

Therapeutic Role of Vitamin D in Cardiovascular Disease: Emerging Evidence

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Abstract

While the importance of Vitamin D for calcium absorption and metabolism and bone health was recognized about a century ago, its importance for non-skeletal effects has only been generally recognized and appreciated in this century. Most of the supporting evidence is either from observational studies, including prospective studies, related to serum 25-hydroxyVitamin D [25(OH)D] concentrations, or studies of Vitamin D mechanisms. A moderate amount of this evidence has been generated from clinical trials.

Keywords: Vitamin D; Cardiovascular diseases; Heart failure; Metabolic syndrome; Hypertension; Erectile dysfunction; Statin tolerance

Introduction

Indirect support comes from the fact that skin pigmentation varies globally in response to prevailing solar ultraviolet-B (UVB) doses as modified by clouds and forest cover [1], and that nearly every cell in the body has Vitamin D receptors. Most of the effects of Vitamin D are mediated through the hormonal metabolite of Vitamin D, 1, 25-dihydroxyVitamin D, binding to a Vitamin D receptor and affecting gene expression, upregulating some, downregulating others; the production of this metabolite in target tissues being regulated mainly through the availability of 25(OH)D in the circulation and by local production of the catabolic 24-hydroxylase enzyme [2].

However, in order for the health systems to recommend higher serum 25(OH)D concentrations appropriate for favorable non-skeletal effects, clinical trials are required. The year 2017 saw some progress along this front.

The consensus among Vitamin D researchers, especially in the United States, is that optimal 25(OH)D concentrations are certainly above 30 ng/ml (75 nmol/l) [3], if not in the range of 40-60 ng/ml (100-150 nmol/l).

This paper is a narrative review of the latest research related to cardiovascular diseases and Vitamin D status in relationship to outcomes. Included are observational trials as well as prospective clinical trials. Databases searched included Pubmed and Google Scholar, with search terms of 25 hydroxy Vitamin D, English language, Vitamin D₃, and from 2015 to the present.

Experimental

Vitamin D metabolism

Solar ultraviolet B (UVB) radiation is the primary precursor of Vitamin D in humans, converting 7-dehydrocholesterol in the epidermis and dermis to previtamin D₃. Isomerization triggered by body temperature converts previtamin D₃ to Vitamin D₃. Many factors affect the ability of skin to convert UVB radiation to Vitamin D, including melanin, age, clothing, and sun blocks. Additionally, the angle of the sun strongly affects Vitamin D production. Little or no Vitamin D is produced when the sun is at an oblique angle in the early and late part of the day. The sun angle is oblique throughout the full day in northern latitudes from October through March [4].

A minimal erythematous whole-body dose of sunlight (amount of sun that causes change in skin color to pink) provides between 10,000-

25,000 IU of Vitamin D [5,6]. Once the Vitamin D₃ is produced in the skin or ingested in the diet, it becomes active after 2 sequential hydroxylation reactions. The first occurs in the liver, forming 25 hydroxyVitamin D [25(OH)D].

It is now known that many tissues, including the heart and vascular smooth muscles [7,8], possess the 1- α -hydroxylase enzyme (CYP27B1). This enzyme is required for the second step of hydroxylation. 25(OH)D is used as a substrate to make the active 1,25 dihydroxyVitamin D (calcitriol), which is then used for various autocrine/paracrine functions. Another ubiquitous enzyme capable of inactivation and degradation of Vitamin D₃ is 24 hydroxylase, also present in the heart [8]. Localized production of calcitriol via CYP27B1 may help provide high concentrations of calcitriol intracellularly. These high concentrations may be necessary to trigger gene expression [9]. The presence of CYP27B1 in the heart suggests that Vitamin D plays an important role in cardiovascular health.

25(OH)D status in serum is dependent either on input from either UVB or derived from food or supplements. The presence of Vitamin D in foods is minimal, ranging from 100 IU per serving of milk to around 300 IU for a serving of oily fish. Vitamin D content may vary widely even within species of fish [10]. Meats do serve as a source of 25(OH)D [4,10]. However, preparation methods of the food may diminish the Vitamin D content by 50% [10]. A relative lack of UV exposure, high prevalence of deficiency, and minimal intake of Vitamin D in food underscores the importance of clinical trials in cardiovascular disease [4,10,11].

Mechanisms of CV action

Vitamin D helps to regulate autophagy, inflammation, oxidative stress, epigenetic changes, DNA disorders, and alterations in reactive oxygen species signaling. This is supported by observations that deficiency exacerbates these processes [12].

In rats, Vitamin D supplementation reduced inflammatory

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markers, including catalase and TNF- α . GPx and SOD improved as well [13].

Twelve c-reactive protein single nucleotide polymorphisms (CRP SNPs) were genotyped in order to determine gene-environment interactions with 25(OH)D. Both 25(OH)D and CRP were quantified, in 505 black South African women. Alarming, 66% and 60% of the women presented with deficient/insufficient 25(OH)D and elevated CRP concentrations, respectively. CRP concentrations were higher in individuals with lower 25(OH)D concentrations. However, no 25(OH)D-CRP genotype interactions were evident [14].

Prevalence/Incidence of Vitamin D deficiency

Vitamin D deficiency, often defined by circulating 25(OH)D levels less than 20 ng/ml (50 nmol/L) is highly prevalent in most populations [15]. Insufficiency is defined as <30 ng/ml (75 nmol/L) and severe deficiency <10 ng/ml (25 nmol/L).

Numerous risks are associated with deficiency. See below for prevalence of deficiency in various population groups:

- Healthy adults (United states): Adults: 65% insufficiency and deficiency [16].
- Older adults (Germany): 62% insufficiency and deficiency [17].
- Obese children 97% deficiency and insufficiency combined [18].
- Pregnant women: 79% [19].
- Healthy women (Korea): 79% [19].
- Metabolic syndrome: 87% deficiency and insufficiency combined [20].

- Heart failure: 87%, with 25% with severe deficiency [21].
- Type 1 diabetes: 78% deficiency [22].

Multiple micronutrient deficiencies are often present in people with cardiovascular risk factors. For example, concomitant magnesium deficiency may have additive or synergistic effects with Vitamin D deficiency in obese individuals, worsening or exacerbating cardiovascular disease risk [23].

Results and Discussion

Current observational trials are summarized in Tables 1-3. Table 1 includes cross-sectional studies, Table 2 includes case-control studies and Table 3 includes longitudinal and prospective studies.

Vitamin D status is associated with cardiovascular risk factors in children

Deficiency of Vitamin D appears to be global and is associated with risk factors even in young children [24]. These risks include higher insulin levels, hemoglobin A1C levels and increased Body Mass Index (BMI).

Observational studies find between 70-97% of obese children in Turkey have insufficient or deficient Vitamin D status. Gull, demonstrated the association between poor Vitamin D status and insulin resistance, dyslipidemia and hypertension [18]. Deficiency in obese children is related to HDL status as well [25].

Serum 25-hydroxyVitamin D<30 ng/mL was documented in 87% of Mexico City children residing in a high polluting, urban environment. This deficiency was associated with 12h fasting hyperleptinemia, altered appetite-regulating peptides, and increases in ET-1 in clinically healthy children [26].

Population	Number	Results	Author
7 year olds, Iceland	159	66% deficient 22% severe deficiency Inverse correlation of vitamin D and BMI, Hgb A1c, insulin and direct correlation with HDL p<0.05	Hannesdottir [24]
Obese children	376	70% insufficiency, 25(OH)D levels related to HDL cholesterol p<0.001	Iqbal [25]
Children ages 3-18	2300	Reduced risk of type 2 diabetes OR 0.73, CI 0.57-0.95 and OR 0.65, CI 0.51-0.84 for each SD increment of vitamin D status	Wu [27]
>20 years, United States	7674	Vitamin D reduced risk of insulin resistance HOMA-IR: 0.70 (0.59, 0.84)	Al-Khalidi [28]
Patients with diabetes and metabolic syndrome	124	59.68% and 27.42% had vitamin D deficiency and insufficiency, respectively. Systolic blood pressure (SBP) Serum log (25-OHD) inversely correlated with SBP, HbA1c, low-density lipoprotein cholesterol (LDL-C), TGs, and total cholesterol and directly correlated with pancreatic β cell function (HOMA- β), p<0.05	Alkhatatbeh [20]
Healthy young adults	196	Plasma 25(OH) D concentration 42.1 (13.0), 72% had vitamin D deficiency (25(OH)D<50 nmol/L); 13/184 (6.6%) were severely deficient (<25 nmol/L). Inverse association 25(OH)D and fasting glucose (r=-0.18; p<0.05). Higher HbA1c and TC:HDL-C ratio and lower HDL-C with 25(OH)D<25 nmol/L (p<0.05).	Wang [31]
Heart failure patients	261	87% with hypovitaminosis D, 25% with severe vitamin D deficiency. Severe deficiency group had lower VO2/kg, peak VO2%, higher NT pro BNP and Metabolic Exercise Cardiac Kidney Index (r=0.16, p=0.008)	Saponaro [21]
Elderly	137	The risk of HF associated with vitamin D deficiency (OR): 12.19; (CI)=4.23-35.16 p<0.001	Porto [32]
42 years mean age	6294	Highest vitamin D intake presented lower levels of triglycerides 14.6 mg/dL (P for trend=0.001); 2.0 cm less in waist circumference (P for trend=0.001) and 0.8 points less in the Framingham cardiovascular disease risk score (P for trend=0.002) compared with the subjects in the lower quintile of vitamin D intake. Highest vitamin D intake less likely to develop elevated 10-year cardiovascular disease risk, compared with those in the lowest quintile (OR=0.51; 95% CI: 0.33, 0.77; P for trend=0.007).	Muñoz-Aguirre [38]
NA	1080	25(OH)D status and not genetic variants of VDBP were significantly associated with CAD (25-hydroxyvitamin D OR [95% CI]=0.99 [0.97-1.0] p=0.05	Daffera [39]
Women, 19-50 years old	200	Vitamin D deficiency 48%, while 38% showed levels higher than 30 ng/ml. Fasting glucose and insulin levels were significantly higher in subjects with vitamin D deficiency/insufficiency (P=0.034 and P=0.049, respectively) 25(OH)D levels inversely correlated with insulin levels (P=0.0001) and intima-media thickness (P=0.015), and directly with serum HDL cholesterol (P=0.010).	Giovinazzo [40]

Table 1: Cross-sectional studies of Vitamin D status and cardiovascular health.

Setting	Number	Type	Results	Author
Twenty-one studies	3,777 Vitamin D deficiency patients and 4,792 controls	Pooled case-control	Compared to controls, Vitamin D deficiency patients showed a significantly higher CCA-IMT 0.043 mm; 95%CI: 0.030, 0.056; P<0.001), and an increased prevalence of carotid plaques (Odds Ratio [OR]: 2.29, 95%CI: 1.03-5.11; P=0.043) with an attributable risk of 35.9%	Lupoli [43]
Multi-Ethnic Study of Atherosclerosis (MESA)	1837	Case cohort	Low total 25(OH) D was differentially associated with CHD events, by race, with or without adjustment for VDBG (P for interaction=0.04 or 0.05, respectively)	Robinson-Cohen [44]
Community-based	8155	Nested case control	At baseline, 592 participants (7.3%) were hypertensive; of those, 71% were no longer hypertensive at follow-up (12 ± 3 months later). There was a significant negative association between BP and serum 25(OH) D level (systolic BP: coefficient=-0.07, p<0.001; diastolic BP: coefficient=-0.1, p<0.001). Reduced mean systolic (-18 vs. -14 mmHg) and diastolic (-12 vs. -12 mmHg) BP, pulse pressure (-5 vs. -1 mmHg) and mean arterial pressure (-14 vs. -13 mmHg) were not significantly different between hypertensive participants who did and did not take BP-lowering medication	Mirhosseini [49]
Adults 40-89 years old	2690 stroke cases, 19538 controls	Nested case control	Calcium supplementation with vitamin D was not associated with an increased risk of ischemic stroke (odds ratio 0.85; 95% confidence interval, 0.67-1.08) Calcium supplement monotherapy was not associated with an increased risk in the population as a whole (odds ratio 1.18; 95% confidence interval, 0.86-1.61), although a significant increased risk at high doses (≥1000 mg/day: odds ratio 2.09; 95% confidence interval, 1.25-3.49;<1000 mg: odds ratio 0.76; 95% confidence interval, 0.45-1.26) compared with nonuse was observed	De Abajo [47]
66 yrs, mean age	180 pts with HF	Case cohort	25(OH) D level in the AF (+) group was significantly lower than in the AF (-) group (11.05 ng/mL vs. 20 ng/mL, p<0.001) and the parathyroid hormone level was significantly higher in the AF (+) group (76.7 vs. 55 pg mL, p<0.001. Independent predictor (based on multiple regression) of AF was vitamin D level (OR=0.854, 95% CI: 0.805-0.907, p<0.001)	Belen [48]
Chronic kidney disease, stage 3	1664	Case cohort	Vitamin D deficiency (HR 1.62, 95% CI 1.01 to 2.58) and elevated PTH (HR 1.42, 95% CI 1.09 to 1.84) were independently associated with all-cause mortality	Shardlow [49]

Table 2: Case-control studies of cardiovascular disease and Vitamin D.

Author	Setting	Number	Year	Type	Results
Samefors [30]	Prospective Study in Primary Care (CARDIPP)	698			Serum 25(OH) D3 inversely associated with CV morbidity and mortality: HR 0.98 (CI) 0.96 to 0.99, P=0.001]. Compared with the fourth quartile (Q4) [25(OH) D3>61.8 nmol/l], HR (with 95% CI) was 3.46 (1.60 to 7.47) in Q1 [25(OH) D3<35.5 nmol/l] (P=0.002)
Wang [31]	Rotterdam longitudinal cohort	6220	18 yrs	Prospective cohort	Lower vitamin D concentrations were associated with higher risk of dementia (adjusted HR, per SD decrease 1.11, 95% CI 1.02; 1.20) and AD (adjusted HR: 1.13, 95% CI 1.03; 1.24).
Sheerah [45]	Adults 49-79 years	58646	19	Prospective cohort	Highest vitamin D intake were 0.70 (0.54-0.91; P for trend=0.04) for total stroke and 0.66 (0.46-0.96; P for trend=0.04) for intraparenchymal hemorrhage
Nie [46]	Patients hospitalized for ischemic stroke	387	1	Prospective cohort	OR for CVD death lowest 25(OH) (D) quartile, 3.06 [95% CI, 2.16-4.95]; all-cause mortality: OR for first quartile, 2.76 [95% CI, 2.01-4.32].
Mastroeni [55]	Pure North Preventive Health Program	6755	Mean 1.1 yr. follow up	Longitudinal	Relative to obese participants without increases in 25(OH)D, those with improvements in 25(OH)D were less likely to have elevated CRP concentrations (≥1 mg/L), compared to those with no improvements
Faridi [56]	Atherosclerosis risk in Communities study, mean age 57 yrs	13039	5.2 yrs.	Longitudinal	Deficient compared with >30ng/ml 25(OH)D had modestly increased risk for incident dyslipidemia in demographic-adjusted models (relative risk [RR], 1.19; 95% CI, 1.02-1.39), which was attenuated in fully adjusted models (RR, 1.12; 95% CI, 0.95-1.32)
Meems [33]	Non-HF patients	7470	12.6	Longitudinal	Calcidiol levels were univariately associated with new onset HF [hazard ratio (HR) 0.82 (95% CI 0.69-0.96)], NS after adjustment
Gaksch [35]	61 years	26916	10.5years	Meta-analysis with standardization of 25(OH)D	Compared to participants with 25(OH)D concentrations of 75 to 99.99 nmol/L, HR for mortality: 25(OH)D groups with 40 to 49.99, 30 to 39.99, and <30 nmol/L were 1.15 (1.00-1.29), 1.33 (1.16-1.51), and 1.67 (1.44-1.89), respectively. CV death: 1.56 (1.38-1.74) 1.19 (1.08-1.31) 1.08 (0.97-1.19) 1.04 (0.95-1.12) 1.00 1.04 (0.89-1.19) 1.10 (0.72-1.49) from lowest to highest tertiles of 25(OH)D respectively
Chen [42]		16434		Met-analysis	Carotid atherosclerosis (odds ratio, 0.95; 95% confidence interval [CI], 0.93-0.96). Hypovitaminosis D was associated with an 0.85-fold decrease of having a higher carotid intima-media thickness (95% CI, 0.76-0.96; P < .05) Vitamin D level was a protective factor against increased carotid plaque (odds ratio, 0.95; 95% CI, 0.93-0.97; P < .05; I2 =29%).

Table 3: Prospective cohort studies and meta-analyses of Vitamin D and cardiovascular disease.

However, a main issue in Mexico City is the pollution. Fine particulate matter (PM 2.5) is a risk factor for cardiovascular disease (CVD).

Vitamin D status and metabolic syndrome

In a 31 year follow up study, both youth and adult blood levels of 25(OH)D is associated with diabetes risk; each 12.5 nmol increase in Vitamin D status in youth reduced risk of diabetes by 26%(CI, 0.57-0.95) [29]. Vitamin D status was also associated with of metabolic

syndrome and insulin resistance in the large NHANES cohort [27,28].

In congruence, a large cohort of Italian workers found that only 11% had ideal circulating 25OHD, and 25OHD was inversely associated with BMI, insulin resistance, inflammation, and fatty liver index [29].

Vitamin D status is associated with cardiovascular events and mortality in patients with type 2 diabetes [30]. Young adults in Hong

Kong had average 25 (OH)D levels of 13 ng/ml, with associations of HDL, and inverse association with TC:HDL and HbA1c [31].

Vitamin D status and heart failure

Recently, the prevalence of Vitamin D deficiency in heart failure patients was found to be between 65- 87%, with 25% having severe Vitamin D deficiency (levels less than 10 ng/ml) [21]. Vitamin D status was inversely related to peak VO₂ and metabolic Exercise Cardiac Kidney Index, a prognostic marker of mortality. Deficiency is strongly predictive of heart failure risk, with over 12 times the risk of heart failure with Vitamin D deficiency [32]. 25 (OH) D levels are associated with heart failure incidence in some, but not all studies [33,34]

Vitamin D status is associated with cardiovascular events and mortality

Two recent meta-analyses found that Vitamin D status was associated with cardiovascular events, including myocardial infarction, cardiovascular mortality, and also all-cause mortality [35,36]. The meta-analysis by Gatsch is the first to look at standardized 25 hydroxyVitamin D concentrations. This is important because variability in measurements likely have affected outcome data up to this point.

The Physician's Health Study did not find associations with Vitamin D intake and CVD risk; however, Vitamin D at >217 IU/day was considered as the high quartile of intake [37].

Increased Vitamin D intake was associated with reduced cardiovascular risk in an urban Mexican population [38]. Vitamin D status was also positively correlated with the presence of coronary lesions [39], and it was also inversely related to carotid intima media thickness [40]. Vitamin D status was shown to be associated with is also of peripheral artery disease [41].

A recent meta-analysis demonstrated that Vitamin D status was inversely related to both carotid artery plaque and carotid intima media thickness [42]. Atherosclerosis is also negatively associated with Vitamin D status [43].

Vitamin D binding protein binds the 25OHD, making it less bioavailable. It is associated with coronary heart disease incidence and appeared to strengthen the association of 25OHD and a reduction in events in the Multi-Ethnic Study of Atherosclerosis [44].

Vitamin D status and stroke risk and hypertension

Vitamin D intake was inversely associated with stroke mortality in a large Japanese cohort study [45] and Vitamin D status was inversely associated with stroke infarct volume after ischemic stroke [46]. Calcium supplements appeared to increase risk of stroke, however, use of Vitamin D with calcium supplements seemed to mitigate the risk of stroke that calcium brings [47]. Low Vitamin D status in heart failure patients may also be associated with atrial fibrillation, a major risk factor for stroke [48].

In a nested case-control study, improvements in 25 (OH) D levels >100 nmol/L (40 ng/ml) was associated a reduced risk of hypertension at a one year follow-up [49]. This community-based study aimed to improve Vitamin D status. Achieving improvement strikingly was associated with normalization of blood pressure measurements.

Vitamin D and statin tolerance

Vitamin D status is associated with statin-induced myopathy, and

statin tolerance rates are also higher among people with 25OHD levels greater than 20 ng/ml [50].

In a retrospective study of Vitamin D replenishment, 40% of previously statin-intolerant individuals were able to tolerate their statin after supplementation, and were more likely to meet their cholesterol goals [51].

Khayznikov demonstrated that up to 95% of individuals with statin intolerance (myalgia, myopathy, myonecrosis) could subsequently tolerate the re-challenged statin after repleting their Vitamin D deficiencies [52].

Vitamin D status and biomarkers

Optimal Vitamin D status (>30 ng/ml) reduced risk of incidental dyslipidemia over a 5 year period in the Atherosclerosis Risk in Communities study. [53]. Vitamin D status was also shown to be inversely related to platelet counts and mean platelet volume [54]. Sequential improvement in Vitamin D status was associated with reduction of c-reactive protein in obese individuals, with greater improvements in 25(OH)D affording further reductions in c-reactive protein [55]. Additionally, Vitamin D status was inversely related to homocysteine, cystatin-C, creatinine, HbA1c and uric acid levels, particularly in women [56].

Vitamin D, endothelial function, and erectile dysfunction

In Korean women, Vitamin D status was inversely related to endothelial dysfunction [57]. Men with Vitamin D insufficiency (<30 ng/ml) were shown to have an increased risk of erectile dysfunction in the NHANES study [58] and also in men with type 2 diabetes (glycaemia, HDL cholesterol, and triglycerides), testosterone plasma levels and endothelial dysfunction may be affected) [59]. Additionally, men with ED are more likely to have Vitamin D deficiency than those who do not [60].

Vitamin D associated with coronary calcification

A controversial topic that remains is the concept of Vitamin D and calcification of soft tissue. Recent observational studies have shed further light on this topic. Coronary calcification scores were higher in Vitamin D insufficient patients than in the sufficient Vitamin D groups ($p < 0.001$) [61]. In fact, middle-aged men with Vitamin D deficiency were over 3 times as likely to have coronary artery calcification [62].

Clinical trials of Vitamin D in cardiovascular disease

Our team conducted a 6-month double-blinded, placebo-controlled trial of Vitamin D₃ 10,000 IU daily supplementation in patients with NYHA II-III heart failure with reduced ejection fraction [63]. Our findings were: that Vitamin D₃ at 6 months improved BNP compared to placebo, reduced circulating PTH levels, and improved quality of life compared to placebo by 10 points in the composite domains on average. While our sample size was small, the results are noteworthy because the dose used was higher than any trial in CHF to date, and because of the significant rise of Vitamin D status. Additionally, in the subset of men, C-reactive protein was significantly reduced with Vitamin D₃ treatment. We also found a large magnitude of change for PTH levels, on average 20 pg/ml decrease. A trial of lower dose, but longer duration found that supplemental Vitamin D₃ at 4000 IU per day improved ejection fraction [64].

Similar to our findings in quality of life, Vitamin D₃ supplementation of 50,000 IU per week improved health-related quality of life in healthy premenopausal urban-dwelling women; at the end of the study they

had significantly greater improvements in 25(OH)D compared to placebo as well [65].

A recent review of Vitamin D supplementation clinical trials found that very few had beneficial effects on direct or surrogate CVD end points [66]. The parameters of the trials such as enrollment criteria for 25(OH)D concentration, Vitamin D dose, number of participants, and trial duration were tabulated. Of the 40 trials, 19 had no restrictions on 25(OH)D concentration, two had <60 ng/ml, one had <40 ng/ml, nine had <30 ng/ml, four had <25 ng/ml, four had <20 ng/ml, and one had <16 ng/ml.

Since that review, at least two Vitamin D clinical trials found reduced risk of surrogate CVD end points. One trial found that high-dose Vitamin D₃ significantly reduced blood pressure for those who had hypertension [67]. At baseline, the mean 25(OH)D concentration of the 8155 participants was 87 ± 37 nmol/l (35 ± 15 ng/ml) and they were taking 1600 ± 2500 IU/d Vitamin D₃. At the end of one year after increasing the Vitamin D₃ intake to 5200 ± 4300 IU/d, the mean 25(OH)D concentration was 113 ± 39 nmol/l while the controls, who did not change Vitamin D₃ intake, 25(OH)D concentrations were relatively unchanged. For the 480 hypertensives that increased their Vitamin D₃ intake, the mean systolic blood pressure dropped from 156 mm to 138 mm and the mean diastolic blood pressure dropped from 96 mm to 84 mm, both independent of whether they were taking blood pressure medication. As a result, 71% of those hypertensive at baseline were no longer hypertensive at the end of the trial. There was no significant reduction in blood pressure for non-hypertensives.

A clinical trial involving overweight African Americans with Vitamin D deficiency (<20 ng/ml) supplemented with 600, 2000, or 4000 IU/d Vitamin D₃ for 16 weeks found that 4000 IU/d was associated with 10% reduction in carotid-femoral pulse wave velocity (PWV) and an 8% reduction in carotid-radial PWV [68].

The 2000 IU/d dose was nearly as effective for carotid-radial PWV but not carotid-femoral PWV. A clinical trial in Iran gave adolescent girls (mean age 14 yrs., mean BMI ~20 kg/m²) nine weekly doses of 50,000 IU Vitamin D₃ [69]. At the end of three months, hs-CRP decreased from 0.98 (95% CI, 0.50-1.85) to 0.86 (0.39, 1.61) ($P = 0.007$) and neutrophil-to-lymphocyte ratio decreased from 1.66 ± 0.72 to 1.53 ± 0.67 ($P=0.002$). 25(OH)D concentration was not measured.

A Vitamin D clinical trial was conducted on children with autism in China [70]. At baseline, the mean 25(OH)D concentration for those with autism was 21 ng/ml while that for controls was 25 ng/ml. After Vitamin D₃ treatment (150,000 IU/m by injection plus 400 IU/d orally for three months) significant improvements were observed for behavioral abnormalities of the autistic children (apart from the sensory subscale).

Proper design of clinical studies

One potential reason for the failure of many Vitamin D clinical trials is that they have been based on the guidelines for trials for pharmaceutical drugs rather than those for nutraceuticals [71]. Two basic assumptions of efficacy or safety drug trials are that the only source (or dose or dosage regimen) of the agent is the trial and that there exists a previously demonstrated a dose-response relationship. Neither assumption is satisfied for Vitamin D. The Vitamin D dose-25(OH)D concentration relationship is very nonlinear [72], as is the 25(OH)D concentration-health outcome relationship as exemplified by that for breast cancer incidence with respect to 25(OH)D concentration found from case-control studies [73,74]. Another reason for failure

of Vitamin D clinical trials to find beneficial effects includes enrolling people with 25(OH)D concentrations high enough that for the Vitamin D doses used, no significant benefit could be expected.

Researchers are beginning to realize that Vitamin D clinical trials should be guided and based on measurements of 25(OH)D concentrations. A recent paper illustrates this point. The trial involved 2300 postmenopausal women in Nebraska [75]. They were given 2000 IU/d Vitamin D₃ plus 1500 mg/d calcium in the treatment arm and placebos in the control arm for four years. Mean baseline 25(OH)D concentration was 33 ng/ml. When the data were analyzed according to intention to treat, the outcome analysis did not reach statistical significance ($P=0.06$ CI, 0.048 to 0.076) due to one too many cancer cases in the treatment arm. However, when cancer incidence rates were analyzed in terms of 25(OH)D concentrations, it was found that achieved serum 25(OH)D concentration between 45 and 85 ng/ml was associated with significantly reduced incidence of cancer. The results of this trial were compared to model calculations based on estimated 25(OH)D concentration among the study population and the 25(OH)D concentration-breast cancer incidence relationship [75]. It was found that the outcome in terms of intention to treat was as expected; suggesting a larger “n” and longer trial duration may have demonstrated that the results would have been significant when analyzed according to intention to treat.

Recently the Heaney guidelines for nutrient studies [71] were extended with respect to Vitamin D trials [76]. The key steps are: 1-develop or obtain a 25(OH)D concentration-health outcome relationship for the expected health outcome. If none is available for that particular outcome, use one developed for other outcomes. 2-measure 25(OH)D concentrations for possible participants and try to enroll those with lower 25(OH)D concentrations. 3-set the Vitamin D₃ dose high enough to achieve significant reduction in risk according to the 25(OH)D concentration-health outcome relationship. 4-if calcium and/or magnesium are given to the treatment group, they should also be given to the control group. Magnesium helps convert Vitamin D to 25(OH)D [76]. 5-measure 25(OH)D concentrations at intervals during the trial and use these values both to titrate/adjust Vitamin D doses to achieve desired 25(OH)D concentrations. 6-analyze results with respect to 25(OH)D concentrations.

Conclusion

Vitamin D deficiency remains a significant problem that appears to adversely affect the cardiovascular system. Measuring 25(OH)D concentrations and supplementation to achieve circulating levels shown to have beneficial effects (>40 ng/ml) should be the goal of future research. Vitamin D₃ screening and supplementation with follow up of circulating 25(OH)D should be considered in all people due to high rates of deficiency, and should be part of future clinical trials in regards to cardiovascular outcomes.

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