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REVIEW

# Vitamin D and the cardiovascular system

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**Abstract** Vitamin D, a secosteroid hormone, affects multiple biological pathways via both genomic and nongenomic signalling. Several pathways have potential benefit to cardiovascular health, including effects on parathyroid hormone, the renin–angiotensin–aldosterone system, vascular endothelial growth factor and cytokine production, as well as direct effects on endothelial cell function and myocyte calcium influx. Observational data supports a link between low vitamin D metabolite levels and cardiovascular health. Cross-sectional data shows associations between low 25-hydroxyvitamin D levels and stroke, myocardial infarction, diabetes mellitus, hypertension, and heart failure. Longitudinal data also suggests a relationship with incident hypertension and new cardiovascular events. However, these associations are potentially confounded by reverse causality and by the effects that other cardiovascular risk factors have on vitamin D metabolite levels. Intervention studies to date suggest a modest antihypertensive effect of vitamin D, no effect on serum lipids, a small positive effect on insulin resistance and fasting glucose, and equivocal actions on arterial stiffness and endothelial function. Analysis of cardiovascular event data collected from osteoporosis trials does not currently show a clear signal for reduced cardiovascular events with vitamin D supplementation, but results may be confounded by the coadministration of calcium, and by the secondary nature of the analyses. Despite mechanistic and observational data that suggest a protective role for vitamin D in cardiovascular disease, intervention studies to date are less promising. Large trials using cardiovascular events as a primary outcome are needed before vitamin D can be recommended as a therapy for cardiovascular disease.

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## Introduction

Following the discovery of vitamin D in the early twentieth century, decades of research have uncovered a myriad of potential biological effects. The traditionally recognised actions on calcium and bone metabolism are well documented, but more recent work has uncovered its potential role in the pathophysiology of cardiovascular disease. Furthermore, longitudinal and cross-sectional studies have shown associations between vitamin D deficiency and a variety of cardiovascular disease states and risk factors. Interventional trials in a variety of cardiometabolic conditions have sought to identify the possible role of vitamin D as a therapeutic agent in the management of cardiovascular risk factors. This review aims to highlight the vascular effects of vitamin D, provide an overview of existing observational data, critically review interventional trials of vitamin D in vascular disease to date and detail future directions in this field.

### What is vitamin D?

Vitamin D is a secosteroid hormone, mainly produced from the action of sunlight on skin. 7-Dehydrocholesterol in skin is converted to vitamin $D_3$ (cholecalciferol) by the action of ultraviolet light. Small amounts of vitamin $D_3$ can be gained from dietary sources and vitamin $D_2$ from plant sources. Vitamins $D_3$ and $D_2$ need to be converted to their active form by the process of hydroxylation. Cholecalciferol is converted to 1α,25-dihydroxyvitamin $D_3$ (1,25OHD, calcitriol) by first undergoing 25-hydroxylation in the liver to form 25-hydroxyvitamin D (25OHD), and then 1α hydroxylation in the kidney. Both processes are dependent on cytochrome CYP450 containing enzymes [1]. 1α Hydroxylase has also

been found in many cells throughout the body, e.g., macrophages, thus conversion to the active form may occur in an autocrine or paracrine manner, rather than occurring only in the kidney as previously thought [2, 3]. 25OHD is the major circulating form of vitamin D bound to vitamin D-binding protein [4], and given its long half-life (several weeks), is the form most commonly measured to ascertain vitamin D repletion status. Controversy exists as to what levels of 25OHD should define repletion, insufficiency, and deficiency. For the purposes of this review, deficiency is used to denote levels below 25 nmol/L (a level associated with an increasing risk of rickets and osteomalacia), and insufficiency for levels below 75 nmol/L, a level postulated to associate with better health outcomes by some authors based on observational data [5].

### Vitamin D receptors

1,25OHD binds to the vitamin D receptor (VDR). VDR is a nuclear steroid receptor expressed on at least 36 different tissues including cardiac muscle, vascular smooth muscle, endothelium, and lymphocytes [4]. Vitamin D receptors have similar features to other ligand-activated receptors such as the retinoic acid receptor and thyroid hormone receptor [1]. Indeed, the vitamin D receptor forms a heterodimer with the retinoic acid receptor, and it is this heterodimeric complex that binds to the vitamin D response element to mediate effects on gene transcription [6]. Vitamin D-responsive elements are found at the start of a large number of target genes [7]. Intriguingly, recent work suggests that vitamin D metabolites may also act via alternative nongenomic pathways, using an alternative binding site on the vitamin D receptor, rapid activation of cell signal pathways at the plasma membrane appear to mediate some effects [4, 6].

The major role of 1,25OHD is in calcium homeostasis. Circulating calcium levels are tightly regulated via the effects of calciotropic hormone systems including vitamin D and parathyroid hormone (PTH). 1,25OHD enhances production and activity of the TRPV6 ion channel and calbindin calcium binding protein in the intestinal epithelium to promote gut absorption of calcium. In the presence of low blood calcium, 1,25OHD and PTH act together to mobilise calcium from the skeleton through stimulating osteoclastogenesis and both act together to increase distal renal tubule reabsorption of calcium [7]. Vitamin D also plays a role in bone remodelling [1]. Calcium sensors in the parathyroid gland closely monitor serum calcium concentrations and PTH stimulates renal production of 1-alpha hydroxylase to increase 1,25OHD production.

Low vitamin D levels are common, especially in countries at latitudes >40° north or south, where insufficient ultraviolet light reaches Earth's surface during winter to allow vitamin D synthesis. Indoor living, extensive skin cover by clothing, old age, obesity, dark skin, and genetic variation in metabolic pathways are all recognised to contribute to low 25OHD levels. Basic science investigation over the last 20 years has elucidated a number of pathways by which vitamin D metabolites might influence cardiovascular health, which are discussed below. We then review the observational data linking low 25OHD levels with cardiovascular disease before examining whether supplementation can ameliorate any deleterious effects on cardiovascular health.

## Vitamin D and vascular biology

### Effects on the renin–angiotensin–aldosterone system

Both animal and human studies have shown that vitamin D acts as a negative regulator of the renin–angiotensin–aldosterone system (RAAS) [8–10]. The RAAS is involved in the maintenance of electrolyte homoeostasis, blood pressure and intravascular volume. Vitamin D receptor knockout mice have markedly increased renin and hence angiotensin II activity [8]. This overproduction leads to hypertension, cardiac hypertrophy, and increased water intake and sodium absorption. Studies in humans also suggest effects of vitamin D on the RAAS, although this may be mediated via more complex effects on angiotensin II production rather than a direct effect on reducing renin levels [10]. Large prospective human studies have also identified a relationship between low vitamin D levels and increased activity of RAAS [9]. Correction of vitamin D insufficiency appears to have similar effects on tissue response to angiotensin II infusion in humans as does administration of captopril, suggesting a mechanism of action for vitamin D in humans that is similar to ACE inhibition [11].

### Direct effects on calcium flux

1,25OHD can act through both genomic and nongenomic mechanisms at cardiac myocytes. Nongenomic pathways are mediated through the action of 1,25OHD at voltage-gated calcium channels in cardiac myocytes, providing a rapid influx of calcium into cells [12]. Animal studies have demonstrated that 1,25OHD directly alters myocyte contractility with accelerated relaxation which may be important for normal diastolic function. This mechanism was shown to occur through phosphorylation of protein kinase C [13]. 1,25OHD can also alter calcium influx into the cell via beta adrenergic mediated adenylate cyclase and cAMP pathways in animal models [12], potentially again enhancing myocyte contractility.

### Vascular calcification

Calcium deposition in atherosclerotic plaques, vessel walls, and valves forms a major cause of cardiac morbidity and

mortality. Arterial calcification is an additional risk factor for cardiovascular events independent of traditional risk factors [14]. Vitamin D has been associated with both increased vascular calcification and evidence conversely supports a protective effect. Observational studies in humans have shown an inverse correlation between vitamin D and coronary artery calcification [15]. Such studies are at variance with results in rat models, where the combination of nicotine and vitamin D can induce vascular calcification [16]. Recent evidence suggests that vascular calcification involves similar regulatory mechanisms and pathways to bone formation [17]. Several bone-associated proteins have been identified in the process of vascular calcification, including osteocalcin, matrix Gla protein, and osteoprotegerin. A number of bone matrix proteins have been identified which lead to osteoblast differentiation and subsequent mineral deposition in vascular endothelial cells [18]. Further research investigating the bone vascular axis is needed to outline the exact mechanisms linking vitamin D with vascular calcification.

### Left ventricular hypertrophy and parathyroid hormone

VDR knockout mice display profound left ventricular hypertrophy at 12 months. VDR is present in myocytes and thus a direct effect on the regulation of myocyte growth is possible [12]. In addition, VDR are present in the parathyroid gland and 1,25OHD suppresses production of PTH and prevents proliferation of parathyroid glands [7]. As such, vitamin D helps to regulate parathyroid levels, and vitamin D insufficiency is associated with elevated PTH levels. Observational studies have suggested that elevated parathyroid hormone levels are associated with left ventricular hypertrophy [19] and patients with primary hyperparathyroidism have impaired endothelial function that can be improved by parathyroidectomy [20]. PTH levels were an independent risk factor for the development of heart failure in a large observational study [21], suggesting that such pathophysiological mechanisms may be clinically relevant.

### Endothelial dysfunction

Endothelial dysfunction is the common end pathway for many cardiovascular risk factors, including smoking, hypertension, and hyperlipidaemia. It is thought to presage atherosclerosis and in turn leads to increased vascular tone. Vascular endothelial cells and smooth muscle cells possess VDR, and in vitro studies have shown that 1,25OHD increases nitric oxide synthase activity and nitric oxide production in human umbilical vein endothelial cells [22]. 1,25OHD also increases the angiogenic potential of endothelial cells; an effect that appears to be mediated in part by autocrine and paracrine effects of enhanced vascular endothelial growth factor (VEGF) production [23, 24]. Vitamin D also reduces the ability of endothelial cells to induce platelet aggregation [25]. Additional potential antithrombotic effects may be mediated by vitamin D-induced alterations in the production of proteins involved in regulation of thrombogenesis, e.g., thrombomodulin and antithrombin [26].

### Insulin and glucose handling

Vitamin D may play a pathophysiological role in the development of both types 1 and 2 diabetes mellitus. Vitamin D receptors are found on islet cells and may stimulate insulin release by beta cells [27, 28]. Early animal studies demonstrated reduced secretion of insulin in response to glucose in vitamin D-deficient rats [29]. This finding may be driven in part by the elevated parathyroid hormone levels seen in vitamin D insufficiency [30]. In addition, the anti-inflammatory and immunomodulatory actions of vitamin D may ameliorate both the autoimmune pathology seen in type I diabetes mellitus and could ameliorate the chronic inflammation thought to contribute to insulin resistance in type 2 diabetes [31].

### Immune/inflammatory modulation

Vitamin D receptors are present on a number of cells in the immune system including lymphocytes, monocytes, macrophages, and the thymus and its role in a number of immune-mediated diseases has been studied [1]. Indeed activated vitamin D preparations have long formed part of the therapeutic armamentarium used by dermatologists to treat immunologically-mediated skin disease. Immune cells play an important role in vascular disease via cytokine production and subsequent immunomodulation and inflammation; factors known to be important in the pathophysiology of heart failure and atherosclerosis. A demonstration of the potential role of vitamin D as an immunomodulator in vascular disease is the increase in interleukin 10 and decrease in tumour necrosis factor (TNF) seen in a randomised controlled trial of vitamin $D_3$ and calcium supplementation in chronic heart failure [32]; both are cytokines thought to play a role in the pathophysiology of heart failure.

Vascular endothelial cells are known to express VDR. Studies have shown the ability of 1,25OHD to regulate innate immunity in endothelial cells when activated by lipopolysaccharides [33]. Jabalonski et al. [34] demonstrated a relationship between low vitamin D levels and increased vascular endothelial cell expression of NF-kappa B and interleukin 6. Such associations do not necessarily infer a causal relationship between low levels of vitamin D metabolites and inflammation, as some clinical studies suggest that the inflammatory response may lead to reduction of 25OHD levels [35]. Thus, a number of potentially plausible biological mechanisms exist

that link vitamin D and its metabolites with effects on the cardiovascular system. These potential mechanisms are summarised in Fig. 1.

## Observational evidence linking vitamin D to vascular disease

### Cross sectional and case–control data

A wealth of cross-sectional data has shown associations between a range of cardiometabolic risk factors and 25 hydroxyvitamin D levels. Cross-sectional data support inverse relationships between 25OHD levels and blood pressure [36, 37]. The degree of difference in blood pressure across the spectrum of 25OHD levels is small (3 mmHg for systolic blood pressure) but potentially significant at a population level. Similar relationships have been shown between higher 25OHD and higher HDL levels, and lower 25OHD and indices of impaired glucose homeostasis [37]. 25OHD levels are lower in patients with type 2 diabetes than in age- and sex-matched patients without diabetes [38] and participants in the 1958 British birth cohort with 25OHD levels in the highest tertile had only one third the prevalence of the metabolic syndrome compared to those with the lower tertile of 25OHD levels [39]. An association between low 25OHD levels and prevalent cardiometabolic risk factors extends even to adolescents [40]. Inverse associations were noted between 25OHD levels and blood pressure and insulin resistance, and higher 25OHD was associated with higher HDL in this population. These associations remained significant after correcting for obesity, age, sex, and ethnicity. Nontraditional risk factors for cardiovascular disease are also related to 25OHD levels in cross-sectional studies, worse endothelial function and arterial stiffness are correlated with lower 25OHD levels in healthy individuals [41], and lower 25OHD levels (<75 nmol/L) are related to worse endothelial function in patients with type 2 diabetes [42].

Patients with myocardial infarction, stroke, and heart failure [43–45] have 25OHD levels that are lower than age and sex matched controls. Patients with type 2 diabetes mellitus [46] with serum 25OHD levels of less than 50 nmol/L were 1.7 times as likely to have an existing diagnosis of cardiovascular disease, despite a similar prevalence of other cardiovascular risk factors. Cross-sectional data is however notoriously prone to confounding and this is a particular problem with vitamin D measurement. Almost any illness will lead to reduced activity, with consequent reduced sun exposure and lower 25OHD levels.

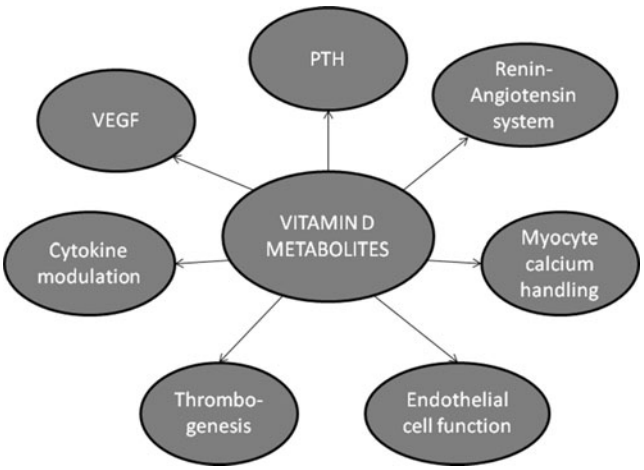


**Fig. 1** Potential biological pathways for effects of vitamin D on the cardiovascular system

### Longitudinal data

A considerable body of longitudinal data also exists supporting an association between 25OHD levels and a range of cardiovascular diseases. Some of the key data are outlined below, and a summary of relevant meta-analyses in this area is given in Table 1.

#### *Hypertension*

Lower baseline 25OHD has been associated with an increased risk of new incident hypertension [47, 48]. Studies followed patients after either direct measurement of 25OHD levels or after algorithm-based prediction of 25OHD levels for between 4 and 8 years. Meta-analysis of several cohort studies, all of which relied on self-reported hypertension, demonstrated that those with the lowest baseline 25OHD level have a 1.8 times higher risk of developing a new diagnosis of hypertension [49, 50]. However, more recent data from the Women's Health Initiative trial found no relationship between baseline 25OHD level and the risk of either incident hypertension or of change in measured blood pressure over a 7-year follow up [51].

#### *Metabolic syndrome and diabetes*

Lower 25OHD levels are associated with a lower risk of progression to diabetes. This risk of progression in the Nurses' Health study [52] was 48 % lower for those in the highest 25OHD quartile compared to those in the lowest quartile. Similar, though less striking, results were also seen in patients enrolled in the Diabetes Prevention Programme [53]. Meta-analysis of eight longitudinal studies confirmed a reduction of 43 % (95 % CI, 24–57 %) in incident cases of diabetes in those with serum 25OHD >62.5 nmol/L compared with those with serum 25OHD<35 nmol/L [54]. Similar results have been seen with similar, updated meta-analyses [55, 56].

**Table 1** Meta-analyses of observational studies linking 25-hydroxyvitamin D levels to cardiovascular disease

| Study | Focus | Main finding | Notes |
|---|---|---|---|
| Pittas [49] | Hypertension | Relative risk of incident hypertension 1.76 (95 % CI, 1.27–2.44) comparing lowest category of 25OHD with highest category | Three cohort studies |
| Mitri [54] | Diabetes | 43 % (95 % CI, 24–57 %) lower incidence of new diabetes in those with baseline 25OHD >62 vs <35 nmol/L | Eight prospective studies |
| Sun [59] | Stroke | Relative risk of stroke 1.52 (95 % CI 1.20–1.85) in lowest 25OHD group compared to highest group | Seven prospective studies. Similar results examining ischaemic stroke alone |
| Chowdhury [61] | Cerebrovascular disease | Relative risk of event 0.60 (95 % CI, 0.48–0.72) for top tertile of baseline 25OHD vs bottom tertile | Seven studies, 49,000 participants. Events defined as any fatal or nonfatal ischaemic or haemorrhagic stroke or transient ischaemic attack |
| Forouhi [55] | Diabetes | Relative risk of new type 2 diabetes 0.59 (95 % CI, 0.52–0.67) comparing highest vs lowest quartile of 25OHD | Eleven studies, 59,000 participants |
| Pilz [65] | Mortality in CKD | Relative risk of death 0.86 (95 % CI, 0.82–0.91) per 25 nmol/L increase in 25OHD level | Ten studies, 6,800 participants. Similar results in dialysis and nondialysis populations |
| Burgaz [50] | Hypertension | Odds ratio for hypertension 0.73 [95 % CI 0.63–0.84] for highest vs lowest category of 25OHD | Eighteen studies, 14 cross-sectional |
| Grandi [64] | CVS events | CV event relative risk 1.54 (95 % CI, 1.22–1.95); CV mortality relative risk 1.83 (95 % CI, 1.19–2.80) for lowest 25OHD category vs highest category | Four prospective cohort studies included for incidence (5,200 participants); five studies for mortality (24,400 participants) |
| Parker [56] | Cardiometabolic disease | Odds ratio, 0.67 (95 % CI, 0.55–0.81) for CV disease, 0.49 (95 % CI, 0.38–0.64) for metabolic syndrome, 0.45 (0.25–0.82) for diabetes mellitus, comparing highest category of 25OHD levels with lowest category | Mixture of 28 cohort, case control and cross-sectional studies |
| Brondum-Jacobsen [60, 62] | Ischaemic heart disease and stroke | 39 % (95 % CI, 25–54) higher rate of ischaemic heart disease and 36 % (9–70) for ischaemic stroke for lowest vs highest category of 25OHD. | Eighteen cohort studies for ischaemic heart disease (82,982 participants); 10 cohort studies for stroke (58,384 participants) |
| Scragg [63] | Cardiovascular events | Risk ratio 1.35 (95 % CI, 1.17–1.56) comparing lowest with highest category of 25OHD | Twelve cohort studies |

*Cardiovascular events*

In the Framingham study, participants without overt cardiovascular disease at baseline were at increased risk of cardiovascular events if their baseline 25OHD level was <37.5 nmol/L, risk of cardiovascular events was doubled at the lowest 25OHD concentrations [57]. The combination of hypertension and low 25OHD level dramatically increased the risk of future cardiovascular events. Interestingly, although progressively lower 25OHD concentrations below approximately 60 nmol/L were associated with a progressive increase in cardiovascular risk, no further reduction in risk was seen above this threshold.

Data from Austria examining the risk of cardiovascular events in patients referred for coronary angiography supports the above findings. In addition, low vitamin D levels in this cohort also predicted future heart failure. The relation between low 25OHD levels and future sudden death or heart failure appeared even stronger in the subgroup with metabolic syndrome at baseline, but interestingly, baseline 25OHD levels did not predict incident myocardial infarction in this subgroup [58]. A recent meta-analysis of studies examining the relationship between 25OHD level and incident stroke is also consistent with the above findings. Low 25OHD levels were associated with a 50 % increased risk of future stroke in a pooled analysis [59–61].

Systematic review of longitudinal data [49] suggests that a 25OHD level below 25 nmol/L is associated with a 1.2- to 2.5-fold increase in the risk of cardiovascular events compared to levels >75 nmol/L. Such associations remain robust

after adjustment for age, sex, body mass index, smoking, and previous cardiovascular events and have been seen in other recent meta-analyses [56, 62–64] including one in patients with chronic kidney disease [65]. However, unmeasured confounders may still exist to explain the association between 25OHD levels and vascular disease.

### Heart failure

Patients with heart failure have particularly low levels of 25OHD [44, 66]. This is almost certainly a reflection of the marked exercise intolerance that is a hallmark of heart failure. This limits physical activity and is thus likely to limit sun exposure; most heart failure patients are over the age of 75, and will therefore be less efficient at generating vitamin D in response to UV light.

Cross-sectional studies have confirmed a relationship between heart failure severity and lower 25OHD levels [67, 68]. Lower 25OHD levels correlate with higher levels of natriuretic peptides in patients with heart failure, lower peak exercise capacity, and worse disease-specific quality of life [44, 69]. Longitudinal data confirms that low 25OHD levels independently predict death in patients with heart failure; the relative risk of death was increased by 1.52 (95 % CI, 1.21–1.92) for heart failure patients with 25OHD levels <25 nmol/L compared to those with 25OHD levels of >75 nmol/L in a large Israeli population study [70].

Data on the relationship between 25OHD levels and incident heart failure are harder to interpret. PTH, but not 25OHD levels, was associated with incident heart failure in the Cardiovascular Health Study [21] despite the fact that 25OHD levels are known to have a major effect on PTH levels. The Intermountain study group [71] examined incidence of heart failure in routinely collected population data; although the incidence of decompensated heart failure episodes was higher in those with 25OHD levels <37.5 nmol/L, this group had a higher prevalence of previously diagnosed heart failure at baseline (19 vs 10 % for those with 25OHD> 75 nmol/L), follow up was short (mean, 1.3 years) and 25OHD sampling was based on clinical indication, rather than being a random population sample. Data from over 3,000 patients referred for coronary angiography [68] who had baseline 25OHD levels measured found that the risk of dying from heart failure was 2.5 times higher in those with baseline 25OHD <25 nmol/L. This result remained robust to multiple risk factor adjustments and benefitted from a longer follow up (median, 7.7 years)

### Atrial fibrillation

Data on the relationship between 25OHD levels and atrial fibrillation are conflicting. Cross-sectional data from the Intermountain study shows a higher prevalence of atrial fibrillation in those with 25OHD levels <37.5 nmol/L [71], but longitudinal data from the same study showed no increase in incidence of new AF in those with baseline 25OHD <37.5 nmol/L compared to those with 25OHD >75 nmol/L. Longitudinal data from the Framingham study also suggests that 25OHD levels do not predict incident atrial fibrillation over and above the effect of traditional cardiovascular risk factors (adjusted hazard ratio 0.99 [95 % CI, 0.88–1.10] per SD increment in 25OHD) [72]. Interestingly, further data from the Intermountain study suggests that patients with very high (>250 nmol/L) 25OHD levels have a significantly increased risk of developing atrial fibrillation (adjusted hazard ratio, 2.5; $p$=0.003) [73].

#### *Problems with observational data*

Any observational data, no matter how well prepared or controlled, is still potentially subject to residual confounding. This is a particular problem for observational studies of vitamin D and cardiovascular disease for several reasons. Firstly, any disease state is likely to reduce activity and therefore sun exposure, leading to reverse causality. This issue dogs all cross-sectional data and potentially confounds any longitudinal data with short follow-up times. Secondly, a number of risk factors for cardiovascular disease are also known to lead to low 25OHD levels. These include older age, obesity, smoking, and physical inactivity [38, 74]. Low 25OHD levels could therefore be a bystander (risk marker) due to the presence of these risk factors, but it is still possible that some of the deleterious effect of these risk factors on cardiovascular health is mediated via reduction of 25OHD levels. Dissecting out the independent effect of vitamin D from these other risk factors in observational studies is probably not possible without some residual confounding. Thirdly, it is entirely possible that other, poorly understood biological factors may confound any relationship. Recent work suggests that 25OHD is a negative acute-phase reactant, falling rapidly and significantly after a systemic inflammatory insult [35]. Therefore, the presence of low-grade chronic inflammation, known to be a pathophysiological contributor to cardiovascular disease and often present years before symptoms become overt, may drive both cardiovascular disease and low 25OHD levels. In a similar vein, ultraviolet light has other biological effects beyond vitamin D synthesis; UV light appears to have vascular effects independent of vitamin D, mediated by release of cutaneous nitric oxide stores [75]. Changes in external temperature with season may influence blood pressure [76], giving yet another biological mechanism for mediating cardiovascular risk that is correlated with, but not caused by, vitamin D. Finally, several different technologies exist for measuring 25OHD levels [77] and measurements have historically suffered from a lack of global standardisation (although this is now being addressed via a

global program based on internal standards from the National Institute of Standards and Technology in the USA). Thus, comparison across studies and across sites is problematic, especially given the nonlinear response of some assays and the frequent reporting of results using cutoff values, which are highly sensitive to differences in assay technique. These potential confounding factors are summarised in Table 2.

## Intervention studies

### Effects of vitamin D on surrogate markers of vascular disease

#### *Blood pressure*

Some, but not all studies investigating the effect of vitamin D supplementation have seen improvements in blood pressure. Meta-analysis [78] confirmed a mean reduction of 3.6/3.1 mmHg with supplementation, but this effect was confined to studies with a mean baseline systolic BP> 140 mmHg. Studies examining normotensive individuals did not show any effect on blood pressure. This meta-analysis also suggested that vitamin $D_2$ or $D_3$ were more effective than 1-alpha hydroxylated versions of vitamin D. Similar small reductions in blood pressure were noted in another meta-analysis [79]. A summary of meta-analyses of intervention trials for blood pressure, other surrogate markers and cardiovascular event data is given in Table 3. Arterial stiffness, a key contributor to both hypertension and left ventricular hypertrophy via increased cardiac afterload, did not improve after 20 weeks of treatment with 3,000 IU of vitamin $D_3$ per day [80] compared with placebo.

#### *Left ventricular hypertrophy*

Left ventricular hypertrophy (LVH) is a major independent risk factor for cardiovascular events and cardiovascular death. High blood pressure is a significant contributor to the development of LVH, but is by no means the only determinant. Few intervention studies to date have examined the effect of vitamin D supplementation on left ventricular mass; the Paricalcitol Capsule Benefits in Renal Failure-Induced Cardiac Morbidity (PRIMO) trial [81], examining the effect of paricalcitol on LV mass in patients with chronic kidney disease (CKD), showed no effect on LV mass after 48 weeks of therapy when compared to placebo. Results from other studies underway evaluating this question are awaited.

#### *Cholesterol*

No overall effect of vitamin D supplementation on cholesterol levels has been seen in studies to date [82]; however, there is an interesting, possibly two-way interaction between vitamin D and statin therapy, perhaps mediated by the cytochrome 3A4 pathway. Statin therapy has been shown to raise vitamin D levels slightly in some but not all studies [83–85]. Conversely, giving vitamin D to patients on atorvastatin with low vitamin D levels may lower cholesterol [86]. Such interactions have not been explored in detail in randomised-controlled trials, and represent an opportunity for future research.

#### *B-type natriuretic peptide*

B-type natriuretic peptide is a marker of future cardiovascular risk. Its utility is not confined to the diagnosis of heart failure. Ventricular wall stress and myocardial ischaemia both elevate

**Table 2** Potential confounders in observational studies of vitamin D

| Confounder | Notes |
|---|---|
| Age | Older skin less efficient at vitamin D manufacture; age-associated changes are risk factor for cardiovascular disease |
| Obesity | Obesity is linked to lower circulating 25OHD levels (possibly due to sequestration) and independently to cardiovascular disease |
| Smoking | Smoking impairs 25OHD synthesis and is a risk factor for cardiovascular disease |
| UV exposure | Not all cardiovascular effects of UV exposure are due to vitamin D; direct effects on blood pressure via nitric oxide release |
| Sedentary lifestyle | Sedentariness is associated with lower levels of sun exposure and hence lower 25OHD levels; sedentariness impairs endothelial function, leads to obesity |
| Chronic inflammation | Inflammatory response may itself lead to lower 25OHD levels, as well as being a risk factor for endothelial dysfunction and cardiovascular disease |
| Medication | Statins (and potentially other cytochrome-metabolised medications with cardiovascular effects) may alter 25OHD levels |
| Genetics | Genetic variations (e.g., skin colour, 7-dehydrocholesterol reductase) involved in regulating 25OHD levels may be part of either causal pathways for cardiovascular disease, or be in linkage with genes involved in cardiovascular disease. |
| Assay heterogeneity | Lack of standardisation in assays until recently makes comparison between laboratories and across studies difficult |

**Table 3** Meta-analyses of intervention studies testing effect of vitamin D analogues on cardiovascular disease

| Study | Focus | Main finding | Notes |
|---|---|---|---|
| Witham [78] | Blood pressure reduction | 3.6 mmHg reduction in systolic blood pressure (95 % CI, −0.7 to 8.0); 3.1 mmHg reduction in diastolic BP (95 % CI, 0.6–5.5) in studies with mean baseline systolic BP>140 mmHg | Eight RCTs included; no effect seen in 4 RCTs with mean SBP<140 mmHg |
| Wu [79] | Blood pressure reduction | 2.4mmHg reduction in systolic BP (95 % CI, 0–4.9). No change in diastolic blood pressure (95 % CI, −4.0 to 4.0 mmHg) | Four RCTs included |
| George [104] | Glycemic control | Small reduction in fasting glucose (0.32 mmol/l; 95 % CI, 0.07–0.57) and small improvement in insulin resistance (standard mean difference 0.25, 95 % CI, 0.03–0.48). No improvement in glycated haemoglobin | Effect confined to those with diabetes or impaired glucose tolerance (6 RCTs) |
| Wang [82] | Lipids | No effect on total cholesterol, HDL or triglycerides. LDL cholesterol increased by 0.08 mmol/L (95 % CI, 0–0.15) | Eleven RCTs |
| Elamin [96] | CVS events | Hazard ratio for myocardial infarction 1.02 (95 % CI, 0.93–1.13); hazard ratio for stroke 1.05 (0.88–1.25) | Population derived from osteoporosis trials |
| Wang [97] | CVS events | Relative risk 0.90 (95 % CI, 0.77–1.05) | Two RCTs included comparing vitamin D vs placebo |
| Pittas [49] | Blood pressure | Systolic BP lower by 1.9 mm Hg (95 % CI, −4.2 to 0.4 mmHg); no difference in diastolic BP. | Ten RCTs |
| Cheng [103] | CVS events | No reduction in endocrine/cardiovascular adverse events (RR, 1.07; 95 % CI, 0.84–1.36) | Population with CKD taking paricalcitol. Combined endocrine/cardiovascular endpoint included for safety, not efficacy |
| Kandula [100] | CVS events | No data found on CVS events | Population with CKD (5 RCTs); compared vitamin $D_2$/$D_3$ with placebo |
| Palmer [101] | Mortality | No reduction in mortality (relative risk, 1.34; 95 % CI, 0.42–4.26) | Population with CKD; comparison of 1 alpha hydroxylated vitamin D vs placebo. Three studies with mortality data included |

levels of B-type natriuretic peptide (BNP), and a range of cardiac pathologies, including coronary artery disease, LVH, and atrial fibrillation can raise BNP levels.

A number of studies have examined the effect of vitamin D supplementation on BNP levels. One study on older heart failure patients [66] did demonstrate a reduction in BNP levels, but a second study in younger patients failed to demonstrate a fall despite a higher dose (2,000 IU per day of vitamin $D_3$) being used [32]. No reduction was seen in patients supplemented with vitamin D after myocardial infarction [87] but bolus supplementation with either 100,000 or 200,000 IU of oral vitamin $D_3$ lowered BNP levels relative to placebo in patients with type 2 diabetes mellitus [88]. One trial of vitamin D supplementation in patients with previous stroke [89] showed an increase in BNP levels in the intervention arm compared to placebo.

*Endothelial function*

Endothelial function denotes the final common pathway to vascular damage and is influenced by multiple vascular insults, including smoking, high cholesterol, elevated blood pressure, and pollution. It is an independent predictor of future cardiovascular events and is thought to represent the pathological lesion that leads to atherosclerotic change. A number of studies have now examined the effect of vitamin D supplementation on endothelial function across a range of diseases. Marked improvement in endothelial function was noted in an uncontrolled trial of supplementation performed in a healthy young Turkish population with 25OHD levels of <25 nmol/L [90]. Results from randomised controlled trials have been mixed: in patients with type 2 diabetes one study showed improvement in endothelial function after a single 100,000 IU oral dose of vitamin $D_2$ [91] but another study showed no benefit after bolus supplementation with high dose vitamin $D_3$ [88]. Bolus (60,000 IU per month) vitamin $D_3$ supplementation also improved endothelial function in overweight African-Americans [92]. Transient improvements 8 weeks after supplementation were seen in patients with stroke, but no improvement was noted at 16 weeks [89]. Measurement of fingertip plethysmography (a correlate of coronary artery endothelial function) showed no improvement

with supplementation in a group of patients with previous myocardial infarction [87]. It is possible that the lack of effect seen was due to the use of fingertip plethysmography as opposed to measurement of brachial artery function used in several other trials. Differences between trials may also be due to the differences in the proportion of patients already receiving therapies known to improve endothelial function, e.g., ACE inhibitors and statins.

A major caveat with most trials examining surrogate markers to date is that they have been of short duration, typically 6 months or less. It is therefore unclear if the effects of vitamin D on such surrogate markers (particularly blood pressure) are sustained in the longer term.

### Cardiovascular outcome trials

To date, no large randomised controlled trials have been published that were designed specifically to test the effect of vitamin D supplementation on cardiovascular events. Some event data are available from existing trials, which were mostly conducted in patients with osteoporosis with the aim of reducing fractures or improving bone mineral density. Although there is a well-known association between osteoporosis and vascular disease, such trial results cannot reliably be generalised to wider populations, were not powered to detect differences in cardiovascular events, and were not designed to accurately classify, record, and adjudicate on the presence of cardiovascular events.

Nevertheless, a number of authors have attempted to analyse existing trials, either singly or in meta-analysis. The Women's Health Initiative (WHI) study [93] randomised 36,000 women to receive 1g calcium plus 400 U vitamin $D_3$ daily for 7 years. No difference was seen in either myocardial infarction or coronary heart disease death (HR, 1.04; 95 % CI, 0.92–1.18), stroke (HR, 0.95; 95 % CI, 0.82–1.10) or new-onset diabetes mellitus [94] (HR, 1.01; 95 % CI, 0.94–1.10). Trivedi et al. [95] randomised 2,286 older people to receive 100,000 IU oral vitamin $D_3$ or placebo every 4 months for 5 years; the relative risk of both cardiovascular death (0.84) and overall mortality (0.88) was non-significantly lower in the treatment arm.

A recent meta-analysis concluded that for the small number of eligible trials ($n$=6 for myocardial infarction, $n$=6 for stroke), no significant difference was detectable between the intervention and control groups for myocardial infarction (HR, 1.02; 95 % CI, 0.93–1.10) or stroke (HR, 1.05; 95 % CI, 0.88–1.25) [96]. Similar effects were seen in another recent meta-analysis [97]. However, these results were heavily influenced by the size of the included WHI trial relative to the other included trials. The dose of vitamin D used in the WHI trial was very small; extrapolation from other published data suggests that this dose was enough to increase circulating 25OHD levels by only 10 nmol/L [98]; smaller than might be needed to detect a reduction in cardiovascular events. Thus, the lack of effect in this trial (and by extension the aforementioned meta-analysis) may be due to inadequate dosing.

### CKD

The effects of vitamin D supplementation in chronic kidney disease are even more complex than for other disease states. Impaired 1 alpha hydroxylation by the failing kidney has led to widespread use of 1-alpha hydroxylated vitamin D analogues and concern about the ability of vitamin D to increase serum calcium and hence calcium × phosphate product has led to development of analogues (e.g., paricalcitol) designed to have less effect on serum calcium. More recent data suggests that 1-alpha hydroxylation may still occur in the failing kidney, as well as occurring in other target tissues (e.g., macrophages) [2, 3]. Thus, it is biologically plausible that giving nonhydroxylated vitamin $D_2$ or $D_3$ to CKD patients could still have beneficial biological effects.

Intervention trials in CKD have shown mixed effects. No effect of 8 weeks of 40,000 IU/week supplementation with vitamin $D_3$ was seen on blood pressure or arterial stiffness [99] despite an increase in 1,25OHD and a decrease in PTH levels in the treatment group. A recent meta-analysis [100] failed to find data on the rates of cardiovascular events from the small number ($n$=5) of RCTs comparing vitamin $D_2$ or $D_3$ with placebo in patients with CKD; no effect on overall mortality was seen in another meta-analysis [101].

Recent trials of paricalcitol (1–2 μg per day for 24 weeks) have shown a reduction in proteinuria in patients with diabetic nephropathy [102], but the PRIMO trial failed to show regression of left ventricular hypertrophy in paricalcitol-treated patients, despite giving 2 μg per day for 48 weeks [81]. A meta-analysis of paricalcitol trials [103] showed no reduction in cardiovascular endpoints, and the effect of 1-alpha hydroxylated vitamin D on CKD progression and all-cause mortality remains unclear from existing trial data [101].

### Diabetes/glycaemic control

Despite a considerable body of observational evidence, trial data examining the effect of vitamin D supplementation on glycaemic control in diabetes, or aiming to prevent onset of diabetes have been disappointing to date. A recent systematic review noted a small (−0.3 mmol/L) reduction in fasting glucose with vitamin D supplementation in patients with diabetes or impaired fasting glucose, no effect on glycosylated haemoglobin, and a modest improvement in insulin resistance [104]. Analyses of data from two osteoporosis trials (WHI and Randomised trial of vitamin D and calcium for the secondary prevention of osteoporosis-related fractures in the elderly (RECORD)) failed to show any reduction in the rate of new

diagnoses of type 2 diabetes mellitus after vitamin D treatment [94, 105].

The existing data pertinent to type 2 diabetes can be legitimately criticised as being derived from trials set up for other reasons. Imprecise definition of new onset diabetes diagnosis and lack of trials targeting groups at high risk of developing new onset type 2 diabetes are potential problems, although the existing evidence does not lend strong support for performing such trials. It is still possible that vitamin D could reduce microvascular or macrovascular adverse outcomes in patients with diabetes even without an effect on glycaemic control. Effects on VEGF system might mediate beneficial effects on microvascular function and improvement in blood pressure and endothelial function could still reduce macrovascular risk, which is less driven by glycaemic control. Trials are needed to test whether vitamin D can reduce the incidence of type 1 diabetes; intervention data are lacking in this area, and the different mechanisms postulated for benefits in type 1 diabetes (i.e., immunosuppression) support further work in this area.

### Heart failure trials

Heart failure is a systemic disease; it affects not only cardiac function, but is accompanied by a syndrome of neurohormonal overactivation (particularly adrenergic and renin–angiotensin–aldosterone systems). Significant chronic inflammation is also a hallmark of heart failure, with elevated levels of proinflammatory cytokines, particularly TNF alpha. Anaemia of chronic disease is also part of the heart failure syndrome, but of even more note is the profound skeletal myopathy that accompanies heart failure. This myopathy affects predominantly type 1 muscle fibres, contributing in large part to the fatiguability and exercise intolerance that is the hallmark of chronic heart failure.

There are thus multiple potential targets on which vitamin D could potentially act in heart failure, given its potential effects on inflammation, muscle function, and endothelial function. Two randomised controlled trials have reported to date on the effects of vitamin D in heart failure. The first trial [32] compared 2,000 IU per day of oral vitamin $D_3$ with placebo, given over a 9-month period. They found no effect of vitamin D on ejection fraction, exercise capacity or B-type natriuretic peptide levels, but did show a significant reduction in TNF alpha levels. The second trial [66] compared the effects of two 100,000 IU oral vitamin $D_2$ doses given 10 weeks apart with placebo in older (mean age, 80 years) heart failure patients. This trial found no effect on exercise capacity, physical function, quality of life, or TNF alpha levels, although BNP levels were reduced in the intervention group.

Although no encouraging signals of effect in heart failure have yet been seen, it is possible that larger doses are needed and beneficial effects on hospitalisation and death may still be produced by vitamin D supplementation even in the absence of benefits on symptoms and physical function.

### Cardiovascular safety

Most observational and interventional data collected to date does not suggest any deleterious effect of vitamin D on cardiovascular health, even with relatively long-term follow up. Animal models (particularly the rat) show that administration of vitamin D analogues may accelerate vascular calcification [16], but observational studies in humans suggest the opposite. Higher 25OHD levels were associated with lower coronary artery calcification scores [15] and no worsening of coronary artery calcification was seen in the WHI trial. One small study performed in rural Indian manual workers [106] suggested that those with very high 25OHD levels (>222 nmol/L) might have an increased risk of myocardial infarction. Such levels appear anomalously high, only being very rarely reached in most populations however, and the methodology of this study has been criticised. Data from the Framingham cohort suggest that the incidence of vascular events in a healthy population follows a U-shaped pattern, with a nadir at around 60 nmol/L [57], albeit with wide confidence intervals; a recent study of 250,000 community-dwelling people in Denmark also suggest a U-shaped pattern, with increasing risk of all-cause mortality above and below 50–60 nmol/L [107]. Other studies have found either linear relationships between 25OHD level and vascular risk or levels that flatten off above approximately 50 nmol/L [108].

### The confounding effect of calcium

A key confounder in much of the interventional data collected to date (which originates from studies designed to improve bone health) is the coadministration of calcium. Recent meta-analyses have suggested that calcium, when compared to placebo, causes an increase in vascular death, particularly that due to myocardial infarction [109]. The interaction between calcium and vitamin D supplementation on cardiovascular disease is currently unclear. Reanalysis of the MRC RECORD trial suggests a trend to lower cardiovascular mortality in those given vitamin D alone vs placebo, higher mortality in those given calcium alone vs placebo, and a neutral effect of combined calcium and vitamin D [110]. Original analysis of the WHI trial data [93] suggested no effect of calcium plus vitamin D supplementation on either myocardial infarction or coronary heart disease death (HR, 1.04; 95 % CI, 0.92–1.18) or stroke (HR, 0.95; 95 % CI, 0.82–1.10), a result that was consistent with a meta-analysis of trials [96] that showed no effect of supplementation on either myocardial infarction or stroke. Reanalysis of the WHI dataset, omitting the 54 % of participants

taking calcium supplements at baseline, suggested that calcium and vitamin D supplementation actually increased the risk of cardiovascular events by 15–20 % [111]. In contrast, one recent individual patient meta-analysis [112] including only large osteoporosis trials concluded that supplementation with vitamin D alone did not alter overall mortality (HR, 0.99; 95 % CI, 0.89–1.09) but combined calcium and vitamin D reduced overall mortality (HR, 0.91; 95 % CI, 0.85–1.00).

How then to interpret these conflicting data? The findings are certainly due in part to variation in the choice of trials to include in the different analyses. The WHI trial was performed on younger, healthier patients, and the vitamin D dose used was very low—possibly too low to produce a sufficient increment in vitamin D levels to affect cardiovascular health. It is therefore possible that the WHI results should be interpreted more as giving unopposed calcium, rather than calcium plus vitamin D. Further trials, rather than reanalysis of existing data, are required to resolve this controversy.

## Future directions

Ultimately, the question of whether vitamin D supplementation improves vascular health can only be settled by performing large randomised controlled trials, specifically designed to answer this question. The concerns that have been raised over the possible deleterious effects of calcium should direct us to compare vitamin D with placebo, without coadministration of calcium. Debate continues as to what dose of vitamin D should be used in such trials; the current obsession with reaching prespecified levels of 25OHD has rather obscured any focus on dose–response relationships with vascular risk factors such as blood pressure and cholesterol. However, doses of vitamin D sufficient to reach mean 25OHD levels of around 75 nmol/L in trial populations will be needed to avoid criticism of insufficient dosing in the event that such trials are negative.

Trials will need to be powered to detect differences in cardiovascular event rates—in particular, cardiovascular death, new myocardial infarction and new stroke; incident heart failure is also an important outcome measure to record. Larger trials in populations with established heart failure are also needed, focusing on hospitalisation and death, but also on symptoms and quality of life. Further trials examining the effect of vitamin D supplementation on blood pressure are also needed, particularly focusing on populations with established hypertension, which have not been well studied to date. Such populations should be subdivided by existing treatment, and also by renin status, given the fact that vitamin D may reduce blood pressure via effects on the renin–angiotensin–aldosterone system. Existing data do not support trials of vitamin D specifically to prevent type 2 diabetes mellitus or to improve glycaemic control in this population, although the immunosuppressant actions of vitamin D still make type 1 diabetes a potential target for intervention trials, and it is still possible that vitamin D might be able to reduce micro and macrovascular complications in type 2 diabetes. Similarly, existing data do not support further trials using vitamin D as an adjunct to weight loss.

The current evidence does not therefore support routine supplementation with vitamin D to reduce cardiovascular risk at the population level. Supplementation for specific cardiovascular disease states cannot be recommended either, even for diseases such as heart failure which are associated with very low 25OHD levels. Until appropriate trial data are available, the balance between described risks (e.g., renal stones, costs, and tablet burden) and any potential benefits remains unclear, and extending the prescribing indications for vitamin D beyond its current use in osteomalacia, osteoporosis, and falls prevention in older patients in institutional care cannot be justified.

## Conclusions

Vitamin D has a myriad of biological effects in addition to its traditionally ascribed roles in calcium metabolism and bone health. A number of biological pathways have been described that may mediate potentially beneficial effects on the cardiovascular system, and a large body of observational evidence supports an association between low 25OHD levels and both cardiovascular risk factors and cardiovascular events. However, such associations are prone to confounding, and existing trial data shows inconsistent effects on cardiovascular risk factors. Meta-analysis of cardiovascular data from osteoporosis trials has given inconsistent results, and large trials using cardiovascular events as a primary outcome are needed before vitamin D can be recommended as a therapy for cardiovascular disease.

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