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

Pamela R. von Hurst¹*, Welma Stonehouse¹ and Jane Coad²

¹Institute of Food, Nutrition and Human Health, Massey University, Private Bag 102 904, North Shore Mail Centre, Auckland, New Zealand
²Institute of Food, Nutrition and Human Health, Massey University, Palmerston North, New Zealand

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Low serum 25-hydroxyvitamin D (25(OH)D) concentrations have been shown to correlate with increased risk of type 2 diabetes. Small, observational studies suggest an action for vitamin D in improving insulin sensitivity and/or insulin secretion. The objective of the present study was to investigate the effect of improved vitamin D status on insulin resistance (IR), utilising randomised, controlled, double-blind intervention administering 100 μg (4000 IU) vitamin D₃ (n 42) or placebo (n 39) daily for 6 months to South Asian women, aged 23–68 years, living in Auckland, New Zealand. Subjects were insulin resistant – homeostasis model assessment 1 (HOMA1) > 1.93 and had serum 25(OH)D concentration < 50 nmol/l. Exclusion criteria included diabetes medication and vitamin D supplementation >25 μg (1000 IU)/d. The HOMA2 computer model was used to calculate outcomes. Median (25th, 75th percentiles) serum 25(OH)D3 increased significantly from 21 (11, 40) to 75 (55, 84) nmol/l with supplementation. Significant improvements were seen in insulin sensitivity and IR (P=0.003 and 0.02, respectively), and fasting insulin decreased (P=0.02) with supplementation compared with placebo. There was no change in C-peptide with supplementation. IR was most improved when endpoint serum 25(OH)D reached ≥80 nmol/l. Secondary outcome variables (lipid profile and high sensitivity C-reactive protein) were not affected by supplementation. In conclusion, improving vitamin D status in insulin resistant women resulted in improved IR and sensitivity, but no change in insulin secretion. Optimal vitamin D concentrations for reducing IR were shown to be 80–119 nmol/l, providing further evidence for an increase in the recommended adequate levels. Registered Trial No. ACTRN12607000642482.

Vitamin D: Type 2 diabetes: Insulin resistance

There is mounting interest in the role of vitamin D in the aetiology of type 2 diabetes, and the most commonly preceding conditions, reduced insulin sensitivity and compromised β-cell function.

Low serum 25-hydroxyvitamin D (25(OH)D) concentrations have been shown to correlate with impaired glucose tolerance and an increased risk of type 2 diabetes¹–⁵, while a correlation between hypovitaminosis D and insulin resistance (IR) has been identified in pregnant women and obese adolescents⁶,⁷. A 10-year prospective study identified an inverse relationship between baseline serum 25(OH)D concentrations and later risk of IR⁸. Administration of supplemental vitamin D to subjects with elevated blood glucose levels has resulted in an improvement in insulin secretion⁹,¹⁰, and similar improvements have been observed in vitamin D-deficient subjects following supplementation¹¹,¹².

Tai et al.¹² found no improvement in glucose tolerance following the administration of two vitamin D doses (2500 μg; 100 000 IU) with an interval of 2 weeks to thirty-seven non-diabetic, vitamin D-deficient adults. Nagpal et al.¹³ reported a randomised, controlled trial of vitamin D₃, three fortnightly doses of 3000 μg (120 000 IU) or placebo, in centrally obese Indian men. The subjects were not necessarily insulin resistant, but there was some improvement in postprandial insulin sensitivity following supplementation. A recent systematic review and meta-analysis on the role of vitamin D and calcium in type 2 diabetes conclude that ‘there appears to be a relationship’ but due to the paucity of data, an understanding of the mechanisms is incomplete⁶,¹⁴.

To date, there have been no randomised, controlled trials with vitamin D supplementation of a dose sufficient to raise serum 25(OH)D to >80 nmol/l in vitamin D-deficient, non-diabetic, insulin-resistant subjects. It has been shown that a dose of vitamin D >50 μg (2000 IU) per day is required to raise and maintain serum concentration to 80 nmol/l¹⁵. However, the increase in serum levels is related to baseline concentration, and where a patient is severely deficient (<12.5 nmol/l), a higher dose may be required¹⁶. Earlier

Abbreviations: FSG, fasting serum glucose; HOMA, homeostasis model assessment; IR, insulin resistance; MMP, matrix metalloproteinases; 25(OH)D, 25-hydroxyvitamin D.

* Corresponding author: Pamela R. von Hurst, fax +64 9 443 9640, email p.r.vonhurst@massey.ac.nz
concerns about the toxic effects of high doses of supplemental vitamin D have been allayed by safety and efficacy tests, which have demonstrated that doses of 100 and 250 μg (4000 and 10 000 IU) per day for 5–6 months resulted in no toxic effects.(16,17)

The population of interest in the present study is women of South Asian origin living in Auckland, New Zealand. There is a threefold higher prevalence of self-reported diabetes in South Asians living in New Zealand compared with the general population(18), and we have previously reported a prevalence of hypovitaminosis D of 84 % in South Asian women.(19)

Aim

The aim was to investigate the effect of improved vitamin D status on markers of metabolic syndrome, primarily IR, in South Asian women who were insulin resistant and vitamin D deficient.

Method

The study protocol is described in greater detail elsewhere(20). In brief, the study design was a randomised, placebo-controlled, double-blind trial with 100 μg (4000 IU) vitamin D₃ (cholecalciferol) per day (four capsules of 25 μg (1000 IU) each) or four capsules of placebo per day for 6 months. Women of South Asian origin (n 235) were recruited and screened for hypovitaminosis D (serum 25(OH)D < 50 nmol/l) plus IR (homeostasis model assessment, HOMA-IR ≥ 1·93) and/or TAG/HDL cholesterol ratio ≥ 3·0. There are no recognised HOMA-IR cut-offs for this population, and the rather arbitrary selection of 1·93 was based on the findings of the Chennai Urban Population Study in India where the upper quartile was found to have a HOMA-IR score ≥ 1·93(21). An elevated TAG/HDL ratio has been identified as a strong predictor of IR and metabolic syndrome(22).

Exclusion criteria included fasting serum glucose (FSG) ≥ 7·2 mmol/l, medication for diabetes and vitamin D supplementation ≥ 25 μg (1000 IU) per day.

Subjects were matched into pairs by age and BMI. Randomisation of the vitamin D/placebo capsules and allocation to the members of each pair were performed by Blackmores Ltd using nQuery Advisor® version 6·0 (Statistical Solutions, Cork, Ireland). Randomisation and allocation were fully concealed from the researchers until after statistical analysis of the data.

Fasting blood samples and anthropometric measurements were obtained at baseline and the end of the study. The intervention in the original cohort commenced in July 2007, which is mid-winter in New Zealand, and a second small cohort (n 7) commenced in October 2007 following the early loss of subjects from the study (see Results section). Subjects were recalled for their final blood test 6 months later i.e. January 2008 (mid-summer) and April 2008. Subjects were also recalled for a blood test at 3 months. This was primarily to check for adverse effects in response to the high dose of vitamin D supplementation. Serum Ca results were immediately checked for abnormality by a colleague not associated with the study, and results were entered into the database at the end of the study. Subjects were advised to contact research staff immediately if they suspected a reaction to the supplements.

The original HOMA1 model for IR was used for subject selection as explained above(23). This model utilises a simple linear equation based on pairing FSG and fasting serum insulin to establish a measure for IR: HOMA1-IR = (fasting serum insulin × FSG)/22·5. The technique is simple and inexpensive with relatively low subject burden, and has been shown to correlate well with the glucose clamp in predicting insulin sensitivity.(24,25)

The revised HOMA2 model was utilised to assess outcomes of the intervention. This is a computer model consisting of non-linear empirical equations that, when solved, allow the determination of insulin sensitivity (HOMA2 %S) from FSG and fasting serum insulin, and β-cell function (HOMA2 %B) from paired FSG and C-peptide. C-peptide is a reliable marker for insulin secretion as, unlike insulin, it is not taken up by the liver(26). Both β-cell function and insulin sensitivity are reported as a percentage, where 100 % is normal(26). IR is the reciprocal of percentage sensitivity, and 1-0 is normal.

Methods for all measurements and laboratory analysis of FSG, fasting serum insulin, lipid profile, high sensitivity C-reactive protein, Ca and serum 25(OH)D are reported in von Hurst et al. (27). C-peptide was measured in EDTA plasma samples stored at –80°C, by Canterbury District Health Board Laboratory (Christchurch, New Zealand), performed on the automated Roche Elecsys 2010 analyser, CV (within batch) of 2·4 % at 620 pmol/l. Insulin and glucose were measured by LabPlus (Auckland, New Zealand). Insulin method: micro-particle enzyme immunoassay technology (Abbott Diagnostics, Abbott Park, Ill., USA); intra-assay CV 4·5 % at 58·3 pmol/l (8·4 mU/l). Glucose method: standard enzymatic colourimetric assay (Roche Diagnostics, Basel, Switzerland); intra-assay CV 0·8 % at 6·6 mmol/l; inter-assay CV 1·8 % at 6·6 mmol/l.

Statistical methods

It was calculated that forty-two subjects would be required for each arm of the trial to demonstrate a significant difference at 80 % power and 5 % significance. Power calculations were based on the results of a lifestyle intervention in obese women, which achieved a reduction in HOMA-IR of 0·98 (sp 0·77)(27). Serum 25(OH)D, high sensitivity C-reactive protein, insulin, glucose, plasma C-peptide and HOMA1 and 2 were not normally distributed and are reported as median (25th, 75th percentiles). Normally, distributed data are reported as means and standard deviations. Non-parametric tests were used to compare groups (Mann–Whitney U test), and to compare baseline and endpoint measures within groups (Wilcoxon). The Kruskal–Wallis test and post hoc tests plus Bonferroni adjustments were used to compare more than two independent groups or conditions, and the Friedman’s ANOVA and post hoc tests plus adjustments to compare more than two related groups. A two-tailed P-value of <0·05 was considered statistically significant. Pearson’s correlations were used for normally distributed data, and Spearman’s correlations used for non-parametric data.

Ethical approval

The present study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures
Results

Two hundred and thirty-five women were recruited and screened for IR and hypovitaminosis D. One hundred and fourteen qualified for selection, and from those 106 women volunteered to take part in the intervention trial. Twelve were lost to the study due to becoming pregnant (n 3), moving overseas (n 4), perceived side effects (headaches and constipation; n 2) and medical practitioner prescribing vitamin D (n 3). Following the early loss of the above-mentioned subjects, the study was reopened for recruitment to ensure sufficient numbers. Seven women joined the RCT 4 months after the initial cohort – their numbers are included in the totals above. A further thirteen women could not be contacted/traced at the end of the trial. The baseline characteristics of this group of twenty-five women (eleven from the vitamin D group and fourteen from the placebo group) did not differ significantly from those participants who remained in the study.

The majority of participants (91%) were Indian, with 6% from Sri Lanka and 3% from Pakistan. Of the participants, 79% had been in New Zealand for ≤ 10 years, and no relationship was seen between time in New Zealand and baseline 25(OH)D.

Baseline characteristics of the vitamin D and placebo groups are shown in Table 1. There were no significant differences between the groups at baseline in any of the measures reported. BMI did not change significantly in either group during the study. While there was no relationship between HOMA-IR and age, a positive correlation was seen between HOMA-IR and BMI at baseline (r 0·316, P = 0·004). No adverse effects were observed in the serum calcium results at 3 months.

Serum 25(OH)D concentrations increased significantly in the vitamin D-supplemented group, from 21 (11, 40) nmol/l at baseline to 93 (69, 103) nmol/l at 3 months and then declined to 80 (67, 94) nmol/l at 6 months. There was also a significant increase in the placebo group between 3 months at 21 (15, 36) nmol/l and 6 months at 29 (23, 46) nmol/l (P = 0·014). A significant inverse relationship was found between baseline concentrations of 25(OH)D and the change in HOMA1-IR and fasting insulin over 6 months in both the vitamin D (r = 0·349, P = 0·023) and placebo (r = 0·456, P = 0·004) groups. There was no relationship between change in vitamin D concentration and BMI.

Significant improvements were seen in insulin sensitivity, IR and fasting insulin (Table 2), with supplementation compared with placebo. There was a significant difference in the change in HOMA1-IR between groups (P = 0·03), with a decrease of −0·25 (0·24, −0·81) in the vitamin D-supplemented group and an increase of 0·36 (1·16, −0·41) in the placebo group. Changes in FSG, HOMA2%B, C-peptide, high sensitivity C-reactive protein, total cholesterol, TAG/HDL cholesterol ratio, HDL cholesterol and TAG were NS within, and did not differ between, groups. In the vitamin D-supplemented group, HOMA2 %S increased (P = 0·01), fasting insulin declined (P = 0·02) and overall IR decreased compared with baseline (P = 0·03). To eliminate regression toward the mean as a confounder, change was calculated by subtracting endpoint values from the mean of baseline and endpoint HOMA2 %S; the difference between groups remained significant (P = 0·003).

Sixteen out of the forty-two women in the vitamin D group achieved serum 25(OH)D concentrations of > 80 nmol/l at both the 3 and 6 month tests. In these women, HOMA2 %S increased from 60·1 (50·9, 70·5) at baseline to 66·4 (55·3, 84·5) at 3 months (P = 0·12), but the increase did not achieve significance until 6 months when HOMA2 %S reached 85·8 (47·3, 103·9; P = 0·013; Fig. 1).

### Table 1. Baseline characteristics of trial participants

<table>
<thead>
<tr>
<th></th>
<th>Vitamin D group (n 42)</th>
<th>Placebo group (n 39)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Age (years)</td>
<td>41·8</td>
<td>10·1</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27·5</td>
<td>5·0</td>
</tr>
<tr>
<td>Waist−hip ratio</td>
<td>0·80</td>
<td>0·07</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>121·6</td>
<td>17·6</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>80·4</td>
<td>8·9</td>
</tr>
<tr>
<td>HOMA-IR (HOMA1 model)</td>
<td>2·70</td>
<td>2·13, 3·61</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>5·1</td>
<td>4·5, 5·5</td>
</tr>
<tr>
<td>LDL-C (mmol/l)</td>
<td>3·2</td>
<td>2·9, 3·5</td>
</tr>
<tr>
<td>HDL-C (mmol/l)</td>
<td>1·2</td>
<td>1·0, 1·4</td>
</tr>
<tr>
<td>TAG (mmol/l)</td>
<td>1·4</td>
<td>0·9, 1·7</td>
</tr>
<tr>
<td>TAG/HDL-C ratio</td>
<td>2·6</td>
<td>1·9, 3·6</td>
</tr>
</tbody>
</table>

HOMA-IR, homeostasis model assessment insulin resistance; LDL-C, LDL cholesterol; HDL-C, HDL cholesterol.

There were no significant differences between the groups in any of the variables reported above (for statistics between groups: non-parametric variables – Mann–Whitney U test; parametric variables – independent t test). Baseline 25(OH)D, fasting serum glucose (FSG), fasting serum insulin (FSI) and high sensitivity C-reactive protein (hs-CRP) are reported in Table 2.

* Data are normally distributed.
† Data are not normally distributed.

Involving human subjects were approved by the Massey University Human Ethics Board (Southern A), Reference no. 06/67. Written informed consent was obtained from all subjects.
in the latter half of the study. Meanwhile, it is likely that the increase in serum 25(OH)D concentrations in the placebo group from mid-study is due to more incidental sun exposure and higher levels of UVB with the approach of summer, to which the supplement group would also have been exposed. We have previously reported a small increase in the 25(OH)D concentrations in South Asian women who were tested during late summer, compared with those tested during winter and spring(19).

The method of administration of the supplement in the present study (daily dose) differed from the large fortnightly doses used in recent studies(12,13) and the approved prescription dose in New Zealand of 1250 μg (50 000 IU) per month(28). While the larger, less frequent doses may be advantageous from a compliance perspective, daily doses have been shown to be more effective than weekly or monthly doses as measured by serum 25(OH)D, parathyroid hormone and bone markers(29). It is possible that a single, large bolus could be detrimental, as the body is not designed to handle large doses of vitamin D. Indeed, there is evidence that a single, large bolus can have negative outcomes; Taylor & Wise(30) treated three vitamin D-deficient individuals with type 2 diabetes with one intramuscular dose of 7500 μg (300 000 IU) of vitamin D2 and saw an increase in hyperglycaemia and lipidaemia(30).

The wide range of endpoint serum 25(OH)D concentrations in the vitamin D group (10–119 nmol/l) suggests variable compliance. Subjects reported finding it difficult to consistently consume four capsules per day for 6 months. The decline in median serum levels from the 3-month tests to endpoint in the vitamin D group probably reflects compliance dropping off during winter and spring.
Vitamin D supplements and insulin resistance

Fig. 1. Changes in serum 25-hydroxyvitamin D (25(OH)D) and HOMA2 %S over time in subjects (n 16) whose endpoint serum 25(OH)D was >80 nmol/l. Mean value was significantly different from that at baseline: * \( P = 0.013, \) ** \( P < 0.001. \) Y-axis values include both serum 25(OH)D (nmol/l) and HOMA2 %S where 100 % is ideal. The Friedman’s ANOVA and post hoc tests with adjustments were used to compare more than two related groups.

The significant inverse relationship between baseline concentration and increase in serum 25(OH)D suggests that baseline values influence the response to both cutaneous synthesis and supplementation. This is in agreement with other evidence that low baseline levels produce a steep dose-response slope in response to supplementation, and is possibly due to feedback regulation, although synthesis of 25(OH)D in the liver is only loosely regulated.

Previous vitamin D supplementation studies, especially those in diabetic subjects, have concentrated on the role of vitamin D in increased insulin secretion. There is also considerable evidence from animal studies that adequate 25(OH)D concentrations are required for normal insulin secretion.

Subjects in the present study were not diabetic, insulin secretion was not compromised and there was no change in C-peptide levels, suggesting that insulin secretion did not increase in response to supplementation. With the increase in serum 25(OH)D concentration in the vitamin D group, IR improved, driven by an upward shift in insulin sensitivity and a corresponding drop in fasting insulin as tissue extraction of insulin increased. Insulin sensitivity was significantly improved when the endpoint serum 25(OH)D concentrations exceeded 80 nmol/l. These findings support those of Chiu et al. (36) in a cross-sectional study, which suggested enhanced insulin sensitivity might be seen if 25(OH)D concentration was to be increased from 25 to 75 nmol/l. The same study also reported a subtle variation in β-cell response to an oral glucose tolerance test at different vitamin D concentrations.

There have been at least two mechanisms postulated for an increase in insulin sensitivity in response to improved vitamin D status – suppression of chronic inflammation and increased expression of the insulin receptor and/or proteins of the insulin-signalling cascade. A mild inflammatory state, marked by the presence of proinflammatory cytokines, is associated with obesity and IR. These cytokines, predominantly TNF-α and IL-6, are known to be released from adipose tissue and increased serum concentrations are known to induce IR in multiple tissues. Vitamin D has recognised anti-inflammatory actions: it has been shown to dose dependently suppress the release of TNF-α and IL-6 while up-regulating synthesis of the anti-inflammatory cytokine IL-10, thus potentially partly counteracting the inflammatory consequences of increased adiposity. Plasma matrix metalloproteinases (MMP) are also inflammatory markers and are associated with vascular damage and unstable angina. MMP2 and MMP9 have been shown to be inversely correlated with vitamin D status, and reduced with vitamin D supplementation.

CRP is considered to be a useful biomarker for the presence of TNF-α and IL-6. In the present study, we measured only high sensitivity C-reactive protein; baseline levels were within the normal range in each group and the reduction following supplementation was NS. Participants, although overweight, were not obese and mean waist/hip ratio did not exceed 0.80. These characteristics are similar to those found in women of other ethnicities, and in centrally obese Indian men (33,43). It is conceivable that the inflammatory effects of increased adiposity were not a major cause of their IR; thus, the anti-inflammatory action of vitamin D is unlikely to explain the improved IR observed in the present study. Future studies, especially in subjects susceptible to inflammation, should measure IL-6, TNF-α, IL-10 and possibly MMP2 and MMP9 to further explore this as a plausible mechanism.

A second potential mechanism for the influence of vitamin D on insulin sensitivity is in the regulation of the insulin-signalling cascade. A vitamin D response element has been identified on the human insulin receptor (IR) gene promoter, and in vitro treatment with 1,25-dihydroxyvitamin D3 resulted in increased transcription of the insulin receptor gene, together with improved insulin-dependent glucose transport. Additionally, 1,25(OH)2D3 appears to stimulate glucose oxidation either via the activation of IR transcription or by a direct regulation of phosphatidylinositol 3-kinase activity. Murine studies have shown phosphatidylinositol 3-kinase and the insulin receptor substrate proteins to be important coordinators of insulin regulation. Insulin-stimulated activity of phosphatidylinositol 3-kinase and other proteins downstream of phosphatidylinositol 3-kinase such as protein kinase C has been shown to be impaired in obese and diabetic human subjects, and improved in obese subjects following weight loss.

Conclusions

Insulin sensitivity did improve in these insulin-resistant women with vitamin D supplementation. No significant change was seen in insulin sensitivity until serum 25(OH)D concentrations reached levels above 80 nmol/l, and despite substantial increase in serum 25(OH)D concentration at 3 months, there was no significant change in insulin sensitivity until 6 months. The findings provide further evidence for an increase in the recommended adequate levels of 25(OH)D from 50 to 80 nmol/l, and suggest the importance of the long-term maintenance of adequate vitamin D levels. Differences in duration of studies could explain the disparity between the present results and those of Tai et al. (12) and Nagpal et al. (13).
Future studies should examine the effect of vitamin D supplementation on both insulin secretion and sensitivity, as well as the potential anti-inflammatory effect of vitamin D, although this is possibly more relevant if the subjects are obese and inflammatory markers are elevated. Further investigation is required into the role of vitamin D in the expression of the insulin receptor gene and in the insulin-signalling pathway.

Interpersonal variations in sun exposure requirements, together with the accepted risks of excessive UV radiation, make it very difficult to give recommendations about sun exposure. Supplementation may be the best solution for some populations, especially those with darker skin living in temperate climates. However, as sufficiency of dose is critical, we need to learn more about the long-term safety and efficacy of high dose vitamin D supplements and the most effective way to deliver them.

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References