

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

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36

37 Abstract

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

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

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

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

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62 1. Introduction

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

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

75 Vitamin D is a pro-hormone which is mostly known for its involvement in the regulation of
76 calcium homeostasis and bone remodelling [5]. However, vitamin D is also essential for
77 several non-musculoskeletal functions including regulation of vascular tone, gluco-insular
78 homeostasis and immunity [5]. Vitamin D receptors (VDRs) are expressed in several tissues
79 notably endothelial cells, vascular smooth muscle cells and cardiomyocytes [6]. The active
80 form of vitamin D ($1\alpha,25$ -dihydroxyvitamin D₃, $1,25(\text{OH})_2\text{D}_3$) is a direct transcriptional
81 regulator of endothelial NO synthase [7]. A recent study has shown that VDR mutant mice
82 have lower NO bioavailability leading to endothelial dysfunction, increased arterial stiffness,
83 increased aortic impedance, structural re-modelling of the aorta, and impaired systolic and
84 diastolic heart function [8]. However, observational studies evaluating the association of
85 vitamin D with CVD risk have reported mixed results. A significant inverse relationship
86 between low vitamin D status, as assessed by serum 25-hydroxy vitamin D (25-OHD) and

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

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

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

112 the effect size was modified by health status, study duration, dose, route of vitamin D
113 administration, baseline vitamin D status and changes in 25-OHD after supplementation,
114 body mass index (BMI), age and type of vitamin D (vitamin D₂ or vitamin D₃).

115 **2. Methods**

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

118 *2.1 Literature search*

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

129 *2.2 Study selection*

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

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

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

147 *2.3 Data extraction and quality assessment*

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

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

157 *2.4 Statistical analysis*

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

162 allow comparison of effect sizes between studies, standardised mean differences (SMDs)
163 were used as a summary statistic. SMD is estimated from the difference between the mean
164 outcome values of the intervention and control groups divided by the pooled standard
165 deviation (SD) of the outcome values; this converts the estimated effect to SD units. SMD of
166 0.2, 0.5 and 0.8 represent small, medium and large effect sizes, respectively[37]. In addition,
167 different methods were frequently used in the same trial to assess EF, as shown in **Table 1**,
168 and therefore this lack of independence of the EF measurement in each trial was taken into
169 consideration in the derivation of the pooled effect size. Statistical analyses were performed
170 by using Comprehensive meta-analysis software (version 2, Biostat, Englewood, New Jersey,
171 USA). Data synthesis, including calculation of effect sizes with 95% confidence intervals,
172 was accomplished by employing a random-effects model using inverse variance weighting.
173 Forest plots were generated for graphical presentation of the effect of supplemental vitamin D
174 on EF. For this purpose, the mean and SD of the EF measure before and after the intervention
175 period (for both vitamin D intervention and control) were extracted and used in the analysis.
176 For studies that reported changes in EF at two or more time-points (e.g. acute and chronic
177 effects of vitamin D supplementation), the last EF measurement was used in the meta-
178 analysis. Data not provided in the main text or tables were extracted from the figures.
179 Subgroup analyses were undertaken to investigate the variables which may have influenced
180 the effects of supplementation on EF. These factors included: health status, type (vitamin D₂
181 or D₃) and the frequency of administration (single dose, daily-weekly or monthly) of vitamin
182 D supplementation. Random effect meta-regression analyses were used to determine whether
183 participant baseline characteristics (age, BMI, systolic and diastolic blood pressure, baseline
184 concentration of 25-OHD) influence the effect of vitamin D supplementation (vitamin D₂ or
185 D₃) on EF. Furthermore, meta-regression analyses were conducted to investigate the
186 influence of other factors including vitamin D dose, baseline 25-OHD, change in 25-OHD

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

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

195 **3. Results**

196 *3.1 Search results*

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

205 *3.2 Studies characteristics*

206 The total number of participants from the 16 studies included in this systematic review was
207 1177 (607 females; 570 males) with median of 73 (range 34 -159) participants per study. The
208 median age was 63.2 (range 30-77) years. All RCTs included in the meta-analysis were
209 parallel, double-blind, placebo-controlled trials. The duration of the trials ranged from 4
210 weeks to 52 weeks (**Table 1**).

211 Three studies investigated the effect of vitamin D in healthy participants [16, 18, 40], two
212 studies were conducted in patients with chronic kidney disease (CKD) [19, 22], four studies
213 in diabetics [15, 17, 25, 30], six studies in patients with CVDs [20, 23, 24, 26, 28, 29] and
214 one study in patients with HIV [21]. All trials supplemented vitamin D orally. Trials however
215 utilised different forms of supplementation including tablets [20, 23, 30], solution [17, 19, 24-
216 26, 28, 40], capsules [15, 16, 21, 22] and fortified biscuits [18]. The majority of the trials
217 utilised vitamin D₃ with daily doses varying from 1000IU/day [15] to 5000IU/day [30].
218 Several methods were used to assess EF in the included trials. The most commonly used
219 methods were FMD [16-19, 21, 22, 25, 29, 30], PWV [18-20, 22, 29, 30, 40] and
220 augmentation index (AIx) [15, 18, 20, 24]. Other methods include laser Doppler flowmetry
221 [40] and digital volume pulse [28] (**Table 1**).

222 3.3 Qualitative analysis

223 Three of the studies included in the present systematic review reported a significant
224 improvement in EF in response to vitamin D administration [15, 17, 41] whereas the other 13
225 studies reported no effect of supplementation [18-30]. Ten studies described the methods of
226 randomisation [18-23, 25, 27, 28, 30] and five studies stated the methods of allocation
227 concealment [20, 21, 25, 27, 28]. The drug history of the participants was reported by all
228 except three studies [15, 16, 27]. With the exception of two studies [16, 19], all other studies
229 reported, and described, participant dropout. The quality of the included studies ranged from
230 3 to 5 (Jadad score) and eleven studies had a low risk of bias (Jadad score ≥ 4) (**Table 1**).

231 3.4 Meta-analysis

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

236 **Supplementary Material**). Heterogeneity between studies was not significant ($Q=21.7$,
237 $I^2=26.4\%$, $P=0.15$). Subgroup analysis showed that vitamin D supplementation improved EF
238 significantly in participants with type 2 diabetes ($N=5$, SMD: 0.31, 95%CI: -0.05, 0.57,
239 $P=0.02$) (**Table 2**). This was confirmed by the significant effect of vitamin D
240 supplementation in type 2 diabetic on changes in FMD% ($N=4$, +0.81%, 95%CI: 0.005, 1.61,
241 $P=0.04$, **Table S1, Online Supplementary Material**). The response of EF to vitamin D
242 supplementation was not significantly modified by type of vitamin D, method of
243 administration, baseline 25-OHD concentrations or baseline health status of the participants
244 (**Table 2**). Meta-regression analyses demonstrated a weak, positive effect of BMI (β : 0.05,
245 SE: 0.02, $P=0.06$) and of baseline diastolic blood pressure (β : 0.02, SE: 0.01, $P=0.07$) in
246 modifying the effect of vitamin D supplementation on EF (**Table 3**). BMI did not modify the
247 association between type 2 diabetes and EF ($N=6$, β : 0.04, SE: 0.04, $P=0.23$) whereas lower
248 baseline 25-OHD concentrations were associated with a greater effect size in type 2 diabetic
249 participants ($N=6$, β : -0.02, SE: 0.01, $P=0.03$) (**Figure S3, Online Supplementary**
250 **Material**). The dose of vitamin D was not associated with significant changes in EF (**Table 3**
251 **and Figure S4, Online Supplementary Material**)

252 3.5 Publication bias

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

256 4. Discussion

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

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

263 Several putative mechanisms could explain the positive effects of vitamin D on EF in some
264 population groups, particularly in those at higher cardiovascular risk. Vitamin D is involved
265 in the regulation of endothelial cell-dependent vasodilation which may be mediated by the
266 effect of vitamin D metabolites on the renin-angiotensin-aldosterone system, a hormonal
267 system that regulates blood pressure and fluid balance. A low plasma 25OHD predisposes to
268 up-regulation of the **renin-angiotensin system**, smooth muscle proliferation and favours a pro-
269 inflammatory state which can increase the risk of hypertension and left ventricle hypertrophy
270 [42]. The improvement in EF through vitamin D supplementation could also be mediated by
271 the local effects of vitamin D metabolites on calcium metabolism in vascular smooth muscle
272 cells and on the release of inflammatory cytokines which may affect vascular contractility
273 [43]. Vascular smooth muscle and endothelial cells express VDR as well as 1α -hydroxylase
274 [44], allowing for autocrine production of $1,25(\text{OH})_2\text{D}$, which may act at the local level to
275 modulate the effects of inflammatory cytokines on the vasculature, such as decreasing
276 endothelial adhesion molecules, increasing NO production [45] and reducing platelet
277 aggregation [46]. The activation of VDRs induces the transcription of a wide range of genes
278 including those coding for **vascular endothelial growth factor** which in turn promotes NO
279 synthesis by endothelial cells. In addition, $1,25(\text{OH})_2\text{D}_3$ is a direct regulator of endothelial
280 NO synthase [8].

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

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

292 In the present meta-analysis, we observed that vitamin D supplementation produced a
293 significant improvement in endothelial function in individuals with type 2 diabetes. While the
294 small number of trials included in the analyses (N=4) call for a cautious and objective
295 interpretation of the results, we believe that they are supported by a robust mechanistic
296 rationale and provide important insights for future studies. This apparent diabetes-specific
297 effect may be explained by several mechanisms including the link between low 25OHD
298 concentrations and i) deterioration of β -cell function, ii) dysregulation of peripheral insulin
299 signalling and iii) altered glucose disposal which are typically involved in the pathogenesis of
300 type 2 diabetes [11, 14, 58]. These effects appear to be supported by the greater effect of
301 vitamin D supplementation on EF in type 2 diabetic patients with insufficient vitamin D
302 status. Vitamin D receptors and 1- α -hydroxylase are expressed in pancreatic β -cells and
303 therefore an involvement in the regulation of insulin secretion may be expected [51]. In turn,
304 1,25(OH)₂D activates transcription of the human insulin receptor gene, stimulates expression
305 of the insulin receptor [59], and enhances insulin-mediated glucose transport in vitro[60]. In
306 addition, insulin secretion is a calcium-dependent process and vitamin D metabolites have
307 been linked to the regulation β -cell calcium pools, which promotes insulin release [61]. The
308 putative beneficial effects of vitamin D metabolites on EF may also be explained by the
309 mechanistic inter-connection between the insulin and NO pathways. The activation of the
310 insulin receptor on the endothelial cells instead induces a vasodilatory response via the

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

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

324 Results may have been affected by the choice of the method used to measure vitamin D
325 concentrations. Unlike chromatographic methods, immunoassays do not measure vitamin D3
326 and vitamin D2 independently and this is a well-recognised limitation of immunoassays. The
327 importance of being able to quantify both metabolites of vitamin D independently is
328 becoming increasingly important in recent years with the evidence that vitamin D3 is more
329 biologically active than vitamin D2 [67] as well as emerging evidence that 25(OH)D2
330 concentrations are in the range of 1.5 to 10.0 nmol/l in several RCT and population based
331 studies, this contributing significantly to total 25(OH)D [68]. It is also important to point out
332 that results of 25(OH)D using chromatographic methods show significant variation, mainly
333 due to extraction and calibration problems associated with these methods. Such assay
334 variation reinforces the need for all users of vitamin D assays to have appropriate QC and
335 standardization protocols in place.

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

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

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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	0.23
• HIV	1	0.009	-0.61 0.62	0.97	
• Diabetes	5	0.31	0.05 0.57	0.02	
• 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	0.71
• Daily-Weekly	7	0.02	-0.17 0.21	0.82	
• Single dose	6	0.09	-0.20 0.40	0.53	
Baseline 25-OHD concentration					
Normal (≥ 50 nmol/L)	4	-0.01	-0.21 0.17	0.84	0.27
Deficient (< 50 nmol/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	

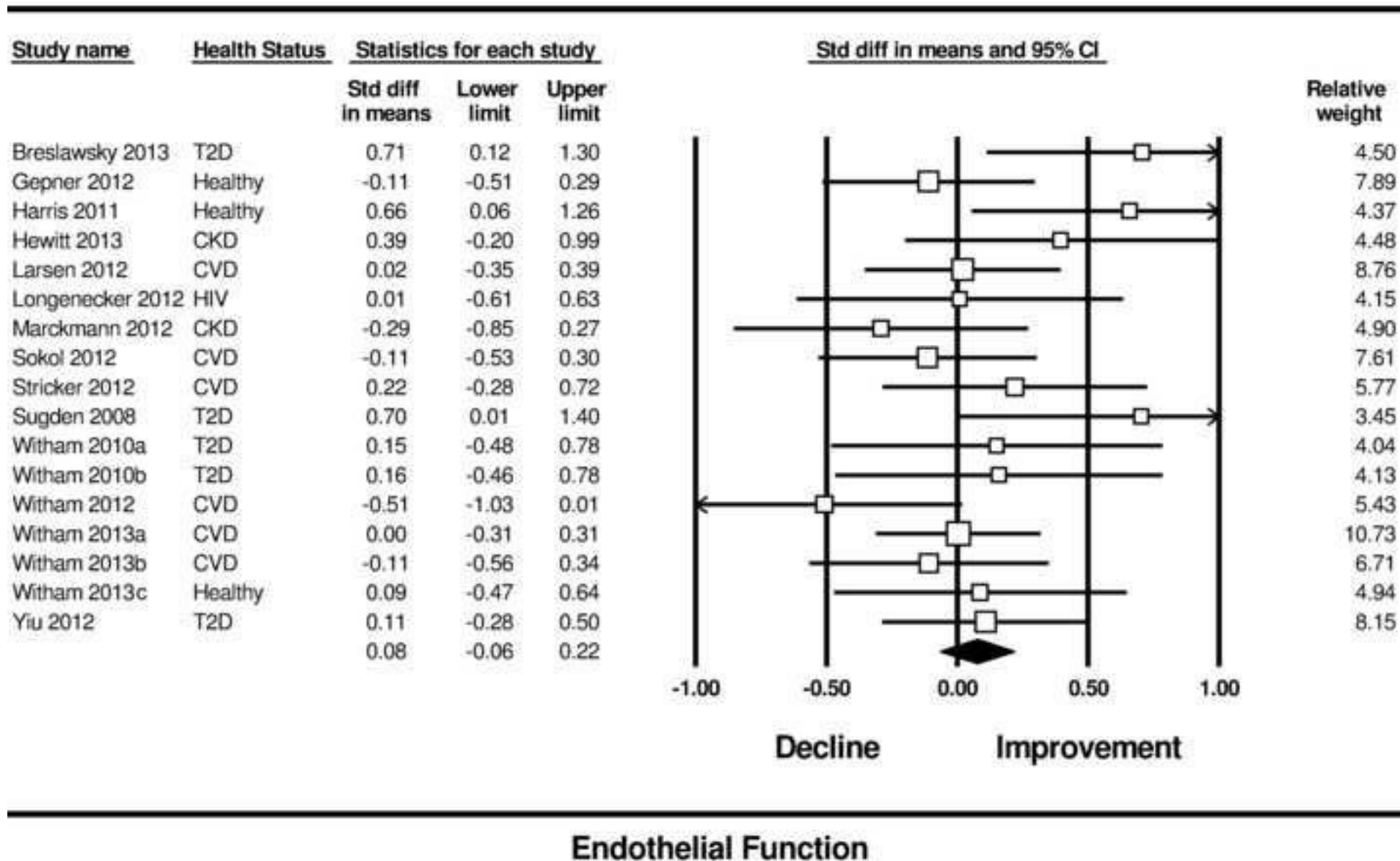
T2D = type 2 diabetes; CVD = cardiovascular disease; CKD = chronic kidney disease; D₂ = ergocalciferol; D₃ = cholecalciferol; 25-OHD = 25 hydroxy vitamin D.

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





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