Vitamin D supplementation has no effect on insulin sensitivity or secretion in vitamin D–deficient, overweight or obese adults: a randomized placebo-controlled trial[1]

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ABSTRACT

Background: Vitamin D supplementation has been proposed as a potential strategy to prevent type 2 diabetes. Existing clinical trials have been limited by short duration, low doses of vitamin D, variability in participants' vitamin D–deficiency status, and the use of surrogate measures of body composition, insulin sensitivity, and insulin secretion.

Objective: To address existing knowledge gaps, we conducted a double-blind, randomized, placebo-controlled trial to investigate whether vitamin D supplementation that is provided in a sufficient dose and duration to vitamin D–deficient individuals would improve insulin sensitivity or secretion as measured with the use of gold-standard methods. We hypothesized that vitamin D supplementation would improve insulin sensitivity and secretion compared with placebo.

Design: Sixty-five overweight or obese, vitamin D–deficient (25-hydroxyvitamin D [25(OH)D] concentration ≤50 nmol/L) adults were randomly assigned to receive either a bolus oral dose of 100,000 IU cholecalciferol followed by 4000 IU cholecalciferol/d or a matching placebo for 16 wk. Before and after the intervention, participants received gold-standard assessments of body composition (via dual X-ray absorptiometry), insulin sensitivity (via hyperinsulinemic-euglycemic clamps), and insulin secretion [via intravenous-glucose-tolerance tests (IVGTTs)].

Results: Fifty-four participants completed the study [35 men and 19 women; mean ± SD age: 31.9 ± 8.5 y; body mass index (in kg/m^2): 30.9 ± 4.4]. 25(OH)D increased with vitamin D supplementation compared with placebo (57.0 ± 21.3 compared with 1.9 ± 15.1 nmol/L, respectively; $P = 0.02$). Vitamin D and placebo groups did not differ in change in insulin sensitivity (0.02 ± 2.0 compared with −0.03 ± 2.8 mg · kg^{-1} · min^{-1}, respectively; $P = 0.9$) or first-phase insulin secretion (−21 ± 212 compared with 24 ± 184 mU/L, respectively; $P = 0.9$). Results remained nonsignificant after adjustment for age, sex, percentage of body fat, sun exposure, physical activity, and dietary vitamin D intake ($P > 0.1$).

Conclusions: Vitamin D supplementation does not improve insulin sensitivity or secretion in vitamin D–deficient, overweight or obese adults, despite using high-dose vitamin D supplementation and robust endpoint measures. Therefore, it is unlikely that vitamin D supplementation would be an effective strategy for reducing diabetes risk even in vitamin D–deficient populations. This trial was registered at clinicaltrials.gov as NCT02112721. *Am J Clin Nutr* 2017;105:1372–81.

Keywords: insulin secretion, insulin sensitivity, obesity, randomized trial, RCT, vitamin D

INTRODUCTION

Vitamin D deficiency has long been implicated in the development and progression of chronic conditions including obesity, insulin resistance, and type 2 diabetes (1, 2). These associations are increasingly clinically relevant with vitamin D deficiency being prevalent worldwide because of increasing sedentary and indoor lifestyles and the use of sunscreen and protective clothing to prevent skin cancer (3). Diet alone is not an adequate source of vitamin D because few foods are naturally high in vitamin D or are vitamin D fortified (3). Vitamin D supplementation is used for treating deficiency while avoiding conflict with public health measures for skin cancer prevention (3). The amount of supplementation that is required to correct vitamin D deficiency also remains controversial (4). Recommended daily oral intake of 200–600 IU vitamin D/d for adults (aged 19–70 y) (5) conflicts with recent studies in which a minimum oral intake of 4000 IU/d was required to raise serum 25-hydroxyvitamin D [25(OH)D][8] concentrations to optimal amounts within 2–3 mo (6, 7).

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[8] Abbreviations used: BP, blood pressure; IVGTT, intravenous-glucose-tolerance test; OGTT, oral-glucose-tolerance test; RCT, randomized controlled trial; 25(OH)D, 25-hydroxyvitamin D.

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Lower serum 25(OH)D concentrations have been correlated with a higher prevalence of obesity (8), higher fasting serum glucose concentrations (9), increased insulin resistance (10), increased first- and second-phase insulin secretion (11), and higher glycated hemoglobin (12) in cross-sectional studies. In prospective studies, low serum 25(OH)D was associated with the development of new-onset obesity (8), insulin resistance, and type 2 diabetes (13). Despite the strength of epidemiologic data, few good-quality clinical trials have assessed the effects of vitamin D supplementation on metabolic features including obesity and glycemic status. Meta-analyses of vitamin D–supplementation trials have revealed substantial heterogeneity in study quality, risk of bias, supplementation dose and duration, participant vitamin D status, and sample sizes and have highlighted the use of indirect surrogate measures of glucose metabolism (14, 15). These limitations have made it difficult to interpret findings. Most trials also have not assessed lifestyle factors (diet and exercise) or dietary vitamin D or calcium intake (14). In this context, it has been hypothesized that beneficial effects may be limited to vitamin D–deficient individuals [25(OH)D concentration $<$50 nmol/L) who receive a sufficient dose ($\geq$4000 IU/d) and duration ($\geq$3 mo) of vitamin D supplementation, and there has been some emerging evidence to support this hypothesis (14).

Although large-scale trials are currently underway to assess whether vitamin D supplementation improves cardiovascular outcomes (16), the effect of vitamin D supplementation on insulin resistance and secretion in individuals who are at high risk of diabetes remains unknown. Good-quality trials that use gold-standard measures are needed to address current knowledge gaps. To this end, we aimed to assess the efficacy of high doses of vitamin D supplementation for a sufficient duration of 16 wk in vitamin D–deficient and overweight or obese, but otherwise healthy, adults and to use gold-standard methods for measuring adiposity and insulin sensitivity and secretion. The primary aim was to determine whether vitamin D supplementation improves insulin sensitivity (with the use of hyperinsulinemic-euglycemic clamps). Secondary outcomes included insulin secretion and cardiovascular disease risk factors that are associated with type 2 diabetes including adiposity, blood pressure (BP), and lipids.

METHODS

Study design and participants

This study was a parallel-group, double-blind, randomized, placebo-controlled trial, and a detailed trial protocol has been published (17). Briefly, 65 overweight or obese, but otherwise healthy, nondiabetic adults were recruited over a 2-y period from the local community in Melbourne, Australia, via posters, flyers, e-mail newsletters, and online social media and community websites. Overweight and obese participants were targeted because of their greater risk of insulin resistance and type 2 diabetes (18). Participants were screened, and individuals with serum 25(OH)D concentrations $\leq$50 nmol/L on screening were recruited if they met the following inclusion criteria: aged 18–60 y; generally healthy on medical screening; overweight or obese [BMI (in kg/m^2) $\geq$25); weight $<$159 kg because of facility restrictions; and a stable weight ($<$5 kg change in the preceding year) and no intention to lose weight or change their diets and physical activity levels for the trial duration. Exclusion criteria included smoking or high alcohol use ($>$4 standard drinks/wk for men; $>$2 standard drinks/wk for women), hypercalcemia, allergies, diabetes [previously diagnosed or based on an oral-glucose-tolerance test (OGTT)], and the use of medications, vitamins, or supplements. On the basis of a medical history and a physical or laboratory examination, participants were excluded if they had major diseases including active cancer within the preceding 5 y or current acute inflammation. Women who were pregnant, lactating, or experiencing menopause were excluded.

Ethics

All participants provided written informed consent before commencing the trial. The trial was conducted according to the principles of the Declaration of Helsinki and received ethical approval from the Monash University Human Research Ethics Committee and Monash Health (protocol CF13/3874–2013001988). The trial was registered at clinicaltrials.gov as NCT02112721.

Intervention and random assignment

Participants were randomly assigned to either the vitamin D group, in which they received an initial bolus dose of 2500 μg cholecalciferol (100,000 IU in 2 capsules) followed by 100 μg cholecalciferol (4000 IU in 4 capsules)/d, or the placebo group, in which they received an equivalent number of identical placebo capsules that were continued daily for a period of 16 wk. The bolus dose that was selected was well below amounts associated with toxicity or adverse effects (19) and was taken orally in front of researchers in the clinic with the aim of achieving elevated serum 25(OH)D concentrations to 100 nmol/L within 1 wk in the intervention group (6), after which the daily dose aimed to sustain serum 25(OH)D concentration at repletion ($\geq$75 nmol/L) for the study period (7). All participants were instructed to consume the 4 capsules daily and to maintain their usual diet and exercise habits.

Random assignment was performed with the use of a computerized random-sequence–generation program and was done in blocks of 4 by sex and time of study entry (seasons) to ensure balance between the sexes in each test group and to control for the effect of seasonal change. Random assignment was performed by an independent researcher who was not involved in the data collection, analysis, or reporting and who received the packaged supplements from an external clinical trials pharmacy (Alfred Hospital Pharmacy). All capsules were identical and tasteless to maintain blinding, and all participants, investigators, and outcome assessors remained blinded until after data lockdown and the analysis of results. Compliance was assessed by the empty pill containers that were returned by participants at the final follow-up visit and by postintervention 25(OH)D concentrations.

Outcome measures

The primary outcome of the trial was the change in insulin sensitivity. Secondary outcomes were changes in insulin secretion, BMI, percentage of body fat (fat mass and fat-free mass), waist-to-hip ratio, resting systolic BP and diastolic BP, pulse

pressure and mean arterial pressure, and fasting plasma lipid profiles.

Outcome measures were obtained at baseline before the initial bolus of vitamin D and were repeated (except for the OGTT) after 16 wk of supplementation. Detailed descriptions of the outcome measures were reported in our published protocol (17). In brief, participants who were eligible on phone screening attended our research center for a medical screening, which included a medical history, physical examination (including BP, anthropometric measures, and pregnancy tests for women), measurement of serum 25(OH)D to document vitamin D status, and an OGTT to exclude diabetes according to WHO guidelines (20). Serum 25(OH)D was measured with the use of direct competitive chemiluminescent immunoassays (DiaSorin Inc.) with interassay and intra-assay CVs of <10% and <4%, respectively. Plasma glucose concentrations were determined via the glucose oxidase method (YSI 2300 STAT; YSI Inc.) (SE of prediction: 0.28 mmol/L; mean percentage of error: 1.79%). Insulin was measured with the use of a simultaneous immunoenzymatic sandwich assay (Access/DXI ultrasensitive insulin assay; Beckman Coulter), with interassay and intra- assay CVs of <5% and <7%, respectively. Fasting venous blood samples were collected and measured with the use of commercial enzymatic immunoassays (Beckman Coulter) for kidney- and liver-function tests, full blood counts, and fasting lipid profiles. All blood samples were analyzed under blinded conditions with the use of standard quality-control systems (all results within ±2 SDs) by an accredited and quality-assured laboratory (Monash Health Pathology).

The primary outcome of insulin sensitivity was measured with the use of a hyperinsulinemic-euglycemic clamp (21), which was initiated by an intravenous bolus injection of insulin (9 mU/kg) after which insulin was constantly infused at a rate of 40 mU · m^{-2} · min^{-1} for ≥120 min, whereas glucose was variably infused to maintain euglycemia. Plasma glucose values were monitored every 5 min, and the variable infusion rate of glucose was adjusted to maintain blood glucose at a constant value of 5 mmol/L for ≥30 min. Acute insulin secretion was measured with the use of an IVGTT whereby 50 mL 50% glucose was delivered intravenously over a 3-min period, and insulin and glucose concentrations were measured at 3, 4, 5, 6, 8, 10, 15, 20, 25, and 30 min to determine the insulin secretory response. Total and first- and second-phase insulin secretions were calculated as the mean incremental plasma insulin concentration from minutes 3–5 and 10–30 after the glucose bolus, respectively. Body composition was measured with the use of dual X-ray absorptiometry (Monash Health Radiology), and participants completed validated questionnaires that assessed self-reported sun-exposure habits, physical activity (International Physical Activity Questionnaire) (22), and diet (3-d food record; Foodworks 8.0 Professional; Xyris Software).

Statistical analysis

On the basis of data from a similar healthy cohort of overweight or obese subjects in our metabolic laboratory with a mean ± SD insulin-mediated glucose uptake value of 8.1 ± 2.0 mg glucose · kg^{-1} · min^{-1}, a sample size of 25 completing each arm was required to detect a 20% change in insulin-mediated glucose uptake between the treatment group and placebo group. The 20% increase was based on effects of vitamin D supplementation in type 2 diabetes with the use of a comparable insulin-sensitivity measurement technique and 4 wk of treatment (23). On the basis of a type I error of 0.05 (2 tail) and a type II error of 0.20 (power: 80%), we required a total of 50 participants to complete the trial. To account for an expected 20% dropout rate, 65 participants were recruited (17).

Analyses were performed per protocol with the use of Stata statistical software (v.12.0; StatCorp LP). Shapiro-Wilk tests, histograms, and scatterplots were used to assess normality with the assistance of an experienced biostatistician. Baseline characteristics are presented as means ± SDs and frequencies (percentages) or as medians (IQRs) if the distribution was skewed. Continuous variables were logarithmically transformed to the base 10 if normality was violated. Differences between treatment groups and between dropouts and nondropouts were assessed with the use of independent Student's *t*-tests and chi-square tests for continuous and categorical variables, respectively. Within-group differences were assessed with the use of paired Student's *t* tests. The efficacy of the intervention on the outcomes (between-group differences) was analyzed via changes in outcome variables and linear regression (ANCOVA). All analyses were adjusted for multiple testing with the use of Bonferroni correction. In a multiple-regression analysis, we adjusted for variables that were significantly correlated with the outcome measure on the basis of Pearson correlations as well as other clinically relevant variables including baseline values, age, sex, ethnicity, sun exposure, diet, and percentage of body fat. Prespecified subgroups such as subjects with baseline 25(OH)D concentrations <30 nmol/L as well as obese (BMI >30) and insulin-resistant [$M < 4.7$ mg · kg^{-1} · min^{-1} (24)] subgroups were assessed in exploratory analyses. All tests were 2 sided, and $P < 0.05$ was considered statistically significant.

RESULTS

Sample and baseline characteristics

The participant flowchart is presented in **Figure 1**. Of 1072 participants who were screened for eligibility, 132 participants attended the initial medical screening, and 65 participants were successfully randomly assigned between September 2014 and July 2016 (33 and 32 participants in the vitamin D and placebo groups, respectively) (Figure 1). By the end of the study, 9 participants had dropped out, and 2 participants were withdrawn (1 participant was withdrawn because of a protocol violation, and 1 participant was withdrawn because of an adverse event of thrombophlebitis after the IVGTT). The remaining 54 participants (28 in the vitamin D group and 26 in the placebo group) completed the study and were analyzed in a blinded fashion as per protocol. Baseline demographic, anthropometric, and biochemical characteristics of both groups are presented in **Tables 1** and **2**. Baseline characteristics did not differ between dropouts and nondropouts (all $P > 0.05$).

Thirty-five men and 19 women with a median age of 30 y (IQR: 25–36 y), median BMI of 30.1 (IQR: 27.7–33.2), and mean ± SD percentage of body fat of 39.6% ± 8.7% completed the study. The mean baseline serum 25(OH)D concentration was 32.7 ± 11.4 nmol/L (range: 9–50 nmol/L) with 43% of participants (n = 23) having a 25(OH)D concentration ≤30 nmol/L.

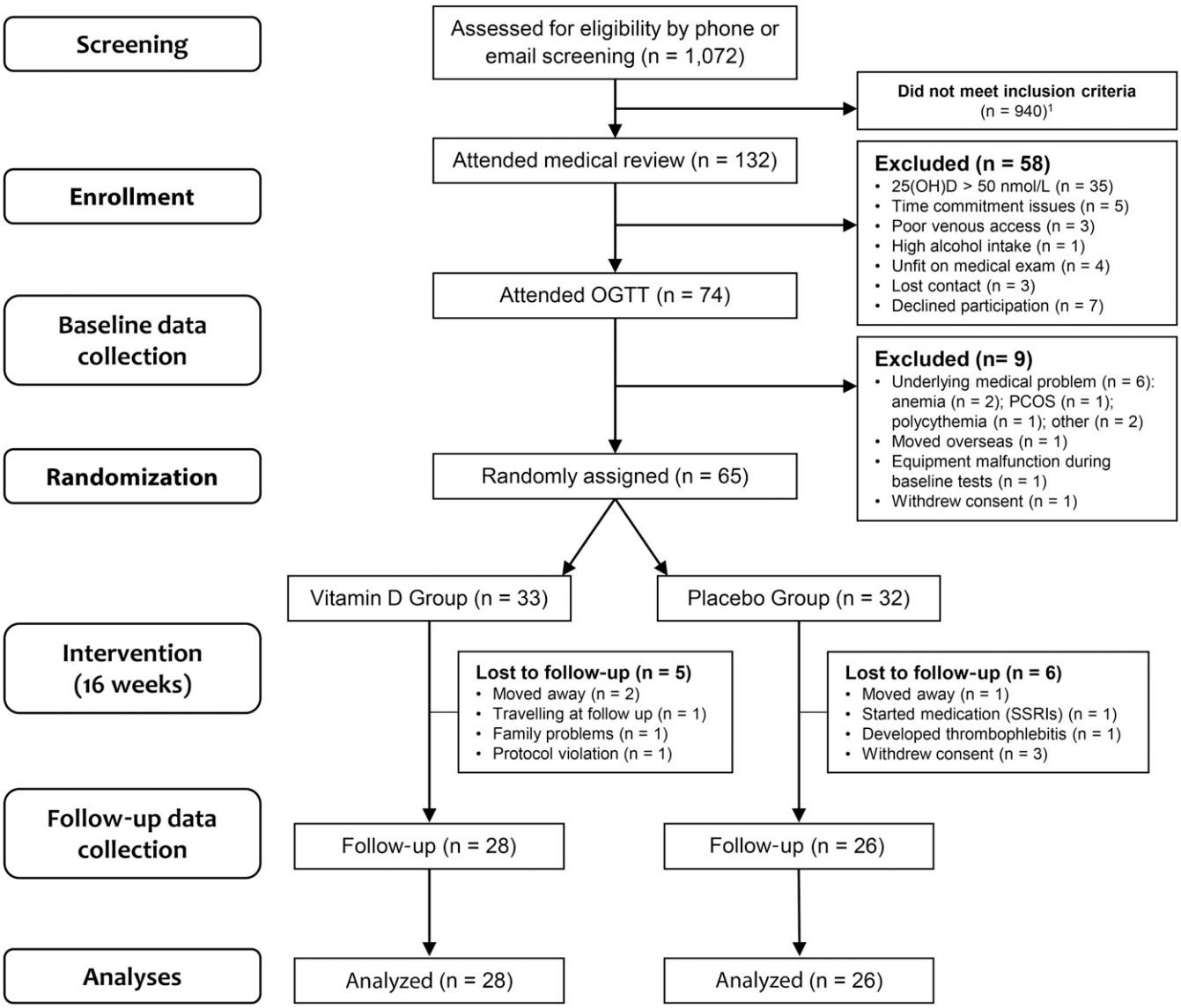

FIGURE 1 Participant flowchart showing numbers of participants who were recruited, were randomly assigned, dropped out, and were analyzed during the trial. [1]The majority of interested participants did not meet the criteria because of taking medication or supplements, not being overweight or obese, or not being interested after receiving a detailed description of study procedures. exam, examination; OGTT, oral-glucose-tolerance test; PCOS, polycystic ovary syndrome; SSRI, selective serotonin reuptake inhibitor; 25(OH)D, 25-hydroxyvitamin D.

Mean baseline insulin sensitivity (*M* value) was 6.7 ± 2.9 mg · kg^{-1} · min^{-1} (IQR: 4.1–8.7 mg · kg^{-1} · min^{-1}). Demographic, anthropometric, and cardiometabolic measures did not differ between groups at baseline nor did physical activity, dietary intake of vitamin D (Tables 1 and 2), or diet composition comprised of total energy, protein, fiber, fat, and carbohydrate intake (data not shown).

Changes in serum 25(OH)D concentrations

After the 16-wk intervention, the mean serum 25(OH)D concentration was 63.2 ± 32.1 nmol/L (range: 9–147 nmol/L). Serum 25(OH)D concentrations increased significantly with vitamin D (from 31.4 ± 12.6 to 88.4 ± 21.0 nmol/L; $P = 0.02$) with no change with the placebo (from 34.2 ± 10.0 to 36.1 ± 15.3 nmol/L; $P = 0.9$). All but one participant in the vitamin D group achieved a 25(OH)D concentration ≥60 nmol/L with 82% of these participants having a 25(OH)D concentration >70 nmol/L at follow-up. The baseline 25(OH)D concentration was inversely associated with the change in 25(OH)D ($r = -0.28$, $P = 0.04$) but not with the change in any of the outcomes (all $P > 0.1$).

Effect of vitamin D supplementation on cardiometabolic outcomes

Changes in anthropometric measures including BMI, waist-to-hip ratio, percentage of body fat, fat mass, and fat-free mass did not differ between vitamin D and placebo groups (all $P > 0.9$) (Table 2). No differences between groups were observed in changes in glucose metabolism including fasting glucose or fasting insulin (both $P > 0.9$) (Table 2).

TABLE 1
Participant demographics and baseline characteristics[1]

Characteristic	Vitamin D group (n = 28)	Placebo group (n = 26)	P
Men, n (%)	17 (60.7)	18 (69.2)	0.99
Age, y	30.5 (25–35)[2]	29.5 (25–41)	0.99
Ethnicity,[3] n (%)			0.99
Caucasian	9	5	
South and Central Asian	8	9	
Southeast and Northeast Asian	4	9	
Other[4]	5	2	
Family history of diabetes,[5] n (%)	11 (39.2)	7 (27.0)	0.99
Sun exposure,[6] index score	4.2 (1.6–6.3)	5.1 (2.0–6.9)	0.99
Physical activity,[6] IPAQ-METS[7] score	1751 (920–3510)	2912 (1485–5544)	0.99
Dietary vitamin D intake,[6] IU	91.1 (54.6–130.9)	73.1 (61.0–110.8)	0.99
Season of blood collection, n (%)			0.99
Winter	5 (17.8)	8 (30.7)	
Spring	11 (39.3)	4 (15.4)	
Summer	8 (28.6)	10 (38.5)	
Autumn	4 (14.3)	4 (15.4)	

[1] P values were determined with the use of Student's t tests, chi-square tests, or ANOVA for baseline differences between treatment groups after adjustment for multiple testing with the use of Bonferroni correction.
[2] Median; IQR in parentheses (all such values for nonnormally distributed variables). Nonnormally distributed variables were log transformed to the base 10 before analysis.
[3] Determined by self-report (n = 51 of 54 reported ethnicity).
[4] Refers to African, Middle Eastern, South American, and Polynesian ethnicities.
[5] Includes only first-degree relative with diabetes.
[6] Calculated from self-reported questionnaires and food records as previously reported (17).
[7] IPAQ-METS, international physical activity questionnaire–multiples of the resting metabolic rate.

The change in insulin sensitivity did not differ significantly between groups (0.02 ± 2.0 and −0.03 ± 2.8 mg · kg^{-1} · min^{-1} in vitamin D and placebo groups, respectively; P = 0.9) (Table 2). Similarly, there were no differences between groups in the change in insulin secretory response. which was measured as the total, first-phase, or second-phase insulin AUC (all $P > 0.9$) (Table 2). With regard to secondary outcomes, there were no differences between groups in cardiovascular disease risk factors including BP or lipids (Table 2).

In the multivariable analysis that was adjusted for baseline values, age, sex, ethnicity, and season of blood collection, differences between groups in changes in insulin sensitivity and secretion remained nonsignificant (**Table 3**). Differences in changes in anthropometric measures, BP, and lipids were also nonsignificant after adjustment (data not shown). Replacing ethnicity and the season of blood collection in the multivariable model with factors that may have affected vitamin D status including dietary vitamin D intake, sun exposure, and physical activity did not alter the results (Table 3). Results remained nonsignificant in a third model that was adjusted for baseline values and factors that are clinically relevant to both vitamin D status and diabetes risk including changes in the percentage of body fat, dietary vitamin D intake, diet composition (as the fat:carbohydrate ratio), physical activity, and sun exposure (Table 3). The addition of protein or fiber intake to the model did not change the results (data not shown).

Subgroup analyses

A prespecified subgroup analysis of participants with baseline 25(OH)D concentrations <30 nmol/L (n = 23) was conducted. Changes in primary and secondary outcomes were not different between the intervention and placebo groups (**Table 4**). No differences in primary or secondary outcomes were shown between groups in further exploratory subgroup analyses of participants who were obese at baseline (BMI >30; n = 23) and those with insulin resistance at baseline [M < 4.7 mg · kg^{-1} · min^{-1}; n = 18 (24)], as well as in further analyses that restricted the vitamin D group to include only subjects who were replete at follow-up [25(OH)D concentration >70 nmol/L (total n = 49) and >80 nmol/L (total n = 44)] (data not shown).

DISCUSSION

This randomized placebo-controlled trial examined the effect of oral cholecalciferol (100,000 IU bolus followed by 4000 IU/d) for 16 wk in overweight or obese, but otherwise healthy, individuals with vitamin D deficiency [25(OH)D concentration <50 nmol/L]. We showed no difference in insulin sensitivity measured with the use of a hyperinsulinemic-euglycemic clamp and insulin secretion measured with the use of an IVGTT after vitamin D supplementation compared with after placebo intake despite a significant increase in 25(OH)D concentrations in the vitamin D group. A subgroup analysis of individuals with 25(OH)D concentrations <30 nmol/L as well as obese or insulin-resistant individuals showed similar results. Furthermore, there were no significant differences in anthropometric measures, BP, or lipid profiles between the vitamin D and placebo groups.

To our knowledge, in healthy individuals, only 2 previous randomized controlled trials (RCTs) have used hyperinsulinemic-euglycemic clamps to investigate the effect of vitamin D supplementation on insulin sensitivity, both of which had smaller sample sizes and shorter durations. One study supplemented 50,000 IU ergocalciferol/wk for 8 wk to 12 participants (25),

TABLE 2
Comparison of outcomes before and after supplementation in both groups[1]

	Vitamin D group (n = 28)				Placebo group (n = 26)					
Outcome variable	Baseline	Follow-up	P^2	Change	Baseline	Follow-up	P^3	Change	P^4	P^5
Serum 25-hydroxyvitamin D, nmol/L	31.4 ± 12.6[6]	88.4 ± 21.0	0.02	57.0 ± 21.3	34.2 ± 10.0	36.1 ± 15.3	0.99	1.9 ± 15.1	0.99	0.02
BMI, kg/m^2	30.2 (28.4–34.5)[7]	30.4 (28.7–34.5)	0.99	0.003 ± 0.9	29.8 (27.6–32.6)	29.2 (27.5–32.6)	0.99	−0.1 ± 1.2	0.99	0.99
Waist-to-hip ratio	0.94 ± 0.08	0.93 ± 0.07	0.99	−0.01 ± 0.04	0.93 ± 0.04	0.94 ± 0.04	0.99	0.001 ± 0.02	0.99	0.99
Body fat, %	40.3 ± 8.2	39.9 ± 8.1	0.99	−0.4 ± 1.6	38.8 ± 9.4	38.5 ± 9.2	0.99	−0.3 ± 1.7	0.99	0.99
Fat mass, %	36.4 ± 9.8	36.0 ± 9.4	0.99	−0.5 ± 2.1	33.7 ± 10.2	33.4 ± 10.6	0.99	−0.3 ± 2.4	0.99	0.99
Fat-free mass, %	53.9 ± 12.9	54.2 ± 12.7	0.99	0.3 ± 1.4	53.1 ± 12.2	53.0 ± 11.9	0.99	−0.9 ± 1.5	0.99	0.99
Fasting glucose, mmo/L	4.6 ± 0.6	4.6 ± 0.5	0.99	0.04 ± 0.5	4.5 ± 0.4	4.7 ± 0.3	0.60	0.2 ± 0.4	0.99	0.99
Fasting insulin, mU/L	10.0 (8.1–15.8)	10.2 (6.9–18.2)	0.99	0.1 ± 5.9	7.1 (5.8–10.5)	7.9 (5.8–13.6)	0.99	2.1 ± 6.3	0.87	0.99
Insulin sensitivity, M, mg · kg^{-1} · min^{-1}	5.7 ± 2.5	5.8 ± 2.8	0.99	0.02 ± 2.0	7.7 ± 3.1	7.6 ± 3.3	0.99	−0.03 ± 2.8	0.29	0.99
Total insulin AUC, mU/L	1760 (1090–3542)	1984 (1171–3156)	0.99	−6.9 ± 843	1515 (976–2174)	1345 (959–2150)	0.99	130 ± 1092	0.99	0.99
First-phase insulin AUC, mU/L	375 (166–577)	348 (166–140)	0.99	−21 ± 212	275 (185–405)	286 (194–467)	0.99	24 ± 184	0.99	0.99
Second-phase insulin AUC, mU/L	1250 (727–2155)	1413 (811–2052)	0.99	13 ± 598	890 (596–1359)	862 (557–1442)	0.99	124 ± 818	0.99	0.99
Systolic blood pressure, mm Hg	117.2 ± 10.4	117.8 ± 11.4	0.99	0.6 ± 6.3	124.7 ± 14.2	121.0 ± 11.1	0.99	−3.7 ± 9.9	0.87	0.99
Diastolic blood pressure, mm Hg	78.8 ± 7.1	79.3 ± 9.2	0.99	0.5 ± 7.7	80.9 ± 10.0	81.9 ± 9.6	0.99	1.0 ± 7.7	0.99	0.99
Pulse pressure, mm Hg	38.4 ± 8.8	38.4 ± 8.7	0.99	0.04 ± 7.7	43.8 ± 11.0	39.0 ± 7.7	0.10	−4.7 ± 7.9	0.99	0.60
Arterial pressure, mm Hg	91.6 ± 7.3	92.1 ± 9.2	0.99	0.5 ± 6.3	95.5 ± 10.4	94.9 ± 9.4	0.99	−0.6 ± 7.6	0.99	0.99
Total cholesterol, mmol/L	5.0 ± 1.0	4.9 ± 1.1	0.99	−0.1 ± 0.5	4.9 ± 0.8	4.7 ± 0.8	0.99	−0.2 ± 0.6	0.99	0.99
HDL cholesterol, mmol/L	1.2 ± 0.2	1.1 ± 0.2	0.99	−0.04 ± 0.1	1.2 ± 0.3	1.1 ± 0.2	0.99	−0.05 ± 0.2	0.99	0.99
LDL cholesterol, mmol/L	3.1 ± 0.8	3.0 ± 0.8	0.99	−0.08 ± 0.5	3.0 ± 0.7	2.9 ± 0.6	0.99	−0.08 ± 0.5	0.99	0.99
Triglycerides, mmol/L	1.6 (1.0–2.0)	1.4 (1.0–2.4)	0.99	0.16 ± 0.6	1.4 (1.1–2.1)	1.5 (1.0–1.8)	0.99	−0.14 ± 0.9	0.99	0.99

[1] All analyses were adjusted for multiple testing with the use of Bonferroni correction.
[2] Determined with the use of paired Student's t tests for differences between baseline and follow-up in the vitamin D group.
[3] Determined with the use of paired Student's t tests for differences between baseline and follow-up in the placebo group.
[4] Determined with the use of independent samples t tests for differences at baseline between vitamin D and placebo groups.
[5] Determined with the use of independent samples t tests for differences in change scores between vitamin D and placebo groups.
[6] Mean ± SD (all such values).
[7] Median; IQR in parentheses (all such values for nonnormally distributed variables). Nonnormally distributed variables were log-transformed to the base 10 before analysis.

TABLE 3
Multivariable regression analysis for differences in selected metabolic variables between vitamin D and placebo groups after adjustment for covariates[1]

Dependent variable (change) and model	R^2	Standardized coefficient, β	t	P
Insulin sensitivity, M, mg · kg^{-1} · min^{-1}				
Model 1	0.25	−0.15	−0.94	0.4
Model 2	0.26	−0.19	−1.10	0.3
Model 3	0.18	−0.13	−0.67	0.5
Total insulin AUC (0–30 min), mU/L				
Model 1	0.41	−0.12	−0.84	0.4
Model 2	0.13	0.03	0.14	0.9
Model 3	0.23	−0.06	−0.35	0.7
First-phase insulin AUC (3–5 min), mU/L				
Model 1	0.43	−0.10	−0.71	0.5
Model 2	0.27	−0.05	−0.28	0.8
Model 3	0.41	−0.12	−0.76	0.5
Second-phase insulin AUC (10–30 min), mU/L				
Model 1	0.39	−0.13	−0.91	0.4
Model 2	0.12	0.03	0.15	0.9
Model 3	0.20	−0.07	−0.36	0.7

[1] Model 1 was adjusted for baseline values, age, sex, ethnicity, and season of blood collection. Model 2 was adjusted for baseline values, age, sex, change in dietary vitamin D intake, physical activity (international physical activity questionnaire–multiples of the resting metabolic rate), and sun-exposure index. Model 3 was adjusted for baseline values, percentage of body fat, dietary vitamin D intake, diet composition (fat:carbohydrate ratio), physical activity (international physical activity questionnaire-multiples of the resting metabolic rate), and sun-exposure index. *P* values were determined with the use of a multiple linear regression analysis (ANCOVA) for differences between groups after adjustment for covariates.

and the other study supplemented 1.5 μg calcitriol/d for 7 d to 18 participants (26). Only one other study used hyperglycemic clamps to measure insulin sensitivity and secretion, in which 20,000 IU cholecalciferol was given 2 times/wk for 6 mo to 104 participants (27). The cohort in the study was older than in our study (mean age: >50 y compared with <35 y in our study), thereby increasing the likelihood of comorbidities and the use of related medications, such as statins, which may have affected insulin sensitivity (27). Moreover, potential confounders such as the percentage of body fat or food composition were not considered (27). Despite these limitations, all of the studies (25–27) showed no effect for vitamin D supplementation on insulin sensitivity or secretion. Here, in a larger, longer trial in which gold-standard methods and higher vitamin D doses in deficient adults were used, we also showed no metabolic effects of vitamin D supplementation.

Other RCTs in healthy individuals have examined the effect of vitamin D supplementation on insulin sensitivity and secretion through the use of indirect measures such as HOMA-IR or HOMA of β cell function, a quantitative insulin-sensitivity check index, fasting insulin, insulin AUC post-OGTT, and C-peptide AUC (28–39). Many of these trials combined calcium with vitamin D, which could have confounded the results because calcium concentrations have been shown to influence both insulin sensitivity and secretion (40). Of these RCTs, only one trial showed a positive effect whereby vitamin D supplementation in the form of cholecalciferol (4000 IU/d for 6 mo) improved insulin resistance (HOMA-IR) and insulin sensitivity (HOMA2%S) calculated by using a computer model of fasting blood glucose and insulin concentrations, and β cell function from paired fasting blood glucose and C-peptide (41). This study included only women with a South Asian background who were living in New Zealand and were vitamin D deficient [25(OH)D concentration <50 nmol/L] and insulin resistant on the basis of HOMA-IR (41) and, thus, may not be generalizable. Furthermore, the study did not adjust for covariates such as adiposity or physical activity. The study also included only insulin-resistant (HOMA-IR >1.93) individuals, which has not been an inclusion criterion in other RCTs (28–38). The findings of the trial suggest that the effect of vitamin D supplementation on insulin resistance may be present only in vitamin D–deficient individuals with high insulin resistance. In the current study, we recruited vitamin D–deficient, and overweight and obese individuals, who are more insulin resistant than lean individuals are, and we conducted a subgroup analysis of insulin-resistant participants [M < 4.7 mg · kg^{-1} · min^{-1} (24)] with our results being consistent with the majority of the literature that vitamin D supplementation does not improve insulin resistance.

In patients with prediabetes, vitamin D supplementation has shown no effect on insulin sensitivity and secretion, glycemic control, or the progression to diabetes. A meta-analysis of RCTs in individuals with normal glucose tolerance (3 RCTs) and impaired glucose tolerance (one RCT) showed no effects of vitamin D supplementation on the progression to diabetes (15). Another meta-analysis of 10 RCTs in patients with prediabetes also showed no significant effect of vitamin D supplementation on insulin resistance (HOMA-IR) or blood glucose concentrations 2 h post-OGTT although there were improvements in fasting blood glucose concentrations and glycated hemoglobin (42). To our knowledge, no studies of vitamin D supplementation in prediabetes have measured insulin sensitivity with the use of a hyperinsulinemic-euglycemic clamp.

We did not find any effect of vitamin D supplementation on measures of adiposity including the percentage of body fat in

TABLE 4
Subgroup analysis of participants with baseline 25-hydroxyvitamin D concentrations <30 nmol/L[1]

	Vitamin D group (n = 14)				Placebo group (n = 9)				
Outcome variable	Baseline	Follow-up	P^2	Change	Baseline	Follow-up	P^3	Change	P^4
Serum 25-hydroxyvitamin D, nmol/L	20.9 ± 5.9	80.9 ± 17.9	0.02	60.0 ± 20.3	23.0 ± 5.6	29.9 ± 21.4	0.99	6.9 ± 18.4	0.02
BMI, kg/m^2	31.32 ± 4.9	31.31 ± 4.7	0.99	−0.01 ± 0.7	29.3 ± 2.7	29.1 ± 3.2	0.99	−0.2 ± 0.9	0.99
Waist-to-hip ratio	0.96 ± 0.06	0.94 ± 0.06	0.99	−0.02 ± 0.04	0.92 ± 0.04	0.94 ± 0.05	0.99	0.02 ± 0.01	0.14
Body fat, %	39.9 ± 7.2	39.8 ± 7.2	0.99	−0.1 ± 1.9	40.8 ± 11.1	40.4 ± 10.9	0.99	−0.4 ± 1.4	0.99
Fat mass, %	34.9 ± 8.9	34.7 ± 7.8	0.99	−0.2 ± 2.2	34.0 ± 9.3	33.5 ± 9.6	0.99	−0.5 ± 1.8	0.99
Fat-free mass, %	52.9 ± 11.8	53.1 ± 12.2	0.99	0.2 ± 1.5	49.5 ± 10.6	49.3 ± 9.9	0.99	−0.2 ± 1.4	0.99
Fasting glucose, mmol/L	4.68 ± 0.8	4.75 ± 0.6	0.99	0.07 ± 0.7	4.56 ± 0.4	4.65 ± 0.3	0.99	0.09 ± 0.57	0.99
Fasting insulin, mU/L	11.0 ± 5.3	12.3 ± 6.3	0.99	1.3 ± 4.3	8.4 ± 3.6	8.2 ± 3.4	0.99	−0.2 ± 1.0	0.99
Insulin sensitivity, M, mg · kg^{-1} · min^{-1}	5.5 ± 2.3	5.6 ± 2.7	0.99	0.05 ± 2.2	7.0 ± 1.7	8.2 ± 3.7	0.99	1.2 ± 2.4	0.99
Total insulin AUC, mU/L	2037 ± 1677	2171 ± 1265	0.99	134 ± 748	1433 ± 572	1529 ± 729	0.99	96 ± 397	0.99
First-phase insulin AUC, mU/L	339 ± 315	355 ± 225	0.99	16 ± 222	260 ± 127	326 ± 183	0.99	66 ± 115	0.99
Second-phase insulin AUC, mU/L	1453 ± 1164	1547 ± 910	0.99	94 ± 470	955 ± 370	975 ± 457	0.99	20 ± 240	0.99
Systolic blood pressure, mm Hg	115.4 ± 9.1	114.9 ± 10.3	0.99	−0.5 ± 4.9	125.2 ± 12.6	125.3 ± 9.7	0.99	0.1 ± 11.1	0.99
Diastolic blood pressure, mm Hg	77.2 ± 8.1	76.9 ± 10.1	0.99	−0.3 ± 9.1	85.1 ± 6.6	85.3 ± 8.5	0.99	0.2 ± 8.3	0.99
Pulse pressure, mm Hg	38.2 ± 7.9	38.1 ± 7.9	0.99	−0.1 ± 7.1	40.1 ± 9.1	40.0 ± 7.6	0.99	−0.1 ± 4.9	0.99
Arterial pressure, mm Hg	89.9 ± 7.6	89.5 ± 9.4	0.99	−0.4 ± 7.2	98.5 ± 8.0	98.7 ± 8.2	0.99	0.2 ± 9.1	0.99
Total cholesterol, mmol/L	5.0 ± 0.8	4.8 ± 0.7	0.99	−0.2 ± 0.5	5.1 ± 0.8	5.0 ± 0.7	0.99	−0.1 ± 0.3	0.99
HDL cholesterol, mmol/L	1.18 ± 0.18	1.15 ± 0.2	0.99	−0.03 ± 0.2	1.2 ± 0.1	1.1 ± 0.2	0.99	−0.1 ± 0.2	0.99
LDL cholesterol, mmol/L	3.1 ± 0.65	2.9 ± 0.58	0.99	−0.2 ± 0.40	3.10 ± 0.4	3.11 ± 0.6	0.99	0.01 ± 0.4	0.99
Triglycerides, mmol/L	1.6 ± 0.6	1.7 ± 0.7	0.99	0.1 ± 0.5	1.74 ± 1.19	1.66 ± 1.05	0.99	−0.08 ± 1.01	0.99

[1] Data are expressed as means ± SDs. All analyses were adjusted for multiple testing with the use of Bonferroni correction.
[2] Determined with the use of paired Student's t tests for differences between baseline and follow-up in the vitamin D group.
[3] Determined with the use of paired Student's t tests for differences between baseline and follow-up in the placebo group.
[4] Determined with the use of independent samples t tests for differences in change scores between vitamin D and placebo groups.

our overweight or obese vitamin D–deficient cohort. A similar study that included 52 obese (BMI >30) individuals with vitamin D deficiency [25(OH)D concentration <50 nmol/L) reported similar findings whereby supplementation with 7000 IU cholecalciferol/d for 26 wk had no effect on adiposity compared with the effect of a placebo (30). The study also reported no effect of vitamin D supplementation on subcutaneous and visceral fat or intrahepatic and intramyocellular lipids that were evaluated with the use of MRI and magnetic resonance spectroscopy (30). Furthermore, a meta-analysis of RCTs showed no effect of vitamin D supplementation on weight or BMI in obese adults (43). Overall, with the inclusion of our data, vitamin D supplementation does not affect total or visceral adiposity in vitamin D–deficient individuals.

In terms of cardiovascular outcomes, a recent meta-analysis of 46 RCTs that investigated the effect of vitamin D for >4 wk showed no effects on systolic BP or diastolic BP, in line with our findings (44). For lipid profiles, a review of 10 RCTs showed similar results to our study, with no effect of vitamin D supplementation on lipid profiles in all but one study (45). Note that the effect of vitamin D on lipid profiles was not a primary outcome in any of these RCTs, and the trials were not sufficiently powered (45). Overall, it appears that vitamin D does not change BP or lipid profiles including in vitamin D–deficient overweight or obese adults.

Our study has several strengths, particularly the use of a rigorous methodology, a double-blind randomized controlled design, and gold-standard methods to measure adiposity, insulin sensitivity, and insulin secretion. Furthermore, we studied participants with vitamin D deficiency who had high BMI and were more likely to be insulin resistant and at risk of developing type 2 diabetes. This group has been proposed to benefit most from vitamin D supplementation. Another strength of our study was that 82% of the intervention group reached a 25(OH)D concentration >70 nmol/L. We also had a well-characterized study sample, and we were able to adjust for possible confounders including physical activity, sun exposure, and dietary composition. A limitation of our study is the small sample size, which could have resulted in insufficient power to detect differences in secondary outcomes or to draw valid conclusions from our subgroup analyses. Moreover, 25(OH)D repletion (>75 nmol/L) was not achieved for all participants in the vitamin D group (82% had concentrations >70 nmol/L and 64% had concentrations >75 nmol/L). Our inclusion of only overweight or obese, but otherwise healthy, individuals means that our results might not be generalizable to other populations. Because of resource constraints, we were not able to measure 25(OH)D with the use of the gold-standard liquid-chromatography–mass spectrometry method, and instead, we used the assay from DiaSorin Inc., which measures both 25(OH)D_2 and 25(OH)D_3 concentrations. We also did not measure liver fat or separate visceral and subcutaneous body fat, and we did not assess the potential contribution of vitamin D axis gene polymorphisms; thus, we were unable to account for these factors in our analyses. Finally, we examined the effect of vitamin D supplementation on factors affecting risk of developing type 2 diabetes, which may not necessarily reflect the incidence of diabetes.

The present study addresses current knowledge gaps through the use of high doses of vitamin D supplemented for a sufficient duration to vitamin D–deficient individuals, all of which are factors that have not been addressed in previous trials to our knowledge. We show no beneficial effect of vitamin D supplementation on insulin sensitivity or secretion, adiposity, or other cardiovascular disease risk factors including BP and lipid profiles in this high risk group of overweight or obese and vitamin D–deficient individuals. In light of these and previous data, it is unlikely that vitamin D supplementation has a role in improving metabolic outcomes or risk factors for type 2 diabetes and cardiovascular disease.

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