Vitamin D and cardiometabolic health: a review of the evidence

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Abstract

The cardiometabolic syndrome (MetS) is a clustering of related metabolic abnormalities including abdominal adiposity, insulin resistance, hypertension, dyslipidaemia and increased inflammatory and thrombotic markers, which is linked to increased risk of type 2 diabetes, CVD and overall mortality. Several cross-sectional and prospective studies have shown an association between low vitamin D status, as indicated by concentrations of serum 25-hydroxyvitamin D (s25(OH)D), and increased prevalence of the MetS and individual CVD risk factors. These epidemiological observations are supported by mechanistic studies but experimental data are limited. The available data from intervention studies are largely confounded as most vitamin D supplementation trials were mainly carried out to explore the role of Ca in CVD and include Ca in the treatment arms. Inadequate consideration of seasonal effects on s25(OH)D concentrations is also a common design flaw in most studies. Further complications arise from shared risk factors such as adiposity and ageing, which predispose individuals to exhibit both a more pronounced risk profile and relatively lower s25(OH)D concentrations. In conclusion, while epidemiological associations are promising and a rationale for low vitamin D status as a potentially modifiable risk factor for CVD is supported by mechanistic data, suitable experimental data from appropriately designed trials are just beginning to emerge. As yet, this body of literature is too immature to draw firm conclusions on the role of vitamin D in CVD prevention. Carefully controlled vitamin D trials in well-described population groups using intervention doses that are titrated against target s25(OH)D concentrations could yield potentially valuable outcomes that may have a positive impact on CVD risk modification.

Key words: Vitamin D; CVD: Metabolic syndrome; Type 2 diabetes; Obesity; Hypertension; Inflammation; Insulin resistance

Introduction

CVD is a major cause of mortality and morbidity. In 2005, CVD accounted for 35·3% of all deaths in the USA\(^1\). Similarly, in Ireland, CVD resulted in 35% of all deaths in 2006\(^2\). Non-modifiable risk factors of CVD include increasing age, sex, family history and ethnicity\(^3\), whereas modifiable risk factors include abnormal blood lipids (low HDL-cholesterol, apoB/apoA1), smoking, diabetes, high blood pressure, abdominal obesity, lack of physical exercise, diet, psychosocial stress and over-consumption of alcohol\(^4\).

The cardiometabolic syndrome (MetS), first described by Kylin\(^5\), is defined as a clustering of metabolic abnormalities including obesity, high fasting blood glucose, insulin resistance, hypertension and dyslipidaemia, which are linked to increased risks of serious health complications such as type 2 diabetes, CVD, stroke and kidney failure. An estimated 47 million US residents have the MetS; the prevalence ranges from 6·7% among young adults aged 20–29 years to 43·5% among older adults aged 60–69 years and 42% among those aged ≥70 years\(^6\). The prevalence of the MetS is 15% in non-diabetic European adults over 30 years\(^7\) and this is expected to rise with increasing prevalence of obesity. In a subsample of middle-aged Irish men and women (n 1018; aged 50–69 years), Villegas et al.\(^8\) estimated the prevalence of the MetS to be 21% using the definition proposed by the WHO\(^9\) or 20·7% according to Third National Cholesterol Education Program Adult Treatment Panel (NCEP/ATP III) criteria\(^10\).

Identification of the multiple genetic and environmental factors that predispose some overweight individuals to developing the various metabolic disorders that constitute the MetS would be a valuable step in helping to address the condition. In this context, nutrition, including vitamin D status, is potentially a modifiable risk factor. Numerous reports have linked low vitamin D status with an increased

Abbreviations: 1,25(OH)\(_2\)D, 1,25-dihydroxyvitamin D; 25(OH)D, 25-hydroxyvitamin D; CRP, C-reactive protein; CYP27B1, 25-hydroxyvitamin D\(_1\)-\(\alpha\)-hydroxylase; DBP, diastolic blood pressure; HOMA-IR, homeostasis model assessment of insulin resistance; HR, hazard ratio; IGF, insulin-like growth factor; MetS, cardiometabolic syndrome; MMP, matrix metalloproteinase; NCEP/ATP III, Third National Cholesterol Education Program Adult Treatment Panel; NHANES, National Health and Nutrition Examination Survey; PAI-1, plasminogen activator inhibitor type 1; PTH, parathyroid hormone; RAS, renin–angiotensin system; RCT, randomised controlled trial; RR, relative risk; RXR, retinoic acid X receptor; s25(OH)D, serum 25-hydroxyvitamin D; SBP, systolic blood pressure; TIMP, tissue inhibitor of metalloproteinase; UVB, UV blue; VDR, vitamin D receptor.

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risk of the MetS and CVD risk\textsuperscript{(11,12)}. Therefore, given both the increasing prevalence of the MetS among adults and evidence of widespread low vitamin D status, exploration of these relationships could be a valuable step towards addressing one (or both) problems.

**Scope of review and approach**

The present review will describe epidemiological associations between vitamin D and cardiometabolic health. It will outline the mechanisms proposed for the association and will evaluate the recent experimental evidence. Searches were made on PubMed and ISI Web of Knowledge\textsuperscript{sm} up to May 2010 using search terms to capture generic and specific words relevant to the topic, including ‘vitamin D’, ‘cholecalciferol’, ‘cardiovascular disease’, ‘metabolic syndrome’, ‘cardio-metabolic syndrome’, ‘blood pressure’, ‘hypertension’, ‘glucose’, ‘diabetes’, ‘insulin’, ‘insulin resistance’, ‘glucose metabolism’, ‘lipids’, ‘dyslipidemia’, ‘cholesterol’, ‘high-density lipoprotein’, ‘HDL’, ‘low-density lipoprotein’, ‘LDL’, ‘inflammation’, ‘C-reactive protein’, ‘CRP’, ‘interleukin-6’, ‘IL-6’, ‘tumour necrosis factor alpha’, ‘TNF-α’, ‘plasminogen activator inhibitor type 1’ (PAI-1), ‘matrix metalloproteinases’, ‘adhesion molecules’ (ICAM-1 and VCAM-1), and combinations of these. We also manually searched bibliography lists of some primary articles for additional studies. The search on randomised controlled trials (RCT) was carried out from January 1990 to May 2010, and excluded RCT that used calcitriol (1,25-dihydroxyvitamin D (1,25(OH)\textsubscript{2}D)) or vitamin D analogues (for example, 1α-calcidiol). RCT using cholecalciferol or ergocalciferol, alone or in combination with Ca, were included; however, no RCT using ergocalciferol were identified in the time-frame specified, except for one which looked primarily at risk of falls. We restricted the search to articles in English.

**Vitamin D endocrine system**

The two most common forms of vitamin D are ergocalciferol (vitamin D\textsubscript{2}) and cholecalciferol (vitamin D\textsubscript{3}). Cholecalciferol is synthesised on skin exposure to UV light (UVB) radiation. In the skin, 7-dehydrocholesterol is converted to precholecalciferol (pre-vitamin D), which is unstable. Through thermal isomerisation, pre-vitamin D is converted to cholecalciferol. Extra vitamin D\textsubscript{3} can be obtained from the diet, although sources are limited and include oily fish, egg yolks and fortified foods. Ergocalciferol is produced by UV irradiation of the fungal steroid ergosterol and is used in some vitamin D supplements. Although the relative ability of vitamin D\textsubscript{2} to increase vitamin D status (as indicated by serum concentrations of 25-hydroxyvitamin D (s25(OH)D)) compared with vitamin D\textsubscript{3} was previously disputed, recent data from Holick et al.\textsuperscript{(13)} showed that vitamin D\textsubscript{2} was as effective as vitamin D\textsubscript{3}.

Both forms of vitamin D eventually enter the circulation and, bound to the vitamin D binding protein, are transported to the liver where the first of two hydroxylation steps occurs, producing the predominant circulating vitamin D metabolite, 25-hydroxyvitamin D (25(OH)D). Still bound to the vitamin D binding protein, 25(OH)D is transported around the body. The enzyme, 25-hydroxyvitamin D\textsubscript{3}-1-α-hydroxylase (CYP27B1), which is a member of the P\textsubscript{450} family, is responsible for the second hydroxylation step, which produces 1,25(OH)\textsubscript{2}D, the active form of vitamin D. While CYP27B1 is located in many cells throughout the body, including smooth muscle cells, immune cells and pancreatic β-cells, the primary site of this hydroxylation step is in the mitochondria of the renal cortex, which appears to be tightly controlled by parathyroid hormone (PTH) for the maintenance of Ca homeostasis. However, non-renal CYP27B1 appears to operate outside this control\textsuperscript{(14)}. The most accurate method of determining an individual’s vitamin D status is to measure 25(OH)D concentrations in serum. Serum 25(OH)D circulates at about 1000 times the concentration of 1,25(OH)\textsubscript{2}D and has a longer half-life of approximately 2 weeks\textsuperscript{(15)}.

The action of vitamin D is achieved through a vitamin D receptor (VDR)-mediated mechanism in which 1,25(OH)\textsubscript{2}D regulates transcription of a wide variety of genes in vitamin D target cells\textsuperscript{(16)}. This mechanism involves forming a heterodimeric complex with a retinoic acid X receptor (1,25(OH)\textsubscript{2}D/VDR/RXR); once this complex binds with a vitamin D-responsive element it can exert various actions. In addition, 1,25(OH)\textsubscript{2}D can act through intra-cellular signalling pathways activated via plasma membrane receptors. The VDR has been identified in most body cells, including the small intestine, colon, brain, skin, prostate, gonads, breast, lymphocytes, macrophages, smooth muscle and β-cells\textsuperscript{(17)}, which has led to the hypothesis that vitamin D is implicated in the pathogenesis of many disorders, including certain types of cancer, diabetes (type 1 and type 2), CVD and several immune and inflammatory-related diseases\textsuperscript{(15,17)}.

**Links between vitamin D and CVD**

The story linking vitamin D and CVD has evolved substantially since the first studies, which date back to the 1960s. Data from animal studies suggested that hypevitaminosis D caused calcification and was involved in the pathogenesis of atherosclerosis\textsuperscript{(18–20)}. In human subjects, Linden\textsuperscript{(21)} showed evidence indicating that supplementary vitamin D was hypercholesterolaemic and increased the risk of myocardial infarction, and few studies found contradictory results\textsuperscript{(22,23)}. Recent research in the field has proceeded along conventional lines, where mechanistic and experimental data seek to explore, explain, prove or dismiss epidemiological associations between low vitamin D status and indicators of metabolic dysfunction, the MetS and CVD.

With the increasing prevalence of obesity and a greater emphasis on the continuum which represents metabolic...
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data from the Health Professionals Follow-up Study, an important target tissue for vitamin D(24). Actions including RAS have all been described(25). Cytokines and regulation of the renin–angiotensin system include smooth muscle proliferation, modulation of inflammatory cytokines and regulation of the renin–angiotensin system (RAS) have all been described(25).

To help the reader to weave through the large body of data available on this topic, we have taken a similar approach for each section of the present review: with respect to CVD, type 2 diabetes and the MetS as primary adverse health outcomes, as well as for contributing clinical risk factors, such as obesity, hypertension, dyslipidaemia, insulin resistance and inflammation, we have summarised epidemiological associations between the prevalence of each outcome or risk factor and vitamin D status, and followed this with an appraisal of the experimental and mechanistic data available.

CVD

It is interesting to note that the rate of CVD-related death is elevated at higher latitudes. However, data directly relating vitamin D status to the risk of CVD are meagre(26). Using data from the Health Professionals Follow-up Study, Giovannucci et al.(27) showed that men (n 18 225) with a 25(OH)D level < 37·5 nmol/l were at increased risk for myocardial infarction (RR 2·09; 95 % CI 1·24, 3·54) compared with those above 75 nmol/l after controlling for age, smoking status, BMI, family history, alcohol consumption, physical activity, history of diabetes mellitus and hypertension, ethnicity, marine n-3 intake, LDL- and HDL-cholesterol and TAG. Similarly, the Ludwigshafen Risk and Cardiovascular Health study reported an independent association of low s25(OH)D (median 19 nmol/l) with all-cause (hazard ratio (HR) 2·08; 95 % CI 1·60, 2·70) and cardiovascular mortality (HR 2·22; 95 % CI 1·57, 3·13) compared with high s25(OH)D (median 70·9 nmol/l)(12). With respect to intervention studies, two large RCT have examined the effect of cholecalciferol (alone(28) or in combination with Ca(29)) on CVD events. In the UK, Trivedi et al.(28) showed no effect of 100 000 IU cholecalciferol every 4 months for 5 years (about 20 μg/d) on mortality from CVD in 2686 elderly adults (although it was suggestive of a reduced risk; RR 0·84; 95 % CI 0·65, 1·0; P = 0·2). It is important, however, to note that the primary outcome of this trial was fracture incidence, for which a protective effect of supplementation was noted. Hsia et al.(29) evaluated the risk of coronary and cerebrovascular events in the Women’s Health Initiative trial of Ca plus vitamin D supplementation. This trial appears several times during the course of the present review. Briefly, 36 282 postmenopausal women aged between 50 and 79 years were randomised to receive either 1000 mg elemental Ca plus 10 μg cholecalciferol per d, or a matching placebo. After 7 years of follow-up, no effect on coronary or cerebrovascular risk was observed(29). Similarly, a more recent analysis of the Women’s Health Initiative reported no difference in total and cause-specific mortality (including CVD and CHD deaths) in those who received the Ca and vitamin D supplementation compared with placebo(30). There are a number of points to note with respect to the conduct of this trial and implications of the study outcomes: vitamin D and Ca supplementation was permitted among participants in addition to the randomisation protocol. Moreover, compliance was low, with approximately 60 % of participants adhering to an 80 % compliance rate. Lastly, habitual intakes of vitamin D and Ca were quite high (about 9·2 μg/d and about 1150 mg/d, respectively). A third RCT, albeit with falls prevention as the primary outcome, looked at the effect of intervening with ergocalciferol (25 μg/d) plus Ca (1000 mg/d) for 1 year in elderly women (aged 70–90 years) with a baseline s25(OH)D < 60 nmol/l(31). While the intervention reduced the risk of falls there was no effect on the risk of IHD or stroke. A recent meta-analysis(32) reviewing whether Ca supplementation increased the risk of myocardial infarction and other cardiovascular events in adults (aged > 40 years) with, or at risk of osteoporosis, showed that Ca supplementation (> 500 mg/d) increased the risk of myocardial infarction (HR 1·31; 95 % CI 1·02, 1·67). These data imply that giving Ca with vitamin D in RCT may obscure any benefits of vitamin D on reducing cardiovascular events and strengthens the need for vitamin D-only RCT to examine CVD incidence and mortality as a primary outcome.

Cardiometabolic syndrome

Several sets of definitive criteria are available to describe the MetS, which are used to guide therapeutic and clinical interventions as well as public health policy. The two most commonly used definitions are provided by the US National Cholesterol Education Program report(10) and the International Diabetes Federation(33). The two definitions are similar and will identify many of the same individuals as having the MetS. However, the International Diabetes Federation regards an increased waist circumference as an essential criterion for diagnosis, while the NCEP/ATP III requires the presence of any three criteria as sufficient, plus, while the International Diabetes Federation uses ethnic-specific cut-offs for waist circumference, the NCEP/ATP III use only one set of cut-offs, regardless of ethnicity.

The importance of identifying subjects with the MetS is because they are at increased risk of developing CVD, type 2 diabetes, stroke and kidney failure and in addition,
each component can be treated on an individual basis. Hu et al.\(^5\), using data from eleven prospective European cohort studies, reported HR for cardiovascular mortality in adults with the MetS (defined by WHO criteria) of 2.26 (95% CI 1.61, 3.17) and 2.78 (95% CI 1.57, 4.94) in men and women, respectively, after controlling for age, blood cholesterol concentration and smoking. Clustering these risk factors has advantages over the analysis of the single entities by themselves. A prospective study, with a 6-year follow-up of first-degree relatives of patients with type 2 diabetes (\(n = 4483\)), showed the relative risk (RR) for having CHD was greater in patients with the MetS (RR 2.96) compared with those in the highest quintile (7), using data from eleven prospective European studies. A recent cross-sectional analysis of 1654 men and women reported a substantially lower OR for the MetS in the lowest quintile (median 26.8 nmol/l; OR 0.46)\(^39\). McGill et al.\(^41\) showed in 243 obese adults, 80% of whom were women (age 47-6 (sd 12) years), that while s25(OH)D concentration was inversely associated with waist circumference, BMI and HbA\(_1c\), there was no association with the MetS. Using data from the Rancho Bernardo Study (\(n = 1070\); aged 44–96 years), Reis et al.\(^42\) showed no association between s25(OH)D and MetS risk despite the strong positive association between PTH and MetS risk (OR 2.02; 95% CI 0.96, 4.24). In this latter study mean s25(OH)D concentration was high (men, 108.9 nmol/l; women, 101.6 nmol/l) which may hide an association, if apparent. A recent systematic review and meta-analysis, using data from eight cross-sectional studies, showed that the prevalence of the MetS was reduced by approximately 50% (OR 0.49; 95% CI 0.38, 0.64) by having high s25(OH)D concentration\(^43\).

**Glucose and insulin metabolism and type 2 diabetes**

Impaired glucose-mediated insulin production and/or insulin resistance are key components of the MetS. Defects in pancreatic \(\beta\)-cell function, insulin sensitivity, and systemic inflammation are often present before glucose intolerance and/or type 2 diabetes develop\(^7,44\). There are several potential mechanisms by which vitamin D may influence glucose tolerance, and insulin release or sensitivity\(^45,46\).

Firstly, pancreatic \(\beta\)-cells contain VDR and express \(\alpha\)-hydroxylase\(^47\). Zeitz et al.\(^48\) reported that circulating glucose concentration was higher and insulin concentration was lower in mice with non-functioning VDR compared with wild-type mice. Local production of 1,25(OH)\(_2\)D enhances the synthesis and release of insulin by \(\beta\)-cells in response to glucose stimulation\(^49\). Secondly, calbindin, a vitamin D-dependent Ca-binding protein, has long been known to be present in the pancreas of various species, including humans\(^50,51\). Sooy et al.\(^52\) showed that calbindin is an important regulator of insulin production, by regulating Ca concentration in the \(\beta\)-cell. Thirdly, s25(OH)D concentration was related to insulin sensitivity in a group of healthy non-diabetic adults\(^53\), and a recent animal study demonstrated that while 1,25(OH)\(_2\)D treatment had no effect on insulin receptor expression in non-diabetic rats, it normalised the number of insulin receptors and improved insulin response to glucose transport in adipocytes in streptozotocin-induced diabetic rats, most probably by direct transcriptional activation of the rat insulin receptor gene\(^54\).

Elevated PTH concentrations have been shown to be inversely associated with insulin sensitivity in healthy adults\(^55\), possibly through its effects on intracellular free Ca concentration of the pancreatic \(\beta\)-cell, adipocytes or...
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In muscle cells, PTH and not vitamin D has been associated with glucose tolerance; however, other studies have found no association.

VDR gene polymorphisms may also have a role to play. Several polymorphisms in the VDR gene have been described, with ApaI, BsmI, TaqI and FokI being the most commonly investigated in relation to type 2 diabetes, glucose homeostasis and insulin release. Numerous case–control studies have found no difference in the distribution of VDR polymorphisms between diabetic patients and control subjects; however, an association with a specific aspect, such as glucose intolerance or insulin secretion has been observed frequently. Hitman et al. showed a strong association between higher insulin secretion and ApaI VDR polymorphism (homozygous for the A allele) in healthy Bangladeshi adults living in London and at risk of type 2 diabetes, independent of vitamin D status. An extension to this study showed that VDR polymorphisms of TaqI and ApaI and BsmI in all subjects showed no difference in glucose intolerance and insulin secretion. These contrasting results should be further explored, including possible interactions with potential confounders such as IGF-1 and its binding proteins, as well as PTH.

Other potential mechanisms may include effects through inflammatory processes. Systemic inflammation is upregulated in type 2 diabetic patients. While systemic inflammation has mainly been implicated in insulin resistance, elevated cytokines may initiate β-cell apoptosis. Vitamin D, through its inhibitory effect on pro-inflammatory cytokines, may improve insulin sensitivity and encourage β-cell survival. Interactions between vitamin D and inflammatory processes are described in more detail below.

Clinical evidence – observational studies. A high dietary intake of vitamin D and/or Ca, and dairy foods has been inversely associated with glucose and insulin concentration and incidence of type 2 diabetes. Using data from the Women’s Health Study, Liu et al. reported that a total vitamin D intake above about 15 μg/d was associated with a lower risk of incident type 2 diabetes compared with intakes below 4 μg/d (P=0.02). Another prospective study, using data from the first Nurses’ Health Study (n 83 779), reported that women who consumed ≥ 20 μg/d of vitamin D had a 23% lower risk of developing type 2 diabetes compared with women who consumed ≤ 5 μg vitamin D/d after controlling for BMI, age, physical activity, alcohol and caffeine use, family history of diabetes, smoking, hypertension and state of residence (additional adjustment for other dietary factors removed significance). These effects reflected intakes from supplements; women who obtained ≥ 10 μg/d vitamin D from supplements had a 13% lower risk of type 2 diabetes than women who took ≤ 2.5 μg/d, after adjusting for the same covariates plus dietary factors including Ca. This prospective study showed the effects of Ca and vitamin D in combination, in that women with the highest vitamin D (≥ 20 μg/d) and highest Ca intakes (1200 mg/d) had the lowest risk of diabetes (RR 0.67; 95% CI 0.49, 0.90).

Using data from NHANES III, Scragg et al. reported that s25(OH)D concentration was inversely related to the presence of type 2 diabetes and increased insulin resistance in US adults (aged 40–74 years); the OR for diabetes was 0.25 (95% CI 0.11, 0.60) in non-Hispanic whites with s25(OH)D concentration ≥ 81 nmol/l compared with those ≤ 43.9 nmol/l. A cross-sectional analysis of the Framingham Offspring Study in 808 non-diabetic subjects (mean age 59.6 (sd 0.3) years) found that plasma 25(OH)D concentration (mean 47.4 (sd 0.6) nmol/l) was inversely associated with fasting plasma glucose, insulin and the homeostasis model assessment of insulin resistance (HOMA-IR, an indicator of insulin resistance), but not 2 h post-oral glucose tolerance test, after adjusting for age, sex, BMI, waist circumference and current smoking habits. Subjects in the top third of vitamin D status (s25(OH)D ≥ 53.4 nmol/l) had a 1.6-fold lower concentration of fasting plasma glucose (P for trend=0.007), a 9.8% lower concentration of fasting plasma insulin (P for trend=0.001) and a 12.7% lower HOMA-IR score (P for trend<0.001) compared with subjects in the bottom third of vitamin D status (s25(OH)D ≤ 38.6 nmol/l). The HOMA-IR was only decreased in subjects with a plasma 25(OH)D concentration > 81 nmol/l. In the Medical Research Council Ely Prospective Study, Forouhi et al. reported that s25(OH)D concentrations at baseline were inversely related with 10-year risk of hyperglycaemia, insulin resistance and the MetS. In a systematic review by Pittas et al. using data from four studies, a summary OR for the association between s25(OH)D and type 2 diabetes of 0.54 (95% CI 0.23, 1.27) was reported for the highest s25(OH)D concentration (62.5–95 nmol/l) v. the lowest (25–57.5 nmol/l).

There are three possible mechanisms for the development of the chronic complications of diabetes, glycation, polyol pathway and oxidative stress. Naturally, Hb is glycated to some extent (5–10%), but this is increased (2- to 3-fold) in diabetics. Glycated Hb (most commonly HbA1c) levels are used as an index of recent blood glucose control. Inverse associations have been observed between s25(OH)D concentration and HbA1c, which may be more marked in obese subjects.

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<td>Nagpal et al. (2009)(83)</td>
<td>India</td>
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<td>Thirty-two active</td>
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<td>Andersen et al. (2009)(161)</td>
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<td>Sixty-one active</td>
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<td>First-phase insulin secretion: improved; second-phase insulin secretion: no change; IR: no change</td>
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<td>Twelve active</td>
<td>12.5 µg/d i.v.</td>
<td>10 d</td>
<td>CRP, IL-6: decreased</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td>Ten active</td>
<td>5 µg/d i.v.</td>
<td></td>
<td>TNF-α, IL-1: no change</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Twenty-two control</td>
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</tbody>
</table>
**Intervention studies.** Data from RCT are relatively rare and to the best of our knowledge have been limited to patients with type 2 diabetes or other underlying diseases (for example, uraemia), patients who are overweight, insulin resistant or vitamin D deficient. RCT with cholecalciferol (as a single treatment; see Table 1) improved insulin response to glucose in some\(^\text{88,89}\), but not all studies\(^\text{90}\). In India, Nagpal et al\(^\text{91}\) showed that large doses of vitamin D\(_3\) (three \(\times\) 3000 \(\mu\)g, fortnightly) improved postprandial insulin sensitivity after 6 weeks in centrally obese healthy men (aged \(\geq\) 35 years). Female type 2 diabetics, the vast majority of whom were vitamin D deficient, had improved first-phase insulin secretion and insulin resistance after supplementation with 33 \(\mu\)g vitamin D\(_3\) per d for 1 month\(^\text{92,93}\). In a more long-term RCT, insulin sensitivity, insulin resistance and fasting insulin concentration were improved after 6 months of supplementation with 100 \(\mu\)g vitamin D\(_3\)/d in South Asian women (\(n=81\)) who were insulin resistant (HOMA-IR \(\geq\) 1.93) and vitamin D deficient (s25(OH)D \(\leq 50 \text{nmol/l}\)) at baseline\(^\text{94}\). In contrast, thirty-six type 2 diabetic patients, without vitamin D deficiency, who were randomised to receive 142.9 \(\mu\)g vitamin D\(_3\)/d or placebo for 6 months, showed no improvement in glycaemic control\(^\text{95}\). Recently we completed an RCT in healthy adults, aged 20–40 years and \(\geq 64\) years, and showed that 15 \(\mu\)g vitamin D\(_3\)/d offset the seasonal increase in fasting glucose concentration in the younger adults (S Muldowney, TR Hill, AJ Lucey, et al., unpublished results).

Another reason why RCT are difficult to interpret is that they have been confounded by the combination of Ca with vitamin D; therefore, in these studies it is not possible to be confident that the response observed is due to the vitamin D and/or Ca. Additionally, if the findings of the recent meta-analysis\(^\text{32}\) with regard to cardiovascular events are correct, giving Ca with vitamin D may occlude the possible beneficial effects of vitamin D. Pittas et al\(^\text{96}\) completed a post hoc analysis of 314 healthy adults, aged \(\geq 65\) years, who had originally completed a double-blind, placebo-controlled trial designed to assess skeletal outcomes, where subjects received either 500 mg calcium carbonate and 17.5 \(\mu\)g vitamin D\(_3\) or a placebo for 7 years\(^\text{97}\). Positive effects of the supplementation compared with placebo on fasting plasma glucose and HOMA-IR were only apparent in subjects who had impaired fasting glucose at baseline (fasting plasma glucose \(\geq 5.6 \text{mmol/l}\)). In a large double-blind RCT (\(n=330\)) in Norway, no effect of 500 mg Ca daily plus vitamin D\(_3\) (500 or 1000 \(\mu\)g/week) was shown in overweight and obese (\(>28–47 \text{kg/m}^2\)) subjects on glucose metabolism after 1 year\(^\text{98}\). Similarly, using data from the Women’s Health Initiative, de Boer et al\(^\text{99}\) showed that the incidence of diabetes at age 7 years did not differ between those receiving Ca and vitamin D compared with those receiving the placebo and there was no difference in fasting plasma glucose, insulin or HOMA-IR. Thus, the association between s25(OH)D concentrations and altered glucose and insulin metabolism reported

<table>
<thead>
<tr>
<th>Study</th>
<th>Country</th>
<th>Type</th>
<th>Gender</th>
<th>Age</th>
<th>Design</th>
<th>Duration</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Timms et al. (2002)(^\text{10})</td>
<td>UK</td>
<td>RDBPCT</td>
<td>M and F, aged 30–60 years</td>
<td>25–40</td>
<td>3-monthly 1250 (\mu)g or 12·5 (\mu)g i.m.</td>
<td>12 months</td>
<td>CRP: decreased, MMP-9, MMP-2, TIMP-1: decreased</td>
</tr>
<tr>
<td>Scragg (1995)(^\text{11})</td>
<td>UK</td>
<td>RDBPCT</td>
<td>M and F, aged 30–60 years</td>
<td>25–40</td>
<td>Six type 2 diabetic patients, without vitamin D deficiency</td>
<td>6 months</td>
<td>No improvement in glycaemic control</td>
</tr>
<tr>
<td>Raghuramulu et al. (1992)(^\text{12})</td>
<td>India</td>
<td>RDBPCT</td>
<td>M, male; F, female; YA, young adults; OA, older adults</td>
<td>30–60</td>
<td>Randomised, double-blind, placebo-controlled trial</td>
<td>30–60 years</td>
<td>CRP: decreased; MMP-9, MMP-2, TIMP-1: decreased</td>
</tr>
</tbody>
</table>

\(\mu\)g = microgram
widely in cross-sectional and prospective studies requires confirmation by RCT using vitamin D only.

**Obesity**

The prevalence of obesity is increasing worldwide, most probably due to positive energy balance associated with increasing sedentary lifestyles. The WHO estimated that 1.6 billion adults were overweight and 400 million adults were obese worldwide in 2005\(^{(91)}\). In the USA, age-adjusted prevalence of obesity increased from 22.9% in NHANES III (1988–94) to 30.5% in 1999–2000\(^{(92)}\). Using data from the North/South Ireland Food Consumption Survey, McCarthy *et al.*\(^{(93)}\) reported that 39% of Irish adults were overweight and 17.8% were obese, which compared with data from the Irish Nutrition Survey, 1990\(^{(94)}\), is nearly a two-fold increase in obesity in 10 years (1990–2000). In recent years, the evidence for excess body weight as a causative agent in the MetS has encouraged the use of an adiposity measure as an essential element of defining the MetS\(^{(53)}\).

Numerous studies have observed that obesity is a negative predictor of vitamin D status (for a review, see Pérez-López\(^{(95)}\)). For example, data from the sixth Tromso study, which was a longitudinal analysis\(^{(96)}\), showed an inverse association between s25(OH)D concentration and BMI in over 10000 adults. Hypponen & Power\(^{(82)}\), using data from the 1958 British birth cohort (n 7189), observed that 80% of subjects (BMI > 30 kg/m\(^2\)) had a s25(OH)D < 75 nmol/l, compared with 68% of the non-obese subjects. In another study on 243 ambulant adults (BMI 28–50 kg/m\(^2\)) enrolled in a weight-loss study, s25(OH)D concentration was inversely associated with weight (P = 0.0009), BMI (P = 0.005) and waist circumference (P = 0.03), but not body fat percentage\(^{(41)}\). This study also estimated a decrease of 0.74 nmol/l in s25(OH)D per 1 kg/m\(^2\) increase in BMI.

Compston *et al.*\(^{(97)}\) suggested that the inverse association between s25(OH)D and adiposity could be explained by the observation that obese individuals spend less time outdoors and are therefore exposed to less UVB radiation. However, a recent cross-sectional analysis of 385 healthy adults, aged \(\geq 65\) years, showed that sun exposure did not explain the inverse association between s25(OH)D levels and adiposity\(^{(98)}\). Bell *et al.*\(^{(99)}\) proposed that the vitamin D endocrine system is altered in obese subjects, with increased production of 1,25(OH)\(_2\)D exerting negative feedback control on the hepatic synthesis of 25(OH)D. In addition, animal studies have shown that 1,25(OH)\(_2\)D increases lipogenesis and decreases lipolysis\(^{(100},(101)}\); and, therefore, may contribute to obesity; however, 1,25(OH)\(_2\)D concentration is not consistently higher in obese subjects\(^{(102},(103)}\). Furthermore, 1,25(OH)\(_2\)D is not consistently related to 25(OH)D status. The efficiency of epidermal vitamin D production does not appear to be impaired in obesity, but adipose tissue appears to sequester cholecalciferol and 25(OH)D, making it unavailable to the circulation; thus obese individuals frequently have low s25(OH)D concentrations\(^{(104)}\). Several years ago, Arunabh *et al.*\(^{(105)}\) concluded that body fatness was inversely related to s25(OH)D levels in healthy women, but more recently, Freedman *et al.*\(^{(106)}\) confirmed a strong inverse association between 25(OH)D and visceral adiposity in African-Americans with diabetes. This latest finding has important implications for study design; if visceral fat is a potentially more powerful determinant of s25(OH)D levels in overweight individuals as well as a prognostic indicator of metabolic dysfunction, it may be more appropriate to measure visceral fat rather than overall body fatness.

**Intervention studies.** There is a plausible explanation to why vitamin D may have a role in weight management; however, the evidence is limited, as most RCT use vitamin D in combination with Ca. In Norway, 334 overweight/obese subjects were randomised to receive 500 mg Ca daily plus vitamin D\(_3\) (500 or 1000 μg/week) or placebo for 1 year\(^{(107)}\). However, there was no change in weight throughout the intervention. Using data from the Women’s Health Initiative, Caan *et al.*\(^{(108)}\) observed that postmenopausal women randomised to receive Ca and vitamin D\(_3\) had smaller average annual weight gain compared with the women taking the placebo; moreover, the favourable effect was strongest among women who had, at baseline, a Ca intake < 1200 mg/d. Another RCT showed healthy, overweight and obese women (n 63), partaking in a 15-week energy restriction (~700 kcal/d; 2930 kJ/d) intervention, who were randomised to receive a daily supplement of 10 μg vitamin D\(_3\) and 1200 mg Ca, improved their blood lipid profile compared with women randomised to receive a placebo\(^{(109)}\); however, no difference in weight loss was observed between treatment groups. A reanalysis of this study, limited to women with a very low Ca intake (baseline Ca < 600 mg/d), showed that women receiving the combined vitamin D and Ca supplement had greater weight loss compared with those receiving the placebo\(^{(110)}\). A more recent study investigated the effects of vitamin D supplementation (83 μg/d) for 1 year on weight loss and cardiovascular risk factors in overweight subjects who were participating in a weight-loss programme\(^{(111)}\). Weight loss was similar in both treatment groups; however, combined vitamin D supplementation with weight loss resulted in a more pronounced improvement in several CVD risk markers than did weight loss alone. This RCT will be described later in the review. On the basis of this evidence, it is impossible to say whether vitamin D has any direct effect on the maintenance of a healthy body weight.

**Elevated blood pressure**

Elevated blood pressure is a common and independent risk factor for CVD, including stroke, heart failure and CHD. The seventh report of the Joint National Committee on prevention, detection, evaluation, and treatment of high blood pressure provides the most recent US classification of blood pressure levels\(^{(112)}\). Using data from NHANES
(1999–2000), Wang & Wang\(^{113}\) reported that 27% of US adults (\(n = 4805\); aged \(\geq 18\) years) had hypertension. The 2007 Survey of Lifestyle, Attitudes and Nutrition (SLÁN) showed in 1207 Irish adults (aged \(\geq 45\) years) that the prevalence of hypertension was 60%\(^{114}\). Hypertension can be treated with antihypertensive medication and improved by changing modifiable risk factors such as body weight, smoking, physical activity and diet. Dietary approaches to reduce blood pressure have focused on the role of Na, fruit and vegetables and Ca. Growing evidence indicates that vitamin D may have an association with blood pressure, although the data are not consistent\(^{115}\).

The role of 1,25(OH)\(_2\)D in the RAS and its association with PTH are potential mechanisms by which vitamin D and blood pressure are linked. The RAS, also known as the renin–angiotensin–aldosterone system, helps regulate blood pressure, electrolyte levels, and volume homeostasis, and excessive RAS stimulation is associated with hypertension. Renin, a protease, primarily synthesised in the juxtaglomerular cell of the kidney, catalyses the conversion of angiotensinogen to angiotensin I, which is further cleaved to angiotensin II by angiotensin-converting enzyme in the capillaries of the lungs. Elevated angiotensin II raises blood pressure in one of two ways: (1) angiotensin II is a potent vasoconstrictor\(^{116}\) and elevates blood pressure by increasing vascular resistance; (2) angiotensin II stimulates aldosterone secretion, which in turn, increases Na reabsorption in the kidney and therefore blood pressure is raised.

Circulating 1,25(OH)\(_2\)D has been shown to play a role in the regulation of the RAS; for example, 1,25(OH)\(_2\)D was negatively correlated \((r = -0.65; P < 0.001)\) with plasma renin activity\(^{117}\). In this study, 1,25(OH)\(_2\)D was elevated in hypertensive subjects with low renin activity but not normotensive or hypertensive subjects with high renin activity. Renin expression and angiotensin II production are elevated in VDR knock-out mice\(^{118}\). However, the expression of angiotensinogen did not differ between VDR knock-out and wild-type mice and this indicates that the elevated production of angiotensin II is primarily due to the increase in renin activity. The observed hypertension, cardiac hypertrophy and increased water intake were corrected by treatment with an angiotensin-converting enzyme inhibitor (captopril) or an angiotensin II AT\(_1\) receptor antagonist (losartan), confirming that it was the RAS responsible for the observed abnormalities. In addition, that study showed that inhibition of 1,25(OH)\(_2\)D led to up-regulation of renin and, furthermore, treatment with 1,25(OH)\(_2\)D suppressed renin expression in wild-type mice. A continuation of this study, using As\(_4\)-1 cells, which are the best available \(in vitro\) model for a juxtaglomerular cell, showed that 1,25(OH)\(_2\)D directly suppresses renin gene transcription, through a VDR-mediated mechanism. These results were supported by a second animal study\(^{119}\), where VDR knock-out mice developed excessive concentrations of renin in their blood, which led to high blood pressure. The molecular effects are still unknown but appear to be independent of both Ca and PTH concentration\(^{119–121}\). Instead, cyclic AMP, which is a major intracellular signal that stimulates renin expression, has been implicated. Yuan \textit{et al.}\(^{122}\) showed that liganded VDR suppresses renin expression by binding to the transcription factor cyclic AMP-response element-binding protein. This inhibits renin transcription, as cyclic AMP-response element-binding protein is no longer available for binding to the cyclic AMP response elements in the promoter region of the renin gene.

Besides its role in Ca homeostasis, PTH has a pro-sclerotic effect on vascular smooth muscle cells\(^{123}\) which may contribute to vessel-wall thickening and subsequent rises in blood pressure. Elevated circulating levels of 1,25(OH)\(_2\)D, as a result of increased PTH, stimulates Ca influx in a variety of cells, including the vascular smooth muscle cells, which results in contraction and increased vascular resistance, i.e. elevated blood pressure\(^{124}\).

**Clinical evidence – observational studies.** The first indication that vitamin D was related to blood pressure in humans was from observations that residents at higher latitudes, who experience decreased UVB exposure as a result, had higher systolic and diastolic blood pressure (SBP and DBP)\(^{125}\). In fact, UVB light was shown, in addition to increasing \(s25(OH)D\) concentration, to lower blood pressure in adults (aged 26–66 years) with essential hypertension\(^{126}\).

Cross-sectional and prospective studies examining the relationship between vitamin D intake and blood pressure have reported conflicting results\(^{127,128}\); however, the evidence appears to be strongest for SBP. Sowers \textit{et al.}\(^{127}\) examined eighty-six normotensive young women (aged 20–35 years) and 222 older normotensive women (aged 55–80 years) to investigate if Ca and vitamin D intakes affected bone density or blood pressure. In the young women, vitamin D intake above 10 \(\mu\)g/d was predictive of a lower SBP \((-6\) mmHg) but no association was apparent with DBP. In the older women, having an intake of both vitamin D and Ca above 10 \(\mu\)g and 800mg/d, respectively, was predictive of lower SBP. In a larger, more recent study of over 15,000 generally healthy Norwegians (aged 25–69 years) who took part in the fourth Tromso study, no association between vitamin D intake and blood pressure was observed after multivariate adjustment\(^{128}\); however, very few subjects had a vitamin D intake \(>10\) \(\mu\)g/d. Results from three large prospective studies, Nurses’ Health Study I (\(n = 77,436\), Nurses’ Health Study II (\(n = 93,803\) and the Health Professionals Follow-up Study (\(n = 38,074\), found no evidence that a higher intake of vitamin D reduced (or increased) the risk of incident hypertension after multiple adjustments and approaches to the analysis\(^{129}\). Diagnosis of hypertension in this study was self-reported, and although self-reported hypertension in a health professionals' study is reasonably reliable, it does cast doubt over the study outcomes. Data from the Women’s Health Study found that intakes of low-fat dairy products, Ca, and vitamin D were
each inversely associated with risk of hypertension in middle-aged and older women\(^{130}\).

The association between \(25(OH)D\) concentration and blood pressure is also inconsistent. Pasco \textit{et al.}\(^{131}\) showed in a sample of Australian women (\(n\) 861; aged 20–92 years) that the odds for having a high blood pressure was reduced in those with a \(25(OH)D\) in the top or middle third compared with those in the lowest third (median 40 nmol/l), after accounting for weight and other potential confounders. Additionally, we recently reported that adolescents with a \(25(OH)D\) < 60·1 nmol/l were more likely (OR 1·94; 95 % CI 1·18, 3·20) to have an elevated blood pressure. Three articles using data from NHANES III have reported on the association between \(25(OH)D\) and blood pressure/hypertension\(^{124–130}\). Scragg \textit{et al.}\(^{134}\) showed that after multivariate adjustment for sex, age, ethnicity and physical activity, mean SBP and DBP were lower in the highest quintile of vitamin D status (\(s25(OH)D > 85·7\) nmol/l) than in the lowest quintile (\(s25(OH)D < 40·4\) nmol/l). Judd \textit{et al.}\(^{135}\) observed an inverse association between \(25(OH)D\) and SBP in white, but not black, men and women; however, adjustment for age removed the association. In addition, this study showed that in white, but not black, subjects a \(25(OH)D\) > 80 nmol/l reduced the age-related increase in SBP by 20 % compared with white subjects with \(25(OH)D < 50\) nmol/l, taking BMI, sex, physical activity and smoking status into consideration. Further analysis of NHANES III indicates that having a \(s25(OH)D\) concentration < 52·5 nmol/l is associated with an increased risk of hypertension (OR 1·30; 95 % CI 1·13, 1·49) compared with having a \(25(OH)D\) \(\geq\) 92·5 nmol/l\(^{136}\). The confounding effects of age and ethnicity in cross-sectional studies could overpower any putative associations between \(25(OH)D\) levels and blood pressure.

The question of whether low vitamin D status is a risk factor for the development of hypertension has been addressed by prospective studies. Using data from the Health Professionals Follow-up Study (\(n\) 613) and the Nurses’ Health Study I (\(n\) 1198), Forman \textit{et al.}\(^{137}\) showed that plasma \(25(OH)D\) concentration was inversely associated with risk of incident hypertension in men and women, after 8 years of follow-up accounting for age, BMI, physical activity, race, menopausal status (for women) and other potential confounders. In analysis of the pooled data (Health Professionals Follow-up Study and Nurses’ Health Study I), after 4 years of follow-up, individuals whose \(25(OH)D\) levels were < 37·5 nmol/l compared with those whose levels were \(\geq\) 75 nmol/l had an RR for incident hypertension of 3·18 (95 % CI 1·39, 7·29). Using data from the Nurses’ Health Study II, Forman \textit{et al.}\(^{138}\) observed that women who were vitamin D deficient (\(25(OH)D < 75\) nmol/l) had a 47 % increased risk (OR 1·47; 95 % CI 1·10, 1·97) of incident hypertension after adjustment compared with those who had \(25(OH)D > 75\) nmol/l. This study estimated that vitamin D deficiency may account for 23·7 % of all new cases of hypertension developing among young women every year. Collectively, these data indicate that the apparent inverse association between vitamin D status and blood pressure and hypertension is of a sufficient magnitude to have a potentially important impact on cardiovascular mortality\(^{139}\).

**Intervention trials.** To date, only one RCT using cholecalciferol only has been completed with blood pressure as the primary outcome (see Table 1). Scragg \textit{et al.}\(^{139}\) randomised elderly (\(n\) 189; aged 63–76 years) subjects to receive a single supra-physiological dose of vitamin D\(_3\) (2·5 mg) or a placebo during the winter; however, it had no effect on SBP or DBP. Unexpectedly, this study did report a decrease in radial pulse rate in subjects receiving the vitamin D, which is a crude measure of cardiac function. The lack of a treatment effect on SBP or DBP in this study may be due to the short follow-up time (5 weeks) but is more probably due to the effect of age itself on blood pressure in subjects who have had hypertension for some time. The vascular damage resulting from long-term hypertension makes older subjects with established hypertension unsuitable candidates for studies of vitamin D supplementation for blood pressure reduction. Some RCT looking at CVD risk factors, or trials more specific to insulin or glucose metabolism, have also reported blood pressure data (see Table 1). We recently used data from a RCT with cholecalciferol to explore its effects on biomarkers of CVD (S Muldowney, TR Hill, AJ Lucey, \textit{et al.}, unpublished results); however, no effect on blood pressure was apparent (in young or older healthy adults). All other vitamin D RCT that reported blood pressure data were in combination with Ca or used UVB therapy\(^{89,126,140–142}\).

In 1990, Orwoll & Oviatt\(^{140}\) reported no effect of Ca (1000 mg/d) and cholecalciferol (25 \(\mu\)g/d) supplementation for 3 years on blood pressure in thirty-five men (aged 30–92 years) compared with thirty age-matched controls, but given the large age range, this study had insufficient power to detect a difference. Similarly, the Women’s Health Initiative found no effect of Ca and cholecalciferol on blood pressure in postmenopausal women\(^{141}\). More recently, Jorde \textit{et al.}\(^{89}\) showed that overweight and obese subjects who were randomised to receive 500 mg Ca daily plus cholecalciferol (500 or 1000 \(\mu\)g/week) for 1 year had a slightly higher increase in SBP compared with those who received the Ca plus a placebo (2·3 mmHg); however, this study was powered to examine an effect on weight loss not blood pressure. On
the other hand, Pfeifer et al.\textsuperscript{(142)} randomised women, aged $\geq 70$ years, who were vitamin D insufficient ($s25(OH)D < 50 \text{ nmol/l}$) to receive daily either 1200 mg Ca or 1200 mg Ca plus 20 $\mu$g cholecalciferol for 8 weeks. SBP fell by 13·1 mmHg in women receiving the vitamin D plus Ca, whereas in women receiving Ca alone, SBP reduced by 5·1 mmHg. No difference in DBP was observed between the two treatment groups.

Mechanistic evidence is strong for an inverse association between vitamin D and blood pressure; however, the data available to date have added little support for a positive effect of vitamin D on blood pressure. A useful approach may be to select slightly younger adults without longstanding hypertension who have low vitamin D status for participation in RCT.

Dyslipidaemia

Cholesterol is an essential substance, synthesised in the body and obtained from the diet, and is necessary for maintaining cell membrane integrity and for the manufacture of hormones\textsuperscript{(143)}. Cholesterol and TAG are transported in the circulation on lipoproteins synthesised in the liver. LDL carries 60–80\% of the body’s cholesterol, while HDL is involved in reverse cholesterol transport. Apoproteins facilitate cholesterol transport by providing recognition sites for cell-surface receptors and acting as cofactors for enzymes involved in the metabolism of lipoproteins. ApoA1 is the major apoprotein on HDL and is anti-atherogenic under most conditions. ApoB is associated with chylomicrons, VLDL (and their remnants) and LDL. All forms of apoB are potentially atherogenic if their metabolism is not regulated properly\textsuperscript{(145–147)}. Lipoprotein(a), a lipoprotein subclass, is found in variable concentrations and its levels are highly heritable. Lipoprotein(a) is a potential risk factor for CHD\textsuperscript{(148)}.

Dyslipidaemia, an elevation in total cholesterol, or LDL-cholesterol, TAG or a reduction in HDL-cholesterol concentration in the circulation, is a potent risk factor for CVD\textsuperscript{(149)}.

Atherosclerosis, the presence of extensive plaque (lipids, cholesterol, Ca and other substances) in the arteries, causes nearly 75\% of all deaths from CVD in the USA. Low HDL-cholesterol and high TAG concentrations are independent predictors of CVD\textsuperscript{(150,151)}. Elevated plasma lipoprotein(a) is an independent risk factor for the development of CHD in men, comparable in magnitude to the total cholesterol concentration $\geq 6·2 \text{ mmol/l}$ or HDL-cholesterol < 0·9 mmol/l\textsuperscript{(152–154)}. Using data from the National Heart, Lung, Blood Institute Family Heart Study, concentrations of lipoprotein(a) > 50 mg/dl (500 mg/l) were shown to increase the risk of early onset of coronary artery disease, especially if combined with other unfavourable lipid levels or non-lipid risk factors, such as hypertension, diabetes or smoking\textsuperscript{(155)}.

Vitamin D status has been associated with dyslipidaemia in some instances but the literature is conflicting and the evidence basis is still being gathered.

Potential mechanisms linking vitamin D and lipids have not been studied in great detail and the focus has been on the effect of Ca, which implicates vitamin D indirectly through its role in maintaining Ca homeostasis. Increased Ca absorption reduces fatty acid absorption and increases faecal fatty acid content, most probably resulting from the formation of insoluble Ca–fatty soaps\textsuperscript{(149–151)}. The decreased absorption of fat, especially saturated fat, reduces serum total cholesterol and LDL-cholesterol. Furthermore, Ca increases the conversion of cholesterol to bile acids for excretion\textsuperscript{(152)} and increased Ca absorption can decrease hepatic TAG formation\textsuperscript{(153)}.

An alternative mechanism may be a consequence of the suppressive effect of 1,25(OH)\textsubscript{2}D on PTH concentration. Raised PTH has been shown to be accompanied by a decrease in plasma post-heparin lipolytic activity\textsuperscript{(154)}; thus the maintenance of appropriate PTH levels with optimal vitamin D status may decrease TAG concentration by increasing peripheral TAG removal.

Clinical evidence – observational studies. Vitamin D status has been inversely related to dyslipidaemia in some instances, but the literature is conflicting. Lipid levels vary seasonally and total cholesterol, LDL-cholesterol and TAG concentrations are higher during the winter\textsuperscript{(155)}.

Many\textsuperscript{(80,136,156)}, but not all\textsuperscript{(53,157,158)}, studies have shown inverse relationships between 25(OH)D and TAG concentrations. Martins et al.\textsuperscript{(156)} showed that in US adults, TAG was lower in those in the top quartile of 25(OH)D ($\geq 92·5 \text{ nmol/l}$) than in those in the bottom quartile (<52·5 nmol/l); however, there was no association between s25(OH)D and total cholesterol. In a smaller sample of 126 glucose-tolerant healthy adults, Chiu et al.\textsuperscript{(53)} observed an inverse association between 25(OH)D and total cholesterol, and LDL-cholesterol (adjusting for sex, age, ethnicity, season, SBP and DBP, BMI, and waist-to-hip ratio); however, no association was apparent between 25(OH)D and HDL-cholesterol or TAG in this study. The sometimes reported association between s25(OH)D and HDL-cholesterol\textsuperscript{(80,157,158)} is not apparent in all studies\textsuperscript{(53)}, but an association between 25(OH)D and apoA1 has been observed\textsuperscript{(157–159)}.

The total cholesterol:HDLC and LDL:HDL ratios are important predictors of increased risk of CVD. Using data from the Framingham Offspring Study ($n$ 1739), Wong et al.\textsuperscript{(160)} observed that the total cholesterol:HDLC ratio was higher in those with s25(OH)D below 37·5 nmol/l. Although we saw no association between vitamin D status and lipid profiles in young European adults (S Muldowney, A Lucey, G Paschos, et al., unpublished results), we did observe associations in a representative sample of 12- and 15-year-old adolescents (M Kiely, S Muldowney, TR Hill, et al., unpublished results); those with a s25(OH)D below about 50 nmol/l were more likely to have elevated total cholesterol (OR 3·09; 95% CI 1·33, 7·18).

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The total cholesterol:HDLC and LDL:HDL ratios are important predictors of increased risk of CVD. Using data from the Framingham Offspring Study ($n$ 1739), Wong et al. observed that the total cholesterol:HDLC ratio was higher in those with s25(OH)D below 37·5 nmol/l. Although we saw no association between vitamin D status and lipid profiles in young European adults (S Muldowney, A Lucey, G Paschos, et al., unpublished results), we did observe associations in a representative sample of 12- and 15-year-old adolescents (M Kiely, S Muldowney, TR Hill, et al., unpublished results); those with a s25(OH)D below about 50 nmol/l were more likely to have elevated total cholesterol (OR 3·09; 95% CI 1·33, 7·18).
7·21) and LDL-cholesterol (OR 2·09; 95% CI 0·95, 4·59) compared with adolescents who had a s25(OH)D > 75 nmol/l. These associations were stronger in boys than girls.

**Intervention trials.** There is a dearth of RCT on the effects of cholecalciferol on lipids (see Table 1). Zittermann et al. (111) showed that treatment with cholecalciferol, parallel with energy restriction (−700 kcal; −2930 kJ) resulted in a more pronounced decrease in TAG concentration (−13·5% compared with +3%), and an increase in LDL-cholesterol than did energy restriction alone. These beneficial effects on TAG concentration were independent of body weight, fat mass and sex. However, supplementation for 1 year with either 10 or 20 µg cholecalciferol in healthy Pakistani immigrants living in Denmark had no effect on total cholesterol, HDL-cholesterol, LDL-cholesterol or VLDL-cholesterol, the LDL:HDL ratio, or TAG concentration164, although the doses were probably not sufficient to achieve a clinically meaningful effect on lipids. At similar doses of between 5 and 15 µg/d, we showed no effect of cholecalciferol supplementation during the winter on lipids (S Muldowney, TR Hill, AJ Lucey, et al., unpublished results) in adults aged 20–40 and > 65 years. On the basis of these data, future interventions need to use doses of vitamin D to achieve targeted s25(OH)D levels, which may have a beneficial effect on lipids. Four other RCT which reported insulin sensitivity/release or blood pressure as the primary outcome reported no effects on lipids(83,85,86,139).

All other RCT investigating effects of cholecalciferol on lipids also gave Ca(89,109,162–164) . Gannage-Yared et al. (162) observed no effect of 1 g Ca plus 20 µg cholecalciferol on LDL-cholesterol, or HDL-cholesterol or TAG concentration in postmenopausal women (n 39); however, this was a short-term trial of 12 weeks with a small number of subjects. Using data from the Women’s Health Initiative, Rajpathak et al. (165) observed no significant effect of 1 g Ca plus 10 µg cholecalciferol on circulating lipids after 5 years. Major et al. (109) showed that 1200 mg Ca plus 10 µg cholecalciferol for 15 weeks lowered total cholesterol:HDL and LDL:HDL ratios and LDL-cholesterol concentrations compared with placebo. These effects were independent of fat mass and waist circumference for the total cholesterol:HDL and LDL:HDL ratio but not for LDL-cholesterol. A recent RCT in which 140 men > 50 years were randomised to receive either milk (400 ml of 1% fat fortified with 1 g Ca and 20 µg vitamin D3) or no milk for 2 years showed no effect on total cholesterol, HDL-cholesterol, or LDL-cholesterol, or TAG concentration164. Jorde et al. (109) showed that 500 mg Ca per d plus 500 or 1000 µg cholecalciferol per week had no effect on lipid profiles after 1 year in 334 overweight and obese subjects.

For many years, treatment for psoriasis has included climate therapy. Osmanacvic et al. (165) showed that psoriasis symptoms improved in twenty patients aged 25–65 years with 15 d of climate therapy in Gran Canaria. The LDL:HDL ratio decreased from 2·4 to 1·9 mmol/l (P<0·001), apoA1 and HDL-cholesterol concentration increased and apoB decreased during the sun exposure period. However, there was no effect on either total or LDL-cholesterol. In contrast, Carbone et al. (158) showed in young healthy adults (n 49) that UVB radiation had no effect on any lipoproteins or apolipoproteins. However, in this study, subgroup analysis showed differential effects of UVB radiation on lipids and lipoproteins according to baseline s25(OH)D concentrations, providing additional evidence for using a modified study design which seeks to achieve specific s25(OH)D levels and accounts for baseline levels in the data analysis.

There is some evidence that vitamin D may have a negative effect on lipids, particularly HDL-cholesterol, which requires further exploration. Postmenopausal women (n 402) in the Kuopio Osteoporosis Study were randomised to receive one of four treatments: (a) sequential combination of 2 mg oestriadiol valerate and 1 mg cyproterone acetate (hormone replacement therapy); (b) 7·5 µg cholecalciferol; (c) hormone replacement therapy and 7·5 µg cholecalciferol; (d) placebo(166). Both the cholecalciferol and placebo groups received 500 mg calcium lactate. The hormone replacement therapy treatment reduced serum total cholesterol, most probably due to the decrease in LDL-cholesterol; however, cholecalciferol supplementation increased LDL-cholesterol by 6% in the 1-year period. In the follow-up study, Heikkinen et al. (167) observed that cholecalciferol supplementation over 3 years lowered HDL-cholesterol. However, as this reduction was also observed in the placebo group, it may not have been an effect of vitamin D. A recent study showed that VDR knock-out mice had higher total and HDL-cholesterol than wild-type mice(168). The higher HDL-cholesterol concentration was only apparent in males(168) and requires replication in further studies before being acknowledged as a real effect. If vitamin D has a negative effect on HDL-cholesterol it may be a consequence of suppressive effects of 1,25(OH)2D on apoA1 gene expression, possibly by altering co-activators and co-repressors(169). Overall, the evidence for a beneficial or negative effect of vitamin D on lipids requires much further exploration. With regard to total cholesterol, LDL-cholesterol and TAG, the evidence leans towards a beneficial association whereas the data on HDL-cholesterol are conflicting.

**Inflammation**

Obesity induces a chronic inflammatory environment, which is implicated in metabolic dysfunction and CVD(170–172). Pro-inflammatory cytokines include IL-1, IL-6 and TNF-α(173), whereas IL-10 has an anti-inflammatory effect(174). C-reactive protein (CRP), an acute-phase reactant, is a clinically validated and widely measured marker of systemic inflammation and increased synthesis can be stimulated by IL-6 and TNF-α. Certain inflammatory cytokines and CRP levels are elevated in adults with obesity, CVD,
type 2 diabetes and the MetS (170,171,175,176). Accumulating evidence for an important role for vitamin D in regulation both of the adaptive and innate immune systems has led to intense interest in this field. Potent immunoregulatory effects of 1,25(OH)2D include anti-proliferative properties, stimulation of production of cathelicidin by macrophages and bacterial killing and modulation of B- and T-lymphocyte proliferation and function (177). Most exciting about the recent discoveries is the role for 25(OH)D in induction of these effects, which means that the potential impact on human health by improving host defence and modulating the inflammatory response is immense (178).

Immune cells not only express the VDR but also CYP27B1 (179,180), allowing them to synthesise and secrete 1,25(OH)2D in a regulated fashion (181). Crucially, as this occurs in an autocrine fashion and not under endocrine control, vitamin D-inactivating hydroxylase is also expressed, which helps to temper the local effects of 1,25(OH)2D, preventing potential damage caused by over-stimulation of the immune system (177,182). 1,25(OH)2D uses several different molecular mechanisms to regulate cytokine expression, either directly by targeting transcription initiation and regulation or indirectly interfering with other intracellular signalling pathways (183). The 1,25(OH)2D/VDR/RXR complex can occupy the nuclear factor of activated T cells at the nuclear factor of activated T cells-1 site, in the promoter region of some cytokines, thereby preventing T cell-specific transcription factor formation with a complex with activator protein 1, which is necessary for cytokine production (184). Zhu et al. (185) showed that in colonic tissue of IL-10 knock-out mice with inflammatory bowel disease, 1,25(OH)2D was capable of down-regulating several genes associated with TNF-α, including TNF-α receptor 1, proteins involved in the transcription of TNF-α, and TNF-α itself. As well as direct effects, vitamin D could exert beneficial effects on cytokine production indirectly by maintaining serum Ca concentrations; in human umbilical vein cord endothelial cells, hypocalcaemia up-regulated endothelial NF-kB activity and IL-6 concentration was elevated (186). In addition, PTH induces IL-6 synthesis in osteoblasts (187).

Clinical data. Data examining the associations between vitamin D and inflammation in relation to CVD are limited and the data are conflicting. In type 2 diabetics (n 459), CRP concentration was higher in those with a s25(OH)D < 50 compared with those above 50 nmol/l (156). A cross-sectional analysis of the Framingham Offspring Study (n 1381; aged 35–89 years) showed that plasma 25(OH)D was not associated with overall inflammation, indicated by the inflammation index, sum of the fourteen individual markers of inflammation used in the study (188). In addition, plasma 25(OH)D was not associated with any of the individual markers except IL-6 (P = 0.02). Peterson & Heffernan (189) showed in healthy women (n 69), aged 25–82 years, that s25(OH)D concentration was inversely associated with TNF-α but no association was apparent between s25(OH)D and IL-6, IL-10 or CRP. Unexpectedly, we showed in young overweight adults that having a s25(OH)D concentration < 50 nmol/l decreased the likelihood (OR 0·35; 95% CI 0·17, 0·73) of having an elevated CRP concentration (S Muldowney, A Lucey, G Paschos, et al., unpublished results).

Intervention trials. Data from RCT looking at the effects of vitamin D3 (as a single treatment) on inflammatory cytokines or CRP in healthy subjects are meagre. Most of the evidence comes from studies where participants have underlying conditions, or vitamin D deficiency (see Table 1). A total of twenty-two intensive care unit patients were randomised to receive daily either 5 μg (low dose) or 12·5 μg (high dose) for 10 d and compared with healthy age-, sex- and BMI-matched controls (190). While treatment had no effect on TNF-α or IL-1, the reduction in CRP was greater in the high-dose group compared with the low-dose group, and a reduction in IL-6 was only apparent in the high-dose group. Timms et al. (171) randomised healthy British adults of Bangladeshi origin, with vitamin D deficiency (s25(OH)D < 27·5 nmol/l), to receive 3-monthly injections of an oily cholecalciferol solution (1250 μg or 12·5 μg) for 1 year. The two treatment groups were combined and CRP concentration was dramatically reduced post-supplementation (~23%). In the Zittermann et al. (111) weight-loss study, compared with placebo, vitamin D supplementation decreased TNF-α concentration, whereas there was no effect on CRP or IL-6 concentration in either group. Most recently, eighty-one South Asian women living in New Zealand, who were insulin resistant and had a s25(OH)D < 50 nmol/l, were randomised to receive either 100 μg cholecalciferol or a placebo daily for 6 months; however, no effect on CRP was apparent (189). Similarly, we observed no effect on CRP after 6 months of cholecalciferol supplementation in healthy adults (S Muldowney, TR Hill, AJ Lucey, et al., unpublished results).

To the best of our knowledge all other vitamin D RCT looking at its effects on inflammatory markers were in combination with Ca. Schleithoff et al. (191) supplemented congestive heart failure patients (n 93) with 500 mg Ca combined with either 50 μg cholecalciferol daily or a placebo for 9 months. In patients who received vitamin D a reduction in TNF-α concentration was apparent; in addition, IL-10 concentration was increased. Multiple sclerosis patients (n 39) with a s25(OH)D concentration < 50 nmol/l were randomised to receive daily 800 mg Ca plus placebo or 800 mg Ca plus 25 μg cholecalciferol for 6 months (192). Vitamin D supplementation increased serum concentrations of transforming growth factor-β1, an anti-inflammatory cytokine, whereas there was no effect in the placebo group. However, mRNA levels of TNF-α, IL-2, interferon-γ and IL-13 concentrations did not change in either group. In a double-blind randomised study, where non-diabetic elderly adults (aged > 65 years) were given either 500 mg Ca and 17·5 μg cholecalciferol or a...
placebo, no effect on CRP or IL-6 concentration was observed over 3 years in either subjects with impaired fasting glucose or normal fasting glucose concentrations(87). A short-term supplementation trial (12-week duration) with 1000 mg Ca and 20 μg cholecalciferol daily of postmenopausal women (n=39) had no effect on circulating concentrations of IL-6, TNF-α or CRP162. More recently, a large RCT on overweight and obese subjects (n=334) showed no effect of supplementation with Ca (500 mg/d) plus cholecalciferol (500 or 1000 μg/week) for 1 year on a wide range of cytokines and other inflammatory markers(193).

In brief, there appears to be no effect of supplementation in healthy adults (S Muldowney, TR Hill, AJ Lucey, et al., unpublished results; Pittas et al.87; Jorde et al.190; Gannage-Yared et al.162); unless vitamin D deficient at baseline(71), this may be related to the low circulating concentration of the cytokines in healthy subjects. Furthermore, the studies by Schleithoff et al.191 and Mahon et al.192 suggest that a high requirement of vitamin D (>25 μg/d) is needed to mediate its immunological protective effects.

Novel markers of CVD risk

Adhesion molecules, PAI-1 and matrix metalloproteinases (MMP) and their inhibitors can be used as markers of CVD risk and, recently, have been associated with vitamin D status. The adhesion molecules, such as intracellular- and vascular-cell adhesion molecules, mediate the migration of inflammatory cells to atherosclerotic lesions. They act as ligands to tightly bind various molecules in the inflammatory process. A small number of studies, with conflicting results, have shown a relationship between vitamin D (1,25(OH)2D) and the adhesion molecules194-197. The positive effect is most probably mediated through 1,25(OH)2D action on suppressing TNF-α secretion190, but further studies are required to elucidate this relationship further.

PAI-1, which is produced by the vascular endothelium and platelets, is an important regulator of fibrinolysis198 as it rapidly binds, and therefore inhibits, tissue plasminogen activator and urinary plasminogen activator. High PAI-1 concentrations have been shown to predict first occurrence of a myocardial infarction199, or reoccurrence before the age of 45 years200,201. Studies have found that 1,25(OH)2D reduces PAI-1 concentrations in various cell types, including vascular cells and smooth-muscle cells202,203. A possible mechanism for the association between PAI-1 and vitamin D may be similar to blood pressure, which involves the RAS. Angiotensin II stimulates the production of PAI-1 in cultured endothelial and vascular smooth-muscle cells204, whereas the inhibition of angiotensin-converting enzyme is associated with a decrease in both plasma PAI-1 concentrations and PAI-1 activity205. Recently, a large RCT with Ca (500 mg daily plus cholecalciferol at 500 or 1000 μg/week) for 1 year showed no effect on PAI-1 or other haemostatic markers in overweight and obese subjects206. However, evidence is limited and more studies (cross-sectional, interventional and mechanistic) are required.

MMP are a family of Zn-containing enzymes that have an important role in extracellular matrix degradation, synthesis and remodelling207. MMP are activated by MMP proenzymes and inhibited by endogenous tissue inhibitors of metalloproteinases (TIMP) and the net proteolytic activity is a function of the balance of MMP to TIMP. MMP degradation of the extracellular matrix may assist the influx of leucocytes through the endothelial layer, contributing to a decrease in endothelial barrier function, and facilitate migration of vascular smooth muscle cells into the intimal space where they proliferate and contribute to plaque formation172,208. MMP-9 and TIMP-1 have been identified as novel predictors of CVD risk. Experimental studies (either animal or cell) have shown that 1,25(OH)2D regulates MMP expression118,207,209. Rahman et al(207) showed that TIMP-1 and TIMP-3 were significantly under-expressed, while MMP-2 and MMP-9 were up-regulated in the cardiac tissue of VDR knock-out mice as compared with wild-type mice. However, human studies supporting an association between vitamin D and the MMP/TIMP system in humans are limited. A cross-sectional study, ran in tandem with an intervention study on healthy Bangladeshi adults living in Britain (n=171; aged 35–65 years), who were vitamin D deficient (s25(OH)D < 27.5 nmol/l) showed that vitamin D status was an independent predictor of MMP-9 concentration and a reduction in MMP-9 (66.8%) and TIMP-1 (39.8%) concentration was apparent following vitamin D supplementation71.

Summary and future directions

There is a considerable body of evidence from cross-sectional and prospective studies showing that low vitamin D status is associated with an increased risk of CVD, the MetS and each of its individual risk factors. Furthermore, there are strong mechanistic data to support the role of vitamin D in the reduction of CVD risk factors, particularly for insulin secretion, insulin resistance and hypertension mediated by the RAS. Evidence is not as robust for the association between vitamin D status and dyslipidaemia and further studies are required. Data from RCT are relatively rare and are confounded by inappropriate study design, inadequate characterisation of subjects, lack of data on season and sunshine exposure and interventions combining Ca and vitamin D.

If vitamin D influences cardiometabolic health, these effects may only be measurable at higher doses than those achievable through diet. This raises several questions about the food/pharma interface with respect to nutrition interventions, the impact that high dose studies have on skewing the literature and how such data should be considered when dietary recommendations are under review.
With the notable exception of folic acid, where supplemental approaches are required to achieve the maximum benefit, nutritional science so far has relied on food-based approaches to achieve optimal health through diet. However, increasing confidence about the safety of vitamin D doses in excess of the current tolerable upper intake levels should enable further RCT to be implemented using vitamin D as a single treatment at doses calibrated to achieve target thresholds of 25(OH)D concentrations, particularly in vitamin D-deficient individuals or patient subgroups with elevated CVD risk biomarkers.

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