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Review

Therapeutic Potential of 25-Hydroxyvitamin D in Promoting Cardiovascular Health

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Abstract:

Introduction: The primary focus on vitamin D therapy was to treat metabolic bone disease. Over the past decade, however, researchers have demonstrated that vitamin D may have a role in other disease processes, particularly cardiovascular diseases (CVDs).

Review: Serum 25-hydroxyvitamin D (25(OH)D) deficiency is widespread, and is extremely common in kidney transplant recipients. In community surveys, lower levels of 25(OH)D were associated with significantly higher adjusted prevalence of cardiovascular risk factors and CVD. CVD patients have lower 25(OH)D levels than controls, which are associated with more severe disease and worse prognosis. 25(OH)D levels are inversely associated with insulin resistance and future risk of hyperglycemia in non-diabetic subjects. Low levels of 25(OH)D were found to be independent predictors of death and end stage renal disease (ESRD) in chronic kidney disease (CKD) patients. Supplementation with vitamin D was found to lower parathyroid hormone and increase anti-inflammatory cytokines in congestive heart failure patient. It was found to improve flow mediated vasodilatation of the brachial artery in asymptomatic vitamin D deficient subjects and diabetic patients. Vitamin D supplementation was found to reduce proteinuria, mortality and progression to dialysis in CKD patients and to reduce cardiovascular mortality in hemodialysis patients. Vitamin D and calcium supplementation was also found to reduce cancer risk in community dwelling healthy postmenopausal women.

Conclusion: Low levels of vitamin D seem to have undesirable effects on health. Based on the results of recent research, the recommended lower value of serum 25(OH)D is likely to be elevated.

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Introduction

The most important physiological source of vitamin D, the "sunshine vitamin", in humans is the production of vitamin D3 (cholecalciferol) in the skin. Vitamin D3 is produced in the skin by the photochemical conversion of its provitamin, 7-dehydrocholesterol. Both vitamin D3 and vitamin D2 (ergocalciferol) are present in small amounts in the diet. However, dietary vitamin D is often insufficient to protect against vitamin D deficiency in the absence of solar exposure.

Both forms of vitamin D undergo hydroxylation, mainly in the liver, to 25-hydroxyvitamin D [25(OH)D]. This is the main circulating storage metabolite of vitamin D. A proportion of 25(OH)D is then converted in the kidney to the active metabolite 1,25 dihydroxvitamin D [1,25(OH) D], or calcitriol. This step is under tight regulation, being enhanced by parathyroid hormone (PTH), hypocalcemia and hypophosphatemia, and suppressed by hypercalcemia, hyperphosphatemia and impaired renal function [1].

Besides its well known role in bone metabolism, vitamin D has documented associations with a number of health conditions. For the purpose of this review, we performed a Medline search for English publications back to 1980, using the words "vitamin D", "human studies", and "Cardiovascular Disease". In this review, we will discuss the mechanism(s) of 25(OH)D actions, prevalence and causes of 25(OH)D deficiencies, clinical syndromes associated with 25(OH)D deficiencies and the therapeutic potential of 25(OH)D to promote cardiovascular health.

Review

Mechanism of 25(OH)D action

Clinical studies have demonstrated an association between vitamin D deficiency and a number of conditions, most prominent of which is cerebrovascular disease. This has encouraged researchers to look for possible mechanisms of action that may explain the anti-inflammatory and atherosclerotic properties of vitamin D (Tables 1 and 2).

Definition and prevalence of 25(OH)D deficiencies

Serum 25-hydroxyvitamin D (25(OH)D) concentration is accepted as the functional indicator of vitamin D status [18]. The normal range of 25(OH)D is 10-55 ng/ml for all gender and life stage groups. However, there is evidence, specially in the elderly, that in order for the PTH to be at the optimum level, a 25(OH)D level of 20 ng/ml or greater may be required [18]. Based on the results of recent research, the recommended lower value of serum 25(OH)D is likely to be elevated. Table 3 demonstrates the serum 25(OH)D concentration levels corresponding to health status in humans. The daily requirement of vitamin D varies between 10 to 20 mcg/day (400-800 IU/day) [18]. At the other extreme, large doses of vitamin D are not recommended. Excessive amounts of vitamin D cause vomiting, constipation, and weight loss. Human data on this specific topic are very limited.

Vitamin D deficiency is widespread amongst children, adolescents, adults, and senior citizens. Its prevalence varies with ethnicity, nationality, and global location.

Causes of 25(OH)D Deficiency

Deficiency in 25(OH)D can result from a number of reasons. The commonest reason is inadequate exposure to the sun accompanied by inadequate dietary intake or malabsorption [1].

Inadequate exposure to sunlight: Ultraviolet B rays are needed for the synthesis of vitamin D in the skin, the prime natural source of this vitamin. Insufficient exposure to sunlight causes deficiency in cutaneously synthesized vitamin D. Adults in nursing homes or health care institutions are at particularly high risk. Patients with large skin grafts also have a marked reduction in their ability to synthesize vitamin D in the skin. The optimal daily sunshine exposure an individual should get is subject to debate. It is recommended that an individual receives10 to 15 minutes of sun exposure, 3 times a week during none peak sun hours. With age, the efficiency of processing vitamin D becomes less and vitamin D supplementation may be necessary. Darker skin individuals living in the northern hemisphere and individuals using sunscreen that reduce absorption of ultraviolet B rays are also likely to need vitamin D supplementation.

Inadequate intake: Minimal amounts of vitamin D exist in human breast milk. Therefore the American Academy of Pediatrics recommended that all children receive 400 IU/day of vitamin D from their first few days of life through adolescence [19].

Vitamin D malabsorption syndrome: People who have undergone resection of the small intestine are at risk for this condition. Other diseases associated with vitamin D malabsorption include celiac sprue and cystic fibrosis.

Obesity: Individuals with obesity have reduced availability of vitamin D in their body fat. Lower levels of 25(OH)D were found in morbidly obese and obese women compared to non-obese women [20].

Medications: Some medications are associated with vitamin D deficiency. Drugs such as dilantin, phenobarbitals and rifampin can induce hepatic p450 enzymes and accelerate the catabolism of vitamin D.

Liver disease: Decreased synthesis of 25(OH)D occurs in patients with liver disease.

Renal diseases: In data from Third National Health and Nutrition Examination Survey, the adjusted odds for 25(OH)D deficiency were 32% higher in chronic kidney disease (CKD) patients [21]. Serum 25(OH) D deficiency is also extremely common among kidney transplant recipients [22, 23], and even kidney transplant recipients with adequate renal function were found to have significantly lower 25(OH)D levels than age and gender-matched controls [24]. Courbebaisse *et al* [25] were able to demonstrate that treating kidney transplant recipients deficient in 25(OH)D with 100,000 units of cholecalciferol every 2 weeks for 2 months may be enough to significantly elevate serum levels of 25(OH)D to above 30 ng/ml and reduce PTH levels without neither consequent hypercalcemia nor increased urinary calcium excretion, which could adversely affect graft function. However, during the less intensive phase of the treatment, when cholecalciferol was given at the same dose every other month, serum levels of 25(OH)D decreased again, though they remained higher than in control untreated patients at 1 year after transplantation. The message to be drawn from this study is that we need to prescribe large doses of cholecalciferol for our patients after renal transplantation. In addition, we need similar bold studies on the correct dose of cholecalciferol or ergocalciferol to prescribe for our patients at the different stages of CKD. Deficiency in the metabolically active form of vitamin D, 1,25(OH)D, is common in patients with CKD. In the early stages of CKD, hyperphosphatemia induces fibroblast growth factor 23 (FGF-23), a phosphaturic hormone which down regulates 25(OH)D hydroxylase activity. In CKD stages 4 and 5, the kidney is unable to produce adequate amounts of 1,25(OH)D [26].

Table 1: In vitro studies exploring the possible mechanisms of action of vitamin D in preventing cardiovascular disease

Possible mechanisms of action of vitamin D in preventing cardiovascular disease
• In cultured rabbit VSMCs, prostacyclin synthesis was significantly increased in the presence of 1,25(OH)D and 1(OH)D. Treament with 1,25(OH)D significantly induced the activity of cyclooxygenase without changing the activity of phospholipase A2 [2].
• In cultured human HUVEC, calcitriol decreased the adhesion molecules expression, as well as LPS induced mRNA expressions of RAGE and IL-6, and IL-6 and LPS induced IL-6 secrtion. Calcitriol also decreased the LPS induced KFkB-p65 DNA-binding activity [3].
• In HMEC, pretreatment with 1,25(OH)D inhibited the enteric gram-negative bacterial LPS activation of transcription factor NFkB and IL-6, IL-8 and RANTES release [4].
• In cultured bovine VSMCs, 1,25(OH)D increased VSMC calcification and alkaline phosphatase activity in a dose-dependent manner. 1,25(OH)D decreased secretion of PTHrP by bovine VSMCs in a dose-dependent manner and depressed its gene expression. 1,25(OH)D also increased the expression of the osteopontin gene, one of the bone matrix proteins in bovineVSMCs, contributing to its stimulatory action on bovine VSMC calcification [5].
• In activated human T lymphocytes, calcitriol markedly inhibited at the transcriptional level the production of IFN-gamma. Calcitriol down regulated both IL-2 and IFN-gamma production as well as TfR expression at the mRNA level [6].
• In rat aortic VSMC, 1,25(OH)D induced a dose-dependent increase in rate of migration. This response required the activation of PI3 kinase because it was completely abolished by the PI3 kinase inhibitors, LY294002 or wortmannin. The RNA polymerase inhibitor, 5,6-dichlorobenzimidazole riboside did not affect the 1,25(OH)D induced VSMC migration, suggesting that gene transcription is not involved in this rapid response [7].
• In rat aorta VSMC, calcitriol treatment induced uniform cellular growth patterns. Elastic fibers were more abundant in treated than in control cultures [8].
• In the CFK2 chondrogenic cell line, 1,25(OH)D directly stimulates transcription of mRNAs encoding VEGF-121 and VEGF-165 isoforms. Enhanced VEGF expression also was evident in growth plate chondrocytes and osteoblasts in the tibia of juvenile mice treated systemically with 1,25(OH)D [9].
• In rat aortic rings and A7r5 cells derived from fetal rat aortic smooth muscle, 22-oxa-1,25(OH)D induced prostacyclin synthesis but did not cause any significant hypercalcemia [10].
• HUVEC are demonstrated to be able to produce active vitamin D, and the endothelial 1-alpha hydroxilase activity is stimulated by inflammatory cytokines. Exogenously added 1,25(OH)D or 25(OH)D significantly decreased HUVEC proliferation and increased the adhesion of monocytic U937 cells to HUVEC [11].
• In primary cultures of ventricular myocytes isolated from neonatal rat hearts, treatment with 1,25(OH)D inhibited cell proliferation. Proliferating cell nuclear antigen protein levels and protooncogene c-myc protein levels were specifically reduced by 1,25(OH)D. 1,25(OH)D increased myocyte protein levels and increased cell size, suggesting that it induces cardiac myocyte hypertrophy [12].
• Functional VDR, 1-alpha-hydroxylase and 24-hydroxylase are demonstrated in both the myocytes and fibroblasts of the heart, as well as in the intact ventricular myocardium. VDR is shown to interact directly with the human B-type natriuretic peptide gene promoter, a surrogate marker of the transcriptional response to hypertrophy [13].
• In HUVEC culture, the increase in protein levels of ICAM-1 and VCAM-1 induced by TNF-alpha was significantly decreased after incubation of the cells with 1,25(OH)D [14].
• 1,25(OH)D induces a dose-dependent increase in VSMC proliferation in quiescent cells and in cells stimulated to grow. This effect is mediated by an increase of the expression of VEGF [15].

Table 2: In vivo studies exploring the possible mechanisms of action of vitamin D in preventing cardiovascular disease

Possible mechanisms of action of vitamin D in preventing cardiovascular disease
• In vitamin D receptor-null mice, renin expression and plasma angiotensin II production were increased several folds, leading to hypertension, cardiac hypertrophy, and increased water intake. In wild-type mice, inhibition of 1,25(OH)D synthesis also led to an increase in renin expression, whereas 1,25(OH)D injection led to renin suppression [16].
• In patients with a variety of kidney diseases, urinary MCP-1 protein and renal macrophage infiltration were each significantly but inversely correlated with serum 1,2-OHD levels. Patients with acute renal inflammation had significantly lower levels of serum 1,25(OH)D in comparison to patients with chronic ischemic disease despite similar levels of renal damage [17].

VSMC: vascular smooth muscle cell; 25(OH)D: 25 hydroxy vitamin D; 1,25(OH)D: 1,25 hydroxyvitamin D; HUVEC: umbilical vein cord endothelial cells; IL-6: interleukin 6; LPS: lipopolysaccharide; mRNA: messenger RNA; RAGE: receptor of advanced glycation end product; KFkB: nuclear factor kappa B; HMEC: microvessel endothelial cells; IL-8: interleukin 8; RANTES: regulated upon activation normal T cell exposed and secreted; PTHrP: parathyroid hormone-related peptide; IFN-gamma: gamma interferon; TfR: transferrin receptor; PI3 kinase: phosphatidylinositol 3-kinase; VEGF: vascular endothelial growth factor; VDR: vitamin D receptor; TNF-alpha: tumor necrosis factor alpha; ICAM-1: intercellular adhesion molecule-1; VCAM-1: vascular cell adhesion molecule-1; VEGF: vascular endothelial growth factor; MCP-1: monocyte chemoattractant protein-1

Table 3: Serum 25-hydroxyvitamin D concentrations and health status [18]

Health Status	25(OH)D level (ng/ml)
Vitamin D deficiency, leading to rickets in infants and children, and osteomalacia in adults	< 10
Vitamin D levels are within normal range, but are likely to b inadequate for bone and overall health	10-20
Adequate for bone and overall health in normal individuals	> 20
Potentially toxic, leading to hypercalcemia and hyperphosphatemia, although human data are limited	Consistently >200

Others: There are several heritable disorders that cause rickets in early infancy and childhood. Other acquired disorders of vitamin D deficiency include tumor-induced osteomalacia, in which tumors secrete FGF-23. Hyperparathyroidism increases the metabolism of 25(OH) D and 1,25(OH)D as well. Granulomatous disorders such as sarcoidosis, tuberculosis, and lymphomas produce a variety of enzymes that accelerate the conversion of 25(OH)D to 1,25(OH)D.

Clinical syndromes associated with deficiency of 25(OH)D

Table 4 summarizes the clinical associations of 25(OH) D deficiencies.

Martin *et al* [27] studied the prevalence of cardiovascular risk factors and serum levels of 25(OH)D among 7186 male and 7902 female adults from the third NHANES survey in the United States. The adjusted prevalence of hypertension (OR 1.30), DM (OR 1.98), obesity (OR 2.29), and high serum triglyceride levels (OR 1.47) was significantly higher in the first than in the fourth quartile of serum 25(OH)D levels (P<.001 for all). Kim *et al* [28] studied the prevalence of hypovitaminosis D in adults with cardiovascular disease (CVD) using data from the NHANES survey between 2001 and 2004. serum 25(OH)D levels were divided into 3 categories (≥30, 20 to 29, and <20 ng/ml). The burden of CVD increased with lower 25(OH)D categories, with 5.3%, 6.7%, and 7.3% CHD; 1.5%, 2.4%, and 3.2% heart failure; 2.5%, 2.0%, and 3.2% stroke; and 3.6%, 5.0%, and 7.7% peripheral arterial disease. Hypovitaminosis D was more prevalent in those at high risk (OR 1.32), with CHD (OR 1.48), and both CHD and heart failure (OR 3.52) after controlling for age, race, and gender. They concluded that low 25(OH)D was highly prevalent in US adults with CVD, particularly those with both CHD and heart failure.

Wong *et al* [30] followed 1739 individuals without prior CVD for a mean of 5.4 years. Individuals with 25(OH)D levels of less than 15 ng/ml at baseline had a multivariable-adjusted hazard ratio (HR) of 1.62 (P=0.01) for incident cardiovascular events compared to those with levels above 15 ng/ml at baseline. This effect was evident in participants with hypertension (HR 2.13) but not in those without hypertension (HR 1.04). They concluded that 25(OH)D deficiency is associated with incident cardiovascular disease, and recommended the study of correction of vitamin D deficiency to prevent cardiovascular disease.

Dobnig *et al* [34] followed a cohort of 3258 patients scheduled for coronary angiography for a median of 7.7 years. Multivariate-adjusted HR for patients in the lower two 25(OH)D quartiles were higher for all-cause mortality (2.08 and 1.53 respectively) and for cardiovascular mortality (2.22 and 1.82 respectively) compared with patients in the highest 25(OH)D quartile at baseline. Low 25(OH)D levels at baseline were significantly correlated with variables of inflammation (CRP and IL-6 levels), oxidative burden (serum phospholipid and glutathione levels), and cell adhesion (VCAM-1 and ICAM-1 levels). In a large cross sectional study, Pilz *et al* [35] evaluated 3299 Caucasian patients referred for coronary angiography between 1997 and 2000. 25(OH) D was negatively correlated with NT-proANP and was also adversely associated with higher NYHA classes and impaired left-ventricular function. After adjustment for cardiovascular risk factors, the HR of death due to heart failure and to sudden cardiac death were 2.84 and 5.05 respectively when comparing patients with severe vitamin D deficiency with persons in the optimal range. Pilz *et al* [36] followed 3,316 patients who were referred for coronary angiography for a median duration of 7.75 years. They found that low levels of 25(OH)D and 1,25(OH)D at baseline were independently predictive for fatal strokes The odds ratio for fatal stroke were 0.58 per Z value of 25(OH)D and 0.62 per z value of 1,25(OH)D (P<0.001).

Zittermann *et al* [37] evaluated 54 heart failure patients that had NYHA class 2 or more and 34 controls, and found in a nonlinear regression analysis that 25(OH)D and calcitriol were inversely correlated with NT-proANP, a predictor of CHF severity. They suggested that low 25(OH)D may have contributed to the pathogenesis of congestive heart failure. Of note, there was no statistical difference in GFR between patients and controls. Boxer

Table 4: Clinical associations of 25(OH)D deficiencies

Reported clinical associations of 25(OH)D deficiencies
• In community surveys, lower levels of 25(OH)D were associated with significantly higher adjusted prevalence of hypertension, DM, obesity and high serum triglyceride levels. They were also associated with an increased burden of CVD: CHD, heart failure, stroke, and peripheral arterial disease [27, 28].
• In individuals without prior CVD, 25(OH)D deficiency at base line was an independent predictor of increasing apo A-I concentrations and was associated with increased risk for future cardiovascular events [29, 30, 31].
• In individuals with moderate risk for CHD, 1,25(OH)D levels were inversely correlated with the extent of vascular calcification. Low levels of vitamin D were independently associated with calcific aortic sclerosis in coronary artery disease patients [32, 33].
• In patients referred for coronary angiography, low levels of 25(OH)D were significantly correlated with variables of inflammation, oxidative burden, and cell adhesion. 25(OH)D level in these patients was negatively correlated with NT-proANP, NYHA classes and LVD. Low 25(OH)D levels were also independently predictive for fatal strokes, and associated with higher all-cause mortality and higher cardiovascular mortality [34, 35, 36].
• In patients with severe CHF, 25(OH)D level was inversely correlated with NT-proANP levels. Low 25(OH)D level in those patients were also associated with "urgent/high urgent" status on cardiac transplantation waiting list and associated with shorter 6-minute walk distance [37, 38, 39].
• In patients presenting to hospital within 12 hours of the onset of MI symptoms and in patients admitted with first-ever stroke, low levels of 25(OH)D were commoner than controls [40, 41]
• In non-diabetic individuals, 25(OH)D levels were inversely associated with ISI and with plasma glucose concentration during the oral-glucose-tolerance test at baseline. Low 25(OH)D levels were associated with higher 10-year risk of hyperglycemia, insulin resistance and metabolic syndrome [42, 43].
• In diabetic patients, low 25(OH)D levels were commoner than controls, and were associated with higher values of HbA1c, triglycerides, CRP and fibrinogen. Low 25(OH)D levels in diabetics were also associated with CVD [44].
• In CKD patients, low 25(OH)D levels are an independent predictor of death or ESRD [45].
• Prolonged dietary deficiency of calcium and vitamin D was reported to cause extensive cardiac calcification in a child that substantially reduced after adequate replacement of calcium and ergocalciferol [46].
• Vitamin D deficiency was reported to cause heart failure in infants, which may improve with subsequent vitamin D and calcium supplementation [47, 48].

CVD: cardiovascular disease; CHD: coronary heart disease; NT-proANP: N-terminal pro-atrial natriuretic peptide ; NYHA: New York Heart Association; LVD: left ventricular dysfunction; CHF: congestive heart failure; MI: myocardial infarction; ISI: Insulin Sensitivity Index; CKD: chronic kidney disease; ESRD: end stage renal disease

et al [39] studied the association between 25(OH)D level and the 6-minute walk distance and frailty level in 60 patients with an ejection fraction of 40%. They found that longer 6-minute walk distance was correlated with higher 25(OH)D level, and that higher frailty score was significantly correlated with lower 25(OH)D levels. They suggested that 25(OH)D levels may contribute to lower aerobic capacity and frailty in patients with heart failure.

Scraggy *et al* [40] evaluated 179 myocardial infarction (MI) patients presenting to hospital within 12 hours of the onset of symptoms. Mean 25(OH)D levels were significantly lower in MI patients than controls. The relative risk of MI for subjects with 25(OH)D levels equal to or above the median was 0.43 compared to subjects below the median.

In another study, Cigolini *et al* [44] studied 459 consecutive type 2 diabetic patients and a control group of 459 age- and sex-matched non-diabetic volunteers. In fully adjusted logistic regression models, low 25(OH)D level (< 20 ng/ml) was commoner in diabetic patients than in control subjects (60.8% versus 42.8%, $p < 0.001$). In addition, diabetic patients with low 25(OH)D had increased prevalence of higher values of HbA1c, triglycerides, CRP, and fibrinogen levels. There was an association between low 25(OH)D and prevalence of CVD (OR 1.7, $p < 0.01$) that remained statistically significant after adjustment for classical risk factors. They concluded the existence of a high prevalence of low 25(OH)D in diabetic patients and a strong association between 25(OH)D concentration and prevalence of CVD.

In an interesting study by Ravani *et al* [45], they enrolled 168 consecutive new referrals to a CKD clinic over a 2 year period and followed them for up to 6 years. 25(OH)D level was as an independent predictor of study outcomes, death or end-stage renal disease (ESRD), when adjusted for age, heart failure, smoking, CRP, albumin, phosphate, use of angiotensin converting enzyme inhibitors (ACEi) or angiotensin receptor blockers (ARBs), and estimated GFR.

Zaidi *et al* [46] reported the case of a 6-year-old girl who presented with extensive cardiac calcifications due to secondary hyperparathyroidism resulting from prolonged dietary deficiency of calcium and vitamin D. Cardiac calcifications substantially reduced after treatment with adequate daily replacement of calcium and ergocalciferol. Also, Kosecik *et al* [48] described a case of dilated cardiomyopathy due to nutritional vitamin D deficiency rickets that improved with subsequent vitamin D and calcium supplementation.

Human studies on the effects of vitamin D supplementation on health

Table 5 presents the protocols and results that evaluated the effect of vitamin D supplementation. In a unique randomized clinical trial, Schleithoff *et al* [49] evaluated the effect of 25(OH)D supplementation on the survival rate and different biochemical variables in patients with CHF. Patients were randomized to receive either 50 mcg of 25(OH)D plus 500 mg calcium supplement or placebo plus 500 mg calcium supplement for 9 months. The pro-inflammatory cytokine tumor necrosis factor alpha (TNF-alpha) increased in the control group but remained constant in the treatment group. They concluded that 25(OH)D therapy reduces the inflammatory milieu in congestive heart failure patients and might serve as a new anti-inflammatory agent for the future treatment of the disease. The survival rate did not differ significantly between the study groups during the follow-up period.

Low 25(OH)D levels are common in CKD pre-dialysis patient [21]. Several data suggested that urinary protein losses may be more important than loss of GFR in inducing 25(OH)D depletion. In fact, consistent with recent analyses using NHANES 3 data, the graded relationship between serum 25(OH)D levels and proteinuria was present even at sub-nephrotic levels. The K-DOQI [59] work group extrapolated from epidemiological data to make opinion-based recommendations to maintain serum 25(OH)D levels above 30 ng/ml in patients with CKD stage 3 or 4. There have been no randomized controlled trials to study the magnitude of benefit of 25(OH)D supplementation on lowering serum PTH levels.

Naves-Díaz *et al* [54] studied the survival benefit of oral active vitamin D in HD patients from six Latin American countries (FME Register as part of the CORES study) followed for a median of 16 months. After adjustment for potential confounders, the 7,203 patients who received oral active vitamin D had significant reductions in overall, cardiovascular, infectious and neoplastic mortality compared to the 8,801 patients that had not received vitamin D. The survival benefit of oral active vitamin D was seen in those patients receiving mean daily doses of less than 1 mcg with the highest reduction associated with the lowest dose.

Kovesdy *et al* [56] studied a historical cohort of incident HD patients who lived throughout the United States between January 1996 and December 1999. During this period, 37,173 HD patients received activated injectable vitamin D and 13,864 did not. At 2 years, mortality rates were 13.8/100 person-years in the group that received injectable vitamin D compared with 28.6/100 person-years in the group that did not ($P < 0.001$). After adjustment for several potential confounders, the 2-year survival advantage associated with the group that did receive injectable vitamin D was 20%. The incidence of cardiovascular-related mortality was 7.6/100 person-years in the injectable vitamin D group, compared with 14.6/100 person-years in the non-vitamin D group ($P<0.001$).

A recent clinical trial by Lappe *et al* [58] was designed to determine the efficacy of calcium alone and calcium plus vitamin D in reducing incident cancer risk in community-dwelling women randomly selected from the population of healthy postmenopausal women. Cancer incidence was significantly lower in the calcium plus vitamin D women than in the placebo control subjects. In multiple logistic regression models, both treatment and serum 25-hydroxyvitamin D concentrations were significant, independent predictors of cancer risk.

Significant gaps in knowledge

The optimum window of serum 25(OH)D level is yet to be determined, benefits and risks of large doses of supplementation of vitamin D need to be assessed, and large randomized clinical testing with sufficiently high doses of vitamin D for CVD prevention do not exist.

Knowledge of the desirable concentration of vitamin D is important, since low levels are associated with disease states and higher levels are associated with hypercalcemia. In fact, there is hesitation to administer large doses of vitamin D supplementation for fear of hypercalcemia and hyperphosphatemia, because these disorders can affect patient function as well as patient survival. On the other hand, high 25(OH)D concentration may in fact

Table 5: Human studies on the effects of vitamin D supplementation on health in general and CVD in particular

Study population	Author	Type of Study	Outcome
Heart failure patients	Schleithoff *et al* [49]	Double blind, randomized placebo controlled trial	One hundred twenty-three CHF patients randomly received either 50 mcg vitamin D or placebo for 9 months. Compared with baseline, PTH was significantly lower and the anti-inflammatory cytokine interleukin 10 was significantly higher in the vitamin D group after 9 months. The survival rate did not differ significantly between the study groups during the follow-up period.
Hypertensive patients	Jespersen *et al* [50]	Controlled clinical trial	The rapid effects of 1,25(OH)D were assessed over 120 min after a bolus injection in eight men with essential hypertension and in nine healthy men, and a placebo group of 10 healthy men was also included. In the hypertensive patients, a transient increase in blood pressure and a reciprocal fall in cardiac output were observed after 1,25(OH)D injection.
Diabetic patients	Sugden *et al* [51]	Double blind, randomized, placebo controlled trial	A single dose of 100,000 IU vitamin D2 or placebo was administered to 34 patients with type 2 diabetes with 25(OH)D levels < 20 ng/ml. Vitamin D supplementation significantly improved flow mediated vasodilatation (FMD) of the brachial artery by 2.3% and significantly decreased systolic blood pressure by 14 mmHg compared with placebo.
	Tarcin *et al* [52]	Prospective cohort	Twenty-three asymptomatic vitamin D-deficient subjects received 300,000 IU IM monthly for 3 months. Brachial artery flow mediated dilatation (FMD) measurements were significantly lower in 25(OH)D deficient subjects than controls (P = 0.001) and improved after replacement therapy (P = 0.002). Post-treatment values of lipid peroxidation as measures of thiobarbituric acid reactive substances (TBARS) were significantly lower than pre-treatment levels (P < 0.001). A positive correlation between FMD and 25(OH)D (r = 0.45; P = 0.001) was observed. There was a significant increase in leptin levels after therapy, and the leptin levels were positively correlated with 25(OH)D levels (r = 0.45; P < 0.05).
CKD and dialysis patients	Agarwal *et al* [53]	Double blind, randomized, placebo controlled trial	Patients in CKD stage 3 and 4 with secondary hyperparathyroidism (SHPT) were randomized to oral paricalcitol (N=107) or placebo (N= 13) and followed for up to 24 weeks. At the final visit 51% of the paricalcitol patients compared to 25% placebo patients had reduction in proteinuria (P= 0.004). Reduction of proteinuria favored patients on paricalcitol, regardless of age, sex, race, diabetes mellitus, hypertension, or use of therapies to block the renin-angiotensin-aldosterone system (RAAS).
	Naves-Díaz *et al* [54]	Prospective cohort	Among HD patients prospectively followed up for a median of 16 months, 7203 HD patients who received oral active vitamin D had significant reductions in overall, cardiovascular, infectious and neoplastic mortality compared to 8801 HD patients that had not received vitamin D. The survival benefit of oral active vitamin D was seen in those patients receiving mean daily doses of less than 1 mcg with the highest reduction associated with the lowest dose.
	Shoji *et al* [55]	Prospective cohort	Among a cohort of ESRD patients undergoing HD, multivariate analysis showed that cardiovascular mortality was significantly reduced with treatment with alfacalcidol (HR 0.377, P = 0.022).
	Teng *et al* [56]	Historical cohort	In a historical cohort of HD patients who lived throughout the United States between January 1996 and December 1999, active injections of vitamin D were given to 37173 patients and 13864 did not receive the drug. The 2-year survival advantage associated with injectable vitamin D was 20%, and cardiovascular-related mortality was 7.6/100 person-years compared to 14.6/100 person-years in the non-vitamin D group (P < 0.001).
	Kovesdy *et al* [57]	Historical cohort	Among 520 male CKD patients in stages 3-5, there were 258 subjects who received treatment with calcitriol for a median duration of 2.1 years. The incidence rate ratios for mortality and combined death and dialysis initiation were significantly lower in treated versus untreated patients (P < .001).
Others	Lappe *et al* [58]	Double blind, randomized, placebo controlled trial	Community-dwelling healthy postmenopausal women were randomly assigned to receive supplemental calcium alone, supplemental calcium plus vitamin D3 or placebo (N=1179). In multiple logistic regression models, both treatment and serum 25(OH)D concentrations were significant, independent predictors of cancer risk.

suppress PTH levels, which is important in the renal patient. Therefore, knowledge of the desirable window for vitamin D level is necessary. Factors that determine this desirable level include the best level to achieve its skeletal and non-skeletal functions. Parenthetically published levels necessary for healthy skeleton seem similar to levels thought necessary to optimize 25(OH) D's cardiovascular protective effects. As such, we believe that there is in fact a wide window of opportunity to achieve this desirable level (30-70 ng/ml) for health. The relationship between 25(OH)D supplement and its levels has been well documented. For each 1000 IU dose of vitamin D, an expected increase of 10 ng/ml in 25(OH) D level is expected. It should be noted, further, that supplements of up to 5000 IU daily are highly unlikely to lead to 1,25(OH)D toxicity.

What remains is to perform a critical double-blinded controlled trial to investigate whether oral 25(OH)D supplementation, to reach the target window, is able to prevent cardiovascular risk factors and CVD mortality, particularly in those with early CKD, hypertension, DM and those with the metabolic syndrome.

Conclusions

This review evaluated the current literature on vitamin D deficiency and its association with multiple disease processes, particularly cardiovascular disease. There is emerging evidence to suggest a need for a randomized prospective study to determine the beneficial effect of Vitamin D therapy above and beyond the knowledge of strong association between health and vitamin D level.

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