Vitamin D and Cardiovascular Prevention

M. Adnan Nadir,[1] Benjamin R. Szwejkowski[1] & Miles D. Witham[2]

1 Department of Clinical Pharmacology, Centre for Cardiovascular and Lung Biology, Division of Medicine, University of Dundee, Ninewells Hospital, Dundee, UK
2 Department of Ageing and Health, Centre for Cardiovascular and Lung Biology, Division of Medicine, University of Dundee, Ninewells Hospital, Dundee, UK

Correspondence

Miles D. Witham, Ph.D., Department of Ageing and Health, Centre for Cardiovascular and Lung Biology, Division of Medical Sciences, University of Dundee, Mailbox 1, Ninewells Hospital and Medical School, Dundee DD1 9SY, UK.
Tel.: (44) 1382-632436;
Fax: (44) 1382-660675;
E-mail: m.witham@dundee.ac.uk

Vitamin D has been known to medical science for almost a century. Yet, it is only in the last 15 years that we have realized that the biological effects of vitamin D extend far beyond the control of calcium metabolism. Recent observational evidence suggests strong links between low vitamin D levels and a range of cardiovascular conditions, including stroke, myocardial infarction, hypertension, and diabetes. Interventional studies are beginning to explore whether vitamin D supplementation can modify vascular health and prevent cardiovascular disease. This article reviews the physiology and function of vitamin D, examines the current observational and intervention data in cardiovascular disease, and discusses future research and current practice recommendations.

Introduction

What is Vitamin D?

Vitamin D is a secosteroid. It is formed in animals from 7-dehydocholesterol, which is converted by ultraviolet (UV) B radiation (290–320 nm) to vitamin D in the skin. 25-hydroxylation then occurs in the liver to form circulating 25-hydroxyvitamin D (25OHD) with a further 1-hydroxylation step to form the biologically active moiety, 1,25 hydroxyvitamin D (1,25OHD). 1,25OHD has a short half-life, thus the longer-lived 25OHD is used to ascertain *in vivo* vitamin D status.

Sources of Vitamin D

Although sunlight provides the vast majority of vitamin D [1], small quantities of vitamin D3 (cholecalciferol) can be obtained through the diet; it is found in foods such as oily fish, liver, or eggs, along with foods fortified with vitamin D3, such as margarine and liquid or dried milk. Vitamin D2 (ergocalciferol that is closely related structurally) is also ingested in small quantities chiefly from fungal and plant sources, and can be manufactured by UV exposure of ergosterols.

Regulation of Vitamin D

Most actions of vitamin D appear to be exerted via binding of 1,25OHD to the nuclear vitamin D receptor. Actions are exerted via nuclear effects of the vitamin D receptor, which heterodimerizes with the retinoic acid X receptor [2]; this combination then binds to control sites on over 60 target genes in over 30 different tissues. Recent work has suggested an additional pathway via a cytoplasmic, possibly membrane-bound receptor, whose functions remain to be characterized [3].

Vitamin D metabolite levels are regulated through a number of feedback loops. 1,25OHD downregulates its own production when levels are high and upregulates production when levels are low. Parathyroid hormone stimulates 1,25OHD production via the kidneys; 1-alpha hydroxylase enzyme activity is also controlled by calcium

level, phosphate level, and fibroblast growth factor 23. Furthermore, it is now clear that 1,25 hydroxylation may occur in a number of other tissues in addition to the kidney, including dendritic cells and endothelial cells [4], thus paracrine effects of 1,25OHD are also likely to be important.

Potential Effects of Vitamin D on Vascular Biology

A number of potential mechanisms have been suggested by which vitamin D may exert effects on the vasculature. Vitamin D has been shown to suppress renin production in animal models [5]. It increases vascular endothelial growth factor expression [6] in some models, but conversely has been shown to inhibit angiogenesis in a variety of cancer models. Vitamin D is well known to have antiinflammatory actions, reducing levels of tumor necrosis factor (TNF) alpha in heart failure patients [7], but may also be able to induce proinflammatory gene expression and upregulate pathways involved in increasing oxidative stress [8]. Vitamin D, acting directly and via increasing circulating calcium levels, leads to reductions in parathyroid hormone (PTH), and PTH itself has been postulated to be vasculotoxic [9]. Although the biological pathways through which vitamin D may affect vascular health are by no means fully elucidated, vitamin D does appear to be able to improve endothelial function, both in patients with diabetes [10] and in healthy vitamin D-insufficient adults [11].

Vitamin D Insufficiency

Despite nearly a century of research, there is still considerable controversy surrounding the level of vitamin D necessary for optimum health, and the amount of vitamin D required to reach these levels. Current recommended daily intakes range from 200 to 1000 IU, and current definitions of insufficiency vary depending on the disease entity under scrutiny—serum 25OHD levels of >25 nmol/L for prevention of rickets and osteomalacia, >50 nmol for prevention of osteoporosis, and levels as high as 75 nmol/L have been postulated as necessary for optimum health based on observational studies [12].

Risk Factors for Vitamin D Insufficiency

As the majority of vitamin D is derived from the effect of UV radiation on skin, people with less sun exposure are prone to lower vitamin D levels. Those living at high latitudes (i.e., above 40 degrees north or south) do not receive sufficient UV exposure in winter to replenish vita-

min D levels. Impaired utilization of UVB occurs in non-Caucasian populations, and older skin is less efficient at producing vitamin D. Those who are not exposed to sunlight, for example, the housebound, those in institutional care, and those who do not expose their skin for cultural reasons, are also at risk. Any factor that forms a barrier to UV light, for example, sunscreen and atmospheric pollution, particularly urban photochemical smog, may reduce vitamin D synthesis. Those with poor nutritional intake or malabsorption are also at an increased risk of vitamin D insufficiency.

As a result of differences in UV irradiation in different seasons, circulating 25OHD levels change markedly through the year. A study of healthy individuals aged between 20 and 94 years found that the mean 25OHD level peaked in October at 96 nmol/L, falling to 73 nmol/L in April [13]. The 1958 British Cohort Study examined 7437 white individuals and found that vitamin D insufficiency was commonest during winter and spring, with 87.1% <75 nmol/L, 46.6% <40 nmol/L, and 15.5% <25 nmol/L [14].

How common is Vitamin D Insufficiency?

Vitamin D insufficiency becomes more common with age. In the Health Survey for England from 2005, between 50% and 60% of people aged 65 and over had 25OHD levels <50 nmol/L, with between 8% and 13% having 25OHD levels below 25 nmol/L [15]. Certain populations are at higher risk; 94% of a cohort of community dwelling young south Asian women living in the UK had 25OHD levels less than 37.5 nmol/L [16] Among women aged 15 to 49 in the National Health and Nutrition Examination Survey (NHANES) from the United States, 4% of white women but 42% of black women had 25OHD levels <37.5 nmol/L [17]. Vitamin D insufficiency is still an issue even in children; 9% of children aged 21 and under had 25OHD levels <37.5 nmol/L [18]; the risk being 20 times higher for blacks than for non-Hispanic whites.

Observational Data

A large body of observational data now links vitamin D insufficiency with cardiovascular disease, hypertension, myocardial infarction, stroke, diabetes, heart failure, and peripheral arterial disease.

Hypertension

The incidence of hypertension increases with higher latitude, and higher recordings are noted in winter than in summer months [19]. Cross-sectional studies have

shown that hypertension is inversely associated with low levels of vitamin D, supporting the suggestion that these epidemiological observations are due in part to a deficiency in vitamin D. The Third NHANES study [20] adjusted for ethnicity, age, sex, and physical activity and showed that vitamin D status was inversely proportional to blood pressure, with the lowest quintile of 25OHD (<40.4 nmol/L) having a blood pressure 3/1.6 mmHg higher than the highest vitamin D quintile (>85.7 nmol/L). Longitudinal studies confirm the association; patients with baseline 25OHD levels below 37.5 nmol/L were three to six times as likely to develop incident hypertension during follow-up as those with baseline vitamin D levels of >75 nmol/L [21]. The pooled relative risk of developing incident hypertension combining men and women using the random-effects model was 3.18 (95% CI 1.39 to 7.29). Low 25OHD levels are also linked to other cardiovascular risk factors. In a cross-sectional study of 15,088 male and female patients, the prevalence of hypertension (odds ratio 1.30), diabetes (odds ratio 1.98) and high triglycerides (odds ratio 1.47) were significantly higher with lower vitamin D levels ($P < 0.001$ for all) [22].

Vitamin D and other Cardiovascular Diseases—Cross-Sectional Data

Low vitamin D levels have also been associated with stroke [23], peripheral vascular disease, chronic heart failure [24], diabetes mellitus, insulin resistance [25], and the metabolic syndrome [26] in cross-sectional studies. In the Nurses' Health Study [27], 83,779 women had baseline vitamin D and calcium intake with a follow-up of 20 years. A combined daily intake of >1200 mg calcium and >800 IU vitamin D was associated with 33% lower risk of type 2 diabetes in women with a relative risk 0.67 (95% CI 0.49 to 0.90). Observational studies have also suggested that vitamin D supplementation may protect against type 1 diabetes [28], and in a study of 459 outpatients with type 2 diabetes, low levels of vitamin D were associated with prevalent cardiovascular disease [29].

Similar data associate low 25OHD levels with myocardial infarction [30] and coronary artery calcification. Acute coronary syndrome patients with the AA genotype of the vitamin D receptor had a worse prognosis than with other genotypes, but only if they also had low baseline 25OHD levels [31]. In a group of 283 patients at high risk of coronary artery disease, low levels of vitamin D were associated with increased coronary calcium scores on computed tomography [32].

The weakness with all cross-sectional studies in this area, however, is confounding by illness; any disease process has the potential to reduce physical activity levels and hence reduce sunlight exposure—in other words, the disease may cause the low vitamin D level rather than *vice versa*.

Vitamin D and Cardiovascular Events—Longitudinal Data

A number of large cohort studies support a temporal association between low 25OHD levels and future cardiovascular events. In the Framingham offspring cohort [33], 1739 subjects with no known cardiovascular disease were followed up for a mean of 5.4 years. 25OHD levels were measured at baseline, and there was an increase in risk of cardiovascular risk across the lower levels of 25OHD, and the association between 25OHD levels and cardiovascular events was particularly strong in those who were hypertensive at baseline (hazard ratio 2.13, 95% CI 1.30 to 3.48). Dobnig et al. [34] found similar correlations between low vitamin D levels and increased cardiovascular risk over a 7-year follow-up in 3258 patients who attended for coronary angiography. They found that patients in the two lower quartiles had an increased cardiovascular mortality compared with those in the highest quartile of serum 25OHD (hazard ratio 2.22; 95% CI 1.57 to 3.13 for the lowest quartile and hazard ratio 1.82; 95% CI 1.29 to 2.58 for the second lowest quartile). Giovannucci et al. [35] followed up 18,225 men in the Health Professionals Follow-up Study for 10 years. Subjects in this study were aged 40 to 75 years, with no diagnosis of cardiovascular disease. A low baseline vitamin D level (<37.5 nmol/L) was a risk factor for future myocardial infarction (relative risk 2.42; 95% CI 1.53 to 3.84), and the association persisted after adjustment for other baseline risk factors (relative risk 2.09; 95% CI 1.24 to 3.54).

Interventional Studies
Effects on Blood Pressure

A small number of randomized controlled trials have reported data on blood pressure with and without vitamin D supplementation. Pfeifer et al. randomized 148 older women to either vitamin D and calcium or placebo and calcium [36]. After 8 weeks of treatment, there was a 7 mmHg reduction in systolic blood pressure in vitamin D group compared to control ($P = 0.02$). Similar effects were seen when the effects of UVB+UVA irradiation were compared with UVA alone. The combination led to a large increase in circulating 25OHD levels and a 6/6 mmHg reduction in blood pressure compared to UVA alone [37]. A recent *meta*-analysis of randomized controlled trials comparing vitamin D with placebo

found a small but statistically significant drop in diastolic blood pressure (3 mmHg) among those taking vitamin D [38]. This effect was seen only in those who were hypertensive at baseline. The reduction on systolic blood pressure (3.6 mmHg) did not reach significance, but appeared greater in those receiving vitamin D2 or D3 rather than 1-alpha hydroxylated vitamin D derivatives.

On the other hand, the largest trial to date, the Women's Health Initiative (WHI), failed to show any significant impact of a small dose of vitamin D on systolic or diastolic BP after a mean follow-up of 7 years in postmenopausal women [39]. The lack of effect seen in WHI may have been due to the low doses of vitamin D used (only 400 IU per day) and poor adherence (59%) to the study medications in a population who were not hypertensive at baseline. The current evidence to support the role of vitamin D supplementation to reduce BP is weak and further randomized trials are needed to explore this particular use of vitamin D.

Effects on Glycemia

Recent data suggest that vitamin D supplementation may have beneficial effects on glucose metabolism and insulin resistance in selected patients. High-dose (4000 units per day) vitamin D improved insulin sensitivity in South Asians with insulin resistance [40]. In another trial, primarily designed for bone-related outcomes, a *post hoc* analysis revealed that for nondiabetic participants who had impaired glucose tolerance at baseline, supplementation with combined vitamin D and calcium resulted in improved insulin resistance and lower rise in fasting glycemia at 3 years (0.4 mg/dL vs. 6.1 mg/dL, $P = 0.04$) compared with controls [41]. Beneficial effects on insulin secretion and glucose uptake have also been found in patients with chronic kidney disease [42,43].

However, trials in which vitamin D has been given to patients with established type 2 diabetes have mostly shown no effect on insulin sensitivity or glycemic control [10,41,44]. The largest study (the WHI study) to report data on effect of vitamin D and calcium supplementation on the risk of developing diabetes mellitus failed to show any beneficial effect in postmenopausal women free of diabetes at baseline after 7 years of follow-up (hazard ratio 1.01, 95% CI 0.94 to 1.10) [45]. A definitive conclusion based on the published data is not possible and further well-designed randomised controlled trials are required to further define the role of vitamin D in the treatment of prediabetic and diabetic states.

Effects on other Risk Factors

The effects of vitamin D on other risk factors for cardiovascular disease have been less well studied. No signifi-

cant effect was evident on serum lipids in healthy older patients or patients with diabetes [10,46], but improvements were seen in patients with chronic kidney disease [43]. Vitamin D added to a weight loss program, led to greater falls in triglycerides than weight loss alone, but paradoxically led to an increase in LDL cholesterol levels compared to placebo [47]. Fibrinogen levels were unaffected by vitamin D supplementation in older, bed-bound patients [48].

Effects on Heart Failure

Supplementation with 2000 units per day of vitamin D3 did not improve left ventricular (LV) ejection fraction, B-type natriuretic peptide levels, or exercise capacity in heart failure patients with LV systolic dysfunction, but did lead to significant reductions in TNF alpha levels, suggesting a potential antiinflammatory effect of vitamin D [7]. Further studies are needed to ascertain whether vitamin D supplementation can improve symptoms and prognosis in heart failure.

Effects on Cardiovascular Events

The only large trial reporting the effect of vitamin D supplementation on cardiovascular events reported lack of any significant impact. Once again these data are from WHI, where over 36,000 postmenopausal women were randomized to either placebo or a small dose of vitamin D and calcium [49]. After a mean follow-up of 7 years, there were a total of 974 cardiac and 739 cerebrovascular events. There was no statistically significant difference between two groups in terms of cardiovascular events (hazard ratio 1.04, 95% CI 0.92 to 1.18). As mentioned earlier, the small dose and poor compliance with vitamin D in this study may explain the lack of effect. Another possibility is that any beneficial effect of vitamin D was neutralized by the potentially deleterious effect of calcium supplementation; recent data suggest that calcium supplementation may lead to an increase in myocardial infarction rates [50].

Effects on All-Cause Mortality

A recent *meta*-analysis of 18 randomized controlled trials examining vitamin D treatment for osteoporosis, with a total of over 57,000 patients, showed that supplementation with vitamin D resulted in statistically significant reduction in mortality from any cause (relative risk 0.93, 95% CI 0.87 to 0.99) [51]. Mean daily dose of vitamin D used was 528 IU and mean follow-up was 5.7 years. In studies where duration of supplementation was at least 3 years, this relative reduction in mortality was slightly better at 8%. Importantly, there was no evidence of any

significant untoward effect of chronic vitamin D intake. Unfortunately, it was not possible from this analysis to ascertain effect of vitamin D on individual causes of death, and the results should be interpreted with the caveats that all-cause mortality was not the primary outcome of the trials studied, and that the trials recruited patients with osteoporosis rather than those at risk of cardiovascular disease. Nevertheless, the reduction in all-cause mortality seen is far higher than can be explained by fracture prevention alone, and suggests that large trials of vitamin D to reduce cardiovascular death are merited.

Unresolved Issues and Future Work

Although the strength of observational data linking low levels of vitamin D to cardiovascular disease are impressive, and early interventional data are encouraging, there are several questions that require answering before vitamin D supplementation finds a role as a routine clinical therapy for cardiovascular disease.

Despite ongoing debate, the optimal dose and optimal serum 25OHD level for preventing cardiovascular disease are not yet clear. Observational studies suggest that very high 25OHD levels may have paradoxically deleterious effects [33,52], perhaps due to vascular calcification, thus further dose-finding studies with surrogate endpoints including vascular stiffness are required. Conversely, relatively large doses of vitamin D may be required to correct the marked degree of insufficiency seen in many people; perhaps as high as 5000 IU per day for those with 25OHD levels <55 nmol/L [53]. This suggests that many studies performed to date may not have given enough vitamin D to be sure of maximizing the effects of supplementation. Doses that are appropriate for preventing vitamin D insufficiency in the population at large may be quite different from those needed to correct the profoundly low levels of 25OHD seen in osteomalacia. It is clear that oral administration of vitamin D produces higher peak 25OHD levels, and has a longer duration of effect, than intramuscular administration [54]. Most, but not all studies, suggest that vitamin D3 produces higher 25OHD levels than the equivalent dose of D2. It is unclear if this is important in determining the efficacy of each preparation on vascular health, however. There have been no studies examining whether daily, weekly, or monthly dosing has a greater effect on vascular health, but monthly dosing appears to raise 25OHD levels slightly less than the equivalent daily or weekly dose [55].

A second issue is disentangling the effect of vitamin D from the effect of calcium, which is often coadministered. Although there is some evidence that calcium can reduce blood pressure [56], there is also evidence that calcium supplementation alone may increase rates of cardiovascular events [50]. Studies designed to examine the effects of vitamin D alone on cardiovascular disease are therefore important.

Many studies have been of relatively short duration, and the effects of multiple intermittent dosing or long-term daily dosing have not been well studied. The available evidence suggests that not all patient groups may benefit from vitamin D supplementation; patients with hypertension, for example, appear to have more to gain [33,38]. This problem is compounded by an incomplete understanding of the mechanisms by which vitamin D might exert beneficial effects on the vasculature. There is therefore a role for further basic science studies in this area as well as studies targeting well-defined subgroups of cardiovascular disease.

Longer-term, large multicentre randomized controlled trials will be needed, whether at population level or in specific disease states, examining not only cardiovascular event rates, but also total mortality and other key health outcomes including infection, allergy, and cancer. As with all potentially efficacious therapies, the potential for harm also exists [57], in the form of increased rates of renal stones, renal impairment (albeit rare), together with the suggestion from animal and observational data that very high vitamin D levels may predispose to vascular calcification [58] and atopy [59,60]. Experience with antioxidant vitamins should provide a warning; despite good observational and *in vitro* evidence, these therapies proved ineffective, and in the case of beta-carotene, a seemingly innocuous therapy led to net harm [61]. Until the results of large trials are available, the case for giving supplementation with vitamin D to prevent cardiovascular disease therefore remains unproven, even in patients with suboptimal circulating 25OHD levels.

Acknowledgments

Funding

Author Contributions

All authors contributed to literature searching, critical appraisal, writing, and revising the manuscript.

Conflicts of Interest

Dr Witham has received research funding into the role of vitamin D in cardiovascular disease from Chest Heart and Stroke Scotland, Diabetes UK, Chief Scientist Office, Scottish Government, Heart Research UK and the ME Society.

References

1. Lamberg-Allardt C. Vitamin D in foods and as supplements. *Prog Biophys Mol Biol* 2006;**92**:33–38.
2. Jones G, Strugnell SA, DeLuca HF. Current understanding of the molecular actions of vitamin D. *Physiol Rev* 1998;**78**:1193–1231.
3. Norman AW, Okamura WH, Bishop JE, Henry HL. Update on biological actions of 1alpha,25(OH)2-vitamin D3 (rapid effects) and 24R,25(OH)2-vitamin D3. *Mol Cell Endocrinol* 2002;**197**:1–13.
4. Norman AW. From vitamin D to hormone D: Fundamentals of the vitamin D endocrine system essential for good health. *Am J Clin Nutr* 2008;**88**:491S–499S.
5. Sigmund CD. Regulation of renin expression and blood pressure by vitamin D(3). *J Clin Invest* 2002;**110**:155–156.
6. Cardus A, Panizo S, Encinas M, et al. 1,25-dihydroxyvitamin D3 regulates VEGF production through a vitamin D response element in the VEGF promoter. *Atherosclerosis* 2009;**204**:85–89.
7. Schleithoff SS, Zittermann A, Tenderich G, et al. Vitamin D supplementation improves cytokine profiles in patients with congestive heart failure: A double-blind, randomized, placebo-controlled trial. *Am J Clin Nutr* 2006;**83**:754–759.
8. Sun X, Morris KL, Zemel MB. Role of calcitriol and cortisol on human adipocyte proliferation and oxidative and inflammatory stress: A microarray study. *J Nutrigenet Nutrigenomics* 2008;**1**:30–48.
9. Rostand SG, Drueke TB. Parathyroid hormone, vitamin D, and cardiovascular disease in chronic renal failure. *Kidney Int* 1999;**56**:383–392.
10. Sugden JA, Davies JI, Witham MD, Morris AD, Struthers AD. Vitamin D improves endothelial function in patients with type 2 diabetes mellitus and low vitamin D levels. *Diab Med* 2008;**25**:320–325.
11. Tarcin O, Yavuz DG, Ozben B, et al. Effect of vitamin D deficiency and replacement on endothelial function in asymptomatic subjects. *J Clin Endocrinol Metab* 2009;**94**:4023–4030.
12. Bischoff-Ferrari HA, Giovannucci E, Willett WC, Dietrich T, Dawson-Hughes B. Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes. *Am J Clin Nutr* 2006;**84**:18–28.
13. Maxwell JD. Seasonal variation in vitamin D. *Proc Nutr Soc* 1994;**53**:533–543.
14. Hypponen E, Power C. Hypovitaminosis D in British adults at age 45 y: Nationwide cohort study of dietary and lifestyle predictors. *Am J Clin Nutr* 2007;**85**:860–868.
15. Hirani V, Tull K, Ali A, Mindell J. Urgent action needed to improve vitamin D status among older people in England! *Age Ageing* 2010;**39**:62–68.
16. Roy DK, Berry JL, Pye SR, et al. Vitamin D status and bone mass in UK South Asian women. *Bone* 2007;**40**:200–204.
17. Nesby-O'Dell S, Scanlon KS, Cogswell ME, et al. Hypovitaminosis D prevalence and determinants among African American and white women of reproductive age: Third National Health and Nutrition Examination Survey, 1988–1994. *Am J Clin Nutr* 2002;**76**:187–192.
18. Kumar J, Muntner P, Kaskel FJ, Hailpern SM, Melamed ML. Prevalence and associations of 25-hydroxyvitamin D deficiency in US children: NHANES 2001–2004. *Pediatrics* 2009;**124**:362–370.
19. Woodhouse PR, Khaw KT, Plummer M. Seasonal variation of blood pressure and its relationship to ambient temperature in an elderly population. *J Hypertens* 1993;**11**:1267–1274.
20. Scragg R, Sowers M, Bell C. Serum 25-hydroxyvitamin D, ethnicity, and blood pressure in the Third National Health and Nutrition Examination Survey. *Am J Hypertens* 2007;**20**:713–719.
21. Forman JP, Giovannucci E, Holmes MD, et al. Plasma 25-hydroxyvitamin D levels and risk of incident hypertension. *Hypertension* 2007;**49**:1063–1069.
22. Martins D, Wolf M, Pan D, et al. Prevalence of cardiovascular risk factors and the serum levels of 25-hydroxyvitamin D in the United States: Data from the Third National Health and Nutrition Examination Survey. *Arch Intern Med* 2007;**167**:1159–1165.
23. Poole KE, Loveridge N, Barker PJ, et al. Reduced vitamin D in acute stroke. *Stroke* 2006;**37**:243–245.
24. Zittermann A, Schleithoff SS, Tenderich G, et al. Low vitamin D status: A contributing factor in the pathogenesis of congestive heart failure? *J Am Coll Cardiol* 2003;**41**:105–112.
25. Scragg R, Holdaway I, Singh V, et al. Serum 25-hydroxyvitamin D3 levels decreased in impaired glucose tolerance and diabetes mellitus. *Diabetes Res Clin Pract* 1995;**27**:181–188.
26. Hypponen E, Boucher BJ, Berry DJ, Power C. 25-hydroxyvitamin D, IGF-1, and metabolic syndrome at 45 years of age: A cross-sectional study in the 1958 British birth cohort. *Diabetes* 2008;**57**:298–305.
27. Pittas AG, Dawson-Hughes B, Li T, et al. Vitamin D and calcium intake in relation to type 2 diabetes in women. *Diabetes Care* 2006;**29**:650–656.
28. Zipitis CS, Akobeng AK. Vitamin D supplementation in early childhood and risk of type 1 diabetes: A systematic

review and meta-analysis. *Arch Dis Child* 2008;**93**: 512–517.

29. Cigolini M, Iagulli MP, Miconi V, et al. Serum 25-hydroxyvitamin D3 concentrations and prevalence of cardiovascular disease among type 2 diabetic patients. *Diabetes Care* 2006;**29**:722–724.

30. Scragg R, Jackson R, Holdaway IM, Lim T, Beaglehole R. Myocardial infarction is inversely associated with plasma 25-hydroxyvitamin D3 levels: A community-based study. *Int J Epidemiol* 1990;**19**:559–563.

31. Barakat K, Hitman GA. The emerging role of vitamin D and its receptor in the pathogenesis of acute coronary syndromes. *Br J Cardiol* 2006;**13**:9–12.

32. Watson KE, Abrolat ML, Malone LL, et al. Active serum vitamin D levels are inversely correlated with coronary calcification. *Circulation* 1997;**96**:1755–1760.

33. Wang TJ, Pencina MJ, Booth SL, et al. Vitamin D deficiency and risk of cardiovascular disease. *Circulation* 2008;**117**:503–511.

34. Dobnig H, Pilz S, Scharnagl H, et al. Independent association of low serum 25-hydroxyvitamin d and 1,25-dihydroxyvitamin D levels with all-cause and cardiovascular mortality. *Arch Intern Med* 2008;**168**:1340–1349.

35. Giovannucci E, Liu Y, Hollis BW, Rimm EB. 25-hydroxyvitamin D and risk of myocardial infarction in men: A prospective study. *Arch Intern Med* 2008;**168**:1174–1180.

36. Pfeifer M, Begerow B, Minne HW, Nachtigall D, Hansen C. Effects of a short-term vitamin D(3) and calcium supplementation on blood pressure and parathyroid hormone levels in elderly women. *J Clin Endocrinol Metab* 2001;**86**:1633–1637.

37. Krause R, Buhring M, Hopfenmuller W, Holick MF, Sharma AM. Ultraviolet B and blood pressure. *Lancet* 1998;**352**:709–710.

38. Witham MD, Nadir MA, Struthers AD. Effect of vitamin D on blood pressure: A systematic review and meta-analysis. *J Hypertens* 2009;**27**:1948–1954.

39. Margolis KL, Ray RM, Van Horn L, et al. Effect of calcium and vitamin D supplementation on blood pressure: The women's health initiative randomized trial. *Hypertension* 2008;**52**:847–855.

40. von Hurst PR, Stonehouse W, Coad J. Vitamin D supplementation reduces insulin resistance in South Asian women living in New Zealand who are insulin resistant and vitamin D deficient – A randomised, placebo-controlled trial. *Br J Nutr* 2010;**103**:549–555.

41. Pittas AG, Harris SS, Stark PC, Dawson-Hughes B. The effects of calcium and vitamin D supplementation on blood glucose and markers of inflammation in nondiabetic adults. *Diabetes Care* 2007;**30**:980–986.

42. Allegra V, Luisetto G, Mengozzi G, Martimbianco L, Vasile A. Glucose-induced insulin secretion in uremia: Role of 1 alpha,25(HO)2-vitamin D3. *Nephron* 1994;**68**:41–47.

43. Mak RH. 1,25-dihydroxyvitamin D3 corrects insulin and lipid abnormalities in uremia. *Kidney Int* 1998;**53**:1353–1357.

44. Jorde R, Figenschau Y. Supplementation with cholecalciferol does not improve glycaemic control in diabetic subjects with normal serum 25-hydroxyvitamin D levels. *Eur J Nutr* 2009;**48**:349–354.

45. de Boer IH, Tinker LF, Connelly S, et al. Calcium plus vitamin D supplementation and the risk of incident diabetes in the women's health initiative. *Diabetes Care* 2008;**31**:701–707.

46. Daly RM, Nowson CA. Long-term effect of calcium-vitamin D(3) fortified milk on blood pressure and serum lipid concentrations in healthy older men. *Eur J Clin Nutr* 2009;**63**:993–1000.

47. Zittermann A, Frisch S, Berthold HK, et al. Vitamin D supplementation enhances the beneficial effects of weight loss on cardiovascular disease risk markers. *Am J Clin Nutr* 2009;**89**:1321–1327.

48. Bjorkman MP, Sorva AJ, Tilvis RS. C-reactive protein and fibrinogen of bedridden older patients in a six-month vitamin D supplementation trial. *J Nutr Health Aging* 2009;**13**:435–439.

49. Hsia J, Heiss G, Ren H, et al. Calcium/vitamin D supplementation and cardiovascular events. *Circulation* 2007;**115**:846–854.

50. Bolland MJ, Barber PA, Doughty RN, et al. Vascular events in healthy older women receiving calcium supplementation: Randomised controlled trial. *BMJ* 2008;**336**:262–266.

51. Autier P, Gandini S. Vitamin D supplementation and total mortality: A meta-analysis of randomized controlled trials. *Arch Intern Med* 2007;**167**: 1730–1737.

52. Rajasree S, Rajpal K, Kartha CC, et al. Serum 25-hydroxyvitamin D3 levels are elevated in South Indian patients with ischemic heart disease. *Eur J Epidemiol* 2001;**17**:567–571.

53. Aloia JF, Patel M, Dimaano R, et al. Vitamin D intake to attain a desired serum 25-hydroxyvitamin D concentration. *Am J Clin Nutr* 2008;**87**:1952–1958.

54. Romagnoli E, Mascia ML, Cipriani C, et al. Short and long-term variations in serum calciotropic hormones after a single very large dose of ergocalciferol (vitamin D2) or cholecalciferol (vitamin D3). *J Clin Endocrinol Metab* 2008;**93**:3015–3020.

55. Chel V, Wijnhoven HA, Smit JH, Ooms M, Lips P. Efficacy of different doses and time intervals of oral vitamin D supplementation with or without calcium in elderly nursing home residents. *Osteoporos Int* 2008;**19**:663–671.

56. Dickinson HO, Nicolson DJ, Cook JV, et al. Calcium supplementation for the management of primary hypertension in adults. *Cochrane Database Syst Rev* 2006;CD004639.

57. Jackson RD, LaCroix AZ, Gass M, et al. Calcium plus

vitamin D supplementation and the risk of fractures. *N Engl J Med* 2006;**354**:669–683.

58. Norman PE, Powell JT. Vitamin D, shedding light on the development of disease in peripheral arteries. *Arterioscler Thromb Vasc Biol* 2005;**25**:39–46.

59. Hypponen E, Berry DJ, Wjst M, Power C. Serum 25-hydroxyvitamin D and IgE – A significant but nonlinear relationship. *Allergy* 2009;**64**:613–620.

60. Gale CR, Robinson SM, Harvey NC, et al. Maternal vitamin D status during pregnancy and child outcomes. *Eur J Clin Nutr* 2008;**62**:68–77.

61. Druesne-Pecollo N, Latino-Martel P, Norat T, et al. Beta-carotene supplementation and cancer risk: A systematic review and meta-analysis of randomized controlled trials. *Int J Cancer* 2010;**127**: 172–184.