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## REVIEW ARTICLE

# Effect of vitamin D supplementation on measures of arterial stiffness: a systematic review and meta-analysis of randomized controlled trials

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## Summary

**Background** Low vitamin D has been associated with poor arterial compliance in observational studies. Arterial stiffness has prognostic value for cardiovascular disease risk. The aim of this systematic review was to clarify the literature surrounding the use of vitamin D to ameliorate arterial stiffness.

**Methods** We conducted a systematic review of the MEDLINE, Scopus and EMBASE databases for randomized controlled clinical trials investigating the effect of vitamin D supplementation on pulse wave velocity (PWV) and/or augmentation index (AI) as indicators of arterial stiffness. We meta-analysed data and calculated standardized mean difference (SMD) and 95% confidence intervals (CI) using inverse-variance models on RevMan v5.3 software. Study quality was assessed using a modified Jadad scale.

**Results** A total of 607 unique records were identified, of which 18 satisfied our inclusion and exclusion criteria. Study quality was high, ranging from 9 to 12 (of 13). Study design in terms of vitamin D dosing protocol (range: 1000–5700 IU/day), follow-up times (range: 1–12 months), sample size (range:  $n = 29$ –183) and recruitment strategies varied markedly. Thirteen studies had data for meta-analysis. Vitamin D was associated with nonsignificant reductions in PWV [SMD =  $-0.10$ ; 95% CI:  $-0.24$ ,  $0.04$ ;  $P = 0.17$ ;  $n = 806$  from ten studies] and AI [ $-0.15$ ;  $-0.32$ ,  $0.02$ ;  $0.08$ ;  $n = 551$  from eight studies].

**Discussion** There is inconsistent evidence to suggest that vitamin D supplementation improves indicators of arterial stiffness. This may be attributable to the heterogeneity in study design. Therefore, large and well-designed randomized studies are

required to determine the casual relationships between vitamin D and arterial stiffness and cardiovascular risk.

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## Introduction

Vitamin D is a fat soluble steroid hormone with pleiotropic effects. Principally, vitamin D regulates calcium homeostasis and mineral metabolism by influencing intestinal absorption, bone resorption and renal retention. Vitamin D also has a number of nonskeletal effects that may favourably influence the cardiovascular system such as downregulation of the renin–angiotensin system,<sup>1</sup> enhancing insulin sensitivity<sup>2</sup> and modulating inflammation.<sup>3</sup> Previous clinical studies have indicated that low vitamin D (defined as serum calcifediol/25-hydroxyvitamin D (25OHD) concentration below 50 nmol/l [ $=20$  ng/ml] as according to The Endocrine Society guidelines<sup>4</sup>) can impair vascular function which may compromise vascular compliance (the elastic property of blood vessels) manifesting as increased arterial stiffness.<sup>5</sup>

Increased arterial stiffness is a marker for atherosclerotic diseases and is associated with a number of other important clinical outcomes including increased calcification,<sup>6</sup> decreased bone mineral density,<sup>7</sup> decreased muscle strength and increased falls risk<sup>8</sup> and reduced quality of life.<sup>9</sup> Pulse wave velocity (PWV) is a simple, robust and validated measure of arterial stiffness.<sup>10</sup> In brief, the arterial pressure wave form is a composite of the forward pressure wave created by ventricular contraction and a reflected wave from a distal site.<sup>10</sup> The gold standard measurement of arterial stiffness is the carotid–femoral PWV. This is estimated using the foot-to-foot velocity method whereby transcutaneously, the right common carotid artery and the right femoral artery and the time delay (or transit time,  $\Delta t$ ) are measured (in seconds, s) between the feet of the two waveforms (Fig. 1). A

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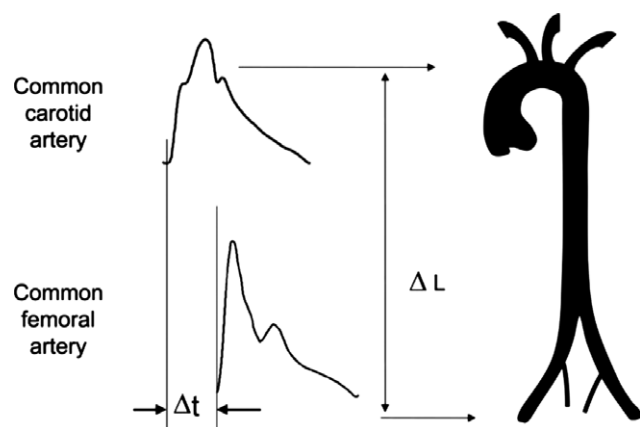


Fig. 1 Carotid–femoral PWV measurement using the foot-to-foot method.<sup>11</sup>

variety of different waveforms can be used including Doppler, pressure and distension. The distance ( $L$ ) covered by the waves between these two sites is measured (in metres, m), and PWV is then calculated as  $\frac{1}{4} L/\Delta t$  with the unit m/s.<sup>11</sup> The less compliant the arteries, the faster the reflected wave returns augmenting systolic pressure interpreted as an increased PWV. The extent of this augmentation in systolic pressure is called the augmentation index (AI).

Observational studies have suggested that vitamin D deficiency or insufficiency is associated with a poorer vascular profile including increased stiffness.<sup>12</sup> No causal relationship has been established. Therefore, naturally, this has stimulated research into improving vitamin D status to determine the impact of vitamin D supplementation on these end-points (PWV and AI). This review will critically analyse randomized controlled studies that utilized vitamin D supplementation and assessed the effect of this intervention on the outcome of PWV and/or AI. We aimed to clarify what effect vitamin D supplementation has on these end-points by way of systematic review and where appropriate data were available, meta-analysis.

## Methods

### Study focus and eligibility criteria

We conducted a systematic review and meta-analysis according to the guidelines outlined in the PRISMA statement (Preferred Reporting Items for Systematic Reviews and Meta-Analyses).<sup>13</sup> We sought original randomized controlled clinical trials that assessed the effects of vitamin D supplementation versus control on the outcome of PWV and/or AI in samples of patients with any pathology. Studies were eligible if the trial was randomized in design, compared vitamin D supplementation to a placebo or control drug and reported descriptive statistics for PWV and/or AI before and following the intervention. Specific exclusion criteria were as follows: studies that were not randomized in design, studies that did not use vitamin D as the treatment arm and studies that did not report outcome of PWV or AI following

vitamin D treatment (i.e. reported some other measure of arterial stiffness) and animal- or cell-based studies.

### Literature search

Relevant studies were retrieved from the MEDLINE (archives from 1966 to 2014), Scopus (1996–2014) and EMBASE (1947–2014) databases by applying a search strategy which broadly followed this protocol: ‘vitamin d’ [Title/Abstract] AND [‘pulse wave velocity’ (Title/Abstract) OR ‘augmentation index’ (Title/Abstract)] with no language restriction on the 16 of May 2015. A detailed search protocol is provided in supplementary materials. Titles and abstracts of identified records were screened. Additionally, we manually scanned the reference lists of eligible texts and the related articles lists that were generated, following a database search for other potential studies of interest. We termed these texts the ‘grey literature’. Following title and abstract screening, the full-text manuscripts were evaluated to determine eligibility. For conference abstracts and other eligible studies otherwise unavailable online as a full-text manuscript, attempts were made to obtain the full-text manuscripts direct from authors and further, and data were also sought direct from authors in order to ensure study eligibility and to complete the data set for comparison and review.

### Data capture and presentation

Data were extracted by a single reviewer (AJR) with the aid of an extraction template. Specifically, we sought information relating to study design, sample demographics, vitamin D regimen, outcome definition and assessment, statistical analyses employed and limitations highlighted by the authors. These data were then tabulated into a format that allowed comparison between trials of the pertinent aspects of the study, namely study design, patient demographics, and effect of vitamin D on study end-points.

### Quality assessment

In addition to data extraction, we performed a quality assessment of included studies. As no standardized quality assessment tool exists for randomized trials of vitamin D on the outcome of PWV or AI, we modified the previously validated Jadad scale to suit our aims.<sup>14</sup> Using a semiquantitative method, each included study was judged in the following areas: study design: sample size and representativeness, outcome definition and assessment, comparability of results, and statistical methods. The maximum possible score that could be achieved was 13 and a score of less than 9 was considered low quality and excluded from analysis.<sup>14</sup> A sample data extraction form and quality assessment tool is provided in Supplementary Materials.

### Statistical analysis

**Meta-analysis eligibility.** Studies were eligible for meta-analysis if they first satisfied the inclusion and exclusion criteria for

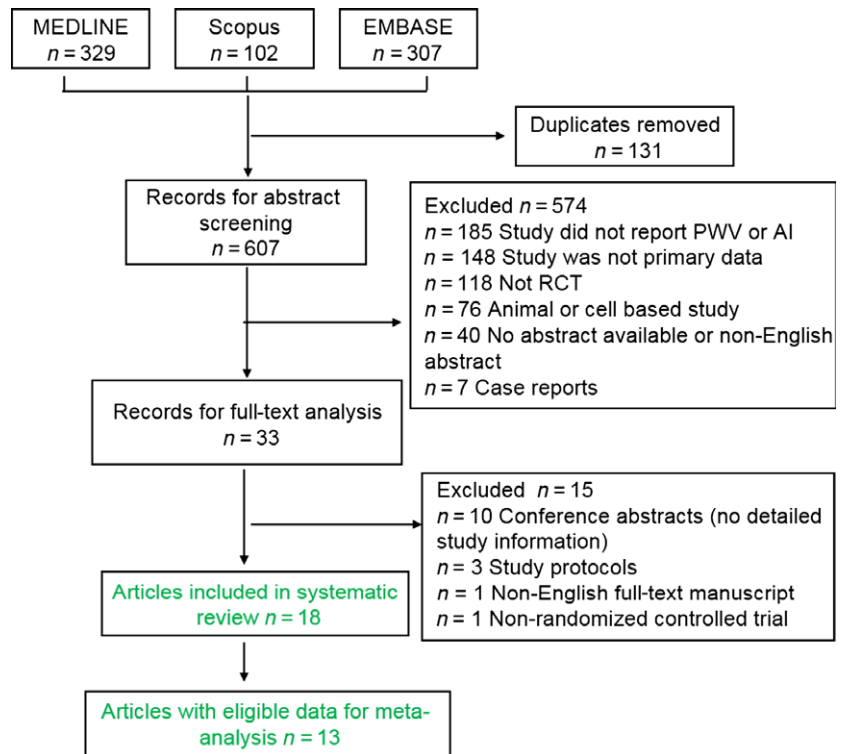


Fig. 2 Study selection flow diagram.

systematic review and reported mean baseline and follow-up data for PWV and/or AI in groups of participants receiving vitamin D (experimental) and placebo (control). Studies were excluded from meta-analysis in instances where the control group did not receive a placebo but instead a lower dose vitamin D.<sup>15,16</sup> For studies that did not specifically report data that we were looking to synthesize into the meta-analysis model [e.g. studies that reported change from baseline to follow-up rather than means at baseline and follow-up,  $n = 2^{17,18}$ ], contact was made with authors requesting these specific data in an attempt to include as much literature as possible but replies were not all forthcoming.

**Meta-analysis.** Data were first tabulated into a format that allowed comparison between mean baseline and follow-up data for PWV and AI comparing groups of patients who received vitamin D relative to patients who received placebo. These data were then synthesized using an inverse-variance method to determine the standardized mean difference (SMD), and 95% confidence interval (95% CI) was calculated. Heterogeneity was determined by the inconsistency percentage ( $I^2$ ) statistic where a random effects model was applied in analyses where  $I^2$  was greater than 50%.<sup>19</sup> Sensitivity analyses restricting the operation to studies employing an equivalent daily dose vitamin D of  $\geq 3000$  IU (arbitrarily defined), studies with a follow-up of  $\leq 3$  months, studies with a follow-up of  $\geq 3$  months (arbitrarily defined), studies involving chronic kidney disease patient, studies involving patients recruited due to hypertension, studies involving patients with insulin resistance (type 2 diabetes [T2D] and polycystic ovarian syndrome [PCOS]), studies with a mean sample age  $< 55$  and studies with a mean sample age  $\geq 55$ . All

statistical operations were performed using RevMan v5.3 software (The Nordic Cochrane Centre, The Cochrane Collaboration 2012).

## Results

### Literature search

The initial database search yielded 783 eligible records, of which 131 were duplicates (that is, appearing in more than one database) leaving 607 unique records for abstract screening. Following screening, a further 574 records were excluded primarily because the study did not assess an outcome of interest, namely PWV or AI (number of records excluded on this basis,  $n = 185$ ). This left 33 records for full-text review and of these 15 records were excluded mainly because the record was a conference abstract and not a full manuscript ( $n = 10$  records) leaving an overall 18 full-text manuscripts included as part of the systematic review and of these, 13 records were included in the meta-analysis as they had eligible data to do so<sup>20–32</sup> (Fig. 2). One author of a conference proceeding provided baseline data but no follow-up data meaning these results could not contribute to our analyses [personal communication, can be provided on request]. Other authors who were contacted directly for their abstract proceedings did not respond to our communications.

### Included Studies

All 18 studies were randomized controlled trials, of which 17 were double-blinded and one other was open label.<sup>15</sup> All studies were placebo-controlled except for two studies,<sup>33,16</sup> which

compared the effects of a high versus low-dose vitamin D supplementation as the trial arms; therefore, 15 studies were eligible for meta-analysis. Study population size varied from a minimum  $n = 29$  participants<sup>22</sup> to maximum  $n = 183$  participants.<sup>28</sup> Recruitment based on deficient or insufficient vitamin D status (10–60 ng/ml) was part of trial inclusion criteria in thirteen studies.<sup>16–18,22,24–26,28–33</sup> Study samples were heterogeneous; four studies were conducted in patients with chronic kidney disease (CKD),<sup>22,24,25,27</sup> four studies were conducted in patients with hypertension,<sup>21,26,28,31</sup> three studies were conducted in patients with T2D,<sup>18,20,32</sup> two studies were conducted in postmenopausal women,<sup>17,33</sup> and other studies were conducted in populations of black youths,<sup>15</sup> older community-dwelling individuals,<sup>16</sup> patients with PCOS,<sup>23</sup> patients with chronic fatigue syndrome (CFS),<sup>30</sup> and peripheral artery disease (PAD).<sup>29</sup> As expected, the inclusion and exclusion criteria for these studies varied considerably and this is summarized in Table 1. All studies reported using vitamin D3 (cholecalciferol) as the vitamin D supplement. Dosing regimens varied considerably. As aforementioned, we defined an equivalent daily dose which ranged from a minimum 1000 IU/day<sup>20</sup> to a maximum 5700 IU/day.<sup>25</sup> One study compared two different single high-dose vitamin D supplementation regimens.<sup>16</sup> Follow-up times varied between a minimum of 2 months<sup>16,25</sup> and maximum of 12 months.<sup>20,31</sup> Six studies had changes in PWV as a study end-point,<sup>15,22,24,28,30,32</sup> four studies had AI as a study end-point,<sup>20,26,29,33</sup> and eight studies had both PWV and AI as study outcomes.<sup>16–18,21,23,25,27,30</sup> Of the studies that had PWV as an outcome, the majority of them assessed either carotid–femoral PWV<sup>16,17,23,27,28,30</sup> or carotid–radial PWV.<sup>15,20,26,29,31</sup> These data are summarized in Table S1.

### Literature quality

Using a modified Jadad scale of randomized controlled trial study quality, scores ranged between 9<sup>24,25,33</sup> and 12<sup>23</sup> of a possible maximum of 13 (Table S2), and thus, all were included in further analyses. All studies reported randomization, inclusion and exclusion criteria, outcome measures, intervention description, control groups and statistical methods. No study reported sample size justification by way of a power calculation or other method. Adverse events and other safety data were reported in only one study.<sup>23</sup>

### Patient demographics

Mean age (in years) ranged from  $16.5 \pm 1.4$ <sup>15</sup> to  $79.3 \pm 7.0$ <sup>16</sup> in patients receiving vitamin D (experimental) and from  $16.3 \pm 1.1$ <sup>15</sup> to  $80.5 \pm 6.6$ <sup>16</sup> in patients receiving control treatments (control). Three studies had samples entirely female.<sup>17,23,33</sup> In other samples, the proportion of males ranged from 19%<sup>25</sup> to 68%<sup>27</sup> in experimental patients and ranged from 20%<sup>25,30</sup> to 73.7%<sup>22</sup> in controls. Mean body mass index (BMI) in kg/m<sup>2</sup> ranged from  $24.0 \pm 4.5$ <sup>27</sup> to  $32.4 \pm 6.4$ <sup>33</sup> in experimental patients and ranged from  $23.8 \pm 4.4$ <sup>27</sup> to  $33.3 \pm 7.3$ <sup>33</sup> in control patients. These data and other important patient characteristics are summarized in Table 2.

### Baseline and follow-up vitamin D

Thirteen studies recruited participants specifically with vitamin D inadequacy (deficiency or insufficiency) defined according to their study protocol (Table S3).<sup>16–18,22,24–26,28–33</sup> Twelve studies measured serum 25-OH vitamin D.<sup>16–18,20,23,24,26,28–31,33</sup> Four studies measured plasma 25-OH vitamin D,<sup>15,21,25,27</sup> and two studies did not specify this information.<sup>22,32</sup> Eleven studies provided follow-up circulating vitamin D concentrations in case and control groups to enable comparison.<sup>16,18,20,23,25–28,30,32,33</sup> For studies that reported group differences in 25-OH vitamin D at baseline (pretreatment), there were no significant differences in mean concentrations between experimental and controls groups. In studies that reported follow-up vitamin D concentrations only one study did not record differences between experimental and control groups.<sup>20</sup> Eight studies reported significantly higher 25-OH vitamin D in experimental groups compared to controls.<sup>16,18,23,25,27,28,30,32</sup> Further, only two studies reported not achieving mean vitamin D adequacy (>20 ng/ml) in the experimental groups following intervention.<sup>16,20</sup> All these data are reported in Table S3.

### Pulse wave velocity

In all studies at baseline, there were no significant differences in PWV between experimental and control groups (Table 3). Specifically, in experimental patients, PWV velocity (m/s) ranged from  $5.41 \pm 0.73$  (measured as carotid–femoral)<sup>15</sup> to  $18.97 \pm 3.38$  (carotid–brachial),<sup>32</sup> and in control patients, it ranged from  $5.38 \pm 0.53$  (measured as carotid–femoral)<sup>15</sup> to  $18.82 \pm 4.14$  (carotid–brachial).<sup>32</sup> At follow-up, only one study<sup>15</sup> reported a significant change in PWV where mean experimental PWV declined 0.08 m/s compared to the control group where mean PWV increased 0.37 m/s. Overall, there was significant heterogeneity in response to vitamin D supplementation where five studies<sup>15,16,22,28,30</sup> reported reductions in PWV and seven reported increases in PWV<sup>17,18,21,23,25,27,32</sup> in patients receiving vitamin D treatment (Fig. 3). One study did not provide sufficient data to enable comparison between baseline and follow-up PWV measurements.<sup>24</sup>

### Augmentation index

At baseline, all studies reported no significant differences in AI (%) between experimental and control groups except for Whitham *et al.*<sup>30</sup> where control group patients had considerably higher AI than experimental patients [27% vs 16%, respectively,  $P = 0.001$ ] (Table 4). In experimental groups, AI (%) ranged from  $8.5 \pm 1.1$ <sup>22</sup> to  $78.8 \pm 13$ <sup>18</sup> and in control groups, the range was from  $8.5 \pm 1.5$ <sup>22</sup> to  $80.5 \pm 11.4$ .<sup>18</sup> At follow-up, three studies reported significant differences in AI between experimental and control groups.<sup>16,20,27</sup> AI was significantly reduced in<sup>16,20</sup> but was increased in<sup>27</sup> (Table 2). Overall, there was significant heterogeneity in response to vitamin D supplementation where four studies reported reductions in AI in patients receiving vitamin D compared to control<sup>16,20–22</sup> and

Table 1. Study details

Study	Design	Cohort	Vitamin D deficient/insufficient at recruitment	Total population (n)	Follow-up (months)	Dose	Daily dose (equivalent IU/day)	Outcome	Inclusion/Exclusion criteria
Breslavsky	RCT, DB, PC	T2D	No	47	12	1000 IU/day	1000	AI	E: Unstable angina, MI, CVA, major surgery, hyperthyroidism, creatinine > 2.5 mg/dl, elevated liver enzymes
Dong	RCT, OL	African American youths	No	49	4	2000 IU v 400 IU	2000	PWV, femoral, radial, distal	I: Normotensive, 14–18 years old, currently not taking medications, can swallow, able to provide blood, not pregnant, not on vitamin D supplements
Dreyer	RCT, DB, PC	CKD 3–4	Yes	29	6	50 000 IU/4 week, 50,000 IU/5 month	1666	PWV	E: Currently on calcium therapy > 10.4 mg/dl, pregnant or lactating, hypercalcaemia, microcirculatory dysfunction
Garg	RCT, DB, PC	PCOS	No	32	6	1 × 120 000 IU/month	4000	PWV, AI	E: Currently on vitamin D supplements, active disease, currently taking medications known to interact with vitamin D, pregnant
Gepner 2012	RCT, DB, PC	Postmenopausal women	Yes	110	4	2500 IU/daily	2500	PWV, AI	I: vitamin D between 10 and 60 ng/mL, otherwise healthy, community-dwelling postmenopausal women. E: History of CVD, calcium > 10.5 mg/dl, hyperparathyroidism, malignancy, tuberculosis, nephrolithiasis, sarcoidosis, Paget's disease, eGFR < 25 ml/min, medications known to interact with vitamin D
Gepner 2015	RCT, DB	Postmenopausal native American women	Yes	98	6	2500 IU v 400 IU daily	2500	AI	I: vitamin D between 10 and 60 ng/ml, no CVD
Hewitt	RCT, DB, PC	HD, CKD5	Yes	60	6	8 × 50 000 IU/weekly, 4 × 50 000 IU/monthly	3611	PWV	I: vitamin D between 10 and 60 ng/mL; Parathyroid surgery, cinacalcet treatment, hypercalcaemia, bisphosphonate therapy, major surgery
Larsen	RCT, DB, PC	Hypertension	No	112	5	3 × 25 µg daily	3000	PWV, AI	E: SBP > 150 mmHg and/or DBP > 95 mmHg, pregnancy/lactating, alcohol abuse, hypercalcaemia, atrial fibrillation, NSAID use, glucocorticoids, current vitamin D intake > 10 µg, tanning bed use, changes in antihypertensive medications during trial
Marckmann	RCT, DB, PC	CKD	Yes	52	2	40 000 IU/weekly	5700	PWV, AI	I: vitamin D < 50 nmol/L; current vitamin D intake, hypercalcaemia, hyperphosphataemia, sarcoidosis, malignancy, psychosis, alcohol/drug abuse, pregnancy/lactating, poor language skills, soy allergy, oestrogen use, contraceptive use

(continued)



Table 1. (continued)

Study	Design	Cohort	Vitamin D deficient/insufficient at recruitment	Total population (n)	Follow-up (months)	Dose	Daily dose (equivalent IU/day)	Outcome	Inclusion/Exclusion criteria
Martins	RCT, DB, PC	Hypertensive African Americans	Yes	115	3	100 000 IU/monthly	3333	AI	I: serum levels of 25(OH)D 10 and 25 ng/mL: Poorly controlled BP, CKD, hypercalcaemia, abnormal liver function tests, MI, stroke, congestive heart failure, kidney stones, allergy to oral vitamin D, current immunosuppressive therapy, current steroid therapy and current NSAID
McGreevy	RCT, DB	Older community-dwelling individuals (>65 years)	Yes	102	2	100 000 IU vs 50 000 IU single	1666	PWV, AI	I: serum 25OHD <50 nmol/L: Currently taking vitamin D supplements, hypercalcaemia, hyperparathyroidism, current malignancy, change in medications during trial
Mose	RCT, DB, PC	HD	No	50	6	75 ug/daily	3000	PWV, AI	E: Malignancy, hypercalcaemia, allergy to vitamin D, inability to give consent (<18 years)
Pilz	RCT, DB, PC	Hypertension	Yes	183	2	2800 IU as oil drops daily	2800	PWV	I: 25(OH)D serum concentration below 30 ng/mL: Hypercalcaemia, pregnancy/lactating, taking drugs from other studies, acute coronary disease, CVD, eGFR<15 mL/min/1.73 m <sup>2</sup> , SBP between 120 and 160 mmHg, DBP >100 mmHg, taking hypertensive drugs, life expectancy <10 years, receiving chemotherapy or radiotherapy, regular vitamin D supplement intake
Ryu	RCT, DB, PC	T2D	Yes	81	6	2000 IU/day (+100 mg/day Ca)	2000	PWV, AI	I: I: 25(OH)D <20 ng/mL: Osteoporosis drugs, insulin use, SB >160 mmHg or DBP >100 mmHg, recent MI, abnormal liver enzymes, alcohol abuse
Stricker	RCT, DB, PC	PAD	Yes	62	1	1 × 100 000 IU	3333	AI	I: serum 25-hydroxyvitamin D level <30 ng/mL: Acute illness, critical ischaemia, thromboangiitis obliterans, renal insufficiency (Cr <130 µmol/L), recent MI, current oral anticoagulants, liver cirrhosis, malignancy
Whitham 2013	RCT, DB, PC	Older individuals (>70 years) with hypertension (>140 mmHg SBP)	Yes	159	12	4 × 100 000 IU monthly	3333	PWV	I: 25OHD level <30 ng/mL: DBP >90 mmHg, SBP>180 mmHg, eGFR <40 mL/min/1.73 m <sup>2</sup> , abnormal liver function tests, metastasis malignancy, sarcoidosis, renal calculi, heart failure, left ventricular dysfunction, atrial fibrillation, already on vitamin D supplements

(continued)

Table 1. (continued)

Study	Design	Cohort	Vitamin D deficient/insufficient at recruitment	Total population (n)	Follow-up (months)	Dose	Daily dose (equivalent IU/day)	Outcome	Inclusion/Exclusion criteria
Whitham 2015	RCT, DB, PC	CFS	Yes	50	6	3 × 100 000 IU (once every 2 months)	3333	PWV, AI	I: serum 25OHD level <75 nmol/L; Osteoporosis, sarcoidosis, renal stones, malignancy, current vitamin D intake, abnormal liver function tests, hypercalcaemia, eGFR < 40 mL/min/1.73 m <sup>2</sup> , no consent given, psychiatric disorders, substance abuse/dependence
Yiu	RCT, DB, PC	T2D	Yes	100	3	5000 IU/daily	5000	PWV	I: serum 25(OH)D concentration <30 ng/mL; HbA1c >11%, pregnancy, lactation, recent MI, angina, Cr >106 µmol/L, liver failure, cancer, uncontrolled hypertension, diabetes complications

E, Exclusion criteria; I, inclusion criteria; RCT, randomized controlled trial; DB, double blind; SB, single blind; CKD, chronic kidney disease; HD, haemodialysis; PC, placebo-controlled; PCS, prospective cohort study; CFS, chronic fatigue syndrome; SBP, systolic blood pressure; PAD, peripheral arterial disease; PCOS, polycystic ovarian syndrome; T2D, type 2 diabetes mellitus; AI, augmentation index; PWV, pulse wave velocity; MI, myocardial infarction.

eight studies reported increases in AI for patients receiving vitamin D<sup>17,18,23,25–27,29,30</sup> (Fig. 4).

### Meta-analysis

Thirteen studies had data that was able to be synthesized into a meta-analysis model.<sup>20–32</sup> The authors of studies that were eligible for meta-analysis but did not report appropriate data for synthesis were contacted directly but did not respond to our communications.<sup>17,18</sup> For the outcome of PWV, vitamin D supplementation produced a nonsignificant reduction in PWV relative to placebo (Table 5, Fig. 3). Similarly for AI, vitamin D supplementation produced a nonsignificant reduction in AI relative to placebo (Table 5, Fig. 4). Additionally, a number of smaller models were constructed to determine the influence of vitamin D dosing, follow-up, age and the sample in which the studies were conducted in. All subanalyses showed nonsignificant reductions in PWV or AI (Table S4), except for an analysis involving two studies of patients with T2D or PCOS (insulin-resistant syndromes) which showed vitamin D to be nonsignificantly related with an increase in PWV (Table S4).

### Discussion

This systematic review of 18 randomized controlled trials assessing the effect of vitamin D supplementation on PWV and AI, two measures of vascular compliance, found three studies that reported significant but conflicting differences in AI and only one study that reported a significant decrease in PWV following vitamin D supplementation. Studies that fulfilled our inclusion and exclusion criteria were largely of high quality but were heterogeneous in terms of study design, patient sample, follow-up times, vitamin D regimen and outcome. A meta-analysis demonstrated that vitamin D supplementation produced small, nonsignificant reductions in PWV and AI relative to placebo. In subanalyses, where we attempted to explore possible explanations for the variable response to vitamin D on these outcomes, we found no significant associations in terms of study follow-up time, study sample, sample age or vitamin D dosing. These results are in contrast to a large amount of observational clinical studies which have shown that low vitamin D (defined as <50 nmol/L) is associated with poor measures of arterial stiffness.<sup>34,35</sup> For example, in the NHANES longitudinal studies from 2001 to 2004, the risk of developing peripheral arterial disease, a condition largely characterized by arterial stiffness, was almost twice as high in subjects with vitamin D < 50 nmol/L compared to those with vitamin D > 75 nmol/L (odds ratio 1.82; 95% CI:1.26–2.61).<sup>5</sup>

Preclinical studies have also provided strong evidence that vitamin D is an important factor in maintaining good vascular compliance and a number of possible mechanisms by which vitamin D may influence vascular compliance have been proposed. In particular, *in vitro* studies have suggested that vitamin D deficiency is associated with increased vascular endothelial cell (EC) expression of nuclear factor κβ (NFκβ) and interleukin-6 (IL-6), two important inflammatory mediators.<sup>36</sup> Further, vitamin D may attenuate the adverse effects of advanced glycation



Table 2. Patient demographics and clinical characteristics

Study	n		Age (years)		Male sex [n(%)]		BMI (kg/m <sup>2</sup> )		P	
	Exp.	Cont.	Exp.	Cont.	P	Exp.	Cont.	P	Exp.	Cont.
Breslavsky	24	23	66.8 ± 9.2	65.8 ± 9.7	0.716	11 (45.8%)	11 (47.8)	0.562	27.9 ± 5.2	30.6 ± 5.1
Dong	23	21	16.5 ± 1.4	16.3 ± 1.1	0.95	10 (43%)	15 (71)	0.08	n/r	n/r
Dreyer	20	18	45.8 ± 10.0	48.8 ± 12.2	0.39	14 (60.9)	14 (73.7)	0.22	30.4 ± 7.1	29.2 ± 3.4
Garg	15	17	22.0 ± 4.61	22.8 ± 4.56	0.64	n/a	n/a	n/a	26.8 ± 4.56	26.7 ± 6.11
Gepner 2012	57	57	64.1 ± 3.0	63.6 ± 3.1	0.419	n/a	n/a	n/a	27.1 ± 4.7	25.3 ± 5.1
Gepner 2015	49	49	60.7 ± 7.7	61.8 ± 7.0	n/r	n/a	n/a	n/a	32.4 ± 6.4	33.3 ± 7.3
Hewitt	30	30	60 (53–71)	67 (54–72)	n/r	53	43	n/r	26.6 ± 6.4	31.3 ± 9.5
Larsen	55	57	60 ± 12	61 ± 9	0.78	17 (30)	18 (32)	0.94	27.7 ± 4.2	28.3 ± 3.7
Marckmann	26	26	71 (62–78)	68 (59–76)	n/r	19	20	n/r	25.9 (22.1–29.7)	24.6 (22.0–27.3)
Martins	60	55	n/r	80.5 ± 6.6	n/r	41 (63.1)	38 (58.5)	n/r	n/r	n/r
Mc Greevy	51	51	79.3 ± 7	80.5 ± 6.6	0.37	28	26	0.16	26.6 ± 6.4	26.9 ± 9.5
Mose	25	25	68 ± 9	67 ± 13	0.794	17 (68)	15 (60)	0.556	24 ± 4.5	23.8 ± 4.4
Pilz	100	100	60.5 ± 10.9	59.7 ± 11.4	0.607	54	52	0.777	30.4 ± 4.4	30.4 ± 6.2
Ryu	40	41	54.5 ± 7.4	56.7 ± 7.9	0.203	n/r	n/r	n/r	24.4 ± 5.0	25.3 ± 3.4
Stricker	31	31	72.9 ± 8.7	74.8 ± 14.6	0.5	19 (61)	19 (61)	0.94	n/r	n/r
Whitham 2013	80	79	76.9 ± 4.8	76.7 ± 4.5	n/r	40 (50)	42 (53)	n/r	28.5 ± 5	27.9 ± 4.5
Whitham 2015	25	25	48.1 ± 12	50.7 ± 13.1	0.47	7 (28)	5 (20)	0.51	28.8 ± 7.9	29.8 ± 5.4
Yiu	50	50	65.8 ± 7.3	64.9 ± 8.9	0.58	27 (54)	23 (46)	0.42	25.8 ± 4.3	25.1 ± 3.4

Study	T2D [n(%)]		Smoker [n(%)]		CVD [n(%)]		Hypertension [n(%)]		Dyslipidaemia [n(%)]	
	Exp.	Cont.	P	Exp.	Cont.	P	Exp.	Cont.	P	Exp.
Breslavsky	n/r	n/r	n/r	6 (25)	3 (13)	0.461	n/r	n/r	n/r	20 (83.3)
Dong	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	20 (87.0)
Dreyer	n/r	n/r	n/r	1 (5)	2 (11.1)	n/r	16 (80)	12 (66.7)	0.33	9 (45)
Garg	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r
Gepner 2012	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r
Gepner 2015	10 (20)	13 (27)	n/r	n/r	n/r	n/r	27 (55)	27 (55)	n/r	23 (47)
Hewitt	15	18	n/r	13	15	n/r	n/r	n/r	n/r	23 (47)
Larsen	4 (7)	5 (9)	0.77	4 (7)	5 (9)	0.77	47 (84)	48 (84)	0.85	17 (30)
Marckmann	8 (31)	10 (38)	n/r	n/r	n/r	n/r	23 (88)	18 (69)	n/r	17 (30)
Martins	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r
Mc Greevy	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r
Mose	2 (8)	5 (20)	0.384	1 (4)	5 (20)	0.22	17 (68)	17 (68)	0.914	n/r
Pilz	32	41	0.186	19	14	0.341	5	5	n/r	n/r
Ryu	100	100	n/a	17 (42.5)	15 (36.6)	0.85	n/r	n/r	n/r	n/r
Stricker	10 (32)	7 (23)	0.57	8n	14n	n/r	n/r	n/r	n/r	n/r
Whitham 2013	11 (14)	11 (14)	n/r	n/r	n/r	n/r	24 (77)	23 (74)	0.77	n/r
Whitham 2015	3 (12)	1 (4)	0.61	4 (16)	6 (24)	0.73	41 (51)	50 (63)	n/r	41 (51)
Yiu	100	100	n/a	15 (30)	13 (26)	0.66	3 (12)	5 (20)	0.71	2 (4)

n.b All values in mean ± SD or mean(SE) or median(interquartile range). exp, Experimental (vitamin D) group; cont., Control (placebo) group; n/a, not applicable; n/r, not reported.  
exp, Experimental (vitamin D) group; cont., Control (placebo) group; n/a, not applicable; n/r, not reported.

Table 3. Outcome of PWV from Vitamin D supplementation

	PWV site	Baseline measurement (m/s)			Follow-up measurement (m/s)		Mean difference (within group)		Mean difference (between group)	
		Experimental	Control	P	Experimental	Control	Experimental	Control	P	
Dong	Femoral	5.41 ± 0.73	5.38 ± 0.53	n/r	5.33 ± 0.79	5.71 ± .075	-0.08	0.37	-0.45	0.019
	Radial	7.83 ± 1.14	7.77 ± 1.64	n/r	7.81 ± 0.98	7.92 ± 0.89	-0.02	0.15	-0.17	0.93
	Distal	6.75 ± 0.64	6.87 ± 0.64	n/r	6.71 ± 0.63	7.22 ± 0.79	-0.04	0.25	-0.29	0.46
Dreyer		8.5 ± 1.1	8.5 ± 1.5	0.66	8.4 ± 1.3	8.5 ± 1.2	-0.1	0	-0.1	0.78
Garg		5.6 ± 1.3	6.5 ± 1.25	0.61	6.2 ± 1.32	6.3 ± 1.04	0.6	-0.2	0.8	0.16
Gepner 2012		7.8 ± 0.9	8.0 ± 1.4	0.426	n/r	n/r	0.05	0	0.05	0.625
Hewitt		n/r	n/r	n/r	9.3 ± 3.3	10.5 ± 2.8	n/r	n/r	n/a	0.76
Larsen		8.5 ± 2.3	8.7 ± 2.1	n/r	9.0 ± 2.5	9.0 ± 2.5	0.4	0.3	0.1	0.66
Marckmann		10.4 ± 4.2	10.7 ± 6.8	n/r	8.9 ± 3.6	8.5 ± 3.5	-1.5	-1.2	-0.3	0.750
Mc Greevy	PWV	12.55 ± 5.36	11.24 ± 2.21	n/r	11.1 ± 2.3	11.1 ± 2.3	-1.45	-0.14	-1.31	0.097
Mc Greevy	PWV (adapted)	11.94 ± 4.75	10.1 ± 2.77	n/r	10.61 ± 1.83	10.22 ± 2.55	-1.33	0.12	-1.45	0.071
Mose		9.7 ± 2.5	10 ± 2	n/r	10.5 ± 4	10.1 ± 2.5	0.8	0.1	0.7	0.269
Pilz		8.41 ± 1.97	8.26 ± 2.06	0.669	8.48 ± 2.22	8.64 ± 2.42	0.07	0.38	-0.31	0.302
Ryu		15.79 ± 2.56	15.74 ± 2.88	0.934	n/r	n/r	-0.16	-0.6	0.44	0.348
Whitham 2013		8.8 ± 1.2	8.7 ± 1.2	n/r	8.8 ± 1.4	9.0 ± 1.4	0.0	0.2	-0.2	0.40
Whitham 2015		7.3 ± 2.6	8.3 ± 1.9	0.13	6.9 ± 2.4	8.1 ± 1.4	-0.4	-0.2	-0.2	0.93
Yiu	Heart-Carotid	9.25 ± 2.67	10.06 ± 3.4	0.17	9.57	9.74	0.32	-0.32	0.64	0.75
	Heart-Ankle	10.71 ± 2.52	11.16 ± 1.45	0.27	11.25	11.11	0.54	-0.05	0.59	0.28
	*Brachial-Ankle	18.97 ± 3.38	18.82 ± 4.14	0.85	18.92	18.7	-0.05	-0.12	0.07	0.80

\*Data used for meta-analysis.

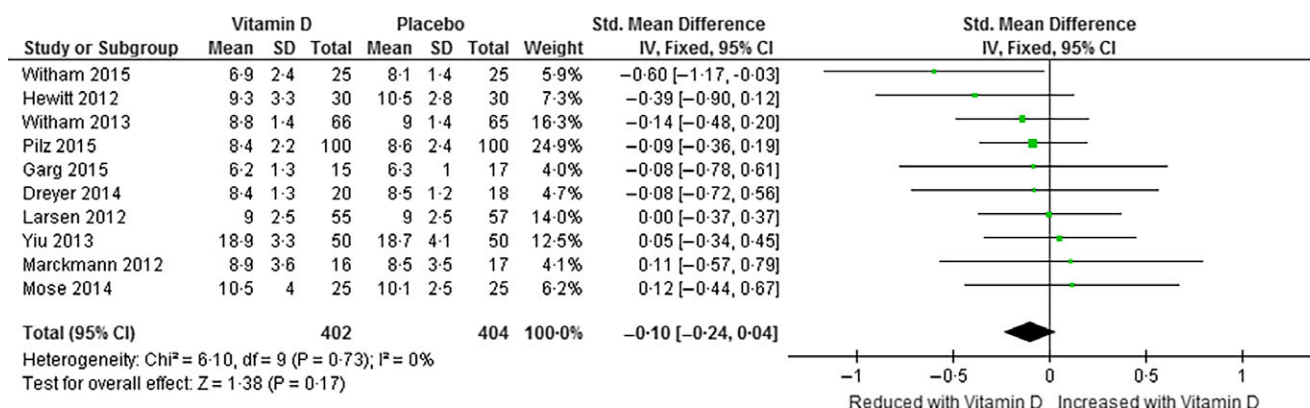


Fig. 3 Forest plot of mean vitamin D- and placebo-treated group data for the outcome of PWV.

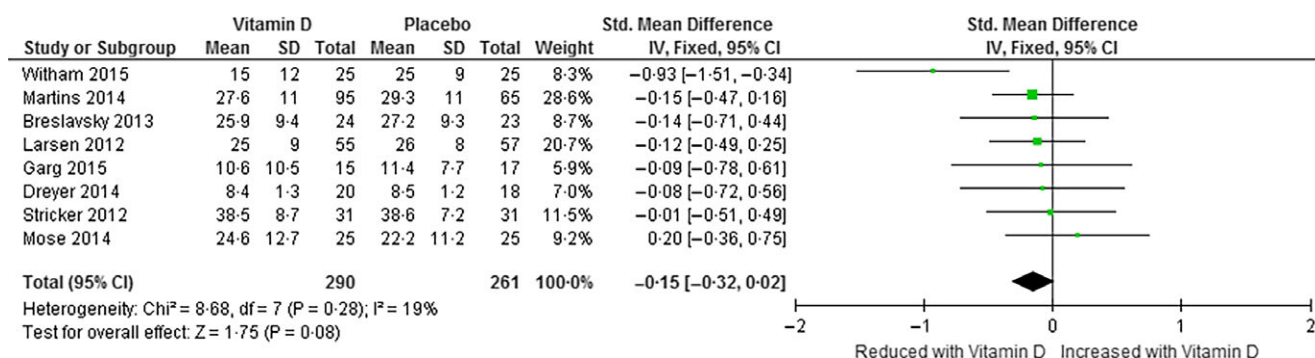
end-products on EC which may precipitate dysfunction.<sup>37</sup> Another proposed mechanism from *in vivo* studies is vascular smooth muscle cell (VSMC) modulation, where vitamin D analogues have been shown to upregulate endothelin gene (which in turn can influence the expression of the powerful vasodilator nitric oxide) and further downregulate oxytocin receptor gene in VSMC, an effect which favours vessel relaxation.<sup>38</sup> Other studies have shown that exogenous supplementation of vitamin D resulted in decreased EC proliferation and that EC stress upregulates vitamin D receptor expression on EC creating an auto-crine/paracrine role for vitamin D with the potential to influence or modulate EC adhesion and VSMC migration and proliferation.<sup>39</sup> These results indicate that vitamin D is, at the molecular/cellular level, critical to the proper working of the

vasculature. Taken together, it is these observational and preclinical data that have provided the evidence to suggest that vitamin D supplementation may improve measures of arterial stiffness. However, the interventional clinical studies surveyed in this review have not demonstrated that vitamin D supplementation results in improved vascular compliance.

This review identified 18 randomized interventional trials that sought to determine whether vitamin D supplementation (using cholecalciferol/vitamin D<sub>3</sub>) would improve PWV or AI. There were significant sources of heterogeneity and study quality between these studies that may explain the apparent lack of, and inconsistent, effects across this literature. Principally, the dosing regimen employed varied substantially and thus may account for the most heterogeneity in effect. Only two studies failed to

**Table 4.** Outcome of AI from Vitamin D supplementation

Study	Baseline (%)			Follow-up (%)		Mean difference (within groups)		Mean difference (between groups)	
	Experimental	Control	P	Experimental	Control	Experimental	Control	P	
Breslavsky	32.9 ± 11.9	29.5 ± 10.9	0.314	25.9 ± 9.4	27.2 ± 9.3	-7	-2.3	-4.7	0.01
Dreyer	8.5 ± 1.1	8.5 ± 1.5	0.66	8.4 ± 1.3	8.5 ± 1.2	-0.1	0	-0.1	0.78
Garg	11.9 ± 10.72	12.1 ± 7.73	0.98	10.6 ± 10.5	11.4	-1.3	-1.5	0.2	0.78
Gepner 2012	n/r	n/r	n/r	n/r	n/r	2.7	0.9	1.8	0.096
Larsen	26 ± 7	26 ± 9	n/r	25 ± 9	26 ± 8	-1	0	-1	0.37
Marckmann	28 (22–31)	26 (18–30)	n/r	n/r	n/r	-1.5	-2	0.5	n/r
Martins	28.2 ± 11.2	31 ± 12	0.1824	27.6 ± 11	29.3 ± 11	-0.6	-1.7	1.1	n/r
Mc Greevy	29.4 ± 6.9	28.5 ± 7.2	n/r	25.6 ± 1.2	28.4 ± 0.9	-3.8	-0.1	-3.7	0.033
Mose	22.1 ± 9.7	26.4 ± 11.5	n/r	24.6 ± 12.7	22.2 ± 11.2	2.5	-4.2	6.7	0.013
Ryu	78.8 ± 13	80.5 ± 11.4	0.595	n/r	n/r	-2.2	-4.3	2.1	0.399
Stricker	38.6 ± 7.3	39.5 ± 7.1	0.77	38.5 ± 8.7	38.6 ± 7.2	-0.1	-0.9	0.8	n/r
Whitham 2015	16 ± 13	27 ± 10	0.001	15 ± 12	25 ± 9	-1	-2	1	0.16

**Fig. 4** Forest plot of mean vitamin D- and placebo-treated group data for the outcome of AI.**Table 5.** Summary of meta-analysis for the outcome of PWV and AI

Analysis	# Studies	# Participants	SMD [95% CI]	P	I <sup>2</sup>	Effects model
Pulse Wave Velocity	10	806	-0.10 [-0.24, 0.04]	0.17	0	Fixed
Augmentation Index	8	551	-0.15 [-0.32, 0.02]	0.08	19	Fixed

SMD, standardized mean difference; 95% CI, 95% confidence interval; I<sup>2</sup>, inconsistency percentage.

achieve mean vitamin D adequacy in their experimental group.<sup>16,20</sup> Participants in these two studies were severely vitamin D deficient, which may explain their failure to achieve adequate vitamin D status during the intervention period. Alternatively, this could be due in part to the relatively low dose employed (1000 IU/day<sup>20</sup>) or the fact vitamin D was given as a single dose at the start of their trial.<sup>16</sup> Indeed, the optimal vitamin D dosing protocol in terms of concentration and frequency is unclear.<sup>40</sup> This is a consistent technical problem in study design, as many interventional studies that are grounded in epidemiological evidence suggesting low vitamin D is associated

with an outcome, show no effect for vitamin D supplementation.<sup>41</sup> However, attempts to overcome this with high-dose vitamin D supplementation may be harmful.<sup>42</sup> Overall, vitamin D supplementation was largely successful in achieving adequate mean vitamin D levels; however, there was no significant difference in outcome in meta-analyses involving only studies with high-dose vitamin D or a low-dose vitamin D. However, for studies that reported decreases in PWV<sup>15,16,22,28,30</sup> or AI<sup>16,20,21</sup> five of these studies employed a vitamin D less than 3000 IU/day<sup>15,16,20,22,28</sup> and four of these studies had follow-up times >6 months.<sup>15,16,21,28</sup> Further evidence of a dose-dependent effect

is supported by the fact that the studies reporting an increase in PWV in experimental groups relative to controls<sup>18,21,23,25,27,32</sup> all but one used a vitamin D dose less than 3000 IU/day<sup>18</sup>; however, this study had the longest follow-up time suggesting that the duration of vitamin D supplementation may be important in influencing the outcome. Indeed, orthodox theory is that studies conducted over relatively short time frames (e.g. 1–3 months) may not be sufficient to witness discernible and significant differences in outcome. However, in subanalyses comparing the outcome in studies with a shorter or longer follow-up time, there were no significant differences in vitamin D-treated groups compared to placebos. In many instances, follow-up vitamin D was not reported making it difficult to comment on the overall efficacy of vitamin D supplementation.<sup>15,17,21,22,24,25,29,31</sup> Genetic differences in the vitamin D receptor between the samples (an aspect not explored in any study) may potentially explain much of the responsiveness to vitamin D supplementation and may help guide dosing protocols. In considering the diversity of the samples that make up the literature aggregated in this review, there is a high likelihood that there are significant genetic differences in vitamin D and receptor biology.<sup>43</sup> Further, recent evidence is emerging to suggest that 1,25-dihydroxyvitamin D, the active metabolite of 25OHD, may be more relevant to cardiovascular disease where it has a positive relationship to the risk of hypertension in contrast to the negative relationship of 25OHD.<sup>44</sup> 1,25-dihydroxyvitamin D is associated with a higher urinary calcium possibly indicating increased calcium absorption that may promote vascular calcification and increase arterial stiffness. Importantly, though, the activity or concentration of enzymes involved in the synthesis pathway of the active vitamin D metabolite were not quantified in this previous study<sup>44</sup> and this may offer another explanation for the contradictory relationships found between 25OHD and 1,25-dihydroxyvitamin D. The age and body composition of the samples considered in this review were heterogeneous between the samples. This means in the meta-analysis we are aggregating samples of older and younger, overweight and normal weight samples which may affect the mechanism of vitamin D. Vitamin D is a fat soluble steroid hormone and is known to become sequestered in adipose tissue as adiposity increases, which may limit vitamin D receptor sensitivity.<sup>45</sup> Therefore, in the light of these aspects, it may be the case that vitamin D dosing may need to be better targeted taking into account the person's adiposity and genetic variants in the vitamin D receptor which have previously been described for the risk of diabetes.<sup>46</sup>

Despite a many number of observational studies showing associations between low vitamin D concentrations and a wide variety of diseases and outcome, an equally large number of randomized interventional trials have not confirmed the hypothesis that raising vitamin D concentrations can modify the occurrence or clinical course of these disorders.<sup>47</sup> Hence, associations between vitamin D and health disorders reported by investigators of observational studies are not causal. Low vitamin D could well be the result of inflammatory processes involved in the occurrence and progression of disease. Evidence in critically ill patients with acute

health conditions characterized by severe inflammation support this, as vitamin D concentrations fall substantially during acute health events.<sup>48</sup> In the light of these aspects, vitamin D as a stand-alone measure may be insufficient and that it may need to be combined with an adjunctive therapy in order to improve arterial stiffness and lower CVD risk. Indeed, as a demonstration of its adjunctive capacity, a study conducted in obese older adults showed 5-year gains in fat tissue were smaller in people with higher concentrations of 25OHD at baseline and had higher levels of physical activity; suggesting that physical activity may be required to enhance the effect of vitamin D.<sup>49</sup>

The current review has some limitations. Firstly, the authors did not have access to primary data and thus were limited in the statistical analyses performed. Secondly, data extraction was performed by a single reviewer only. Thirdly, we considered PWV and AI measurements based on assessments of different vascular regions (e.g. brachial–ankle and carotid–radial) using different devices. This is significant as some sites are better predictors of CVD outcome than others, and indeed, more standardized measurement protocols are required.<sup>50</sup> Finally, during screening, we excluded a number of records based on the fact that the record existed only in conference proceedings/abstract form and has not been published as a full-text manuscript. This means that there may be a significant amount of data, not yet publically available, that could potentially contribute to the conclusions reached in this review. However, some data were obtained directly from authors although as these results are not yet published, we cannot guarantee these results were subject to full peer review and thus it was prudent to not include them in our critique of the literature.

In conclusion, this review sought to clarify the literature surrounding the use of vitamin D supplementation on the outcome of arterial stiffness in randomized controlled trials. We found no conclusive evidence to suggest that vitamin D supplementation is beneficial despite overwhelming observational evidence suggesting low vitamin D is a risk factor for increased arterial stiffness. Indeed, there was significant heterogeneity in the effect of vitamin D supplementation. This may be related to study design in terms of dosing protocol and the samples in which these studies were conducted. Due to its pleiotropic effects, low vitamin D is associated with a number of diseases, but few casual associations have been established.<sup>51</sup> Therefore, large, robust and well-designed RCTs and prospective longitudinal studies are required to determine the potential casual nature of vitamin D on arterial compliance and CVD risk independent of known risk factors.

## Author contributions

AJR designed the study, performed the literature searches, extracted data, performed quality assessment, tabulated important information, wrote the manuscript and reviewed the final draft. DS provided expert opinion on vitamin D biology, significantly contributed to manuscript development, advised on information tabulation and reviewed the final draft. VS provided expert opinion on vascular compliance, contributed to manuscript development and reviewed the final draft. PRB provided

expert opinion on vitamin D biology, contributed to manuscript development and reviewed the final draft.

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## Declaration

The authors declare no conflicts of interest.

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