Title: Effects of vitamin D supplementation on endothelial function: a systematic review and meta-analysis of randomised controlled trials

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Running title: Vitamin D supplementation and endothelial function

Keywords: vitamin D, nitric oxide, flow-mediated dilation, forearm blood flow, pulse wave velocity, cardiovascular risk

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The material presented in this manuscript is original and it has not been submitted for publication elsewhere while under consideration for European Journal of Nutrition.

Abstract word count: 247
Total word count: 3657
References: 68
Tables: 3
Figures: 1
OSM: 1

PROSPERO Database registration: **CRD42014009668**, http://www.crd.york.ac.uk/prospero/

Abstract

Background: In addition to regulating calcium homeostasis and bone health, vitamin D influences vascular and metabolic processes including endothelial function (EF) and insulin signalling. This systematic review and meta-analysis of randomized clinical trials (RCTs) was conducted to investigate the effect of vitamin D supplementation on EF and to examine whether the effect size was modified by health status, study duration, dose, route of vitamin D administration, vitamin D status (baseline and post-intervention), body mass index (BMI), age and type of vitamin D.

Methods: We searched the Medline, Embase, Cochrane Library, and Scopus databases from inception until March 2015 for studies meeting the following criteria: 1) RCT with adult participants, 2) vitamin D administration alone, 3) studies that quantified EF using commonly applied methods including ultrasound, phlethysmography, applanation tonometry, laser Doppler.

Results: Sixteen articles reporting data for 1177 participants were included. Study duration ranged from 4 to 52 weeks. The effect of vitamin D on EF was not significant (SMD: 0.08, 95%CI:-0.06, 0.22, *P*=0.28). Subgroup analysis showed a significant improvement of EF in diabetic subjects (SMD: 0.31, 95%CI: 0.05, 0.57, *P*=0.02). A non-significant trend was found for diastolic blood pressure (β=0.02; *P*=0.07) and BMI (β=0.05; *P*=0.06).

Conclusions: Vitamin D supplementation did not improve EF. The significant effect of vitamin D in diabetics and a tendency for an association with BMI may indicate a role of excess adiposity and insulin resistance in modulating the effects of vitamin D on vascular function. This remains to be tested in future studies.

1. Introduction

Cardiovascular diseases (CVDs) are a major public health concern and contribute to >30% of overall mortality worldwide[1]. The pathogenesis of CVDs is multifactorial and a critical step in the onset and advancement of CVDs is the formation of atherosclerotic lesions [2]. One of the earliest stages of the atherosclerosis process is the impairment of endothelial function (EF) [3].

The pathophysiology of endothelial dysfunction is complex and involves multiple mechanisms including over-production of reactive oxidative species, inflammatory cytokines and pro-atherogenic lipoproteins together with an imbalance between vaso-dilating and vaso-constricting molecules. Impairment of vasodilatation may be due to reduced bio-availability of nitric oxide (NO), which is produced by the endothelial cells and which is involved in multiple physiological processes including vasodilation, inflammation and platelet aggregation[4].

Vitamin D is a pro-hormone which is mostly known for its involvement in the regulation of calcium homeostasis and bone remodelling [5]. However, vitamin D is also essential for several non-musculoskeletal functions including regulation of vascular tone, gluco-insular homeostasis and immunity [5]. Vitamin D receptors (VDRs) are expressed in several tissues notably endothelial cells, vascular smooth muscle cells and cardiomyocytes [6]. The active form of vitamin D (1α,25-dihydroxyvitamin D_3, $1,25(OH)_2D_3$) is a direct transcriptional regulator of endothelial NO synthase [7]. A recent study has shown that VDR mutant mice have lower NO bioavailability leading to endothelial dysfunction, increased arterial stiffness, increased aortic impedance, structural re-modelling of the aorta, and impaired systolic and diastolic heart function [8]. However, observational studies evaluating the association of vitamin D with CVD risk have reported mixed results. A significant inverse relationship between low vitamin D status, as assessed by serum 25-hydroxy vitamin D (25-OHD) and

increased risk of major cardiovascular events and chronic diseases such as myocardial infarction (MI), stroke, hypertension and type 2 diabetes has been reported [9-11], but this has not been confirmed in other cohorts [12, 13]. These discrepant results may be ascribed to the differences between study designs and phenotypic characteristics of study participants including 1) duration of follow up, 2) cut-off values for the definition of deficient vitamin D status, 3) diagnostic criteria for the identification and classification of cardiovascular outcomes, 4) confounding factors (i.e., diet, sun exposure, seasonality, physical activity) and 5) health status of the participants in the cohorts [14]. Randomised controlled trials (RCTs) examining the effects of vitamin D supplementation on EF have also reported contradictory results; whilst some studies have reported improvement in EF [15-17] others have observed no effect of vitamin D supplementation [18-30]. A recent meta-analysis has showed a non-significant effect of vitamin D supplementation on changes in flow mediated dilation measured by ultrasound after post-occlusion hyperaemia. The study showed that effects was greater in short studies (<16 weeks) and in subjects with raised systolic and diastolic blood pressure (BP)[31].

The method for the assessment of EF in humans depends on the availability of resources and equipment, technical and research expertise and, most importantly, by the research question under investigation. The most commonly used methods to measure dynamic vascular responses are: i) ultrasound to assess the increase in diameter of large arteries following post-occlusive hyperaemia, ii) phlethysmography to assess changes in forearm blood flow during infusion of pharmacological agents targeting endothelial-related mechanisms (e.g. acetylcholine or sodium nitroprussiate) and iii) applanation tonometry by measuring pulse wave velocity (PWV) of peripheral arteries [32].

We aimed to conduct a systematic review and meta-analysis of RCTs investigating the effect of supplemental vitamin D on EF. The secondary aim of the study was to determine whether

the effect size was modified by health status, study duration, dose, route of vitamin D administration, baseline vitamin D status and changes in 25-OHD after supplementation, body mass index (BMI), age and type of vitamin D (vitamin D_2 or vitamin D_3).

2. Methods

The present systematic review was conducted according to the Cochrane guidelines [33] and it is reported according to PRISMA guidelines [34].

2.1 Literature search

Four databases (Medline, Embase, Scopus, and Cochrane Library) were used to search for articles from inception until March 2015. In addition, a manual search of reference lists of relevant reviews and articles included in the systematic review was performed. The search was conducted based on pre-defined search terms [Ergocalciferol OR Cholecalciferol OR vitamin D OR Vitamin D2 OR vitamin D3 OR 25(OH)D] And [Endotheli* OR Endotheli* dysfunction OR FMD or Hyperaemia OR Plethysmography OR Flow mediated OR Endothelial-dependent OR Vasomotor or Vasoacti* OR Blood flow OR Brachial OR Vasodilat* OR Dilat* OR Vascular resistance OR Pulse Wave OR Augmentation index OR Arterial stiffness OR Digital volume pulse OR Pulse amplitude tonometry OR Arterial compliance].

2.2 Study selection

The following criteria were applied to identify articles to be included in this systematic review and meta-analysis: 1) RCTs (no further exclusion criteria were applied in relation to study design or blinding); 2) studies involving adults aged 18 years or more and no exclusion criteria were applied for health status, smoking history or body size; 3) vitamin D administered alone i.e. not combined with other drugs or nutritional interventions; studies were not excluded on the basis of the dose, duration of follow up, route of administration of vitamin D or type of administration (i.e. tablet, capsule, solution or as fortified food) and type

of assay used for the determination of 25-OHD concentrations; 4) studies reporting changes in EF measured by ultrasound, venous-occlusion phlethysmography, photo phlethysmography, pulse wave velocity, pulse amplitude tonometry, laser Doppler flowmetry; 5) no language or time restrictions were applied in searching the databases.

Two investigators (AMH, MS) independently screened the titles and abstracts of the articles to evaluate eligibility for inclusion. If consensus was reached, articles were either excluded or moved to the next stage (full-text). If consensus was not reached the articles was moved to the full-text stage. The full-texts of the selected articles were appraised critically to determine eligibility for inclusion in the systematic review. Disagreements were resolved by discussion among the authors until the consensus was reached.

2.3 Data extraction and quality assessment

The following information was extracted from the eligible articles: 1) authors, journal details and year of publication; 2) participants (total number, male/female ratio, age, health status); 3) study characteristics (country, design, inclusion/exclusion criteria, description of measurement protocols; 4) vitamin D intervention (type, formulation, dose, duration of follow up, route of administration); 5) EF measurement (instrument, position, duration of cuffing) and 6) circulating concentrations of vitamin D before and after intervention.

In addition, we adopted the modified Jadad score to assess the risk of bias of the included studies; possible scores ranged from 0 to 5 and a score of ≤ 3 indicates high risk while a score of > 3 indicates low risk of bias[35].

2.4 Statistical analysis

Serum concentrations of 25-OHD given in ng/mL were converted to nmol/L (1 ng/mL=2.496 nmol/L)[36]. Several methods were used to assess EF in humans including flow mediated dilation (FMD), forearm blood flow (FBF), pulse wave analysis (PWA) and laser Doppler (LD) with the results obtained from these methods reported on different scales. Therefore, to

allow comparison of effect sizes between studies, standardised mean differences (SMDs) were used as a summary statistic. SMD is estimated from the difference between the mean outcome values of the intervention and control groups divided by the pooled standard deviation (SD) of the outcome values; this converts the estimated effect to SD units. SMD of 0.2, 0.5 and 0.8 represent small, medium and large effect sizes, respectively[37]. In addition, different methods were frequently used in the same trial to assess EF, as shown in **Table 1,** and therefore this lack of independence of the EF measurement in each trial was taken into consideration in the derivation of the pooled effect size. Statistical analyses were performed by using Comprehensive meta-analysis software (version 2, Biostat, Englewood, New Jersey, USA). Data synthesis, including calculation of effect sizes with 95% confidence intervals, was accomplished by employing a random-effects model using inverse variance weighting. Forest plots were generated for graphical presentation of the effect of supplemental vitamin D on EF. For this purpose, the mean and SD of the EF measure before and after the intervention period (for both vitamin D intervention and control) were extracted and used in the analysis. For studies that reported changes in EF at two or more time-points (e.g. acute and chronic effects of vitamin D supplementation), the last EF measurement was used in the meta-analysis. Data not provided in the main text or tables were extracted from the figures.

Subgroup analyses were undertaken to investigate the variables which may have influenced the effects of supplementation on EF. These factors included: health status, type (vitamin D_2 or D_3) and the frequency of administration (single dose, daily-weekly or monthly) of vitamin D supplementation. Random effect meta-regression analyses were used to determine whether participant baseline characteristics (age, BMI, systolic and diastolic blood pressure, baseline concentration of 25-OHD) influence the effect of vitamin D supplementation (vitamin D_2 or D_3) on EF. Furthermore, meta-regression analyses were conducted to investigate the influence of other factors including vitamin D dose, baseline 25-OHD, change in 25-OHD

concentration after supplementation, duration of interventions, sample size and quality score (Jadad score) on the effect of vitamin D supplementation on EF.

Heterogeneity between studies was evaluated using Cochrane Q statistics; $P > 0.1$ indicates significant heterogeneity. The I^2 test was also used to evaluate consistency between studies where a value < 25% indicates low risk of heterogeneity, 25-75% indicates moderate risk of heterogeneity, and >75% indicates high risk of heterogeneity[38]. The evidence of publication bias was assessed by visual inspection of the funnel plots and by the Egger's regression test[39].

3. Results

3.1 Search results

The process of screening and selection of studies is summarised in **Figure S1 of the online supplementary material**. The primary search of the four databases produced 4159 articles after removal of duplicates. After title and abstract screening, 22 full-text papers were retrieved for further evaluation. Additionally, one study was found by manual searching references of the relevant reviews and studies. Examination of the full text of 23 articles yielded 16 studies which were eligible to be included in this systematic review and meta-analysis. One trial [25] included two independent arms supplementing different vitamin D doses which resulted in 17 independent interventions entered in the final meta-analysis.

3.2 Studies characteristics

The total number of participants from the 16 studies included in this systematic review was 1177 (607 females; 570 males) with median of 73 (range 34 -159) participants per study. The median age was 63.2 (range 30-77) years. All RCTs included in the meta-analysis were parallel, double-blind, placebo-controlled trials. The duration of the trials ranged from 4 weeks to 52 weeks (**Table 1**).

Three studies investigated the effect of vitamin D in healthy participants [16, 18, 40], two studies were conducted in patients with chronic kidney disease (CKD) [19, 22], four studies in diabetics [15, 17, 25, 30], six studies in patients with CVDs [20, 23, 24, 26, 28, 29] and one study in patients with HIV [21]. All trials supplemented vitamin D orally. Trials however utilised different forms of supplementation including tablets [20, 23, 30], solution[17, 19, 24-26, 28, 40], capsules [15, 16, 21, 22] and fortified biscuits [18]. The majority of the trials utilised vitamin D_3 with daily doses varying from 1000IU/day [15] to 5000IU/day[30].

Several methods were used to assess EF in the included trials. The most commonly used methods were FMD [16-19, 21, 22, 25, 29, 30], PWV [18-20, 22, 29, 30, 40] and augmentation index (AIx) [15, 18, 20, 24]. Other methods include laser Doppler flowmetry [40] and digital volume pulse [28] (**Table 1**).

3.3 Qualitative analysis

Three of the studies included in the present systematic review reported a significant improvement in EF in response to vitamin D administration [15, 17, 41] whereas the other 13 studies reported no effect of supplementation [18-30]. Ten studies described the methods of randomisation [18-23, 25, 27, 28, 30] and five studies stated the methods of allocation concealment [20, 21, 25, 27, 28]. The drug history of the participants was reported by all except three studies [15, 16, 27]. With the exception of two studies [16, 19], all other studies reported, and described, participant dropout. The quality of the included studies ranged from 3 to 5 (Jadad score) and eleven studies had a low risk of bias (Jadad score ≥4) (**Table 1**).

3.4 Meta-analysis

Meta-analysis of the 16 studies (1177 participants) showed that, overall, vitamin D supplementation did not improve EF (SMD: 0.08, 95%CI: -0.06, 0.22, *P*=0.28) (**Figure 1**). The effect of supplemental vitamin D on post-occlusive vasodilation of the brachial artery was not significant (FMD%, N=10, +0.27%, 95%CI: -0.36, 0.91, *P*=0.39, **Table S1, Online**

Supplementary Material). Heterogeneity between studies was not significant (Q=21.7, I^2=26.4%, P=0.15). Subgroup analysis showed that vitamin D supplementation improved EF significantly in participants with type 2 diabetes (N=5, SMD: 0.31, 95%CI: -0.05, 0.57, P=0.02) (**Table 2**). This was confirmed by the significant effect of vitamin D supplementation in type 2 diabetic on changes in FMD% (N=4, +0.81%, 95%CI: 0.005, 1.61, P=0.04, **Table S1, Online Supplementary Material**). The response of EF to vitamin D supplementation was not significantly modified by type of vitamin D, method of administration, baseline 25-OHD concentrations or baseline health status of the participants (**Table 2**). Meta-regression analyses demonstrated a weak, positive effect of BMI (β: 0.05, SE: 0.02, P=0.06) and of baseline diastolic blood pressure (β: 0.02, SE: 0.01, P=0.07) in modifying the effect of vitamin D supplementation on EF (**Table 3**). BMI did not modify the association between type 2 diabetes and EF (N=6, β: 0.04, SE: 0.04, P=0.23) whereas lower baseline 25-OHD concentrations were associated with a greater effect size in type 2 diabetic participants (N=6, β: -0.02, SE: 0.01, P=0.03) (**Figure S3, Online Supplementary Material**). The dose of vitamin D was not associated with significant changes in EF (**Table 3 and Figure S4, Online Supplementary Material**)

3.5 Publication bias

Visual inspection of the funnel plot showed modest evidence of asymmetric distribution of the effect size ((**Figure S2 of the online supplementary material**), which was confirmed formally by the lack of significance of the Egger's test (P=0.08).

4. Discussion

Overall, our meta-analysis demonstrated no effect of vitamin D supplementation on EF. In addition, baseline vitamin D and change in vitamin D concentration after supplementation were not associated with effects of vitamin D supplementation on EF. However, vitamin D supplementation resulted in a significant improvement in EF in patients with diabetes and

there was a positive trend towards greater effects of vitamin D on EF with increasing baseline BMI and diastolic blood pressure.

Several putative mechanisms could explain the positive effects of vitamin D on EF in some population groups, particularly in those at higher cardiovascular risk. Vitamin D is involved in the regulation of endothelial cell-dependent vasodilation which may be mediated by the effect of vitamin D metabolites on the renin angiotensin-aldosterone system, a hormonal system that regulates blood pressure and fluid balance. A low plasma 25OHD predisposes to up-regulation of the renin-angiotensin system, smooth muscle proliferation and favours a pro-inflammatory state which can increase the risk of hypertension and left ventricle hypertrophy [42]. The improvement in EF through vitamin D supplementation could also be mediated by the local effects of vitamin D metabolites on calcium metabolism in vascular smooth muscle cells and on the release of inflammatory cytokines which may affect vascular contractility [43]. Vascular smooth muscle and endothelial cells express VDR as well as 1α-hydroxylase [44], allowing for autocrine production of 1,25(OH)2D, which may act at the local level to modulate the effects of inflammatory cytokines on the vasculature, such as decreasing endothelial adhesion molecules, increasing NO production [45] and reducing platelet aggregation [46]. The activation of VDRs induces the transcription of a wide range of genes including those coding for vascular endothelial growth factor which in turn promotes NO synthesis by endothelial cells. In addition, 1,25(OH)2D3 is a direct regulator of endothelial NO synthase [8].

Vitamin D may also have beneficial effects on cardio-metabolic health in those with hypertension [47-50], type 2 diabetes [11, 30, 51] and cardiovascular disease [52-54]. A meta-analysis of data from 21 prospective studied showed an inverse association between vitamin D status and risk of type 2 diabetes [55]. In addition, cardiovascular disease is the main cause of premature mortality and morbidity in patients with CKD[22]. These

cardiovascular complications may be related to hypovitaminosis D [56], which may be linked to the inability of renal mass to convert 25OHD to the active form of vitamin D, 1,25-dihydroxyvitamin D[57]. However, our results did not show a significant effect of vitamin D supplementation on EF in patients with CKD which could be explained by several factors including the small number of studies (only two trials), the short duration (8 weeks), the inadequacy of the vitamin D dose or the advanced stage of endothelial dysfunction.

In the present meta-analysis, we observed that vitamin D supplementation produced a significant improvement in endothelial function in individuals with type 2 diabetes. While the small number of trials included in the analyses (N=4) call for a cautious and objective interpretation of the results, we believe that they are supported by a robust mechanistic rationale and provide important insights for future studies. This apparent diabetes-specific effect may be explained by several mechanisms including the link between low 25OHD concentrations and i) deterioration of β-cell function, ii) dysregulation of peripheral insulin signalling and iii) altered glucose disposal which are typically involved in the pathogenesis of type 2 diabetes [11, 14, 58]. These effects appear to be supported by the greater effect of vitamin D supplementation on EF in type 2 diabetic patients with insufficient vitamin D status. Vitamin D receptors and 1-α-hydroxylase are expressed in pancreatic β-cells and therefore an involvement in the regulation of insulin secretion may be expected [51]. In turn, $1,25(OH)_2D$ activates transcription of the human insulin receptor gene, stimulates expression of the insulin receptor [59], and enhances insulin-mediated glucose transport in vitro[60]. In addition, insulin secretion is a calcium-dependent process and vitamin D metabolites have been linked to the regulation β-cell calcium pools, which promotes insulin release [61]. The putative beneficial effects of vitamin D metabolites on EF may also be explained by the mechanistic inter-connection between the insulin and NO pathways. The activation of the insulin receptor on the endothelial cells instead induces a vasodilatory response via the

activation of the phosphoinositol-3-phosphate - Akt pathway which increases NO production by the enzyme endothelial nitric oxide synthase [62].

Our meta-regression analysis showed a trend for a greater improvement of EF in response to vitamin D supplementation in participants with high BMI. Growing evidence has shown that there is an inverse association between plasma 25OHD concentrations and BMI [63, 64]. Decreased bioavailability of vitamin D was found in obese subjects [63-65], which may be explained by adipose tissue sequestration and/ or volumetric dilution of 25OHD [66], and may explain the tendency towards a greater effect of supplemental vitamin D on EF in subjects with greater adiposity. In addition, obesity and excess visceral adiposity are closely associated with insulin resistance and development of type 2 diabetes which may explain the almost significant effect of vitamin D supplementation on EF in obese subjects. This may indirectly suggest that the magnitude of the effect size of vitamin D on EF may be correlated with the degree of metabolic derangement of the insulin signalling pathway.

Results may have been affected by the choice of the method used to measure vitamin D concentrations. Unlike chromatographic methods, immunoassays do not measure vitamin D3 and vitamin D2 independently and this is a well-recognised limitation of immunoassays. The importance of being able to quantify both metabolites of vitamin D independently is becoming increasingly important in recent years with the evidence that vitamin D3 is more biologically active than vitamin D2 [67]as well as emerging evidence that 25(OH)D2 concentrations are in the range of 1·5 to 10·0 nmol/l in several RCT and population based studies, this contributing significantly to total 25(OH)D[68]. It is also important to point out that results of 25(OH)D using chromatographic methods show significant variation, mainly due to extraction and calibration problems associated with these methods. Such assay variation reinforces the need for all users of vitamin D assays to have appropriate QC and standardization protocols in place.

Our meta-analysis has some limitations. First, the available trials had relatively small sample sizes with samples sizes of <100 in about 75% of the trials included in the meta-analysis. Second, the variability in duration, dose and type of vitamin D supplementation, the different methods used to assess EF and the diversity in participant characteristics (age, sex and health status) may have introduced significant heterogeneity and have militated against observation of overall effects of vitamin D supplementation on EF in our meta-analysis. Third, not all studies adjusted for potential confounding factors that may have influenced the effect of vitamin D on EF such as sun exposure, seasonality, physical activity or dietary patterns. Finally, most of the study participants were aged between 40 to 77 years old, thus limiting the applicability of the findings to other life stages. Finally, studies have used different assays to measure 25-OHD concentrations (Immuno-Assay, N=13; Liquid Chromatography Mass Spectrometry, N=3), which may have introduced a measurement bias. However, the exclusion of the three studies using LC-MS from the analysis did not modify the results, which provides support to the importance of vitamin D status in influencing the efficacy of vitamin D supplementation on vascular outcomes (data not showed).

We believe that the current evidence base is inadequate to draw firm conclusions about the protective role of supplemental vitamin D on EF and as a pharmaco-nutritional strategy for CVD prevention. However, our study provides important information on the effects of vitamin D supplementation on EF and shows that benefit may be anticipated in diabetics. This may indicate a potential role of insulin resistance in modulating the effects of vitamin D on vascular function. This hypothesis remains to be tested in future studies.

Acknowledgements

The systematic review was conceived by JCM, TH and MS. AM, AA and MS searched, collected and analysed the data and all authors contributed to data interpretation. AM, AA, IS and MS drafted the manuscript. All authors contributed to the final version of the manuscript.

The Corresponding Author (AM) is the guarantor for the manuscript and had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. All authors read and approved the final version of the paper.

Conflicts of Interests: None to Declare

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Figure Legends

Figure 1: Forest plot showing the effect of vitamin D supplementation on endothelial function. T2D = type 2 diabetes; CVD = cardiovascular disease; CKD = chronic kidney disease. Relative weight for a random model allows for small size studies contributing in a similar magnitude to the pooled estimate. The marker may vary in size according to the weights assigned to the different studies. The pooled effect is represented using a diamond.

Table 1: Summary of findings

Author	Country	Compliance	Health Status	Outcome	Sample Size	Male (N)	Age (years)	BMI (kg/m^2)	SBP/DBP (mmHg)	Vit D Dose (IU)	Duration (Frequency)	Formulation (Route)	Baseline 25-OHD (Assay)	Assay name, company	Δ 25-OHD	Vit D/day (IU)	Jadad Score
Breslavsky et al. 2013 [15]	Israel	Not reported	T2D	AI	47	22	67	29	153/74	1000 (D_3)	52w (D)	Capsule (Oral)	29 (IA)	Not stated	17	1000	3
Gepner et al. 2012 [18]	US	Not reported	Healthy	PWV, FMD,AI	109	0	64	26	122/72	2500 (D_3)	16w (D)	Biscuits (Oral)	78 (LC-MS)	Not stated	39	2500	5
Harris et al. 2011 [16]	US	Not reported	Healthy OW	FMD	45	21	29	30	123/74	60000 (D_3)	16w (M)	Capsule (Oral)	36 (IA)	Immunodiagnostic systems, Fountain Hills, AZ	66	2000	4
Hewitt et al. 2013 [19]	Australia	100% compliance	CKD	PWV, FMD	60	29	60	29	131/76	50000 (D_3)	8w (WK)	Solution (Oral)	42 (IA)	DiaSorin Inc, Stillwater, MN	42	7142	4
Larsen et al. 2012 [20]	Denmark	99% compliance	Ht (CVD)	AI, PWV	130	35	60	28	131/77	3000 (D_3)	20w (D)	Tablet (Oral)	57 (IA)	Liaison; DiaSorin, Saluggia, Italy	52	3000	5
Longenecker et al. 2012[21]	US	99% compliance	HIV	FMD	45	35	47	27	118/80	4000 (D_3)	12w (D)	Capsule (Oral)	19 (IA)	Immunodiagnostic Systems, Fountain Hills, AZ, USA	12	4000	5
Marckmann et al. 2012 [22]	Denmark	100% compliance	CKD	PWV, FMD	52	39	71	25	135/72	40000 (D_3)	8w (WK)	Capsule (Oral)	28 (LC-MS)	(LCMSMS 1, Applied Biosystems, Dionex, Sunnyvale, California, US	118	5714	4
Sokol et al. 2012 [23]	US	99% compliance	CHD	RH-PAT	90	66	55	30	133/76	50000(D_2)	12w (WK)	Tablet (Oral)	84 (LC-MS)	Quest Diagnostics, Teterboro, NJ, USA	67	7142	3
Stricker et al. 2012 [24]	Switzerland	100% compliance	PAD (CVD)	AI	62	38	72.9	27	136/74	100000 (D_3)	4w (SD)	Solution (Oral)	41 (IA)	DiaSorin, Saluggia, Italy	19	3571	4
Sugden et al. 2008 [17]	UK	100% compliance	T2D	FMD	34	18	65	31	141/80	100000 (D_2)	8w (SD)	Solution (Oral)	38 (IA)	I.D.S., Tyne & Wear, UK	23	1785	3
Witham et al. 2010 [25]	UK	100% compliance	T2D	FMD	61	41	G1:65 G2:63*	G1:31 G2:32	G1:141/76 G2:128/72	G1:100000 (D_3) G2:200000(D_3)	16w (SD)	Solution (Oral)	G1: 46 G2: 43 (IA)	IDS, Boldon, UK	28 18	G1:892 G2:1785	5
Witham et al. 2012 [26]	UK	100% compliance	Stroke (CVD)	FMD	58	42	66	27	129/72	100000(D_2)	16w (SD)	Solution (Oral)	38 (IA)	DiaSorin Ltd, Bracknell, UK	12	892	3
Witham et al. 2013 [27]	UK-South Asian	100% compliance	Healthy	FMD, PWV, AI, LD-ION	50	0	41	27	121/78	100000 (D_3)	8w (SD)	Solution (Oral)	27 (IA)	IDS Ltd UK	10	1785	5
Witham et al. 2013 [28]	UK	100% compliance	MI (CVD)	RHI	75	52	64	27	128/72	100000(D_3)	24w (2M)	Solution (Oral)	47 (IA)	I.D.S, Bachem UK, Merseyside, UK	13	1785	4
Witham et al.2013 [29]	UK	99% compliance	ISH (CVD)	FMD, PWV	159	82	77	28	163/78	100000(D_3)	52w (3M)	Solution (Oral)	45 (IA)	Not stated	25	1190	5
Yiu et al. 2012 [30]	China	Not reported	T2D	FMD, PWV	100	66	50	25	146/81	5000(D_3)	12w (D)	Tablet (Oral)	54 (IA)	I.D.S, (company not stated)	92	5000	3

N= number of subjects; OW, Overweight; IR, Insulin Resistance; AI, Augmentation Index; PWV, Pulse Wave Velocity; FMD, Flow Mediated Dilatation; RH-PAT, Reactive Hyperaemia Peripheral Arterial Tonometry ; RHI, Reactive Hyperaemia Index; LD-ION: Laser Doppler Iontophoresis; SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure; BMI= Body Mass Index; EF, Endothelial Function; *Different doses of vitamin D , Group 1: 100 000 IU , Group 2: 200 000 IU; w= weeks; D, daily; WK, weekly; M, monthly; SD, single dose; 2M, every 2 months; 3M, every 3 months. Δ= changes in vitamin D concentrations after supplementation. Vitamin D concentrations are reported in nmol/L. IA; Immuno Assay; LC-MS; liquid chromatography mass spectrometry. CVD= cardiovascular disease group. US= Unites States; UK= United Kingdom; Ht, Hypertension; MI, Myocardial Infraction; ISH, Isolated Systolic Hypertension; CHD, Coronary Heart Disease; CKD, Chronic Kidney Disease; PAD; Peripheral Arterial Disease; T2D; Type 2 Diabetes; HIV, Human Immunodeficiency Virus
Study designs for all of the studies are parallel, double blind – placebo controlled randomized trial. I.D.S; Immunodiagnostic system.

Table 2: Sensitivity analysis to evaluate the influence of health status, administration of vitamin D and type of vitamin D dose on the effect of vitamin D supplementation on endothelial function

Group	No of trials or subgroup	Effect size	95% CI	P	P between Groups
Health status					
• Healthy	3	0.15	-0.28 0.59	0.47	
• HIV	1	0.009	-0.61 0.62	0.97	
• Diabetes	5	0.31	0.05 0.57	0.02	0.23
• CKD	2	0.04	-0.62 0.71	0.89	
• CVD	6	-0.05	-0.22 0.11	0.51	
Frequency of Dose Administration					
• 1-3 month	4	0.17	-0.14 0.48	0.29	
• Daily-Weekly	7	0.02	-0.17 0.21	0.82	0.71
• Single dose	6	0.09	-0.20 0.40	0.53	
Baseline 25-OHD concentration					
Normal (≥50nmol/L)	4	-0.01	-0.21 0.17	0.84	0.27
Deficient (<50nmol/L)	13	0.13	-0.06 0.32	0.17	
Vitamin D type					
• D_2	3	-0.02	-0.61 0.58	0.95	0.72
• D_3	14	0.09	-0.03 0.22	0.15	

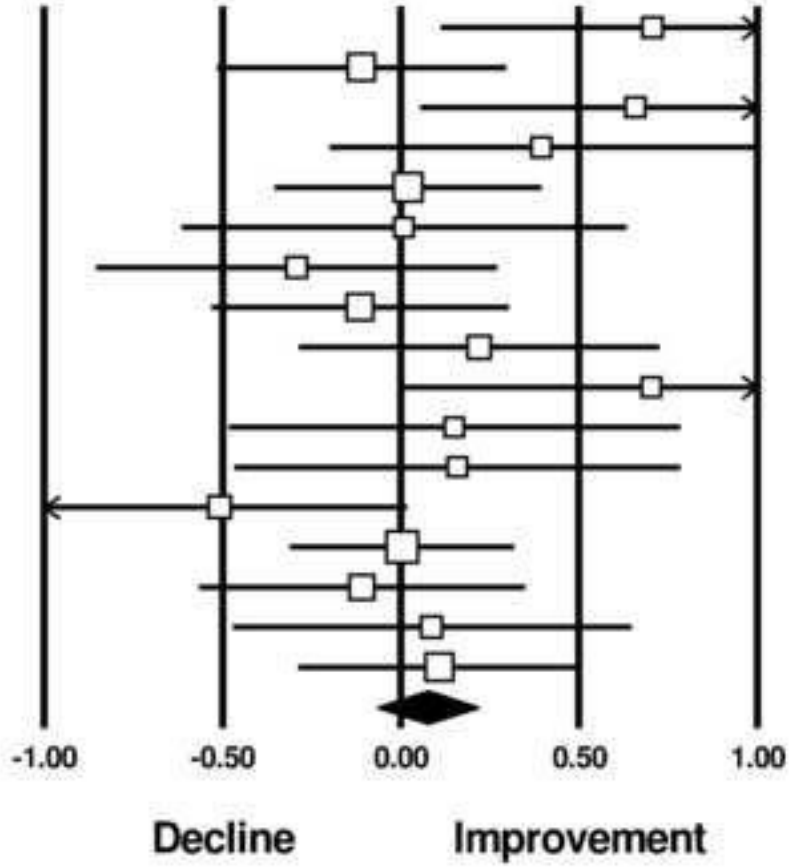
T2D = type 2 diabetes; CVD = cardiovascular disease; CKD = chronic kidney disease; D_2 = ergocalciferol; D_3 = cholecalciferol; 25-OHD = 25 hydroxy vitamin D.

Table 3: Meta-regression analysis to evaluate the association of potential modifiers of the effects of vitamin D supplementation on endothelial function				
Covariates	**Slope**	**SE**	**Q (df=1)**	**P Value**
Baseline Systolic BP (mmHg)	0.002	0.003	0.60	0.43
Baseline Diastolic BP (mmHg)	0.02	0.01	3.1	0.07
Serum 25(OH)D at baseline (nmol/L)	-0.003	0.002	2.47	0.11
Change in serum 25-OHD after supplementation (nmol/L)	-0.001	0.001	0.77	0.37
Study Duration (weeks)	0.001	0.003	0.16	0.68
Vitamin D Dose (IU)	-0.0001	0.00001	0.12	0.71
Age (years)	-0.003	0.004	0.95	0.32
BMI (kg/m^2)	0.05	0.02	3.50	0.06
Study Sample Size (N)	-0.001	0.001	1.43	0.23
Jadad Score	-0.02	0.05	0.28	0.59

BP = blood pressure; BMI = body mass index; N = number of study participants; 25-OHD = 25 hydroxy vitamin D.

Figure 1

Study name	Health Status	Std diff in means	Lower limit	Upper limit	Relative weight
Breslawsky 2013	T2D	0.71	0.12	1.30	4.50
Gepner 2012	Healthy	-0.11	-0.51	0.29	7.89
Harris 2011	Healthy	0.66	0.06	1.26	4.37
Hewitt 2013	CKD	0.39	-0.20	0.99	4.48
Larsen 2012	CVD	0.02	-0.35	0.39	8.76
Longenecker 2012	HIV	0.01	-0.61	0.63	4.15
Marckmann 2012	CKD	-0.29	-0.85	0.27	4.90
Sokol 2012	CVD	-0.11	-0.53	0.30	7.61
Stricker 2012	CVD	0.22	-0.28	0.72	5.77
Sugden 2008	T2D	0.70	0.01	1.40	3.45
Witham 2010a	T2D	0.15	-0.48	0.78	4.04
Witham 2010b	T2D	0.16	-0.46	0.78	4.13
Witham 2012	CVD	-0.51	-1.03	0.01	5.43
Witham 2013a	CVD	0.00	-0.31	0.31	10.73
Witham 2013b	CVD	-0.11	-0.56	0.34	6.71
Witham 2013c	Healthy	0.09	-0.47	0.64	4.94
Yiu 2012	T2D	0.11	-0.28	0.50	8.15
		0.08	-0.06	0.22	



Endothelial Function