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Vitamin D and risk of CVD: a review of the evidence

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This review summarises evidence for an association between vitamin D status and CVD and the mechanisms involved. Vitamin D_3 is predominantly provided by the action of UVB from sunlight on skin. Average UK diets supply 2–3 µg/d vitamin D but diets containing at least one portion of oily fish per week supply about 7 µg/d. Pharmacological doses of vitamin D_2 (bolus injection of 7500 µg or intakes >50 µg/d) result in a smaller increase in plasma 25 (OH)D than those of D_3 but physiological doses 5–25 µg/d seem equivalent. Plasma 25 (OH)D concentrations are also influenced by clothing, obesity and skin pigmentation. Up to 40 % of the population have plasma 25(OH)D concentrations <25 nmol/l in the winter compared with <10 % in the summer. The relative risk of CVD death is 1·41 (95 % CI 1·18, 1·68) greater in the lowest quintile of plasma 25(OH)D according to meta-analysis of prospective cohort studies. Acute deficiency may inhibit insulin secretion and promote inflammation thus increasing the risk of plaque rupture and arterial thrombosis. Chronic insufficiency may increase arterial stiffness. There is no evidence to support claims of reduced CVD from existing trials with bone-related health outcomes where vitamin D was usually co-administered with calcium. Although several trials with cardiovascular end-points are in progress, these are using pharmacological doses. In view of the potential toxicity of pharmacological doses, there remains a need for long-term trials of physiological doses of D_2 and D_3 with CVD incidence as the primary outcome.

Ergocalciferol: Cholecalciferol: Cardiovascular risk

Vitamin D deficiency causes rickets in children and osteo-malacia in adults and may contribute to the causation of CVD. Cholecalciferol (vitamin D_3) is made by the action of UVB light on the skin but is also provided in the diet from foods of animal origin (eggs, oily fish and meat). Ergocalciferol (vitamin D_2) is present in fungi that have been exposed to UVB irradiation. Both forms of vitamin D undergo 25-hydroxylation in the liver to form 25(OH) metabolites, most of which circulate in plasma bound to the vitamin D binding protein (VDBP). In the kidney, 25 hydroxyvitamin D (25(OH)D) is converted to the biologically active metabolite 1,25 dihydroxyvitamin D (1,25 (OH)$_2$D) by the action of 1α-hydroxylase (Fig. 1). This then binds to the vitamin D receptor, which is a high-affinity nuclear hormone receptor that regulates gene expression[1]. Measurement of serum 25(OH)D to assess vitamin D status has predominantly used immunoassays, which show lower sensitivity (about 30 %) to D_2 metabolites than gold-standard methods such as HPLC/tandem MS. Formerly, a lack of standardisation of methods and appropriate quality control made comparisons between studies difficult[2]. The situation has been remedied by the availability[3] of standard reference serum (SRM 972, National Institute of Standards and Technology) and a quality control programme in the UK organised by the Vitamin D External Quality Assessment Scheme[4]. It has been suggested that the free, unbound form of 25(OH)D may provide a more accurate index of vitamin D status[5,6] than the total 25(OH)D[7,8]. Powe *et al.* found that black Americans had lower total 25(OH)D concentrations
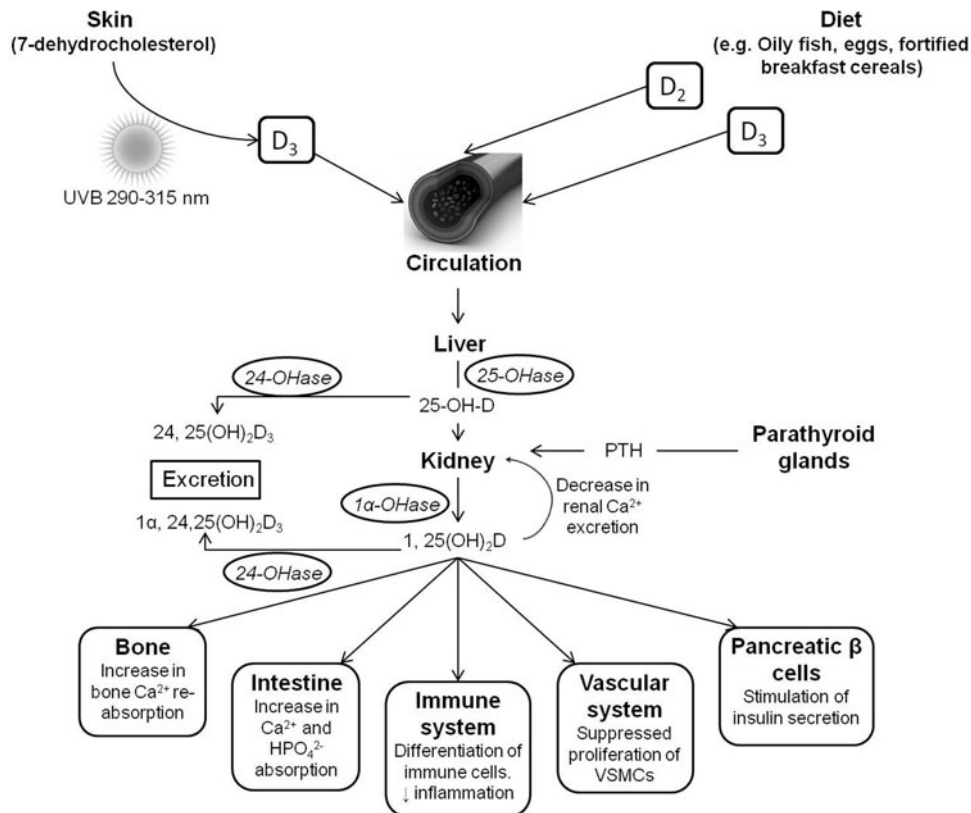

Fig. 1. Metabolism of vitamin D in the body. Vitamin D from exposure of the skin to UVB radiation and dietary sources is transported in the circulation to the liver where 25 hydroxyvitamin D (25(OH)D) is formed by the action of 25-hydroxylase (25-OHase). 25(OH)D is then transported to the kidneys where the enzyme 25-hydroxyvitamin D-1α-hydroxylase (1α-OHase) converts it to the active form, 1,25 dihyroxyvitamin D (1,25(OH)$_2$D). When serum calcium levels are low, parathyroid hormone (PTH) is released which stimulates a greater production of 1,25(OH)$_2$D. This has the effect of increasing serum calcium (Ca^{2+}) concentrations by increasing bone Ca^{2+} re-absorption, increasing the intestinal absorption of Ca^{2+} and phosphate, and decreasing the renal excretion of calcium. 24-hydroxylase (24-OHase) converts both 25(OH)D and 1,25(OH)$_2$D to metabolites which can be excreted from the body. 1,25(OH)$_2$D has also been shown to have effects on pancreatic β cells, immune cells and vascular smooth muscle cells (VSMC).

compared with whites, but because they also had lower concentrations of VDBP, the amount of bioavailable 25 (OH)D for both ethnic groups was similar[9]. The same group found free 25(OH)D concentrations to be better correlated with bone mineral density than the total 25(OH)D concentrations in young adults[10]. Prospective cohort studies have provided evidence that indicates that low concentrations of total 25(OH)D are associated with an increased risk of CVD[11,12]. The discovery that 1α-hydroxylase is expressed in extra-renal tissues, including the vascular endothelium[13–16] may help provide a mechanistic basis for an effect of vitamin D status on the processes associated with the development of CVD.

Sunlight exposure *v.* dietary vitamin D

Exposure to UVB irradiation increases plasma 25(OH)D concentrations[17] and explains the seasonal variations observed in vitamin D status. Skin pigmentation may reduce the capacity to synthesise vitamin D as melanin skin pigmentation competes for and absorbs UVB photons that are responsible for the photolysis of 7-dehydrocholesterol to previtamin D. It has been suggested that an individual with heavily melanised skin may need greater sunlight exposure than one with white skin to make the equivalent amount of vitamin D[18]. Migrants with darker skin moving to northern latitudes are particularly at risk of developing vitamin D deficiency[19]. The use of a sunscreen or clothing/headscarves which cover the skin can also reduce skin synthesis of vitamin D[20–22]. Older age may lead to a decrease in 25(OH)D concentrations as ageing has been shown to be associated with decreased concentrations of 7-dehydrocholesterol in the skin[21] and older people may be less likely to spend time outdoors, particularly if they are institutionalised.

Diet has a lesser effect on vitamin D status and median intakes from food sources in the most recent National Diet and Nutrition Survey Rolling Programme for which the data have been published on information collected between 2008 and 2012 were 2·5 µg/d in men (n 1126) and 2·1 µg/d (n 1571) in women for adults aged 19–64 years[23]. These

intakes are well below the recommendation of 10 μg/d for those confined indoors[24] which becomes relevant for most of the UK population in the winter months when there is no UVB exposure. The recommendation assumed that UVB exposure in the summer months would be sufficient to tide most people over the winter months, but up to 40 % of adults and adolescents in the UK have 25(OH)D concentrations <25 nmol/l from January to March[23].

There is some controversy as to the bioequivalence of D_2 and D_3. Animal studies find D_2 to be less potent than D_3 in curing rickets[25], but D_3 is more toxic than D_2[26]. In the chick bioassay, vitamin D_2 had only 8–11 % of the activity of D_3 in preventing rickets[25]. However, there are large species differences and D_2 has been used effectively in infant formula and to treat hypovitaminosis D in young children[27] for many decades and pharmacopoeias still regard the two forms as equivalent and interchangeable[28,29]. Houghton and Vieth dispute this view arguing that D_3 is more effective than D_2 and have recommended that D_2 should no longer be regarded as suitable for supplementation[30]. In support of this, a recent meta-analysis of seven randomised controlled trials (RCT) which gave vitamin D as a bolus or daily dose concluded that D_2 was less effective in raising plasma 25(OH)D metabolite levels than D_3 (weighted mean difference of 15·2 nmol/l(95 % CI 6·1, 24·3))[31]. However, the comparison for daily vitamin D supplementation in the meta-analysis found no significant difference between the two forms (weighted mean difference 4·8 nmol/l(95 % CI −1·0, 10·6)). There was substantial heterogeneity in the design and dose formulation in the studies included though and some did not adjust for sunlight exposure[32,33] or used assays that were unable to satisfactorily discriminate between 25(OH)D_2 and 25(OH)D_3[33–36]. Some studies also gave pharmacological doses of vitamin D by non-oral routes (i.e. intramuscular administration of 7500 μg[37]) or in tablet or capsule form (i.e. a single dose of 7500 μg[34] or weekly dose of 1250 μg[36]) which are not relevant to dietary intake. We showed that intakes of 5 and 10 μgD_2/d for 4 weeks led to mean increments in 25(OH)D_2 of 9·4 (SE 2·5) and 17·8 (SE 2·4) nmol/l, respectively, and increments in 25(OH)D_3 of 15·1 (SE 4·7) and 22·9 (SE 4·6) nmol/l following 5 and 10 μgD_3/d, respectively[38] which are equivalent to an increase of approximately 2 nmol/l per μg vitamin D. Including our results with comparable trials (Fig. 2) with daily intakes in the nutritional range (5–25 μg/d)[39,40] shows no major difference with regard to effects on plasma 25(OH) metabolite concentrations between D_2 and D_3.

Influence of obesity, inflammation and genetic influences on vitamin D status

Obesity is associated with lower 25(OH)D concentrations[41]. It has been suggested that vitamin D is sequestered in body fat compartments leading to reduced bioavailability[41], but animal adipose tissue is generally a poor source of the vitamin, so this may not explain the association. Increased sedentary behaviour resulting in less sunlight exposure, or inflammation induced by
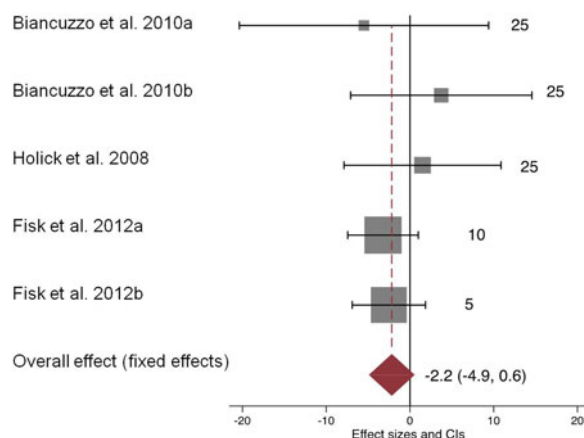

Fig. 2. (Colour online) Meta-analysis of placebo-controlled trials[38–40] comparing the effect of vitamin D doses ranging from 5 to 25 μg/d on total 25(OH)D concentrations measured by HPLC/tandem MS. A negative effect size favours vitamin D_3.

obesity are other possible explanations. VDBP is a negative acute phase protein (i.e. its concentration falls when there is systemic inflammation)[42]. As plasma concentrations of VDBP are a major determinant of plasma 25(OH)D concentrations, disorders that are characterised by chronic systemic inflammation may lower plasma 25(OH)D concentrations. Certain drugs also increase the catabolism of 25(OH)D such as phenytoin, an anticonvulsant drug. Heredity may be important as twin studies find 40 % of the variation in 25(OH)D concentrations[43] to be attributable to additive genetic factors. Some of this variation may be due to different VDBP genotypes[44].

Plasma 25 hydroxyvitamin D concentrations and risk of CVD

A meta-analysis of eight large prospective cohort studies in which 2624 participants died of CVD during follow-up found that individuals in the lowest quintile of 25(OH)D concentrations had an increased risk of all-cause mortality and cardiovascular mortality with or without a history of CVD; relative risk (RR;95 % CI) 1·57 (1·36, 1·81), 1·65 (1·22, 2·22) and 1·41 (1·18, 1·68), respectively[45]. An umbrella review of meta-analyses of observational studies also found significant reductions in the risk of prevalent CVD, IHD and stroke; RR (95 % CI) 0·67 (0·55, 0·82), 0·72 (0·65, 0·81) and 0·61 (0·50, 0·75), respectively[46]. However, these observations may be confounded by other aspects of lifestyle, such as involvement in physical work outdoors, or disease processes resulting in inflammation which influence plasma 25(OH)D concentrations.

Potential mechanisms for an effect of vitamin D on CVD risk

CVD results from the inter-related processes of atherosclerosis and thrombosis. Atherosclerosis develops over several decades, whereas thrombosis is an acute process
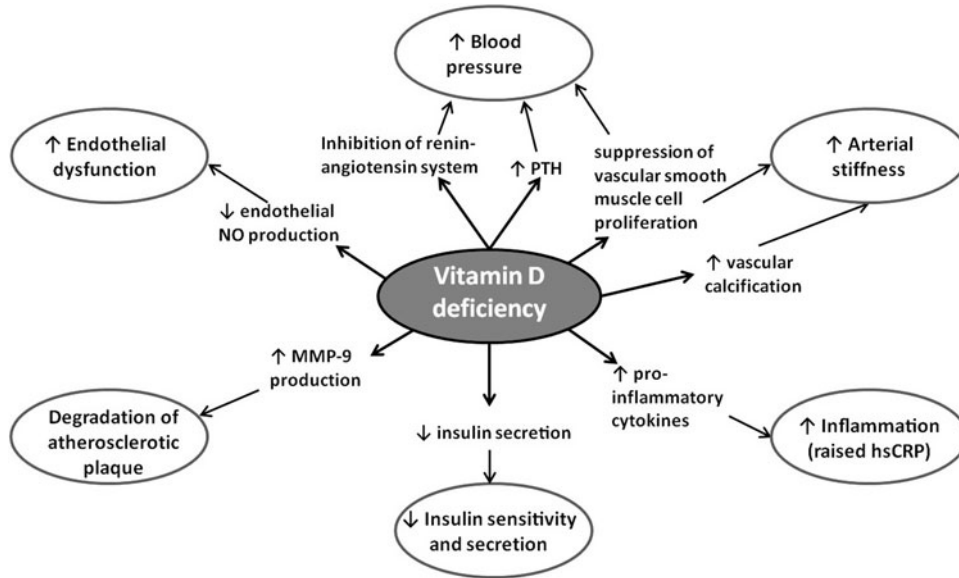
Fig. 3. Potential mechanisms for an effect of vitamin D on CVD. hsCRP, high-sensitivity C-reactive protein; MMP-9, matrix metalloproteinase-9; NO, nitric oxide; PTH, parathyroid hormone.

usually resulting from the rupture of an atherosclerotic plaque that causes a clinical event (heart attack or stroke). Elevated serum LDL-cholesterol and atherogenic dyslipidaemia (low HDL-cholesterol and a predominance of small dense LDL particles) result in atherosclerosis and so are key risk factors for CVD, especially CHD. Elevated blood pressure (BP) is the major risk factor for stroke but it increases the risk of CHD to a lesser extent. Vitamin D may influence the classical risk factors (BP and lipids) as well as other processes (plaque rupture, endothelial dysfunction, arterial stiffening, inflammation and thrombosis; Fig. 3).

Effects of vitamin D on vascular function

$1,25(OH)_2D$ suppresses renin secretion by the kidney which results in decreased conversion of angiotensinogen to angiotensin I. Angiotensin 1 is converted in the pulmonary circulation to the vasoconstrictor angiotensin II which causes blood vessels to constrict, thus increasing BP[47,48]. Vitamin D may also affect BP via changes in parathyroid hormone which has been found in the vascular endothelium and smooth muscle cells[49]. Observational studies have found a positive association between parathyroid hormone and systolic and diastolic BP[50], and parathyroid hormone intravenous infusion has been shown to increase BP in normal subjects[51]. Alternatively, $1,25(OH)_2D$ may have direct effects on the vasculature which is supported by the expression of 1α-hydroxylase in endothelial cells and evidence from experimental studies that $1,25(OH)_2D$ may affect rat vascular smooth muscle cell growth[52].

Disruption of the functional integrity of the vascular endothelium is an important process in the development of atherosclerosis[53]. Impairment of endothelium-dependent vasodilation occurs due to a decrease in the bioavailability of vasodilators, including nitric oxide, and an increase in endothelium-derived contracting

factors[54]. It has been shown in human umbilical vein endothelial cells that $1,25(OH)_2D_3$ can cause a significant concentration-dependent increase in endothelial nitric oxide production via endothelial nitric oxide synthase activation[55]. This is supported by experimental work, which has found that mice carrying a mutant, functionally inactive vitamin D receptor have a lower bioavailability of nitric oxide as a result of a reduced expression of endothelial nitric oxide synthase[56].

Arterial stiffness is determined by structural and functional components of the artery[57]. Normally the balance of collagen and elastin in the vascular wall is kept in a stable state[58], but elastin can be degraded by proteases such as matrix-metalloproteinases (MMP) which have been shown to be inhibited by $1,25(OH)_2D$ in experimental studies in Myobacterium tuberculosis-infected human leucocytes[59] and suppressed in studies in pulmonary tuberculosis patients[60]. This can result in the production of more collagen fibres which are 100–1000 times stiffer than elastic fibres, and therefore the mechanical properties of the artery are shifted into the stiffer range. Vitamin D deficiency may lead to arterial stiffening via increased vascular calcification[61] whereby calcium deposits build up in the media of large arteries[58]. This may also be mediated by MMP as MMP-2 and MMP-9 knockout mice did not develop abdominal aortic calcification after treatment with $CaCl_2$, whereas wild-type mice developed severe calcification[62]. Furthermore, as vitamin D has been shown to suppress smooth muscle cell proliferation[63] it may help prevent an increase in smooth muscle tone or smooth muscle cell hypertrophy which contributes to an increase in vascular stiffness[57,64].

Effects of vitamin D on inflammation

Experimental studies have shown that treatment with $1,25(OH)_2D$ inhibits the production of several pro-inflammatory

cytokines, including IL-6 and TNF-α in partially purified monocytes[65,66] and down-regulates IL-10 expression via the binding of the vitamin D receptor to the promoter region of the IL-10 transcription start site[67]. $1,25(OH)_2D$ has been shown to inhibit NF-κB activation in passively sensitised human airway smooth muscle cells[68] and murine macrophage cells[66]. Therefore it is possible that reductions in C-reactive protein (CRP) concentrations could occur via effects of $1,25(OH)_2D$ on the NF-κB pathway, which plays an important role in regulating the inflammatory response. A proinflammatory environment also promotes the production of MMP which mediate a matrix remodelling process in which the fibrous cap in atherosclerotic lesions is degraded leading to an increased likelihood of plaque rupture[69].

Effects of vitamin D on insulin secretion

1α-hydroxylase and receptors for $1,25(OH)_2D$ have been found in pancreatic β cells[70] and vitamin D appears necessary for normal insulin release[71]. Insulin secretion may be affected by changes in the balance between extracellular and intracellular pools of calcium as insulin secretion is a calcium-dependent process[72].

Epidemiological and clinical trial data on the association between vitamin D and CVD risk factors

Blood pressure

Many cross-sectional and prospective studies have investigated a possible association between vitamin D status and BP. A meta-analysis of four prospective and fourteen cross-sectional studies found a pooled OR of 0·73 (95 % CI 0·63, 0·84) for hypertension in participants in the highest 25(OH)D category compared with those in the lowest category[73]. A number of RCT have measured changes in BP (primarily clinic BP) in response to vitamin D supplementation and shown mixed results[74–80]. A recent meta-analysis of sixteen RCT found no significant treatment effects; weighted mean differences were −0·94 (95 % CI−2·98, 1·10) mm Hg and −0·52 (95 % CI−1·18, 0·14) mm Hg for systolic BP and diastolic BP, respectively[81]. However, 24 h ambulatory BP monitoring has been shown to be better at predicting cardiovascular risk compared with clinic BP[82,83] and the evidence has demonstrated that it is able to more accurately measure the size of reduction in BP brought about by a treatment compared with clinic BP, due to the results being more reproducible over time[84], and the unlikelihood of a 'white coat' or placebo effect[82,85]. Only a few RCT have used 24 h ambulatory BP monitors[86–89] (Table 1) and these were in participants who had hypertension[86,88,89] or had previously suffered from a stroke[87]. None showed a significant difference in the change in 24 h BP between treatment and placebo groups after treatment with 75 µgD$_3$/d for 20 weeks[86], a single oral dose of 2500 µg D$_2$ with 24 h BP measured after 16 weeks[87], 2500 µg D$_3$ taken every 3 months for a year[88] or 2500 µg D$_3$ taken every 2 months for 6 months[89]. There is a need for further well-conducted

vitamin D RCT measuring 24 h ambulatory BP in healthy older participants.

Endothelial function

Endothelial function can be measured non-invasively using flow-mediated dilation of the brachial artery[90]. A couple of cross-sectional studies in healthy adults show lower serum levels of 25(OH)D to be associated with endothelial dysfunction[91,92]. A few trials show an improvement in flow-mediated dilation with vitamin D supplementation[93–95], but most show no effect[79,96–98] (Table 2).

Arterial stiffness

Arterial stiffness is most reliably measured by determining the carotid–femoral aortic pulse wave velocity[99,100]. Many cross-sectional studies have shown inverse associations between vitamin D and arterial stiffness[91,101–107], but there are relatively few clinical trials that have investigated the effect of vitamin D supplementation (Table 2). Gepner et al. conducted a study in which healthy postmenopausal women were randomised to 62·5 µg vitamin D$_3$ daily (n 57) or placebo (n 57) for 4 months. No significant differences were found between groups in the change in pulse wave velocity (P = 0·65) measured using SphygmoCor. This lack of an effect may be due to the women's baseline concentrations of total 25(OH)D being quite high: mean 78·1 (SD 26·5) nmol/l[98]. However, an 8-week RCT in South Asian women with mean baseline serum 25(OH)D concentrations of 27 nmol/l also found no change in pulse wave velocity measured using SphygmoCor in a group given a single oral dose of 250 µg D$_3$/d compared with placebo (P = 0·046)[96]. Two other trials have been conducted in subjects with type 2 diabetes mellitus[97,108] or hypertension[86], but both showed no change in arterial stiffness in response to vitamin D treatment[86,97]. None of the trials have lasted longer than 20 weeks and if vitamin D prevents the age-related increase in arterial stiffness[109] due to inhibition of vascular calcification[61,110], it is likely that longer term studies are needed to see a change.

Inflammation

Inflammation plays an important pathogenic role in all stages of atherosclerosis. Currently, the best available inflammatory biomarker is CRP, an acute phase reactant that is a reliable, independent predictor of the risk of myocardial infarction, stroke and cardiovascular death[111]. Clinical trials of vitamin D supplementation show inconsistent results regarding an effect on CRP concentrations: most report no effect[77,80,97,112–116], whereas some show reductions in CRP concentrations[74,117,118]. Some trials have measured high-sensitivity CRP (hsCRP), which can detect low-grade inflammation. Witham et al. gave patients with a history of myocardial infarction 2500 µg D$_3$ or placebo at baseline, 2 months and 4 months and found that after 6 months CRP had decreased significantly in the D$_3$ group compared with placebo (−1·3 v. 2·0 mg/l, P =

$0.03)^{(118)}$. Similarly, a significant decrease in hsCRP concentrations was observed after 9 weeks of 10 µg/d D_3 (mean −1·41(SE 0·55) mg/l), compared with placebo (mean 1·50(SE 0·94) mg/l; $P = 0.01$) in forty-eight pregnant women aged 18–40 years at week 25 of gestation[74]. In contrast, two larger placebo-controlled RCT that supplemented type 2 diabetic patients with 125 µg/d D_3 for 12 weeks[97], or overweight and obese subjects with 1000 or 500 µg D_3 per week for 1 year[114], found no significant difference in the change in hsCRP between groups. Furthermore, after smaller vitamin D_3 doses of 10 or 25 µg/d for 1 year[80], or 5, 10 or 15 µg/d for 22 weeks in healthy young and older adults[77], hsCRP levels were unaffected. However, a meta-analysis, including ten RCT in 924 participants published between 2009 and 2014 concluded that vitamin D supplementation (median dose 100 µg/d with a range of 10–179 µg/d) significantly reduces hsCRP by 1·08 mg/l (95 % CI −2·13, −0·03) compared with the control[119] but there was significant heterogeneity between trials.

Insulin sensitivity and secretion

Several large epidemiological studies have found 25(OH)D concentrations to be associated with improved insulin resistance and sensitivity[120–122], but RCT have found mixed results; some have found a significant beneficial effect of supplementation[78,116,123], while others have shown no effect[76,124]. An RCT in 100 apparently healthy, but centrally obese men observed an improvement in postprandial insulin sensitivity measured by the oral glucose insulin sensitivity index after supplementation with 3000 µg D_3 once a fortnight for 6 weeks: mean difference in change in oral glucose insulin sensitivity between placebo and the D_3 group was 41·1 (SE 15·5), $P = 0.01$[78]. Similarly, Nikooyeh et al.[123] conducted a 12-week D_3 supplementation trial in ninety diabetic subjects and showed a significant decrease in the homeostasis model assessment of insulin resistance after 25 µg/d D_3 compared with placebo ($P < 0.001$). In contrast to these findings, an RCT of 438 overweight or obese subjects found no significant difference in insulin sensitivity in the fasting state from baseline to 1 year between the placebo group and two D_3 groups that received either 500 µg or 1000 µg per week[76]. Furthermore, 10 or 30 µg/d of D_3 did not lead to significant improvements in insulin sensitivity in a 4-month RCT of patients with type 2 diabetes[124]. A meta-analysis has been published to summarise the effects of vitamin D supplementation on glycaemic control and insulin resistance[125]. For insulin resistance, ten studies were included, four of which were in individuals with normal fasting glucose and six in individuals with abnormal glucose tolerance. When all ten studies were combined there was no effect of vitamin D supplementation on insulin resistance; the standard mean difference favouring vitamin D supplementation was −0·07 (95 % CI −0·20, 0·06). This was also the case when looking at only patients with normal fasting glucose, but in patients with diabetes or impaired glucose tolerance, there was a small improvement in insulin resistance (standard mean difference favouring vitamin D

Table 1. Summary of clinical trials investigating the effect of vitamin D supplementation on 24-h ambulatory blood pressure

Study	Population characteristics	Treatment groups	No of participants completed	Mean age (years)	Duration of study	Mean change (Δ) in 25(OH)D (nmol/l) Treatment	Mean change (Δ) in 25(OH)D (nmol/l) Control	Effect of treatment v. control
Larsen et al.[86]	Hypertensive adults with 24 h SBP ⩽150 mm Hg and/or 24 h DBP ⩽95 mm Hg diastolic	Placebo or 75 µg vitamin D_3/d	112	61	20 weeks	+52·4	−7·5	No significant treatment effects. Between group differences: SBP −3 mm Hg ($P = 0.26$), DBP −1 mm Hg ($P = 0.18$)
Witham et al.[87]	Stroke patients	Placebo or single oral dose of 2500 µg D_2 at baseline or placebo	55	67	16 weeks	+12·3	+2·2	No significant treatment effects at 8 or 16 weeks. At 8 weeks between group differences: −0·7 mm Hg (95 % CI −5·5, 4·2) for SBP and −1·1 mm Hg (95 % CI −4·1, 1·8) for DBP
Witham et al.[88]	Older patients with SBP >140 mm Hg and DBP <90 mm Hg	Placebo or 2500 µg D_3 every 3 months	142	77	1 year	+17·5	NA	No significant treatment effects. Between group differences: SBP 0 mm Hg (95 % CI −2, 2), DBP 0 mm Hg (95 % CI −1, 2)
Witham et al.[89]	Patients with hypertension resistant to conventional treatment	Placebo or 2500 µg vitamin D_3 every 2 months	61	63	6 months	NA	NA	No significant treatment effects. Between group differences: SBP +3 mm Hg (95 % CI −4, 11), DBP −2 mm Hg (95 % CI −6, 2)

NA, not available; SBP, systolic blood pressure; DBP, diastolic blood pressure.

Table 2. Summary of clinical trials investigating the effect of vitamin D supplementation on endothelial function measured using flow-mediated dilation (FMD) and arterial stiffness measured as pulse wave velocity (PWV) in individuals without pre-existing CVD

Study	Population characteristics	Treatment groups	No of participants completed	Mean age (years)	Duration of study	Mean change (Δ) in 25(OH)D (nmol/l) Treatment	Control	Relevant outcomes	Effect of vitamin D v. control
Dong et al.[140]	Normotensive adolescents (boys and girls)	Experimental group receiving 50 µg D_3/d or control group receiving 10 µg/d	44	16	16 weeks	+52·2	+26·5	Arterial stiffness	↓ PWV (7·6 %)
Gepner et al.[98]	Post-menopausal women with serum 25(OH)D 25–150 nmol/l	Placebo or 62·5 µg D_3/d	109	64	4 months	+39·2	−0·5	FMD, PWV	None
Harris et al.[94]	Overweight African–American men and women	Placebo or 1500 µg monthly oral vitamin D_3	45	30	16 weeks	+66·6	+10·5	FMD	↑ FMD (3·1 %)
Larsen et al.[86]	Hypertensive men and women	Placebo or 75 µg vitamin D_3/d	112	61	20 weeks	+52·4	−7·5	PWV	None
Sugden et al.[95]	Men and women with type II diabetes mellitus and serum 25(OH)D <50 nmol/l	Placebo or single oral dose of 2500 µg vitamin D_2	34	64	8 weeks	+22·9	+7·6	FMD	↑ FMD (2·3 %)
Tarcin et al.[93]	Healthy men and women	Treatment group with 25(OH)D < 25 nmol/l 7500 µg D_3 intra-muscularly monthly for 3 months, or control group with 25(OH)D ≥75 nmol/l no supplementation	46	23	3 months	+96·5	NA	FMD	↑ FMD (3·4 %) compared with baseline
Witham et al.[79]	Men and women with type 2 diabetes and baseline 25(OH)D < 100 nmol/l	Placebo or two treatment groups receiving a single dose at baseline of either 2500 µg or 5000 µg D_3	58	66	16 weeks	+18·0 in the 2500 µg group and +28·0 in the 5000 µg group	+8	FMD	None
Witham et al.[96]	Healthy South Asian women with baseline 25(OH)D < 75 nmol/l	Placebo or single oral dose of 2500 µg D_3 at baseline	49	41	8 weeks	NA	NA	FMD, PWV	None
Yiu et al.[97]	Men and women with type 2 diabetes and serum 25(OH)D levels <75 nmol/l	Placebo or 125 µg D_3/d	99	65	12 weeks	+93·6	+4·7	FMD, PWV	None

NA, not available.

supplementation −0·25(95 % CI −0·48, −0·03))[125]. In conclusion, it would appear that vitamin D does not affect insulin sensitivity and that the association of plasma 25(OH)D with insulin resistance may be because VDBP is a negative acute phase reactant.

Randomised controlled trials of the effect of vitamin D supplementation on mortality/clinical endpoints for CVD

A meta-analysis of fifty-six RCT found that vitamin D supplementation slightly decreased mortality risk (RR 0·97 (95 % CI 0·94, 0·99))[126], but other meta-analyses suggest the protective effects against all-cause mortality if supplementation was given for longer than 3 years (RR 0·95 (95 % CI 0·90, 0·98))[127], and in D_3 trials, but not D_2 trials; RR were 0·89 (95 % CI 0·80, 0·99) and 1·04 (95 % CI 0·97, 1·11), respectively[128]. No significant effects on myocardial infarction or IHD, or stroke or CVD were found in a meta-analysis of RCT by Bolland et al.; RR were 1·02 (95 % CI 0·93, 1·13) for the former outcome (nine trials, 48 647 patients) and 1·01 (95 % CI 0·90, 1·13) for the latter outcome (eight trials 46 431 patients)[129]. However, some of the trials included had compared calcium and vitamin D with a placebo or control and it is not always possible

to disaggregate the calcium/vitamin D effects. If calcium has a negative effect on CVD, it could mask a potential protective effect of vitamin D[130]. Ford *et al.*[131] also found no significant effects in a meta-analysis of twenty-one vitamin D supplementation RCT in participants aged ≥60 years which attempted to adjust for calcium use: hazard ratios for cardiac failure, myocardial infarction and stroke were 0·82 (95 % CI 0·58, 1·15), 0·96 (95 % CI 0·83, 1·10) and 1·07 (95 % CI 0·91, 1·29), respectively. The findings of these meta-analyses are inconclusive and the potential effect size is much smaller than indicated by the observational studies. However, the trials were not designed and powered to assess the effects of vitamin D on CVD as a primary outcome. There are a few large trials in progress mostly using high doses of vitamin D_3 which will report in a few years time (Finnish Vitamin D Trial NCT01463813, Vitamin D and Omega-3 Trial NCT01169259 and Vitamin D Assessment Trial ACTRN12611000402943). As high doses of vitamin D have potential for toxicity, there is also a need for trials using more physiological doses of vitamin D. Trials involving appropriate sun exposure would ensure that toxic levels of vitamin D are not reached as any excess previtamin D_3 or vitamin D_3 from UVB exposure is destroyed by sunlight, but concerns over the risk of skin cancer would make these difficult to implement safely.

Risks and benefits of obtaining vitamin D from food, fortified foods, dietary supplements and sunlight/UVB irradiation

Although exposure to UVB irradiation is the most effective means of increasing vitamin D status, it is associated with a major risk of skin cancer[17]. Many authorities now advise restricting exposure to sunlight and promote the use of UVB filters in cosmetics and sun lotions, which decrease the capacity to synthesise vitamin D[132]. For certain at-risk groups such as people with melanised skin, those confined indoors and individuals who cover up their skin when outdoors, dietary intake of vitamin D is particularly important for maintaining an adequate vitamin D status, especially during the winter months when there is negligible UVB radiation in the UK. We have shown that increasing oily fish intake can significantly increase vitamin D status. In the CRESSIDA RCT (ISRCTN92382106), a group following UK dietary guidelines, including advice to consume one to two portions of oily fish per week for 12 weeks were compared with a control group based on a conventional British dietary pattern that included less than one serving per month of oily fish. Mean vitamin D intake increased from 3·0 to 6·6 µg/d in the dietary guideline group and serum 25(OH)D concentrations at 12 weeks *v.* control were 9·2 nmol/l (95 % CI 4·2, 14·2; *P* < 0·001) greater[133]. However, it has been suggested that intakes of 9 and 28 µg vitamin D /d would be needed to maintain winter 25(OH)D concentrations above 25 or 50 nmol/l, respectively, in 97·5 % of the population[134,135].

Diet alone may be insufficient to meet needs in all people and consideration needs to be given to supplementation and fortification of foods. Supplementation requires remembering to take the supplement on a regular basis or having it regularly administered, but it can be an effective targeted intervention that is possible to adapt to an individual's needs. Massive dosage typically by intramuscular injection can also be a remedy for low vitamin D status in at-risk groups, although it does require medical personnel to administer the injection. Fortification of food can be a very effective means of delivering vitamin D providing the food is consumed regularly by a large proportion of the population. Although it may not result in a sufficient status, even low dose vitamin D provided in a fortified malted milk drink at 5 or 10 µg/d is able to significantly increase serum 25(OH)D concentrations[38]. In the UK, foods tend to be fortified with small quantities based on the serving size, for example, breakfast cereals contain approximately 1·3 µg per 30 g portion and margarine 0·75 µg for every two teaspoons. Whilst the current levels of fortification do make a difference to vitamin D status as shown in the most recent National Diet and Nutrition Survey in adults aged 19–64 years where 19 % of vitamin D in the diet was coming from fat spreads and 13 % from cereals and cereal products, fortification at higher levels and in a product which is consumed widely in a high proportion of the population may be needed to help bring a greater proportion of the population to an adequate vitamin D status. We have demonstrated that a malted milk drink is an effective vehicle for vitamin D, but a product such as milk which is consumed extensively may be more effective. However, the potential hazards of inducing toxicity in a small minority have to be considered when fortifying foods. Although an upper level has not yet been established in the UK due to a lack of data, 25 µg/d has been considered as safe and unlikely to cause adverse effects in the general population[136]. This is low though compared with the tolerable upper intake level of 50 µg/d in North America and Europe[137,138]. Before policies could be introduced for fortification of a widely consumed product nationwide, it would be necessary to assess the risk and benefits in different vehicles. One approach is to use that employed by the EU Benefit-Risk Analysis of Foods project which included an estimation of the risk and benefits of universal folic acid fortification of flour/bread[139]. At present, foods are fortified with either D_2 or D_3, although most often D_3 is used. As the forms seem to be equipotent at low doses, D_2 is likely to be important for widespread fortification as it is more acceptable to vegetarians, vegans and religious groups who may not consume D_3 due to it being from animal sources. Another advantage of fortifying foods with vitamin D_2 is that it would allow levels of consumption to be monitored by measuring 25(OH)D_2 concentrations, whereas D_3 is also produced on exposure of the skin to UVB radiation. However, data are lacking on the bioavailability of vitamin D_2 and D_3 in different matrices and there is a need for further research in this area.

Conclusions

Although the observational evidence suggests that a low vitamin D status may increase the risk of CVD by about 40 %, this size effect is not supported by current evidence from clinical trials. Long-term, good quality, sufficiently powered and well-controlled RCT of several years duration are needed to examine the effect of vitamin D supplementation on clinical CVD endpoints in at risk populations.

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Conflicts of Interest

None.

Authorship

C. M. F. drafted the present paper and T. A. B. S. discussed and modified it.

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