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Vitamin D and Cardiovascular Disease: Is There Evidence to Support the Bandwagon?

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Abstract In the last 3 years, more evidence accumulated that vitamin D (vitD)deficiency associates with cardiovascular disease (CVD) and risk factors. The association with higher cardiovascular (CV) mortality was stronger than with nonfatal CVD events. A higher incidence of type 2 diabetes was also shown. Many factors related to lifestyle (physical activity in particular) influence both vitD levels and CVD, and may contribute to explain these observational data. Whether the association between vitD and CVD is causal can only be established through randomized controlled trials (RCTs), and to date the results of the randomized trials, which were not designed for investigating CV outcomes, do not support the association data. Answers on the effects of vitD supplementation on primary and secondary prevention of CV may be found in the specifically designed ongoing RCTs. In the mean time, low vitamin D levels should be regarded as a marker of unhealthy lifestyle, requiring a more aggressive attempt at modifying individual lifestyle.

Keywords Vitamin D · Serum 25(OH)D levels · Cardiovascular disease · Cardiovascular mortality · Diabetes mellitus · Hypertension · Insulin resistance · RCTs

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Introduction

In the last decade the relationship between vitamin D (vitD) and cardiovascular disease (CVD) and its risk factors has been widely explored. The research has also moved forward on the basis of the detection of vitamin D receptors (VDRs) in multiple cells and tissues, in addition to bone, intestine, and kidney, and the 1-alpha hydroxylase enzyme in cells of the cardiovascular system, suggesting that this vitamin has biological effects beyond the simple regulation of mineral bone metabolism. The abundance of publications on vitD (PubMed search: 2,844 in 2000–2001 increasing to 6,635 in 2010–2011), shows the complexity of this issue, which has been addressed in many reviews related to CVD.

The present article will consider the studies on vitD and CVD published in the last 3 years, which add evidence on (a) strength and type of the associations, (b) possible mechanisms, and (c) effects of vitD supplementation on CV disease and CV risk factors.

Source and Circulating Levels of Vitamin D

The main source of vitamin D, accounting for 80–90 % of circulating metabolites, is ultraviolet (UV) B radiation-induced conversion of 7-dehydrocholesterol to provitamin D_3, which spontaneously isomerizes to vitamin D_3 (cholecalciferol). Food such as fatty fish also contain vitamin D_3, while vitamin D_2 (ergocalciferol) is produced by UVB-induced conversion of ergosterol in plants and fungi. Vitamin D_2 and vitamin D_3 are hydroxylated to 25-hydroxyvitamin D (25[OH]D) in the liver and then further hydroxylated to 1,25-dihydroxyvitamin D (1,25[OH]2D) in most tissues and cells of the body, mainly in the kidney. 1,25[OH]2D, which is a steroid hormone, is the most active

vitD metabolite. Circulating 25[OH]D levels are the most utilized in epidemiological and clinical studies, because they best express extrarenal tissue levels of 1,25[OH]2D and, therefore, indicate whole body vitD status [1]. A large part of the population does not meet vitD requirements, because contemporary life is associated with reduced sun exposure and use of UVB-blocking sunscreens. Dark-skinned individuals require more sun exposure to have the same vitD production as people with less skin pigmentation, and the same is true for older versus younger individuals [2].

New Observational Evidence on Vitamin D Levels and Incidence of CV Events

Evidence has accumulated in the last 3 years that vitD deficiency associates with CVD. Four epidemiological prospective studies have reported a higher incidence of CV death in individuals with low serum 25(OH)D concentrations at baseline (Table 1). In the prospective cohort of 3,408 elderly participants at the NHANES-III, during the median follow-up of 7.3 years, there were 1,493 (44 %) all-cause deaths, including 767 CVD-related deaths [3]. Adjusting for demographics, season, and CV risk factors, baseline 25 (OH)D levels were inversely associated with all-cause mortality risk (Hazard ratio=1.83, 95 % CI=1.14–2.94, for subjects with 25(OH)D levels less than 25.0 nmol/L vs. subjects with 100 nmol/L or higher). The association was stronger for CVD mortality (adjusted HR 2.36, 95 % CI= 1.17–4.75). In a large Finnish prospective study, including 6,219 men and women free from CVD, 640 coronary disease deaths and 293 cerebrovascular disease deaths were identified during a follow-up of 27 years [4•]. After adjustment for potential confounders, the hazard ratio for total CVD death was 0.76 for the highest quintile of baseline serum 25(OH)D level vs. the lowest. The association was significant for cerebrovascular death, but not coronary death. In the Uppsala Longitudinal Study of Adult Men (ULSAM), in which 1,194 elderly men were followed for 12 years, participants with serum concentrations of 25 (OH)D below the 10th percentile were at significantly increased risk for CV death (HR=1.89) than participants above the 90th percentile [5]. A U-shaped pattern was observed for overall mortality that was associated with both high and low concentrations of plasma 25(OH)D. A reverse J-shaped association between serum level of 25(OH)D and mortality was also observed in a general practice study in Denmark [6]. In the Ludwigshafen Risk and Cardiovascular Health (LURIC) study, in the cohort of 1,801 participants with the metabolic syndrome referred for coronary angiography, 267 deaths of cardiovascular cause were recorded during the 7.7-year follow-up [7]. After full adjustment, including anthropometric, metabolic, CV parameters, and physical activity, the subjects with severe vitD deficiency (25(OH)D levels<25 nmol/l) showed a substantial increase in CVD mortality (HR=3.03, CI 1.32–5.88), compared with those with optimal levels (> 75 nmol/l).

Reports on the association with nonfatal CV disease are less consistent. In the cross-sectional analysis from the Korean National Health and Nutrition Examination Survey (KNHANES) IV on self-reported diagnosis of angina, myocardial infarction, and stroke, unadjusted serum 25(OH)D levels did not differ significantly between the CVD and non-CVD groups [8]. However, after adjusting for age, gender, body mass index (BMI), education level, residence location, region, and energy intake, subjects in the lowest category (< 25 nmol/l) of serum 25(OH)D levels had a higher prevalence of CVD, about two-fold higher than subjects in the highest category (≥ 75 nmol/l). In a case–control study of 387 survivors of a first myocardial infarction (MI) and 387 controls, VitD insufficiency was not independently related to premature MI [9]. Serum concentrations of 25(OH)D were lower in cases than controls: 55.0 vs. 60.5 nmol/l. Standardized odds ratio (OR) for MI with 95 % confidence interval in univariable analysis was 0.80 (0.69–0.93); p= 0.003). The 25(OH)D association with MI disappeared after adjustment for established and emerging risk factors (OR: 1.01, 0.82–1.25). Also the "Psychological, social and biological determinants of ill health" (pSoBid) study in Glasgow showed a not-so-strong association between 25(OH)D levels and measures of atherosclerotic disease [10]. One unit increase in log 25(OH)D was not significantly associated with either carotid intima media thickness or plaque presence, in univariable or adjusted models. This was observed along with a strong association of 25(OH)D levels with lifestyle factors and socioeconomic deprivation.

The 34-year incidence of stroke in 8,006 Japanese-American men associated with dietary vitD intake in the Honolulu Heart Program [11]. Using Cox regression, adjusting for age, total kilocalories, BMI, hypertension, diabetes, smoking, physical activity, serum cholesterol, and alcohol intake, those in the lowest quartile had a significantly increased risk of incident stroke compared with the highest quartile (all stroke HR 1.22, 95 % CI 1.01–1.47; thromboembolic stroke HR 1.27, 95 % CI 1.01–1.59).

In a large medical records database of a general healthcare population (41,504 patient records), the vitD levels were highly associated with coronary artery disease, myocardial infarction, heart failure, peripheral vascular disease, and stroke (all p<0.0001), as well as with incident death, heart failure, coronary artery disease/myocardial infarction (all p<0.0001), and stroke (p=0.003); however, this analysis was not adjusted for possible confounders [12].

Therefore, the association between low levels of vitD and the hardiest CV outcome, i.e. CV mortality has consistently been shown in these recent prospective studies. The

Table 1 Observational studies published in the last 3 years on the association between circulating 25(OH)D levels (nmol/L) and risk of cardiovascular (CV) mortality and disease and incidence of type 2 diabetes

STUDY Reference, Country	PARTICIPANTS Gender (F&M), Mean age *or* range (y)	STUDY DESIGN Follow-up (years)	OUTCOME	HR/OR (95%CI)* Low vs. High
NHANES-III	1,908 F & 1,500 M,	Prospective (7.3 y)	CV death	2.36 (1.17–4.75)
Ginde et al., [3]	73 y			<25 *vs.* >100
GAJC 2009, USA				
Mini-Finland Health Survey	3,402 F & 2,817 M,	Prospective (27 y)	CV death	1.31 (1.05–1.67)
Kilkkinen et al. [4•]	49 y			<22 *vs.* >69
AJE 2009, Finland				
ULSAM	1,194 M,	Prospective (12.7y)	CV death	1.89 (1.21–2.96)
Michealsson et al.[5]	71 y			<46 *vs.* >93
AJCN 2010, Sweden				
LURIC study	3,402 F & 2,817 M,	Prospective (7.7 y)	CV death	3.03 (1.32–5.88)
Thomas et al.[7]	49 y			<25 *vs.* >75
Diab Care 2012, Germany				
KNHANES	2,863 F & 2,695 M,	Cross-sectional	Angina/MI/Stroke	2.12 (1.51–2.67)
Park et al.[8]	63 y			<25 *vs.* >75
Nutr Res Prat 2012, S.Korea				
Deleskog et al.[9]	387 MI & 387 controls	Case–control	MI	1.01 (0.82–1.25)
Atherosclerosis 2012, Sweden	54 y			
pSoBid study	613 F & M	Cross-sectional	Carotid plaque	0.88 (0.75–1.03) per
Knox et al. [10]	35–64 y			1 unit increase in log 25OHD
Atherosclerosis 2012, Scotland				
Nurse Health Study	608 T2D, 559 control, F	Prospective (5 y)	Incident DM	1.92 (1.20–3.03)
Pittas et al. [14]	56 y			<35 *vs.* >82
Diab Care 2010, USA				
Framingham Offspring Study	442 F & 363 M,	Prospective (14 y)	Incident DM	1.67 (1.03–2.70)
Liu *et al.* [15]	59 y			<41 *vs.* >62
J Clin Nutr 2010, USA				
Tromsø Study	3,754 F & 2,365 M,	Prospective (11 y)	Incident DM	1.05 (0.95–1.16)
Grimnes et al. [16]				per 10 nmol/l decrease
Diab Med 2010, Norway	59 y			
Women's Health Initiative	5,140 F	Prospective (7.3 y)	Incident DM	0.95 (0.57–1.62)
Robinson et al. [17]				<35 *vs.* >64
Diab Care 2011, USA				
AusDiab study	2,860 F & 2,340 M,	Prospective (5 y)	Incident DM	1.32 (1.09–1.59)
Gagnon et al. [18]	55 y			<58 *vs.* >65
Diab Care 2011, Australia				
MONICA-KORA study	1,683 F	Case-cohort	Incident DM	1.37 (0.95–2.00)
Thorand. et al. [19]	52 y			1st vs. 3rd tertile
Diab Care 2011, Germany				
Gonzales-Molero et al. [20]	698 F & 528 M,	Prospective (4 y)	Incident DM	5.88 (1.64–20.0)
Clin. Nutr 2012, Spain	50 y			<45 *vs.* >45
EPIC-Norfolk study	14,714 F & 10,564 M,	Case-cohort	Incident DM	2.00 (1.32–3.12)
Forouhi et al. [21]	58 y			<48 *vs.* >80
Diabetologia 2012, UK				
The Hoorn study	179 F & 172 M	Prospective (7.5 y)	Incident DM	0.44 (0.14–1.37)
Pilz et al. [22]	68 y			<50 *vs.* >75
NMCD 2012, The Netherlands				
Inter99 study	4,296 F&M	Prospective (5 y)	Incident DM	1.06 (0.97–1.16)
Husemoen et al. [23]	30–65 y			per 10 nmol/l decrease
Diab Care 2012, Denmark				

*The ratio most adjusted for confounding variables is reported in the table.

HR = Hazard ratio; *OR* = Odds ratio; *CI* = confidence interval; *DM* = Diabetes mellitus; *NS* = non significant; *MI* = myocardial infarction.

observational studies indicate that results differ with different CVD endpoints. The relationship with CVD may be the end result of the aggregation of multiple established and new CV risk factors, and vitD has been linked to the main CV risk factors, in particular to type 2 diabetes (T2D) mellitus, hypertension, and metabolic syndrome, as discussed in the next section of this paper.

New Observational Evidence on Vitamin D and Type 2 Diabetes or Metabolic Syndrome

Vitamin D has been repeatedly linked to type 2 diabetes in humans, as reviewed by Pittas et al. in 2010 [13]. Since then, other longitudinal studies have associated vitD status with the incidence of type 2 diabetes (Table 1). A nested case–control analysis in the Nurse Health Study after adjusting for diabetes risk factors including BMI, indicated that higher levels of plasma 25(OH)D were associated with a lower risk for type 2 diabetes (OR 0.52, top vs. bottom quartile of 25(OH)D) [14].

In a subsample of the Framingham Offspring Study participants (n=1,972), a total of 133 diabetic subjects were identified over a 7-year average follow-up [15]. In comparison with individuals in the lowest tertile, those in the highest tertile had a 40 % lower incidence of type 2 diabetes after adjustment for age, sex, waist circumference, parental history of type 2 diabetes, hypertension, HDL cholesterol, plasma triglycerides, and impaired fasting glucose. In the Tromsø Study, among 6,119 participants without diabetes at baseline, the 11-year incidence of type 2 diabetes increased in the lowest vs. the highest quartile of serum 25(OH)D concentrations, but this was no longer significant after adjustment for BMI [16]. However, a significant decrease in the risk of developing type 2 diabetes with increasing serum 25(OH)D concentrations was observed in the participants in the lowest BMI quartile. A lack of association between serum 25(OH)D levels and risk of type 2 diabetes after adjustment for BMI and other risk factors such as physical activity was also shown in the Women's Health Initiative (WHI) Clinical Trials and Observational Study in a racially and ethnically diverse population of postmenopausal women [17].

A 22–29 % risk reduction of type 2 diabetes was instead associated with each 25 nmol/l increment in serum 25(OH)D in the Australian Diabetes, Obesity and Lifestyle (AusDiab) study at the 5-year follow-up [18]. Serum 25(OH)D was also positively and independently associated with HOMA-S, a marker of insulin sensitivity. In the MONICA-KORA, a German case–control study in 1,683 women, after adjustment for diabetes risk factors and season, the hazard ratio (HR) for incident type 2 diabetes comparing extreme tertiles of serum 25(OH)D was 1.59 (1.11–2.27) [19]. The association was no longer significant after further adjustment for C-reactive protein, interleukin-6, soluble intercellular adhesion molecule-1, and interferon-γ-inducible protein-10. Similarly, in the prospective evaluation of a representative sample of the Spanish population, Gonzales et al. found an inverse association between plasma 25-hydroxyvitamin D levels and the incidence of type 2 diabetes [20].

Recently, Forouhi et al. presented the data from the European Prospective Investigation of Cancer (EPIC)-Norfolk study [21]. Comparing the highest and lowest quartiles of 25(OH)D levels at the baseline study visit (1993–1997), the multivariable adjusted HR for incident type 2 diabetes occurring until 2006 was 0.50 (95 % CI 0.32–0.76). In the same paper, the authors also presented a meta-analysis, including nine published prospective studies and the previously unpublished EPIC-Norfolk and Ely studies, with a total of 3,612 type 2 diabetes cases and 55,713 non-cases. Individuals in the top quartile of baseline 25(OH)D showed a relative risk (RR) of 0.59 (95 % CI 0.52–0.67) compared with those in the bottom quartile.

In contrast, there was no cross-sectional association of 25(OH)D with type 2 diabetes, or with parameters of glucose metabolism in an elderly subsample of 351 participants in the population-based Hoorn study [22]. In the prospective analyses on the 280 participants without diabetes at baseline, there was no significant association of 25(OH)D with incident diabetes, fasting or 2-hour post-load glucose levels. Similar results were shown by Husemoen et al. in a larger cohort in Denmark, wherein low 25(OH)D status was not significantly associated with incident diabetes after adjustment for confounders, although it was associated with significant deterioration of insulin resistance [23]. In the Intermountain Heart Collaborative (IHC) Study, individuals with severe vitD deficiency in the medical records database had an increased likelihood of developing diabetes, hypertension, and hyperlipidemia in the mean follow-up of 1.5 years [12]. The association between vitD and type 2 diabetes and metabolic syndrome has been partially confirmed in diverse ethnical populations, including Chinese, Thai, Arab Americans, Malay, and African American, although few of the studies adjusted for potential confounders, such as physical activity.

In the last decade there have been numerous reports on the association between vitD and blood pressure/hypertension. The Fourth Tromsø Study has provided further cross-sectional evidence on this association [24]. In fact, in the 4,125 subjects not using blood pressure medication at baseline, after adjusting for sex, age, BMI, and physical activity, the difference in systolic blood pressure between the lowest and highest serum 25(OH)D quartile was 3.6 mmHg (p< 0.001, trend across quartiles). However, in the 14-year prospective analysis of 2,385 of these subjects, after adjustment for potential confounders, baseline serum vitD levels did not predict future hypertension or increases in blood pressure.

In conclusion, the recent observational studies substantially confirm the association between vitD levels and main CV risk factors, although results are discordant because of a number of different factors. First of all, results differed by gender and age, with stronger associations shown in men and in older individuals. Cohorts that differed for baseline metabolic profiles were evaluated, with higher incidence of metabolic diseases in individuals at higher risk, e.g. more insulin resistant. The populations studied also differed for baseline serum 25 (OH)D concentrations. In this respect, repeated measurements of 25(OH)D levels and a greater standardization of the methods for measuring blood 25(OH)D levels would be helpful. The association seems stronger in the presence of, or restricted to, severe vitD deficiency. Seasonal variability of 25(OH)D levels is very high and difficult to take care of. Although data are usually adjusted statistically for season or, sometimes, month of blood sampling, this correction may not be always appropriate. In a simulation by Wang et al. [25], creating 25 (OH)D categories without accounting for time of sampling and then adjusting for season or month of blood collection resulted in bias away from the null. This was corrected by using season-specific or month-specific quartile categories, but this correction may be less effective in real life, because the variability of 25(OH)D levels over the course of the year varies widely between individuals, depending on their seasonal patterns of outdoor activities and diet, for example.

It is now recognized, and partially shown in the Health 2000 Survey, a cross-sectional analysis on 5,174 Finnish men and women, that serum 25(OH)D concentration is associated with a large number of sociodemographic factors, lifestyle, and metabolic health [26••]. Such factors may confound the associations between serum 25(OH)D concentration and chronic diseases, and the strength of the associations vary to a great extent by type and amount of statistical adjustments. Problems may arise when adjustments are made for variables that could eventually not be confounders, but rather the result of vitD action. The equilibrium between the risk of over-adjustment, which will not allow observation of a real association, and the lack of adequate adjustment for known or unknown factors influencing both vitD levels and cardiometabolic parameters, is therefore delicate. For example, adiposity is strictly related to both cardiometabolic variables and vitD levels, but is not clear whether the excess fat is causing low circulating vitD levels due to its sequestration of vitD, or, less likely, vitD deficiency induces adiposity. Physical activity is also a strong confounder, as it relates to cardiometabolic risk as well as to vitD levels, although the nature of this latter association is not clear, reflecting either a direct relationship with vitD metabolism or an indirect one through changes in body fat or sun exposure.

The high number of factors associated with circulating vitD may suggest that low vitD levels are a noncausal indicator of chronic illness related to a lower exposure to sunlight; on the other hand, higher vitD levels may reflect changes to a healthier lifestyle, as suggested by trends towards more former and less current smoking, and increased physical activity in individuals with high vitD levels.

Possible Mechanisms Linking Vitamin D with CV Disease and Risk Factors

Causality implies a pathogenetic link between vitD deficiency and CVD, and various mechanisms have been proposed to explain this association. Some recent studies have confirmed the proposed most plausible biological explanations, i.e. alterations of the renin-angiotensin-aldosterone system (RAS) and insulin resistance. Since vitD seems to inhibit the production of renin, vitD deficiency could lead to overproduction of renin and therefore increased angiotensin II, and, consequently, hypertension. The inverse relation between vitD and RAS activity has been confirmed in patients referred for coronary angiography [27], as well as in non-hypertensive individuals [28].

In addition to potential effects on the RAS, the link between vitD and hypertension has been hypothesized to be mediated by other direct effects on vascular endothelium and smooth muscle. Serum 25(OH)D status was associated with vascular endothelial function among middle-aged/older adults in the absence of clinical disease [29]. These authors also reported that lower 25(OH)D status was associated with increased vascular endothelial cell expression of NFkB and IL-6 and increased NFkB-related suppression of vascular endothelial function, indicating that inflammation could play a role in the relationship [30].

Vitamin D may influence glycemic homeostasis through different mechanisms, including VDRs and 1-α- hydroxylase in pancreatic β-cells, the insulin receptor, and the glucose transport, through a direct action or, indirectly, through its effects on intracellular calcium. However, recent reports on insulin secretion and sensitivity have been discordant. In the Survey in Europe on Nutrition and the Elderly, a Concerted Action (SENECA study) there was no association between 25 (OH)D and fasting plasma insulin or HOMA-IR after full adjustment for demographic factors, lifestyle factors, and calcium intake in elderly individuals [31]. In agreement with this, in individuals with no known history of diabetes, at multivariate analysis BMI was the most powerful predictor of serum 25(OH)D concentration ($r=-0.52$; $p<0.01$), whereas insulin-sensitivity measured by hyperinsulinemic euglycemic clamp was not significantly associated, indicating the lack of an independent relationship between vitD and insulin-resistance [32•]. In contrast, in the Korean Sarcopenic Obesity (SO) Study, serum 25[OH]D levels were positively correlated, while both hsCRP and HOMA-IR levels were negatively

correlated, with skeletal muscle mass index in both men and women [33]. Multiple binary logistic regression analysis showed that HOMA-IR and 25[OH]D levels were independently associated with SO in men. Moreover, in a cross-sectional study in adult Canadian women of white and Aboriginal ancestry, the Aboriginal women had higher BMI, fat, and markers of dysglycemia and lower values of serum 25 (OH)D than the white women [34]. After accounting for age, ethnicity and adiposity using regression analyses, serum 25 (OH)D levels were inversely related to glucose, HbA1c, HOMA-IR, and C-peptide levels.

Although controversial, the link between vitD and CVD seems biologically plausible, and the results of mechanistic studies do not help in demonstrating whether the association between vitD and CVD is causal, which may only be established through randomized controlled trials (RCTs).

Randomized Controlled Trials (RCTs)

Most intervention trials involving vitD supplementation were designed to assess the role of vitD in bone health. Very few trials were designed to look primarily at cardiovascular outcomes or risk factors. Evidence from RCTs published in the last three years (Table 2) was not as promising as could be expected, based on the associations highlighted by the observational studies. In the largest of these trials, the Women's Health Initiative Calcium–Vitamin D, which randomized 36,282 postmenopausal women to either 400 IU of vitamin D_3 + 1 g calcium daily or placebo, nonsignificant trends toward decreases in overall mortality and CV death (HR 0.92, CI=0.77–1.10) were observed in the supplementation group, but the effects of calcium and vitamin D could not be differentiated [35••]. In accordance, a meta-analysis of The Cochrane Collaboration in 2011 on 42,589 participants in ten trials showed that vitD supplementation for a median duration of two years compared to no treatment or placebo had no significant effect on CV mortality (RR 1.01, CI=0.91–1.13) [36••]. This meta-analysis also showed that vitamin D_3 reduced all-cause mortality by about 6 % predominantly in elderly (mean age 74 years) women.

Studies on softer CV endpoints relate to endothelial dysfunction, a condition eventually leading to atherosclerosis. In post-menopausal women, brachial artery flow-mediated vasodilation (FMD), and other arterial indexes did not change after a 4-month daily administration of 2,500 IU vitamin D_3 [37]. Similarly, 100,000 units of vitamin D_3 given every 2 months for 6 months did not improve reactive hyperaemia index on fingertip plethysmography in patients with a history of myocardial infarction [38]. Equally, no significant change in FMD was observed after a single dose of 100,000 or 200,000 IU D_3 in patients with type 2 diabetes [39].

As for CV risk factors, generally vitD administration showed no effects on blood lipids levels, which were not significantly changed after 5 years of treatment in a subgroup of the Women's Health Initiative [40]. This was confirmed with higher doses of vitamin D_3 for shorter treatment periods in individuals overweight/obese [41], with type 2 diabetes [42], or with a history of myocardial infarction [38]. Instead, a decrease in serum triglycerides and an increase in LDL-cholesterol were observed in overweight/obese subjects after one year vitD supplementation, compared with placebo [43].

As for hypertension, RCTs published in the last three years showed that vitD supplementation (D_2 or D_3) had no effect on blood pressure in individuals overweight/obese [41, 43, 44], with type 2 diabetes [42], or with a history of myocardial infarction [43]. These results are concordant with a meta-analysis published in 2009 [45]. In RCTs with a mean baseline blood pressure more than 140/90 mmHg, vitD supplementation compared with placebo showed a non-significant reduction in systolic blood pressure [−3.6 mmHg], and a slight statistically significant reduction in diastolic blood pressure (−3.1 mmHg). No reduction in blood pressure was shown in the studies examining individuals normotensive at baseline.

Recent RCTs evaluating glycemic homeostasis and insulin resistance, which are both related to CV risk and vitD insufficiency in observational studies, have shown weak or no effect of vitD supplementation. VitD did not affect insulin sensitivity in overweight/obese individuals [41, 43, 44] and overweight women with polycystic ovary syndrome [46], nor beta cell function assessed as C-peptide levels at fasting [47] or in a mixed meal tolerance test in healthy nondiabetic individuals [48]. In contrast with these results, a significant improvement in insulin sensitivity with supplementation of vitD compared with placebo was found in South Asian women who showed some degree of insulin resistance at baseline [47]. No substantial effects of vitD supplementation on glycaemic control and insulin resistance were seen in patients with type 2 diabetes [39, 42, 49], apart from a significant reduction in insulin resistance (Matsuda index, p=0.034) and an increase in insulin secretion (p=0.024) in the latter trial [49].

A recent meta-analysis on vitD supplementation and glycemic control and insulin resistance showed a small effect on fasting glucose (−0.32 mmol/l, 95 % CI −0.57 to −0.07) and a small improvement in insulin resistance, while no effect was seen on glycated haemoglobin in patients with diabetes [50].

Evidence on inflammatory markers is inconsistent, some trials showing no effect on C-reactive protein (CRP) levels in overweight individuals [37, 43, 44], another a significant reduction [47]. Moreover, CRP, but not tumor necrosis factor α (TNFα), decreased significantly in patients with a history of myocardial infarction after 6 months of vitD

Table 2 Effects of vitamin D supplementation on cardiovascular mortality or metabolic outcomes: evidence from randomized controlled double blind trials published in the last 3 years

STUDY Reference, Country	PARTICIPANTS Gender (F/M), Age (y)	STUDY DESIGN Dosage, Duration	OUTCOME	p value
LaCroix et al. [35••] 2009, USA	36,282 F Age: 51–82 y Postmenopausal	1 g Ca+400 UI D_3/day *vs.* placebo 7 years	CVD mortality	N.S.
Gepner et al. [37] 2012, USA	114 F Mean age: 64 y Postmenopausal	2,500 UI D_3/day *vs.* placebo 4 months	Endothelial function Arterial stiffness CRP	N.S.
Witham et al. [38] 2012, UK	23 F & 52 M Mean age: 66 y Myocardial infarction	100,000 UI D_3/every 2 months *vs.* placebo 6 months	Endothelial function TNFα, Blood pressure, Blood lipids ↓ CRP	N.S 0.03
Witham et al. [39] 2010, UK	61 M&F Mean age 65 y Type 2 diabetes	200,000 IU D3 vs. 100,000 IU D3 vs. placebo, single dose 16 weeks	Endothelial function HOMA-IR HbA1c	N.S.
Rajpathak et al. [40] 2010, USA	1,291 F Age: 50–79 y Postmenopausal	1 g Ca+400 UI D_3/day *vs.* placebo 5 years	Blood lipids	N.S.
Jorde et al. [41] 2010, Norway	282 F & 156 M Age: 21–70 y Overweight/obese	40,000 UI D_3/week *vs*. 20,000 UI D_3/week *vs.* placebo 1 year	Blood lipids Blood pressure, HOMA	N.S.
Nagpal et al. [44] 2009, India	100 M Mean age: 44 y Overweight	120,000 UI D_3/fortnightly *vs.* placebo 50 days	Blood lipids Blood pressure, CRP, HOMA-IR	N.S.
Zitterman et al. [43]	111 F & 54 M	3,332 UI D_3/day	↓ S-Tg ↑↑LDL-C	<0.001
2009, Germany	Age: 18–70 y	*vs.* placebo	↓ TNFα	0.049
	Overweight	12 months	Blood pressure, IL-6, CRP, FSG	N.S.
von Hurst et al. [47]	81 F	4,000 UI D_3/day	↓ HOMA2-IR, ↓ CRP	<0.05
2010, New Zealand	Age: 23–68 y South Asians Overweight	*vs.* placebo 6 months	C peptide	N.S.
Harris et al. [49]	45 F & 44 M	4,000 UI D_3/day	↑ C peptide	<0.05
2012, USA	Mean age: 57 y	*vs.* placebo	HOMA-IR	
	Afro-Americans, Obese, Prediabetes or Early diabetes	12 weeks		N.S.
Bock et al. [48] 2011, Austria	29 F & 30 M Mean age: 34 y Healthy	140,000 UI D_3/month *vs.* placebo 3 months	FSG, FSI, C-peptide	N.S.
Ardabili et al. [46] 2012, Iran	60 F Age: 20–40 y Overweight, PCOS	50,000 UI D_3/every 20 days *vs.* placebo 2 months	HOMA-IR	N.S.

N.S. = not significant; *BP* = blood pressure; *CRP* = C-reactive protein; *S-Tg* = serum triglycerides; *FSG* fasting serum glucose; *FSI* = fasting serum insulin; *HOMA-IR*=Homeostasis Model Assessment Insulin Resistance; *IL-6* = interleukin-6; *PCOS* = polycystic ovary syndrome; *TNFα* tumor necrosis factor α.

supplementation [38], while, on the opposite, Zitterman and colleagues found a significant reduction in TNFα but no effects on CRP and interleukin 6 (IL-6) [43].

Three main randomized controlled trials, which specifically aim at evaluating the effects of vitD on the incidence of CV events, are currently active. The Physician's Health Study II is investigating whether vitamin E, ascorbic acid, β-carotene or a multivitamin complex containing 500 IU vitD can reduce the risk of myocardial infarction and stroke in healthy male doctors older than 50 years. The estimated

study completion date was June 2011. [Available at: http://clinicaltrials.gov/ct2/show/NCT00270647].

The "Role of Vitamin D in Secondary Prevention of Cardiovascular Events" is investigating whether 150,000 IU of vitamin D_3 every 2 months reduces the incidence of CV events in men or women with a past diagnosis of unstable angina, or myocardial infarction, or at least one coronary stenosis (> 50 %). The estimated study completion date is July 2013 [Available at: http://clinicaltrials.gov/ct2/show/NCT01018849].

The VITamin D and OmegA-3 TriaL (VITAL) is investigating whether vitamin D_3 (2000 IU) and omega-3 fatty acid (840 mg) daily supplementation reduces the risk of developing heart disease and stroke. The study plans to enroll 20,000 subjects, men older than 60 years and women older than 65 years, with no medical history of CVD. The estimated study completion date is June 2016. [Available at: http://clinicaltrials.gov/ct2/show/NCT01169259].

Conclusion

The results of the randomized trials to date do not support the data from observational studies that report beneficial associations with CVD. The first possible explanation for this discrepancy is that the association between vitD and CVD may not be causal. Several sociodemographic, lifestyle, and metabolic factors are recognized as possible confounders, and their strict interrelationship may not have allowed a proper statistical correction in some studies. Among these factors, strictly related to both vitD levels and cardiometabolic risk, a major role is likely played by adiposity, the excess fat likely causing low circulating vitD levels for its sequestration of vitD, and physical activity, acting either directly on vitD metabolism or indirectly through changes in body fat or sun exposure.

A second possible explanation for the discordance between observational data and RCTs is that trials were not adequate to discover effects, for different reasons, including the fact that trials had not been designed for investigating CV outcomes, some of them lacked adequate statistical power, and vitD doses were inadequately low. Moreover, they differed in type of vitamin supplement—25(OH) D_3 or 25(OH) D_2—, concomitant calcium supplementation, and in populations studied not sufficiently "at risk" for vitD status or age, obesity, insulin resistance. Therefore, whether vitD supplementation can be effective for the primary and secondary prevention of CV disease remains unproven. Some of these questions may be answered by the specific ongoing trials. It may be effective only in specific responsive individuals, e.g. with very low vitD levels, high RAS activity, high insulin resistance, heart failure.

While we wait for the outcome of the ongoing trials, it may be wise to consider low vitamin D levels as a marker of unhealthy lifestyle, requiring a more aggressive attempt at modifying individuals' way of life, i.e. sunlight exposure, weight reduction, physical activity, healthy nutrition, smoking cessation, all factors positively influencing both vitamin D status and cardiovascular risk.

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- • Of importance
- •• Of major importance

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