DOI: 10.1111/j.1464-5491.2008.02636.x

Original Article: Metabolism

A double-blind, randomized, placebo-controlled trial of the short-term effect of vitamin D_3 supplementation on insulin sensitivity in apparently healthy, middle-aged, centrally obese men

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Accepted 31 October 2008

Abstract

Aim To determine the short-term effect of vitamin D_3 supplementation on insulin sensitivity in apparently healthy, middle-aged, centrally obese men.

Subjects and methods A double-blind randomized controlled trial was conducted at a tertiary care facility in which 100 male volunteers aged ≥ 35 years received three doses of vitamin D_3 (120 000 IU each; supplemented group) fortnightly or placebo (control group). Hepatic fasting insulin sensitivity [homeostasis model assessment (HOMA), quantitative insulin-sensitivity check index, HOMA-2], postprandial insulin sensitivity [oral glucose insulin sensitivity (OGIS)], insulin secretion (HOMA%B, HOMA2-%B), lipid profile and blood pressure were measured at baseline and at 6 weeks' follow-up.

Results Seventy-one of the recruited subjects completed the study (35 in supplemented group, 36 in control group). There was an increase in OGIS with supplementation by per protocol analysis ($P = 0.038$; intention-to-treat analysis $P = 0.055$). The age- and baseline 25-hydroxyvitamin D level-adjusted difference in change in OGIS was highly significant (mean difference 41.1 ± 15.5; $P = 0.01$). No changes in secondary outcome measures (insulin secretion, basal indices of insulin sensitivity, blood pressure or lipid profile) were found with supplementation.

Conclusion The trial indicates that vitamin D_3 supplementation improves postprandial insulin sensitivity (OGIS) in apparently healthy men likely to have insulin resistance (centrally obese but non-diabetic).

Diabet. Med. 26, 19–27 (2009)

Keywords insulin sensitivity, obese, vitamin D_3

Abbreviations 1,25-$(OH)_2$-D, 1,25-hydroxyvitamin D; BP, blood pressure; HDL, high-density lipoprotein; HOMA, homeostasis model assessment; hs-CRP, high-sensitivity C-reactive protein; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; IRMA, immunoradiometric assay; ISI, Insulin Sensitivity Index; LDL, low-density lipoprotein; MMP, matrix metallopeptidase; OGIS, oral glucose insulin sensitivity; OGTT, oral glucose tolerance test; PTH, parathyroid hormone; QUICKI, quantitative insulin-sensitivity check index; RCT, randomized controlled trial; WC, waist circumference; WHR, waist–hip ratio

Introduction

Type 2 diabetes is a major public health problem, with an estimated 32.7 million patients in India alone [1]. Accumulating evidence suggests that serum vitamin D concentration may be inversely related to the prevalence of diabetes [2], to the plasma concentration of glucose [3], insulin resistance [3,4] and metabolic syndrome [4,5]. These observational studies suggest a role of vitamin D in the pathogenesis of Type 2 diabetes. Furthermore, the prevalence of vitamin D deficiency in Asian Indians is high [6]. However, there is a paucity of intervention trials on the effect of vitamin D supplementation on insulin resistance or glucose metabolism. The available trials, conducted using small sample sizes in different clinical settings [haemodialysed patients [7], healthy volunteers [8], gestational diabetes [9], healthy older adults with impaired fasting glucose (IFG) [10] and post menopausal women [11]] using

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different agents, regimens and outcome parameters document inconsistent results {significant decrease in HbA_{1c} with stable insulin concentration in haemodialysis patients; no effect of 7-day calcitriol supplementation in healthy volunteers; insulin concentration lower with oral 1,25-hydroxyvitamin D [1,25-$(OH)_2$-D] in gestational diabetes; lower rise in homeostasis model assessment (HOMA) over time in healthy older adults with IFG; no effect of vitamin D on serum insulin concentration in post menopausal women}. Unpublished data from a short-term pre- and post-cholecalciferol supplementation pilot trial conducted on 30 volunteers at our Institute had suggested an improvement in insulin sensitivity as measured by the oral glucose insulin sensitivity (OGIS) [12] index. We therefore designed this double-blind randomized controlled trial (RCT) to determine the effect of vitamin D supplementation on peripheral insulin sensitivity using this index as the primary outcome variable. As vitamin D (serum 25(OH)D concentration) status is associated with insulin secretion [13], lipid concentrations [14] and blood pressure [15], we also measured the changes in these variables.

Subjects and methods

A pilot trial was conducted on 30 apparently healthy centrally obese men as a prelude to this RCT. This was a pre- and post-cholecalciferol supplementation study to determine the most suitable oral glucose tolerance test (OGTT)-based index of insulin sensitivity [from amongst HOMA-IR, quantitative insulin-sensitivity check index (QUICKI), Insulin Sensitivity Index (ISI)-Stumvoll, ISI-Composite, ISI-Gutt and 3-h OGIS (Mari's formula [12])] and to calculate the sample size requirements for an RCT using the selected index. The results of the pilot trial indicated that HOMA, QUICKI and the other 2-h OGTT-based indices (ISI-Gutt, ISI-Composite, ISI-Stumvoll and 2-h OGIS) did not show any significant difference before and 4–6 weeks after a single dose of 240 000 IU of vitamin D, but the 3-h OGIS showed an improvement [mean (SD) difference of 38.8 ml min^{-1} kg^{-1} (77.3); $P = 0.017$]. A visual comparison of OGTT curves before and after the intervention revealed that the slope of fall in glucose concentration between 120 and 180 min was steeper after supplementation, possibly explaining the improvement in 3-h OGIS (higher OGIS corresponds to better insulin sensitivity). On regression analysis the change in OGIS (Δ-OGIS) was directly correlated with change in 25(OH)D and inversely correlated with change in parathyroid hormone (PTH) concentration after adjusting for the baseline OGIS. Changes in all other indices were not related to changes in 25(OH)D or PTH. OGIS index was thus selected as the primary outcome variable for the current study. Potential changes in HOMA-IR, HOMA% B [16], HOMA-2 [17], QUICKI, lipid profile, high-sensitivity C-reactive protein (hs-CRP) and blood pressure (BP) were selected as secondary outcome variables. 25(OH)D, PTH, serum creatinine and liver enzymes were identified as potential explanatory variables.

On the basis of the pilot trial it was estimated that a sample size of 40 subjects in each group would be required to detect a difference in Δ-OGIS (primary outcome variable) of 10% with 95% confidence and 80% power based on data {OGIS [mean (SD) = 495 ml min^{-1} kg^{-1} (90.3)], Δ-OGIS [mean (SD) = 38.88 ml min^{-1} kg^{-1} (77.73)]; $n = 31$} from the pilot trial. To account for attrition, 100 subjects were recruited. Post-trial estimates of power indicated that the completed study could detect a difference of 10% in OGIS between the two groups with 95% confidence and 77.7% power.

The current double-blind RCT was conducted between August 2006 and March 2007 at a tertiary care facility in New Delhi, India. Apparently healthy, centrally obese (waist circumference ≥ 78 cm as per the criteria suggested for Indians [18]), male volunteers aged ≥ 35 years were recruited. The above criteria were chosen to increase the possibility of including subjects who had some impairment in insulin sensitivity without frank diabetes. We restricted the recruitment to men for convenience (easier for men to stay overnight and allow blood sampling in the Indian context; higher proportion of working men in possible catchment areas) and to minimize sample size requirements (gender was considered an important confounder and therefore interpretation of the results would require stratification by gender). Exclusion criteria were: (i) fasting plasma glucose > 7.0 mmol/l measured by a home glucose monitoring device (One-Touch-Ultra Model No. AW-060-388-02A) after a supervised overnight fast) or who reported themselves to have diabetes or who were taking any oral glucose-lowering medication or insulin; (ii) resting BP > 140/90 mmHg or those who reported themselves to be hypertensive or those on any antihypertensive medication; (iii) cholecalciferol or calcium supplementation in last 6 months; (iv) chronic renal, hepatic, malignant or intestinal disease (self reported or any suggestive medical documents) or renal stones; (v) any medication within the last month that could influence insulin secretion, insulin sensitivity, vitamin D or calcium metabolism (e.g. theophylline, phenytoin, β-blockers, diuretics, statins or renin–angiotensin system inhibitors, etc.); (vi) febrile illness or infective morbidity in the last 10 days; and (vii) grossly deranged liver (serum bilirubin > 34 μmol/l and serum glutamic pyruvic transaminase more than four times upper limit of normal) or kidney function (serum creatinine > 177 μmol/l). An institutional ethics committee approved the study and informed written consent was obtained prior to recruitment.

A summary of the study design is depicted in Fig. 1. Recruited subjects were randomized into two groups. The supplemented group (S-group) received three doses of 120 000 IU oral cholecalciferol under direct supervision using unlabelled sachets of Calcirol (brand name of cholecalciferol from Cadila Pharmaceuticals, Ahmedabad, India) 2 weeks apart (±2 days), whereas the control group (C-group) received identical unlabelled sachets containing a placebo that was identical to the vitamin D_3 granules in colour, taste and external appearance. Both groups were advised to continue with their normal diet and exercise and to report any interval morbidity including nausea, vomiting, abdominal pain, urinary complaints or muscle cramps or diagnosis of hypercalcaemia (> 2.62 mmol/l) during treatment of any illness. Final assessment was done at 42 days (with margin of +7 days) after baseline. Follow-up assessment for any subject with fever was postponed until 5 days after fever. All investigations at baseline were repeated at follow-up. Allocation concealment and double blinding were ensured and the randomization code was broken only after complete data entry.

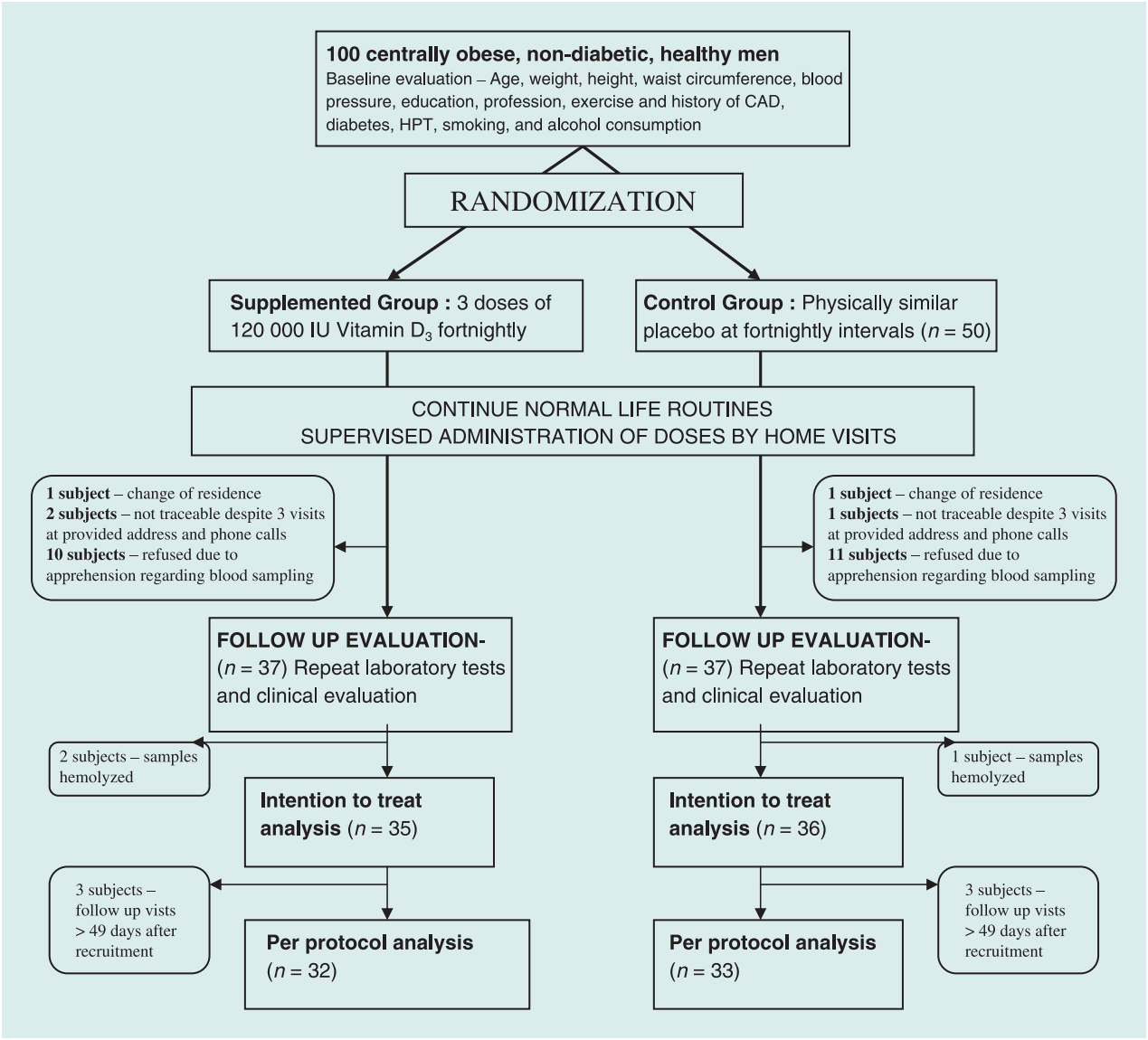

FIGURE 1 Summary of study design.

A standard pretested proforma was used for recording baseline information including age, education, annual household income, family history, betel-nut chewing, smoking and alcohol consumption. A complete physical examination was done in the morning, including height, weight, waist circumference (WC) and BP. Weight was recorded on a manual weighing scale (sensitivity 500 g), height using a SECA stadiometer (sensitivity 0.1 cm; SECA, Birmingham, UK), WC at the midpoint between the lower rib and iliac crest using a measuring tape (sensitivity 0.1 cm), and BP using an automated OMRON electronic instrument (sensitivity 1 mmHg; accuracy ±3 mmHg; OMRON, Milton Keynes, UK) validated in an earlier trial [19]. Height, WC and weight were recorded with light clothing and without shoes. Three serial BP recordings from the right arm were taken after 10 min rest at 10-min intervals in the sitting posture as per World Health Organization recommendations, and the mean used for analysis.

Consenting subjects were admitted around 17.00 h on the day prior to blood sampling. A standard 10 kcal/kg meal (55% carbohydrate, 30% fat, 15% protein) was given under supervision between 17.00 and 18.30 h. Fasting blood sampling was started between 09.00 and 10.00 h, and 10 ml of blood was drawn to estimate 25(OH)D, PTH, lipid profile including total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), very-low-density lipoprotein, hs-CRP and triglyceride, serum calcium and phosphorus, liver function tests, serum creatinine, and plasma glucose and insulin.

Glucose (75 g) in 200 ml water was administered immediately after baseline sampling and blood samples for plasma glucose and insulin estimation were drawn at 5, 10, 30, 90, 120 and 180 min for calculation of indices of insulin sensitivity. For this an intravenous cannula was inserted retrogradely and the sampling arm was warmed during the 3 h of the test to obtain arterialized venous samples (arterialized samples prevent

confounding via local blood flow changes or metabolic activity). The samples were immediately transported to the laboratory at 4°C to separate plasma or serum.

Biochemical analysis

The samples were centrifuged at 1207 ***g*** (4°C) and the serum/plasma was stored at –70°C. Lipid profile (serum) was estimated using the Hitachi 902 analyser (Roche Diagnostics, Basel, Switzerland) based on the cholesterol oxidase-p-aminophenazone principle (total cholesterol normal range < 4.6 mmol/l). Fasting plasma glucose was estimated in the same analyser based on the glucose oxidase-peroxidase principle (normal range 3.9–6.2 mmol/l). Serum 25(OH)D was measured by solvent extraction followed by radioimmunoassay (Diasorin, Stillwater, MN, USA). Plasma insulin was measured by double antibody immunoradiometric assay (IRMA) (Immunotech, Marseilles, France). Serum PTH concentration was also estimated using the double antibody IRMA (Immunotech). Five percent of the samples were re-run for quality control. Mean-centred coefficients of variation for 25(OH)D, PTH, LDL-cholesterol, insulin and glucose were 8.0, 7.0, 6.7, 8.7 and 5.2%, respectively. Insulin and glucose at 0 and 120 min and glucose at 180 min were analysed to enable calculation of OGIS. Samples at other time points are currently pending statistical analysis and modelling to allow calculation of some minimal model-based OGTT indices (Disposition Index and Sensitivity Index Oral—$S_{I\,oral}$).

Data analysis

Data entry and analysis was done using SPSS v 13.0 software (SPSS Inc., Chicago, IL, USA). The crude mean difference of change was tested for normality and compared using the unpaired *t*-test. 'Intention-to-treat' and 'Per-protocol' analysis were used. Post-intervention changes in vitamin D and PTH were analysed to document the efficacy of intervention.

Results

One hundred subjects who fulfilled the selection criteria and consented to participate were enrolled (50 each in the S- and C-groups). Seventy-four of the recruited subjects completed follow-up (37 in each group). Details of the study design and subjects lost to follow-up are depicted in Fig. 1. Thus data from 35 subjects in the S-group and 36 in the C-group were available for analysis. The baseline characteristics of subjects completing the study were comparable to those of subjects 'lost to follow-up' (data not presented). The baseline characteristics of subjects who completed follow-up were comparable except for 25(OH)D level (higher for the S-group; $P = 0.046$; Table 1). All subjects who accepted the second vitamin D dose also accepted the third dose and completed the study.

There was a statistically significant increase in 25(OH)D and decrease in PTH concentration in the S-group, whereas changes in the C-group were not significant, thus confirming the efficacy of the intervention. At follow-up the crude mean change Δ-OGIS was positive in the S-group and negative in the C-group (Tables 2 and 3). This difference was statistically significant ($P = 0.038$) on per-protocol analysis and of borderline significance on intention-to-treat analysis ($P = 0.055$) (Table 2). Changes in the secondary outcome measures including homeostasis model-based indices, BP, hs-CRP and lipid profile did not differ significantly.

Age, betel-nut chewing and baseline 25(OH)D were identified as potential confounders on the basis of baseline differences (Table 1). Correlation analysis with OGIS at baseline identified waist–hip ratio (WHR) ($r = –0.344$, $P = 0.001$), baseline 25(OH)D ($r = 0.156$, $P = 0.131$) and age ($r = –0.153$, $P = 0.138$), where correlation analysis with Δ-OGIS identified WHR ($r = 0.249$, $P = 0.036$) and baseline 25(OH)D ($r = –0.205$, $P = 0.086$) as potential confounders. Review of existing literature also revealed that age, betel-nut chewing [20], central obesity [21] and vitamin D status could potentially influence insulin sensitivity and/or vitamin D metabolism and hence the outcome [4]). To account for potential confounding due to the above correlations and differences, multiple regression analysis was done. We used Δ-OGIS by intention-to-treat analysis as the dependent variable with the assigned group, age, WHR, baseline 25(OH)D level and betel-nut chewing as the independent variables. The covariate adjusted mean difference in Δ-OGIS between the two groups was 41.1 ± 15.4 ($P = 0.01$; $r^2 = 0.204$) (Table 4). Higher WHR ($P = 0.029$) and lower baseline 25(OH)D concentration ($P = 0.010$) were significant predictors of greater improvements in OGIS.

No significant correlation of Δ-OGIS or 25(OH)D concentration was found with other baseline factors such as hours of television viewing, exercise, smoking, alcohol use, income, education, liver enzymes, serum creatinine or hs-CRP. The interval morbidity reported by the study subjects was comparable between the two groups (two vs. one case of cough/cold in the S- vs. C-group, $P = 0.538$; one vs. three cases of febrile illness in S- vs. C-group, $P = 0.317$). No symptoms related to hypercalcaemia were reported in either group. None of the patients had biochemical hypercalcaemia at baseline or at follow-up.

Discussion

Our results indicate that short-term oral supplementation with cholecalciferol for 6 weeks in middle-aged, centrally obese, non-diabetic, apparently healthy volunteers results in improvement in 3-h OGIS, whereas other insulin sensitivity indices (HOMA and QUICKI), B-cell function (HOMA%B and HOMA%2B), lipid profile and BP remain unaffected. The explanation for the discrepancies found is not clear, but may be interpreted as an improvement in postprandial glucose disposal, whereas the basal fasting hepatic insulin sensitivity remain unaffected. The underlying physiological mechanisms that differentiate fasting insulin sensitivity from postprandial glucose disposal have been well documented [IFG is due to

Table 1 Baseline characteristics

Variable	Supplemented group (mean ± SD; $n = 35$)	Control group (mean ± SD; $n = 36$)	Mean diff. ± SE of diff.	*P*-value
Age (years)	42.4 ± 6.6	45.0 ± 9.2	–2.57 ± 1.90	0.181
Education (years)	10.2 ± 2.9	11.5 ± 4.3	–1.27 ± 0.87	0.150
Income (Rs. per month)‡	8914 ± 8196	10466 ± 9931	–1551 ± 2195	0.482
Smoking*	0.43 ± 0.50	0.36 ± 0.49	0.07 ± 0.12	0.567
Alcohol†	0.51 ± 0.51	0.53 ± 0.51	–0.01 ± 0.12	0.911
Body mass index (kg/m^2)	26.7 ± 4.54	26.0 ± 3.46	0.66 ± 0.96	0.494
Waist circumference (cm)	92.2 ± 10.61	91.0 ± 7.43	1.19 ± 2.17	0.586
Waist–hip ratio	0.98 ± 0.06	0.97 ± 0.06	0.00 ± 0.01	0.750
TV viewing (h)	2.4 ± 2.88	3.1 ± 4.15	–0.73 ± 0.85	0.393
Exercise (h)	0.25 ± 0.34	0.19 ± 0.46	0.06 ± 0.10	0.536
Betel nut	18.4 ± 47	33.3 ± 39	14.9 ± 8.8	0.092
SBP (mmHg)	124 ± 11	124 ± 10	0.50 ± 2.5	0.842
DBP (mmHg)	78 ± 8	77 ± 9	1.0 ± 1.9	0.624
25(OH)D (nmol/l)	36.5 ± 14.55	30.0 ± 12.50	6.54 ± 3.22	**0.046**
PTH (ng/l)	33 ± 14.7	38 ± 23.0	–5.10 ± 4.59	0.270
Serum calcium (mmol/l)	2.3 ± 0.15	2.3 ± 0.23	0.02 ± 0.05	0.763
Serum phosphorus (mmol/l)	1.16 ± 0.19	1.16 ± 0.19	–0.02 ± 0.05	0.706
Serum creatinine (μmol/l)	81 ± 15.0	80 ± 20.3	0.88 ± 4.42	0.810
hs-CRP (*n* [%])				
< 0.3 mg/l	31 (88.6)	30 (83.3)	—	0.428
0.3–1.0 mg/l	3 (8.6)	5 (13.8)	—	
> 1.0 mg/l	1 (2.8)	1 (2.7)	—	
Liver function				
SGOT (IU/ml)	46 ± 23.8	37 ± 12.1	8.7 ± 4.5	0.058
SGPT (IU/ml)	48 ± 40.8	46 ± 31.3	2.8 ± 8.6	0.742
ALP (IU/ml)	101 ± 27.2	108 ± 35.1	–6.6 ± 7.5	0.377
Insulin sensitivity				
QUICKI	0.16 ± 0.02	0.16 ± 0.02	0.00 ± 0.004	0.927
HOMA-IR	1.47 ± 1.16	1.33 ± 0.85	0.14 ± 0.21	0.489
HOMA-2-IR	0.79 ± 0.56	0.68 ± 0.39	0.11 ± 0.11	0.325
OGIS (ml min^{-1} kg^{-1})	412 ± 68.8	434 ± 80.7	–21.27 ± 17.82	0.237
B-cell function				
HOMA%B	68.4 ± 49.2	64.4 ± 52.1	4.01 ± 12.04	0.740
HOMA2-%B	66.9 ± 28.95	60.5 ± 28.98	6.33 ± 6.87	0.360

*Current smoking (within last 1 month; any frequency).
†Current alcohol use (within last 1 month; any frequency).
‡The current exchange rate is ~1 US$ = Rs. 41.
SBP, systolic blood pressure; DBP, diastolic blood pressure; PTH, parathyroid hormone; hs-CRP, high-sensitivity C-reactive protein; SGOT, serum glutamic oxaloacetic transaminase; SGPT, serum glutamic pyruvic transaminase; ALP, alkaline phosphatase; QUICKI, quantitative insulin-sensitivity check index; HOMA, homeostasis model assessment; OGIS, oral glucose insulin sensitivity.

impaired basal insulin secretion and preferential resistance of glucose production to suppression by insulin, as reflected by fasting hyperglycaemia, despite normal plasma insulin concentrations and increased HOMA-IR, whereas impaired glucose tolerance (IGT) mainly results from reduced second-phase insulin release and peripheral insulin resistance, as reflected by reduced clamp-determined insulin sensitivity [22]]. The potential roles of the liver, kidney and muscle tissues in determination of HOMA and postprandial indices is also well described (HOMA-IR, which is solely based on fasting plasma glucose and insulin concentrations, is an index of the resistance of hepatic and renal glucose release to suppression by insulin [22], whereas the studies using frequent sample intravenous glucose tolerance tests or glucose clamp experiments [23,24] probably primarily measure the ability of insulin to stimulate muscle glucose uptake). The generally low levels of CRP observed in our study compared with subjects of White, Hispanic or African-American ethnicity [25] may indicate that low-grade inflammation in Indian, centrally obese, non-diabetic subjects is not an important factor.

The strength of the present study is that it is a double-blinded RCT conducted in a country with high prevalence of diabetes and hypovitaminosis D [5] using an accepted surrogate index of insulin sensitivity [26] selected on the basis of a pilot trial. The sample size was sufficient to detect changes in OGIS by per-protocol analysis but not by intention-to-treat analysis. The limitations of the study include the high drop-out rate (26%), small sample size, short duration of follow-up,

Table 2 Comparison of change in outcome parameters (follow-up minus baseline)—intention-to-treat analysis

Variable	Supplemented group (mean ± SD; $n = 35$)	Control group (mean ± SD; $n = 36$)	Mean diff. ± SE of diff.	*P*-value
Weight (kg)	0.03 ± 1.82	–0.38 ± 1.70	0.42 ± 0.42	0.417
Body mass index (kg/m^2)	–0.02 ± 0.62	–0.04 ± 0.68	0.02 ± 0.15	0.895
Waist circumference (cm)	–0.40 ± 3.83	–0.15 ± 2.85	–0.25 ± 0.80	0.756
Waist–hip ratio	–0.01 ± 0.03	–0.004 ± 0.03	0.006 ± 0.008	0.441
SBP (mmHg)	0.60 ± 9.82	–3.35 ± 7.21	3.95 ± 2.05	0.058
DBP (mmHg)	0.43 ± 7.66	–1.26 ± 5.97	1.69 ± 1.64	0.305
TV viewing (h)	–0.10 ± 1.01	–0.17 ± 1.01	0.07 ± 0.24	0.787
Exercise (h)	0.01 ± 0.49	–0.04 ± 0.34	0.05 ± 0.10	0.657
PTH (ng/l)	–5.10 ± 11.25	4.11 ± 19.75	–9.21 ± 3.83	**0.019**
25(OH)D (nmol/l)	35.1 ± 27.28	0.60 ± 11.61	34.52 ± 4.94	**0.000**
hs-CRP (n [%])				
< 0.3 mg/l	32 (91.4)	28 (77.7)	—	0.576*
0.3–1.0 mg/l	3 (8.6)	6 (16.6)	—	
> 1.0 mg/l	0	2 (5.5)	—	
Lipid profile				
LDL (mmol/l)	0.03 ± 0.47	–0.15 ± 0.69	0.18 ± 0.14	0.207
HDL (mmol/l)	0.08 ± 0.16	0.00 ± 0.24	0.08 ± 0.05	0.105
TG (mmol/l)	0.13 ± 0.70	–0.05 ± 0.51	0.18 ± 0.14	0.208
Total cholesterol (mmol/l)	0.18 ± 0.70	0.04 ± 0.90	0.14 ± 0.19	0.474
VLDL (mg/dl)	2.37 ± 12.35	–0.61 ± 8.73	2.98 ± 2.53	0.244
Insulin sensitivity				
HOMA-IR	0.14 ± 0.9	0.16 ± 1.0	–0.02 ± 0.2	0.947
HOMA-2-IR	0.12 ± 0.45	0.08 ± 0.44	0.05 ± 0.11	0.672
QUICKI	0.00 ± 0.02	0.00 ± 0.02	0.00 ± 0.00	0.737
OGIS (ml min^{-1} kg^{-1})	21.17 ± 67.86	–8.89 ± 61.10	30.06 ± 15.42	0.055
B-cell function				
HOMA-%B	33.5 ± 103.94	–0.55 ± 53.56	34.07 ± 19.54	0.086
HOMA2-%B	14.3 ± 41.59	2.27 ± 29.05	12.00 ± 8.49	0.162

*Since many of the CRP values were below the minimum recordable value for the equipment, comparisons were done using χ^2 test at baseline and at follow-up. The numbers and *P*-value reported represent results at follow-up.
SBP, systolic blood pressure; DBP, diastolic blood pressure; PTH, parathyroid hormone; hs-CRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; HDL, high-density lipoprotein; TG, triglyceride; VLDL, very-low-density lipoprotein; QUICKI, quantitative insulin-sensitivity check index; HOMA, homeostasis model assessment; OGIS, oral glucose insulin sensitivity.

restriction of the study population to men and that most of the subjects were severely vitamin D deficient at the start and most of the S-group did not reach normal levels (> 75 nmol/l). The high drop-out rate may be attributable to the lack of perceived benefit in the trial, requirement for overnight stay, loss of wages and fear of repeated blood sampling. However, the likelihood of this loss to follow-up biasing the results is low, because (i) none of the baseline characteristics was significantly different between those reporting and those lost to follow-up, (ii) the proportions of subjects lost to follow-up in both groups were comparable, and (iii) interval morbidity in the two groups did not differ. Nevertheless, the drop-outs constitute a limitation to the interpretation of the results. The significant but lower than anticipated rise in 25(OH)D concentration with supplementation may be due to ethnicity-related differences in vitamin D absorption [27], preferential uptake of vitamin D by adipose tissue in obese subjects [28], altered vitamin D metabolism [29], pharmacokinetic differences in a severely deficient population or a lower than expected potency of the sachet.

As presented earlier, several observational studies have reported that serum vitamin D concentration is inversely related to the prevalence of diabetes [2], to the plasma concentration of glucose [3], insulin resistance [3,4] and metabolic syndrome [4,5]. It is noteworthy that Chiu *et al.* [4] in an observational study on 126 glucose-tolerant subjects using the hyperglycaemic clamp documented a strong positive correlation of 25(OH)D levels with insulin sensitivity ($P < 0.0001$). The results of the current study are also consistent with those of intervention trials conducted in healthy older adults with IFG [10], postmenopausal women [11], gestational diabetes [9] and haemodialysis patients [7], but not with one in healthy volunteers [8]. However, all these trials were conducted on small sample sizes in widely disparate settings using different adducts of vitamin D, regimens and outcome measures resulting in poor comparability. In a recently published, double-blind RCT, long-term daily supplementation with vitamin D (700 IU) and calcium (500 mg) in 92 healthy older (≥ 65 years) adults with IFG led to a lower rise in HOMA-IR compared with placebo [10]. However, that trial was not specifically

Table 3 Comparison of change in outcome parameters (follow-up minus baseline)—per protocol analysis

Variable	Supplemented group (mean ± SD; $n = 32$)	Control group (mean ± SD; $n = 33$)	Mean Diff. ± SE of Diff.	*P*-value
Weight (kg)	0.35 ± 1.60	–0.41 ± 1.73	0.76 ± 0.41	0.070
Body mass index (kg/m^2)	0.08 ± 0.56	–0.05 ± 0.69	0.13 ± 0.16	0.399
Waist circumference (cm)	0.10 ± 3.61	–0.10 ± 2.91	–0.21 ± 0.81	0.794
Waist–Hip ratio	–0.012 ± 0.03	–0.004 ± 0.03	–0.008 ± 0.008	0.373
SBP (mmHg)	0.60 ± 9.82	–3.09 ± 7.14	3.68 ± 2.06	0.079
DBP (mmHg)	0.43 ± 7.66	–1.11 ± 5.99	1.55 ± 1.65	0.353
TV viewing (h)	–0.10 ± 1.01	–0.20 ± 1.00	0.10 ± 0.25	0.680
Exercise (h)	0.01 ± 0.49	–0.04 ± 0.34	0.05 ± 0.10	0.654
25(OH)D (nmol/l)	35.1 ± 27.28	0.65 ± 11.78	34.44 ± 5.02	**0.000**
PTH (ng/l)	–5.10 ± 11.25	4.52 ± 19.88	–9.62 ± 3.86	**0.015**
hs-CRP (n [%])				
< 0.3 mg/l	29 (90.6)	27 (81.8)	—	0.507*
0.3–1.0 mg/l	3 (9.4)	4 (12.1)	—	
> 1.0 mg/l	0	2 (6.1)	—	
Lipid profile				
LDL (mmol/l)	0.03 ± 0.47	–0.15 ± 0.70	0.18 ± 0.14	0.218
HDL (mmol/l)	0.08 ± 0.16	0.01 ± 0.24	0.07 ± 0.05	0.130
TG (mmol/l)	0.13 ± 0.68	–0.04 ± 0.50	0.02 ± 0.14	0.229
Total cholesterol (mmol/l)	0.18 ± 0.70	0.05 ± 0.91	0.14 ± 0.19	0.484
VLDL (mg/dl)	2.37 ± 12.35	–0.50 ± 8.83	2.86 ± 2.57	0.268
Insulin sensitivity				
HOMA-IR	2.58 ± 16.99	2.61 ± 18.29	–0.03 ± 4.22	0.995
HOMA-2-IR	0.81 ± 0.58	0.70 ± 0.39	0.11 ± 0.12	0.355
QUICKI	–0.00 ± 0.02	–0.00 ± 0.02	–0.00 ± 0.00	0.673
OGIS (ml min^{-1} kg^{-1})	21.17 ± 67.86	–11.43 ± 60.97	32.60 ± 15.42	**0.038**
B-cell function				
HOMA-%B	12.6 ± 61.17	–1.35 ± 54.93	13.93 ± 14.40	0.337
HOMA2-%B	69.0 ± 29.19	62.4 ± 28.74	6.61 ± 7.19	0.361

*Since many of the CRP values were below the minimum recordable value for the equipment, comparisons were done using χ^2 test at baseline and at follow-up. The numbers and *P*-value reported represent results at follow-up.
SBP, systolic blood pressure; DBP, diastolic blood pressure; PTH, parathyroid hormone; hs-CRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; HDL, high-density lipoprotein; TG, triglyceride; VLDL, very-low-density lipoprotein; QUICKI, quantitative insulin-sensitivity check index; HOMA, homeostasis model assessment; OGIS, oral glucose insulin sensitivity.

Table 4 Factors affecting the change in OGIS

	Unstandardized coeff.	Standardized coefficients			
	B	SE	Beta	*t*	Significance
Group*	41.084	15.38	0.312	2.670	**0.010**
Waist–hip ratio	302.176	135.03	0.254	2.238	**0.029**
Basal 25(OH)D (nmol/l)	–1.499	0.561	–0.313	–2.672	**0.010**
Age (years)	0.552	0.95	0.067	0.579	0.564
Betel nut†	–9.116	17.57	–0.060	–0.519	0.606

*Supplemented vs. control group.
†Chewers vs. non-chewers.
Dependent variable: change in OGIS; $r^2 = 0.204$.
OGIS, oral glucose insulin sensitivity.

designed to study insulin sensitivity, and the reported outcomes were analysed post hoc. In comparison, the current study reports on short-term intermittent supplementation with only cholecalciferol, without calcium, and documents no change in HOMA-IR over 6 weeks but an improvement in OGIS. The results are concordant with the 3-year trial [10] in showing a positive impact on insulin sensitivity. However, there is lack of improvement in HOMA in our trial, which

may be explicable by the short duration of supplementation in our trial [if basal hepatic sensitivity takes longer than postprandial sensitivity (OGIS) to respond]. Similarly, in 12 women with gestational diabetes [9] there was no difference between insulin concentrations during OGTT prior to and after intravenous or oral (14 days) 1,25-$(OH)_2D_3$, which is consonant with the current trial. Also, in 47 postmenopausal women [11], a 12-week course of 1000 mg/day calcium and 800 IU/day cholecalciferol did not significantly change inflammatory markers, triglyceride, LDL- and HDL-cholesterol or insulin concentration. However, the total dose of vitamin D delivered in that trial was 72 000 IU over 3 months (one-fifth of the current study) and 'fasting levels' were measured over a short period of supplementation without improvement, in accordance with the current study.

In contrast, in a double-blind study on healthy volunteers, 18 healthy male subjects [8] received in random order either placebo or 1.5 μg calcitriol per day orally for 7 days; insulin-mediated glucose uptake, i.e. insulin sensitivity, was assessed using the euglycaemic clamp technique. Mean glucose disposal rate was not significantly affected by placebo or calcitriol treatment. Discordance with our results may be due to differences in subject characteristics, form of vitamin D used for supplementation, or the shorter duration of that study.

The lack of benefit of vitamin D supplementation on pancreatic insulin secretion as estimated by HOMA%B and HOMA2-%B in our study is in agreement with a previously reported human trial. In a 3-month RCT [30] on 65 volunteers with IGT and normal vitamin D concentration, no effect of 0.75 μg 1-α-OH-D_3 per day was documented on insulin secretion. However, this is in contrast to the results of *in vitro*, *in vivo* and animal experiments [13].

Existing literature on the impact of vitamin D on lipids and BP is primarily observational, with studies documenting an association between hypovitaminosis D and high LDL concentration [14] and higher risk of hypertension [15]. A possible mechanism for the effect on BP through the renin–angiotensin axis has been reported by Li *et al.* [31]. The absence of improvement in lipid profile in the current trial is not in agreement with the results of a recent randomized control trial in which weight loss intervention was undertaken for 63 overweight or obese women [32] where a greater reduction in LDL and LDL:HDL ratio was observed in the calcium + D group. This is different from the results of the current trial. However considering the limitations of the current trial, including the severity of the deficiency, with many of the subjects from the S-group not reaching repletion, this would require further exploration in future trials. No difference was observed in BP between the two groups, which is consistent with the present trial.

Timms *et al.* [33] in a cross-sectional study documented that 25(OH)D concentration was independently and inversely correlated with matrix metallopeptidase (MMP)-9 levels and subsequently, in a prospective 1-year vitamin D supplementation trial, documented significant reductions in MMP-9 (–68%), tissue-inhibitor metalloproteinase-1 (–38%), and CRP (–23%). The lack of improvement in CRP levels in our study may be related to the relatively short duration of our study, the severity of the deficiency or the lower baseline levels of CRP in our subjects.

In conclusion, the present trial has indicated that vitamin D_3 supplementation increases the OGIS index in centrally obese men. The response is better in subjects with lower serum 25(OH)D concentrations and in those with greater central adiposity. The results should be validated in diverse settings with other OGTT-based indices with longer follow-up.

Competing interests

Nothing to declare.

Acknowledgements

The study was supported by funding from the Indian Council of Medical Research, New Delhi. We are grateful to Cadila Pharmaceuticals, India for providing the placebo sachets. We also appreciate the suggestions from Professor H. P. S. Sachdev (SBISR, New Delhi), Dr Pankaj Shah (M. D. Anderson Cancer Center, USA) and Dr Barbara Boucher (Royal London Hospital, UK). We are also indebted to Anita Manoharan and Nidhi Gupta for their efforts.

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