 wks) p=0.53 (16 wks) |
| Gepner et al. 2012 [51] | Postmenopausal women 10 ng/ml < 25(OH)D < 60 ng/ml Control n=57 Treatment n=57 | Randomized, double-blind, placebo-controlled | Control: placebo Treatment: Vit D ₃ 2.5K IU 4 mos Outcome measure: FMD | Control: 0.3 +/- 3.4% Treatment: 0.3 +/- 2.6%, p=0.77 |

DM, diabetes mellitus; PAD, peripheral artery disease; Vit D, vitamin D; K, 1000; IU, international unit; mo, month; wk, week; FMD, flow mediated dilation; CI, confidence interval; LDF, post ischemic laser Doppler flux.

adherence is difficult. The body of clinical evidence relating to vitamin D supplementation in endothelial dysfunction is less than that for hypertension.

In small-scale clinical studies, 25-hydroxyvitamin D replacement significantly improved flow-mediated vasodilation (FMD), a measurement of endothelial function, in patients with type 2 diabetes mellitus whose baseline levels of 25-hydroxyvitamin D were below optimal. Additionally, the degree of systolic blood pressure reduction was not correlated with FMD measurement after vitamin D supplementation [44].

Subjects with subclinical (asymptomatic) endothelial dysfunction and concurrent vitamin D deficiency were given 25-hydroxyvitamin D for 3 months; 25-hydroxyvitamin D supplementation significantly ameliorated the FMD measurement, and a positive correlation between FMD and 25-hydroxyvitamin D levels ($r=0.45$; $p<0.05$) was noted. Lipid peroxidation as a surrogate marker was assessed and compared before and after treatment. Post-treatment values of lipid peroxidation were significantly lower than baseline values, and a negative correlation between FMD and lipid peroxidation was reported [44]. There is limited research available that links surrogate markers for endothelial dysfunction and major cardiovascular events or mortality. The summary of clinical trials evaluating vitamin D supplementation on endothelial dysfunction is displayed in Table 2.

It is a plausible hypothesis that vitamin D would modulate human endothelial function based on findings from experimental laboratory and animal studies, especially as epidemiological studies have suggested that vitamin D is associated with atherosclerotic endothelial function. Nevertheless, the putative causal relationship or exact mechanism relating to the role of vitamin D on endothelial function remains to be elucidated due to the lack of more definitive clinical data. As noted in the trials relating to blood pressure, it is important to note that the small sample size, differing vitamin D doses, varying inclusion criteria, and short follow-up durations may limit the comprehensive understanding of vitamin D on endothelial function.

CONCLUSION

It would be premature to conclude that the maintenance of optimal vitamin D levels normalizes blood pressure or ameliorates endothelial dysfunction at this time. Discordant results relating to decreases in blood pressure between the epidemiological or observational studies and the randomized, controlled trials should be carefully explored. For endothelial dysfunction, the outcomes of the clinical trials revealed a lack of consistency. Overall, the epidemiological and the observational studies suggested an association between vitamin D and cardiovascular disease, including hypertension and atherosclerosis. However, the salutary effects of vitamin D in the observational studies were not necessarily replicated in the studies investigating vitamin D supplementation.

The average reduction in blood pressure achieved with vitamin D supplementation was by 2~6 mmHg. More importantly, data on cardiovascular outcomes as hard clinical endpoints, such as mortality or morbidity, are lacking. It is necessary to choose appropriate patients and treat with vitamin D supplementation, as recommended. According to the Endocrine Society's Clinical Guidelines Subcommittee, Clinical Affairs Core Committee, 25-hydroxyvitamin D mea-

surement is recommended to determine vitamin D status, and a level <20 ng/ml (50 nmol/l) is considered as vitamin D deficiency while a level of 21~29 ng/ml (525~725 nmol/l) is defined as vitamin D insufficiency. Although the committee recommends treatment with either vitamin D₂ or vitamin D₃ for deficient individuals, it has concluded that no sufficient evidence is available to prescribe vitamin D for cardiovascular benefits without the presence of biochemical vitamin D deficiency [9]. As such, we should wait until more definitive outcomes are available. Fortunately, well-designed large-scale clinical trials to evaluate the effects of vitamin D supplementation on mortality and morbidity are planned (NCT01169259, NCT01145703) [52,53]. Eventually, we will have more options available than the current armamentarium for the management of cardiovascular disease risk.

ACKNOWLEDGEMENTS

This research was supported by research grants from Catholic University of Dauge in 2010.

REFERENCES

1. DeLuca HF. Overview of general physiologic features and functions of vitamin D. *Am J Clin Nutr.* 2004;80(6 Suppl): 1689S-1696S.
2. Bouillon R, Carmeliet G, Verlinden L, van Etten E, Verstuyf A, Luderer HF, Lieben L, Mathieu C, Demay M. Vitamin D and human health: lessons from vitamin D receptor null mice. *Endocr Rev.* 2008;29:726-776.
3. Ferder M, Inserra F, Manucha W, Ferder L. The world pandemic of vitamin D deficiency could possibly be explained by cellular inflammatory response activity induced by the renin-angiotensin system. *Am J Physiol Cell Physiol.* 2013;304: C1027-1039.
4. Kim DH, Sabour S, Sagar UN, Adams S, Whellan DJ. Prevalence of hypovitaminosis D in cardiovascular diseases (from the National Health and Nutrition Examination Survey 2001 to 2004). *Am J Cardiol.* 2008;102:1540-1544.
5. Holick MF. Vitamin D deficiency. *N Engl J Med.* 2007;357: 266-281.
6. Kamen DL, Tangpricha V. Vitamin D and molecular actions on the immune system: modulation of innate and autoimmune. *J Mol Med (Berl).* 2010;88:441-450.
7. Suzuki Y, Ichiyama T, Ohsaki A, Hasegawa S, Shiraiishi M, Furukawa S. Anti-inflammatory effect of 1 α ,25-dihydroxyvitamin D(3) in human coronary arterial endothelial cells: Implication for the treatment of Kawasaki disease. *J Steroid Biochem Mol Biol.* 2009;113:134-138.
8. Wong MS, Delansorne R, Man RY, Vanhoutte PM. Vitamin D derivatives acutely reduce endothelium-dependent contractions in the aorta of the spontaneously hypertensive rat. *Am J Physiol Heart Circ Physiol.* 2008;295:H289-296.
9. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, Murad MH, Weaver CM. Endocrine Society. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab.* 2011;96:1911-1930.
10. Cheng JB, Motola DL, Mangelsdorf DJ, Russell DW. De-orphanization of cytochrome P450 2R1: a microsomal vitamin D 25-hydroxylase. *J Biol Chem.* 2003;278:38084-38093.
11. Pera L, Sygitowicz G, Bialek S, Lukaszkiwicz J, Sitkiewicz D. Vitamin D and statins: action in preventing cardiovascular events. *Kardiol Pol.* 2012;70:1296-1298.
12. Yavuz B, Ertugrul DT, Cil H, Ata N, Akin KO, Yalcin AA, Kucukazman M, Dal K, Hokkaomeroglu MS, Yavuz BB, Tatal E. Increased levels of 25 hydroxyvitamin D and 1,25-dihydro-

- xyvitamin D after rosuvastatin treatment: a novel pleiotropic effect of statins? *Cardiovasc Drugs Ther.* 2009;23:295-299.
13. **Schwartz JB.** Effects of vitamin D supplementation in atorvastatin-treated patients: a new drug interaction with an unexpected consequence. *Clin Pharmacol Ther.* 2009;85:198-203.
 14. **Brewer LC, Michos ED, Reis JP.** Vitamin D in atherosclerosis, vascular disease, and endothelial function. *Curr Drug Targets.* 2011;12:54-60.
 15. **Brandenburg VM, Vervloet MG, Marx N.** The role of vitamin D in cardiovascular disease: from present evidence to future perspectives. *Atherosclerosis.* 2012;225:253-263.
 16. **Nibbelink KA, Tishkoff DX, Hershey SD, Rahman A, Simpson RU.** 1,25(OH)₂-vitamin D₃ actions on cell proliferation, size, gene expression, and receptor localization, in the HL-1 cardiac myocyte. *J Steroid Biochem Mol Biol.* 2007;103:533-537.
 17. **Kozewski NJ, Herberth J, Malluche HH.** Retinoic acid receptor gamma 2 interactions with vitamin D response elements. *J Steroid Biochem Mol Biol.* 2010;120:200-207.
 18. **Al Mheid I, Patel RS, Tangpricha V, Quyyumi AA.** Vitamin D and cardiovascular disease: is the evidence solid? *Eur Heart J.* 2013. [Epub ahead of print]
 19. **Yang L, Ma J, Zhang X, Fan Y, Wang L.** Protective role of the vitamin D receptor. *Cell Immunol.* 2012;279:160-166.
 20. **Haussler MR, Jurutka PW, Mizwicki M, Norman AW.** Vitamin D receptor (VDR)-mediated actions of 1 α ,25(OH)₂ vitamin D₃: genomic and non-genomic mechanisms. *Best Pract Res Clin Endocrinol Metab.* 2011;25:543-559.
 21. **Swapna N, Vamsi UM, Usha G, Padma T.** Risk conferred by FokI polymorphism of vitamin D receptor (VDR) gene for essential hypertension. *Indian J Hum Genet.* 2011;17:201-206.
 22. **Kammerer CM, Dualan AA, Samollow PB, Périssé AR, Bauer RL, MacCluer JW, O'Leary DH, Mitchell BD.** Bone mineral density, carotid artery intimal medial thickness, and the vitamin D receptor BsmI polymorphism in Mexican American women. *Calcif Tissue Int.* 2004;75:292-298.
 23. **Abd El Gawad SS, Abdul Samee ER, Metwali AA, Abd El Gawad MS.** Vitamin D receptor gene polymorphism and its association with 1,25-dihydroxyvitamin D₃ in patients with Graves disease in an Egyptian population: a pilot study. *Endocr Pract.* 2012;18:132-139.
 24. **Györfy B, Vásárhelyi B, Krikovszky D, Madácsy L, Tordai A, Tulassay T, Szabó A.** Gender-specific association of vitamin D receptor polymorphism combinations with type 1 diabetes mellitus. *Eur J Endocrinol.* 2002;147:803-808.
 25. **Seals DR, Jablonski KL, Donato AJ.** Aging and vascular endothelial function in humans. *Clin Sci (Lond).* 2011;120:357-375.
 26. **Deanfield J, Donald A, Ferri C, Giannattasio C, Halcox J, Halligan S, Lerman A, Mancia G, Oliver JJ, Pessina AC, Rizzoni D, Rossi GP, Salvetti A, Schiffrin EL, Taddei S, Webb DJ.** Working Group on Endothelin and Endothelial Factors of the European Society of Hypertension. Endothelial function and dysfunction. Part I: Methodological issues for assessment in the different vascular beds: a statement by the Working Group on Endothelin and Endothelial Factors of the European Society of Hypertension. *J Hypertens.* 2005;23:7-17.
 27. **Csiszar A, Smith KE, Koller A, Kaley G, Edwards JG, Ungvari Z.** Regulation of bone morphogenetic protein-2 expression in endothelial cells: role of nuclear factor-kappaB activation by tumor necrosis factor-alpha, H₂O₂, and high intravascular pressure. *Circulation.* 2005;111:2364-2372.
 28. **Zhou C, Lu F, Cao K, Xu D, Goltzman D, Miao D.** Calcium-independent and 1,25(OH)₂D₃-dependent regulation of the renin-angiotensin system in 1 α -hydroxylase knockout mice. *Kidney Int.* 2008;74:170-179.
 29. **Resnick LM, Müller FB, Laragh JH.** Calcium-regulating hormones in essential hypertension. Relation to plasma renin activity and sodium metabolism. *Ann Intern Med.* 1986;105:649-654.
 30. **Li YC, Kong J, Wei M, Chen ZF, Liu SQ, Cao LP.** 1,25-Dihydroxyvitamin D(3) is a negative endocrine regulator of the renin-angiotensin system. *J Clin Invest.* 2002;110:229-238.
 31. **Xiang W, Kong J, Chen S, Cao LP, Qiao G, Zheng W, Liu W, Li X, Gardner DG, Li YC.** Cardiac hypertrophy in vitamin D receptor knockout mice: role of the systemic and cardiac renin-angiotensin systems. *Am J Physiol Endocrinol Metab.* 2005;288:E125-132.
 32. **Yuan W, Pan W, Kong J, Zheng W, Szeto FL, Wong KE, Cohen R, Klopot A, Zhang Z, Li YC.** 1,25-dihydroxyvitamin D₃ suppresses renin gene transcription by blocking the activity of the cyclic AMP response element in the renin gene promoter. *J Biol Chem.* 2007;282:29821-29830.
 33. **Tedgui A, Mallat Z.** Cytokines in atherosclerosis: pathogenic and regulatory pathways. *Physiol Rev.* 2006;86:515-581.
 34. **Ginde AA, Liu MC, Camargo CA Jr.** Demographic differences and trends of vitamin D insufficiency in the US population, 1988-2004. *Arch Intern Med.* 2009;169:626-632.
 35. **Melamed ML, Michos ED, Post W, Astor B.** 25-hydroxyvitamin D levels and the risk of mortality in the general population. *Arch Intern Med.* 2008;168:1629-1637.
 36. **Scragg R, Sowers M, Bell C.** Serum 25-hydroxyvitamin D, ethnicity, and blood pressure in the Third National Health and Nutrition Examination Survey. *Am J Hypertens.* 2007;20:713-719.
 37. **Forman JP, Giovannucci E, Holmes MD, Bischoff-Ferrari HA, Tworoger SS, Willett WC, Curhan GC.** Plasma 25-hydroxyvitamin D levels and risk of incident hypertension. *Hypertension.* 2007;49:1063-1069.
 38. **Pfeifer M, Begerow B, Minne HW, Nachtigall D, Hansen C.** Effects of a short-term vitamin D(3) and calcium supplementation on blood pressure and parathyroid hormone levels in elderly women. *J Clin Endocrinol Metab.* 2001;86:1633-1637.
 39. **Dong Y, Stallmann-Jorgensen IS, Pollock NK, Harris RA, Keeton D, Huang Y, Li K, Bassali R, Guo DH, Thomas J, Pierce GL, White J, Holick MF, Zhu H.** A 16-week randomized clinical trial of 2000 international units daily vitamin D₃ supplementation in black youth: 25-hydroxyvitamin D, adiposity, and arterial stiffness. *J Clin Endocrinol Metab.* 2010;95:4584-4591.
 40. **Witham MD, Nadir MA, Struthers AD.** Effect of vitamin D on blood pressure: a systematic review and meta-analysis. *J Hypertens.* 2009;27:1948-1954.
 41. **Pittas AG, Chung M, Trikalinos T, Mitri J, Brendel M, Patel K, Lichtenstein AH, Lau J, Balk EM.** Systematic review: Vitamin D and cardiometabolic outcomes. *Ann Intern Med.* 2010;152:307-314.
 42. **Wu SH, Ho SC, Zhong L.** Effects of vitamin D supplementation on blood pressure. *South Med J.* 2010;103:729-737.
 43. **Elamin MB, Abu Elnour NO, Elamin KB, Fatourehchi MM, Alkatib AA, Almandoz JP, Liu H, Lane MA, Mullan RJ, Hazem A, Erwin PJ, Hensrud DD, Murad MH, Montori VM.** Vitamin D and cardiovascular outcomes: a systematic review and meta-analysis. *J Clin Endocrinol Metab.* 2011;96:1931-1942.
 44. **Sugden JA, Davies JL, Witham MD, Morris AD, Struthers AD.** Vitamin D improves endothelial function in patients with Type 2 diabetes mellitus and low vitamin D levels. *Diabet Med.* 2008;25:320-325.
 45. **Tarcin O, Yavuz DG, Ozben B, Telli A, Ogunc AV, Yuksel M, Toprak A, Yazici D, Sancak S, Deyneli O, Akalin S.** Effect of vitamin D deficiency and replacement on endothelial function in asymptomatic subjects. *J Clin Endocrinol Metab.* 2009;94:4023-4030.
 46. **Yiu YF, Yiu KH, Siu CW, Chan YH, Li SW, Wong LY, Lee SW, Tam S, Wong EW, Lau CP, Cheung BM, Tse HF.** Randomized controlled trial of vitamin D supplement on endothelial function in patients with type 2 diabetes. *Atherosclerosis.* 2013;227:140-146.
 47. **Harris RA, Pedersen-White J, Guo DH, Stallmann-Jorgensen IS, Keeton D, Huang Y, Shah Y, Zhu H, Dong Y.** Vitamin D₃ supplementation for 16 weeks improves flow-mediated dilation in overweight African-American adults. *Am J Hypertens.* 2011;24:557-562.
 48. **Stricker H, Tosi Bianda F, Guidicelli-Nicolosi S, Limoni C, Colucci G.** Effect of a single, oral, high-dose vitamin D supplementation on endothelial function in patients with peripheral

- arterial disease: a randomised controlled pilot study. *Eur J Vasc Endovasc Surg.* 2012;44:307-312.
49. **Witham MD, Dove FJ, Dryburgh M, Sugden JA, Morris AD, Struthers AD.** The effect of different doses of vitamin D(3) on markers of vascular health in patients with type 2 diabetes: a randomised controlled trial. *Diabetologia.* 2010;53:2112-2119.
 50. **Witham MD, Dove FJ, Sugden JA, Doney AS, Struthers AD.** The effect of vitamin D replacement on markers of vascular health in stroke patients - a randomised controlled trial. *Nutr Metab Cardiovasc Dis.* 2012;22:864-870.
 51. **Gepner AD, Ramamurthy R, Krueger DC, Korcarz CE, Binkley N, Stein JH.** A prospective randomized controlled trial of the effects of vitamin D supplementation on cardiovascular disease risk. *PLoS One.* 2012;7:e36617.
 52. Effects of Vitamin D and Omega-3 Fatty Acids on Infectious Diseases and hCAP18. Available at <http://clinicaltrialsfeeds.org/clinical-trials/show/NCT01758081> [Accessed on Jun 23, 2013]
 53. Vitamin D Supplementation and Metabolism in Vitamin D Deficient Elderly (VitD). Available at <http://clinicaltrials.gov/show/NCT01145703> [Accessed on Jun 23, 2013]