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# Vitamin D improves endothelial function in patients with Type 2 diabetes mellitus and low vitamin D levels

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## Abstract

**Aims** To test whether a single large dose of vitamin D2 can improve endothelial function in patients with Type 2 diabetes mellitus and low serum 25-hydroxyvitamin D levels.

**Methods** Double-blind, parallel group, placebo-controlled randomized trial. A single dose of 100 000 IU vitamin D2 or placebo was administered to patients with Type 2 diabetes over the winter, when levels of circulating 25-hydroxyvitamin D were likely to be lowest. Patients were enrolled if their baseline 25-hydroxyvitamin D level was < 50 nmol/l. Endothelial function and blood pressure were measured and fasting blood samples were taken at baseline and 8 weeks after administration of vitamin D.

**Results** Forty-nine per cent of subjects screened had 25-hydroxyvitamin D levels < 50 nmol/l. Thirty-four subjects completed the study, with a mean age of 64 years and a baseline 25-hydroxyvitamin D level of 38.3 nmol/l. Vitamin D supplementation increased 25-hydroxyvitamin D levels by 15.3 nmol/l relative to placebo and significantly improved flow mediated vasodilatation (FMD) of the brachial artery by 2.3%. The improvement in FMD remained significant after adjusting for changes in blood pressure. Vitamin D supplementation significantly decreased systolic blood pressure by 14 mmHg compared with placebo; this did not correlate with change in FMD.

**Conclusions** Vitamin D insufficiency is common in patients with Type 2 diabetes during winter in Scotland. A single large dose of oral vitamin D2 improves endothelial function in patients with Type 2 diabetes and vitamin D insufficiency.

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**Keywords** blood pressure, endothelial function, vitamin D

**Abbreviations** FMD, flow-mediated vasodilation; GTN, glyceryl trinitrate; $HbA_{1c}$, glycated haemoglobin; HOMA, homeostatic model assessment; PTH, parathyroid hormone; VEGF, vascular endothelial growth factor

## Introduction

Vitamin D insufficiency, defined as serum 25-hydroxyvitamin D levels < 50 nmol/l [1], is common in patients with Type 2 diabetes [2,3]. Low 25-hydroxyvitamin D levels are also associated with an increased rate of cardiovascular events [4]. It has been demonstrated that patients at high latitudes are particularly prone to low 25-hydroxyvitamin D levels during winter months, and cardiovascular deaths are known to peak during winter in these populations [4]. Low levels of 25-hydroxyvitamin D are associated with many markers of cardiovascular disease; for example, hypertension [5], increased vascular resistance [6] and increased left ventricular mass index [7]. In addition, 25-hydroxyvitamin D levels correlate inversely with coronary calcification, an indicator of atherosclerosis and a precursor of cardiovascular events [8].

In several small supplementation studies, interventions to increase 25-hydroxyvitamin D has been shown to reduce blood pressure in populations at risk of cardiovascular disease [9,10]. There is also evidence that vitamin D supplementation increases pancreatic insulin release and improves insulin resistance [11] plus impaired glucose tolerance [12] in patients with Type 2 diabetes.

Endothelial function is a powerful surrogate marker of cardiovascular risk [13], which is impaired in patients with Type 2 diabetes [14]. Impaired endothelial function has been

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postulated to provide a final common pathway by which multiple risk factors exert their deleterious effects on cardiovascular health [15]. The primary aim of this study was therefore to ascertain whether a single large dose of oral vitamin D could improve endothelial function in patients with Type 2 diabetes and vitamin D insufficiency.

## Patients and methods

Eighty-seven patients with Type 2 diabetes mellitus diagnosed using World Health Organization (WHO) criteria by diabetes specialist physicians were recruited from hospital and community clinics and general practitioner (GP) surgeries from the Dundee area, Scotland, between November 2005 and March 2006. Tayside Committee on Medical Research Ethics gave approval, and written consent was obtained from each patient. The study was conducted in accordance with the Declaration of Helsinki.

Subjects were included in the study if their medications were stable for 6 weeks and their serum 25-hydroxyvitamin D level was < 50 nmol/l. Subjects were excluded if taking vitamin D supplements, serum creatinine > 200 μmol/l, liver function tests (bilirubin, aminotransferases and alkaline phosphatase) > 3 times upper limit of normal or if corrected calcium > 2.55 or < 2.15 mmol/l. As per the study protocol, subjects were excluded from analysis if their medications changed during the 8-week study period.

Subjects attended the Department of Clinical Pharmacology, Ninewells Hospital, for the measurement of outcome measures at baseline and 8 weeks later. At each visit, the primary outcome measure of endothelial function was assessed non-invasively by flow-mediated vasodilation (FMD) of the brachial artery in response to hyperaemia (endothelium-dependent vasodilation) and sublingual glyceryl trinitrate (GTN; endothelium-independent vasodilation). Subjects were examined in a quiet, temperature-controlled room after fasting for 8 h and were instructed not to take exercise, smoke or consume caffeine, high-fat foods or vitamin C for 4 h prior to testing [16]. Brachial artery diameter and flow were determined by M-mode and Doppler ultrasound using a Philips iE33 ultrasound machine (Philips Medical Systems, Reigate, Surrey, UK) with a video link to Lead Capture software (Lead Technologies Inc., Charlotte, NC, USA). Concurrent electrocardiogram (ECG) monitoring enabled the resulting image to be gated to the R wave of the QRS complex. The brachial artery was longitudinally imaged above the elbow using an 11.3-MHz probe. The image was recorded for 2 min, followed by induction of forearm ischaemia. This was induced by inflating a cuff below the elbow to 200 mmHg (or 50 mmHg above systolic blood pressure, whichever was higher) for 5 min and deflating rapidly (Hokanson Rapid Cuff Inflator; Hokanson Inc., Belleview, WA, USA). The resulting reactive hyperaemia was recorded for 2 min. After a rest period of 10 min, the image was again recorded for 2 min before 0.4 mg GTN (Nitrolingual Pumpspray; Lipha Pharmaceuticals, Middlesex, UK) was administered sublingually and the image was recorded for a further 5 min. Changes in artery diameter were expressed as percentage change relative to vessel diameter before cuff inflation or GTN administration. Blood flow was measured prior to cuff inflation and immediately after deflation. The coefficient of variation of the technique in our laboratory is an 8.9% relative change, equivalent to 0.87% absolute change on a series of test subjects.

Fasting blood was drawn for measurement of glycated haemoglobin ($HbA_{1c}$), serum calcium, phosphate, 25-hydroxyvitamin D, creatinine, glucose, insulin, parathyroid hormone (PTH), renin and angiotensin II. Serum 25-hydroxyvitamin D was measured by ELISA (I.D.S., Tyne & Wear, UK), the assay has 75% cross-reactivity for 25-hydroxyvitamin D2 compared with 25-hydroxyvitamin D3. Intra-assay coefficients of variation were 6.8% for 25-hydroxyvitamin D, 7.8% for renin and 6.4% for angiotensin II. From fasting glucose and insulin levels, homeostatic model assessment (HOMA) insulin sensitivity (IS) [10 000/(fasting insulin, μmol/ml × fasting glucose, mmol/l)] was calculated. Creatinine clearance was calculated using the Cockcroft–Gault formula [17]. Standard office blood pressure measurements were taken seated, at rest using an Omron HEM-705CP; the average of three readings was taken.

The study was of double-blind, randomized, parallel group design. After baseline measurements, a single dose of 100 000 U ergocalciferol (vitamin D2) or matching placebo (DHP Clinical Trial Supplies, Powys, UK) was administered and ingested in the presence of the researcher, thus ensuring 100% compliance. Doses were contained in sequentially numbered bottles with treatment codes generated by DHP from computerized random number tables. These codes were concealed from the researcher until after the study was completed.

The study was powered to detect change in the primary outcome measure of FMD. An absolute change in FMD of 2% is regarded as being clinically significant [16] and we calculated that, in a parallel group study, 40–60 patients were required to detect this change. Assuming a standard deviation of 2.5%, a 40-patient sample would have a power of 75% to detect a 2% change in FMD at the 5% confidence level using a two-tailed test. SPSS version 11.5 (SAS Institute, Cary, NC, USA) was used for statistical analyses. After ensuring that the data was normally distributed, an independent *t*-test was used to compare change from baseline between placebo and vitamin D groups. When comparing baseline parameters, continuous variables were compared using independent *t*-test and categorical variables were compared using $\chi^2$-test.

## Results

Eighty-seven subjects were screened, of whom 43 (49%) had 25-hydroxyvitamin D levels < 50 nmol/l. There was no significant difference in 25-hydroxyvitamin D levels between those screened in the first and second halves of the recruitment period. Of the 43 patients with subnormal 25-hydroxyvitamin D levels, three patients withdrew and six (three in each treatment group) had their cardiovascular medications changed by their usual physician during the study period. Baseline details of the patients who completed the study are presented in Table 1. At baseline there was no significant difference in 25-hydroxyvitamin D levels, PTH, calcium, phosphate, $HbA_{1c}$, HOMA, FMD or blood pressure between those randomly allocated to placebo or vitamin D. There was no significant difference in baseline brachial artery diameter in those receiving

 

**Table 1** Baseline parameters, medical history and drug history

| Baseline parameters | Vitamin D group ($n$ = 17) | Placebo group ($n$ = 17) | $P$ |
|---|---|---|---|
| Age (years) | 64.9 ± 10.3 | 63.5 ± 9.5 | 0.68 |
| Male sex (%) | 10/17 (59) | 8/17 (47) | 0.49 |
| Body mass index (kg/m$^2$) | 31.7 ± 6.4 | 31.7 ± 6.5 | 0.98 |
| Office systolic BP (mmHg) | 145 ± 9.2 | 137 ± 14.1 | 0.07 |
| Office diastolic BP (mmHg) | 82 ± 10.5 | 79 ± 6.0 | 0.31 |
| Office pulse (b/min) | 76 ± 14 | 69 ± 11 | 0.15 |
| FMD response to hyperaemia (%) | 6.38 ± 4.31 | 7.28 ± 2.93 | 0.49 |
| 25-hydroxyvitamin D (nmol/l) | 40.2 ± 10.3 | 36.4 ± 8.5 | 0.25 |
| $HbA_{1c}$ (%) | 7.5 ± 1.6 | 7.3 ± 1.4 | 0.69 |
| HOMA (IS) | 164.5 ± 145.4 | 176.6 ± 135.9 | 0.80 |
| Calcium* (mmol/l) | 2.42 ± 0.07 | 2.42 ± 0.09 | 1.00 |
| Phosphate (mmol/l) | 1.11 ± 0.15 | 1.13 ± 0.14 | 0.69 |
| Creatinine clearance (ml/min) | 80.5 ± 33.8 | 87.7 ± 24.4 | 0.50 |
| PTH (pmol/l) | 4.29 ± 1.79 | 4.94 ± 1.97 | 0.32 |
| Smoker (yes/no/ex) | 3/10/4 | 2/7/8 | |
| History of myocardial infarction | 3 (18) | 2 (12) | 0.50 |
| History of angina pectoris | 3 (18) | 0 | 0.11 |
| History of stroke | 1 (6) | 1 (6) | 1.00 |
| Metformin | 12 (71) | 6 (35) | 0.04 |
| Sulphonylurea | 6 (35) | 5 (29) | 0.50 |
| Thiazolidinedione | 4 (24) | 5 (29) | 0.50 |
| Insulin | 3 (18) | 3 (18) | 1.00 |
| Statin/fibrate | 13 (76) | 9 (53) | 0.14 |
| ACE inhibitor/angiotensin blocker | 14 (82) | 7 (41) | 0.02 |
| Aspirin | 10 (59) | 5 (29) | 0.06 |
| B-blocker | 3 (18) | 5 (29) | 0.34 |
| Calcium channel blocker | 3 (18) | 2 (12) | 0.05 |

Differences assessed by Student's $t$-test and by $\chi^2$-test for categorical values.
*Corrected for albumin level.
Mean ± SD, number (%).
ACE, angiotensin-converting enzyme; BP, blood pressure; FMD, flow-mediated vasodilation; $HbA_{1c}$, glycated haemoglobin; HOMA, homeostatic model assessment; IS, insulin sensitivity; PTH, parathyroid hormone; SD, standard deviation.

vitamin D compared with those receiving placebo. At baseline, 25-hydroxyvitamin D levels correlated only with PTH ($r$ = –0.49, $P$ = 0.001) and not with other measures, including body mass index (BMI).

Vitamin D administration significantly increased 25-hydroxyvitamin D levels compared with placebo (+22.9 nmol/l vs. +7.6 nmol/l, $P$ = 0.02). Serum 25-hydroxyvitamin D levels at follow-up were in the normal range (> 50 nmol/l) in 13/17 of the treatment group and 4/17 of the placebo group ($P$ = 0.002, $\chi^2$-test). Changes in outcome measures between groups are shown in Table 2. There was no significant difference in change in calcium, phosphate, PTH or $HbA_{1c}$ between the groups. Patients in the vitamin D group demonstrated significant improvement in FMD compared with the placebo group (2.35 vs. 0.06%, $P$ = 0.048); there was no significant change in blood flow in response to reactive hyperaemia and no change in response to GTN, confirming that the observed change in FMD was endothelium dependent. Absolute values for FMD reactivity were similar in the two groups at baseline (7.28 vs. 6.38%, $P$ = 0.49) and at follow-up (7.34 vs. 8.73%, $P$ = 0.35).

Vitamin D administration significantly decreased office systolic blood pressure (–7.3 vs. +6.6 mmHg, $P$ = 0.001). Change in systolic blood pressure did not correlate with change in FMD (Pearson's $r$ = –0.05, $P$ = 0.77). To ascertain whether this change in blood pressure accounted for the improvement in FMD seen in the vitamin D-treated group, change in FMD was adjusted for the change in systolic blood pressure. After adjustment, the difference in FMD change between placebo and vitamin D-treated patients was 3.01% (95% CI 0.28 to 5.74%, $P$ = 0.03). Analysis including only the 13 patients not taking angiotensin-converting enzyme (ACE) inhibitors or angiotensin receptor blockers revealed a smaller change (1.07% in placebo vs. 2.52% in treatment arm, $P$ = 0.39), rendered non-significant by the small numbers.

No significant changes in markers of glycaemic control or insulin sensitivity were seen between groups, however, a post-hoc analysis combining both groups showed that insulin sensitivity as measured by HOMA was significantly improved in those who had a 25-hydroxyvitamin D increase of 11 nmol/l or more; the mean increase in vitamin D required to raise


 

**Table 2** Change from baseline of parameters during treatment with vitamin D or placebo, $n = 34$

| Parameter | Change with vitamin D | Change with placebo | $P$ (between groups) |
|---|---|---|---|
| *Vitamin D homeostasis* | | | |
| Vitamin D (nmol/l) | 22.9 ± 16.6 | 7.6 ± 12.5 | 0.02 |
| Calcium (mmol/l) | 0.01 ± 0.08 | −0.04 ± 0.10 | 0.07 |
| Phosphate (mmol/l) | 0.05 ± 0.14 | −0.04 ± 0.14 | 0.08 |
| PTH (pmol/l) | −0.14 ± 0.99 | −0.18 ± 0.94 | 0.89 |
| *Glycaemic control* | | | |
| $HbA_{1c}$ (%) | 0.01 ± 0.60 | −0.05 ± 0.39 | 0.74 |
| HOMA (IS) | −39.7 ± 79.3 | −25.6 ± 139.0 | 0.72 |
| *Endothelial function* | | | |
| FMD response to hyperaemia (%) | 2.35 ± 3.12 | 0.06 ± 3.39 | 0.048 |
| FMD flow (%) | 1.17 ± 26.68 | 3.55 ± 21.55 | 0.78 |
| FMD response to GTN (%) | −1.33 ± 2.72 | −0.98 ± 5.65 | 0.82 |
| *Blood pressure* | | | |
| Systolic BP (mmHg) | −7.3 ± 11.8 | 6.6 ± 9.7 | 0.001 |
| Diastolic BP (mmHg) | −2.2 ± 8.6 | 2.3 ± 5.7 | 0.08 |
| *Renin–angiotensin levels* | | | |
| Renin (ng/ml) | 1.85 ± 0.48 | −0.79 ± 2.04 | 0.06 |
| Angiotensin II (pg/ml) | −6.3 ± 19.0 | 6.8 ± 30.0 | 0.14 |

Mean ± SD.
BP, blood pressure; FMD, flow-mediated vasodilation; GTN, glyceryl trinitrate; $HbA_{1c}$, glycated haemoglobin; HOMA, homeostatic model assessment; IS, insulin sensitivity; PTH, parathyroid hormone; SD, standard deviation.

serum levels into the normal range (HOMA +15 vs. −98, $P = 0.003$).

## Discussion

Our first finding of note is that vitamin D insufficiency is remarkably common during winter in patients with Type 2 diabetes in Scotland. Our second main finding is that vitamin D supplementation improves endothelial function in this group of patients. Endothelial function is a powerful surrogate marker of cardiovascular risk [13]. Changes in endothelial function are the best available surrogate to predict what effect a new therapy will have on cardiovascular events [18]. Treatment-induced changes in endothelial function have produced a few false positive results (e.g. hormone replacement therapy and antioxidant vitamins) but have not produced a false negative result. Endothelial function is thus a highly sensitive, albeit not perfectly specific, test for predicting which interventions are likely to reduce cardiovascular events [13]. Thus, the improvement in endothelial function seen in this study provides evidence that high-dose vitamin D therapy may be able to reduce cardiovascular events in patients with Type 2 diabetes, and merits further study.

There are several possible mechanisms by which vitamin D could improve endothelial function. Vitamin D may improve endothelial function indirectly by reducing blood pressure, which may in turn be due to its suppressing renin [19] and/or to its decreasing vascular resistance [20]. Vitamin D may favourably alter coronary calcification, which is a precursor of vascular events and a common finding in Type 2 diabetes. This possibility arises because of previous observational data linking low 25-hydroxyvitamin D levels with coronary calcification [8]. Vitamin D supplementation has been shown to reduce levels of tumour necrosis factor (TNF)-α, a pro-inflammatory cytokine, in patients with chronic heart failure [21]. Vitamin D also reduces activation of a key cellular component of the atherosclerotic response—the macrophage [22]. Human endothelial cells are able to synthesize the active form of vitamin D, which may act at the local level to modulate the effects of inflammatory cytokines on the vasculature [23]. The vitamin D receptor activates a wide variety of other genes [20] including vascular endothelial growth factor (VEGF). VEGF receptor expression is impaired in patients with Type 2 diabetes [24] and VEGF in turn promotes nitric oxide synthesis by endothelial cells [25]. It has also been hypothesized that low vitamin D levels and subsequent secondary hyperparathyroidism may promote an acute-phase response which could explain why low levels of vitamin D may be a risk factor for increased cardiovascular events [26]. Many hypotheses are possible and further detailed studies are now warranted to elucidate the pathways by which vitamin D improves endothelial function in patients with Type 2 diabetes.

Interpretation of the blood pressure changes in this study is made difficult because of near-significant differences at baseline, but vitamin D supplementation did produce a highly significant fall in systolic blood pressure ($P < 0.001$) and a non-significant fall in diastolic blood pressure. However, the effect of vitamin D on endothelial function appeared to be independent of its effect on blood pressure; the improvement in endothelial function increased slightly after adjustment for

 

change in blood pressure. Although the changes in renin and angiotensin II levels did not reach significance, they suggest a reduction in angiotensin II with escape from negative feedback of renin levels in the vitamin D-treated patients. This mechanism is somewhat different from that previously proposed for the effect of vitamin D on the rennin–angiotensin system [19].

Although insulin sensitivity showed no overall significant changes, it is intriguing that it improved in those who achieved the largest increase in 25-hydroxyvitamin D levels, suggesting a possible therapeutic effect. These data are also consistent with observational data linking low 25-hydroxyvitamin D levels to impaired insulin sensitivity [26–28] and suggest that low 25-hydroxyvitamin D levels could be causally linked to impaired insulin sensitivity. Elevated PTH levels are associated with insulin resistance; the dose of vitamin D used in our study did not suppress PTH levels and higher doses may be needed to show this effect.

A large vitamin D supplementation study (36 282 postmenopausal women) recently showed no decrease in cardiovascular risk with vitamin D supplementation [29]. However, this study had major limitations; firstly, the dose of vitamin D used (400 IU per day) was very low (800 IU per day is recommended for older people at risk of falls [30]); secondly, patients were not recruited only if they were vitamin D insufficient; indeed, baseline or post-treatment serum 25-hydroxyvitamin D levels were not measured at all. Thirdly, all individuals, including the placebo group, were permitted to take vitamin D supplements. Thus, the negative results from this study are unsurprising and certainly do not exclude a clinically useful effect of adequate doses of vitamin D given to vitamin D-insufficient patients.

The limitations of our study were low subject numbers and the near-significant differences in blood pressure at baseline between subjects randomly allocated to the vitamin D or the placebo group, which made interpretation of blood pressure changes difficult. We used only a single dose of vitamin D during the winter; further studies are needed to assess the effect of repeated doses, the effects in other seasons and the effect in patients who are not vitamin D insufficient. The increase in serum 25-hydroxyvitamin D levels throughout this study is likely to have been underestimated by 25%, as the assay used has lower reactivity for 25-hydroxyvitamin D2 compared with 25-hydroxyvitamin D3.

In conclusion, this small pilot study has shown that a single, large dose of vitamin D2 improves endothelial function in patients with Type 2 diabetes and insufficient levels of 25-hydroxyvitamin D. This finding suggests that vitamin D, which is a cheap, safe and inexpensive medication that can be taken once every few weeks, could provide a novel way of reducing cardiovascular events in patients with Type 2 diabetes mellitus. Given the small and limited nature of our study, further research is needed to test the reproducibility of our findings, define the optimum dose and frequency, and explore whether clinically important end points are affected by vitamin D.

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## Competing interests

None to declare.

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