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Effects of 6-Month Vitamin D Supplementation on Insulin Sensitivity and Secretion: A Randomized, Placebo-Controlled Trial

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Abstract

Objective: To determine whether vitamin D_3 supplementation improves insulin sensitivity, using the hyperinsulinemic-euglycemic clamp.

Design: This single-center, double-blind, placebo-controlled trial randomized 96 participants at high risk of diabetes or with newly diagnosed type 2 diabetes to vitamin D_3 5,000 IU daily or placebo for 6 months.

Methods: We assessed at baseline and 6 months: 1) primary aim: peripheral insulin sensitivity (M-value using a 2-h hyperinsulinemic-euglycemic clamp); 2) secondary aims: other insulin sensitivity (HOMA2%S, Matsuda) and insulin secretion (insulinogenic index, C-peptide area under the curve, HOMA2-B) indices using a 2h-oral glucose tolerance test (OGTT); ß-cell function (disposition index: M-value x insulinogenic index); fasting and 2-h glucose post-OGTT; HbA_{1c}; anthropometry.

Results: Baseline characteristics were similar between groups (% or mean±SD): women 38.5%; age 58.7±9.4 years; BMI 32.2±4.1 kg/m^2; prediabetes 35.8%; diabetes 20.0%; 25-hydroxyvitamin D (25(OH)D) 51.1±14.2 nmol/L. At 6 months, mean 25(OH)D reached 127.6±26.3 nmol/L and 51.8±16.5 nmol/L in the treatment and placebo groups, respectively ($p<0.001$). A beneficial effect of vitamin D_3 compared with placebo was observed on M-value (mean change (95% CI): 0.92 (0.24 to 1.59) versus -0.03 (-0.73 to 0.67); p=0.009) and disposition index (mean change (95% CI): 267.0 (-343.4 to 877.4) versus -55.5 (-696.3 to 585.3); p=0.039) after 6 months. No effect was seen on other outcomes.

Conclusions: In individuals at high risk of diabetes or with newly diagnosed type 2 diabetes, vitamin D supplementation for 6 months significantly increased peripheral insulin sensitivity and ß-cell function, suggesting that it may slow metabolic deterioration in this population.

Introduction

Low serum 25-hydroxyvitamin D (25(OH)D) has been associated with an increased risk of developing insulin resistance and type 2 diabetes in prospective observational studies (1, 2). Since low vitamin D status is highly prevalent worldwide (3), the potential role of vitamin D supplementation in improving glucose homeostasis generated great enthusiasm among scientists and clinicians. However, randomised controlled trials (RCTs) of vitamin D supplementation have shown inconstant effects on measures of insulin sensitivity, insulin secretion and β-cell function. A meta-analysis concluded that variable results among RCTs could be explained by heterogeneous study populations in terms of ethnicity, glucose tolerance and vitamin D status, by variations in vitamin D dosage and duration of treatment, and by use of surrogate measures of insulin sensitivity (4, 5). Yet, the five studies that used the gold-standard method to assess insulin sensitivity, the hyperinsulinemic-euglycemic clamp, found no effect of vitamin D supplementation on insulin sensitivity (6-10). However, three of these trials were limited by small sample size (n=12-18) (6-8). Despite adequate sample size (n=62-65) and robust methodology, the two other studies failed to show benefits of vitamin D in metabolically-healthy overweight or obese subjects (10) and in individuals with long standing type 2 diabetes (9).

The primary aim of this 6-month trial was to determine whether vitamin D_3 supplementation improves peripheral insulin sensitivity (M-value) in individuals at high risk for type 2 diabetes or with newly diagnosed type 2 diabetes, using the gold-standard hyperinsulinemic-euglycemic clamp. Secondary aims were to evaluate the effects of vitamin D_3 on: fasting- and oral glucose tolerance test (OGTT)-derived indices of insulin sensitivity, insulin

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secretion, β-cell function, metabolic markers, blood pressure and anthropometric measurements.

Methods

Study design

This randomized, double-blind, placebo-controlled, parallel-group trial was conducted at a single site (Centre Hospitalier Universitaire (CHU) de Québec-Université Laval) located in Québec City, Canada. After screening, eligible participants were randomly assigned, in a 1:1 ratio, to receive vitamin D_3 or placebo for 6 months. Randomisation, computerised based on the Pocock and Simon minimisation method (11), was conducted by a biostatistician to balance treatment arms according to sex, body-mass index (BMI) (< or ≥30 kg/m^2) and age (< or ≥50 years old). The biostatistician sent treatment allocation directly to the pharmacist who was in charge of dispensing the study medication. The investigators, research personnel, care providers and participants involved in this study were blinded to the treatment assignment until the last participant completed the study.

The study protocol and all amendments were approved by the CHU de Québec-Université Laval ethics committee. The study was registered at clinicaltrials.gov before starting recruitment (NCT01779908) and conduct of the trial followed the 2010 Consort guidelines (12). This study was also conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving patients were approved by the CHU de Québec-Université Laval ethics committee (Project number B12-12-1095). Written informed consent was obtained from each participant before any trial-related activities.

Participants

Participants were recruited between January 2013 and October 2016 via flyers in hospitals and public places, through newspapers and radio advertisements and via emails sent to employees of the CHU de Québec-Université Laval and Université Laval. Initially, eligible participants included vitamin D-deficient (25(OH)D ≤50 nmol/L) Caucasian men and postmenopausal women aged ≥50 years, meeting the criteria of the hypertriglyceridemic waist phenotype (waist circumference ≥90 cm plus fasting triglycerides ≥2.0 mM in men or waist circumference ≥85 cm plus fasting triglycerides ≥1.5 mM in women). During the trial, inclusion criteria were modified a few times to improve the recruitment rate. Eligible participants finally included Caucasian men and women aged ≥25 years with a serum 25(OH)D at screening ≤55 nmol/L, an increased waist circumference (≥102 cm for men and ≥88 cm for women) and at least one metabolic abnormality or risk factor associated with insulin resistance. Those were defined as: 1) fasting triglycerides ≥1.7 mM or dyslipidemia treated with lipid-lowering drugs, 2) prediabetes or type 2 diabetes not on glucose-lowering drugs: fasting glucose ≥5.6 mM or 2h-glucose post OGTT ≥7.8 mM or HbA_{1c} ≥5.7%, 3) first-degree relative with type 2 diabetes, 4) history of gestational diabetes.

Participants were excluded if they had any of the following conditions: type 2 diabetes under drug therapy, HbA_{1c} >7%, BMI ≥40 kg/m^2, medication influencing vitamin D or glucose metabolism in the last 3 months, regular consumption of supplements containing > 400 IU/d of vitamin D_3 over the last 2 months, renal insufficiency (estimated glomerular filtration rate <60 ml/min), pregnancy, cirrhosis, intestinal malabsorption (e.g. bypass surgery, celiac

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disease) or osteoporosis, history of nephrolithiasis, hypercalcemia (>2.6 mM), hypercalciuria (>0.6 fasting urine calcium/creatinine ratio), >5% change in weight in the last 3 months, diseases affecting glucose metabolism (e.g. hyperthyroidism), pacemaker (for bioimpedance only); inability to provide informed consent and complete questionnaires due to physical or mental problems.

Intervention

The treatment group received 5,000 IU of vitamin D_3 once a day (half a tablet of D-tabs 10,000 IU, Riva Laboratory, Québec, Canada), aimed to reach serum 25(OH)D ≥75 nmol/L (13), whereas the control group received placebo daily, for 6 months. Placebo pills were produced by the hospital pharmacy using lactose monohydrate powder. To ensure blinding, both the vitamin D and placebo pills were enrobed in capsules of identical shape and color. The pharmacy department dispensed study pills and asked participants to take the pill with the morning meal. The pharmacist assessed compliance with treatment at 3 and 6 months using pill count. Participants were questioned about adverse effects or change in their health condition during the intervention. All participants were instructed to maintain their usual diet and exercise habits throughout the study. Medication was assessed at baseline and any further change was documented during follow-up. An independent blinded clinician performed safety monitoring of serum calcium at 3 months.

Outcome measures

Hyperinsulinemic-euglycemic clamp

The primary endpoint was change between baseline and 6 months in peripheral insulin sensitivity, measured by M-value, using a 2-h hyperinsulinemic-euglycemic clamp.

Participants were instructed to eat the same dinner the night before the clamp at 0 and 6 months and to avoid physical activity (except walking), alcohol and caffeine for 24 h prior to the procedure. After a 12-h overnight fast, an intravenous catheter was placed in an antecubital vein for the administration of regular insulin (Humulin® R) and dextrose 20%. A second catheter was placed in the other arm for blood sampling. Insulin was infused at a fixed rate of 40 mU/m^2/min. Dextrose infusion rate was adjusted every 5 minutes to maintain capillary glucose measurement (using Xceed Pro and Accu-Chek Inform II glucose meters) between 5.0 and 5.5 mM. Serum insulin was measured every 5 minutes during the last 30 minutes of the clamp. M-value, an index of stimulated glucose disposal rate, was obtained by dividing exogenous glucose infusion rate by kilogram of body weight during the last 30 minutes (mg/kg x min). Another index of peripheral insulin sensitivity (M/I), defined as M-value divided by the mean serum insulin concentration during the last 30 minutes of the clamp, was calculated.

Oral glucose tolerance test

After a 12-h fast, a 75g 2-h OGTT was performed in the morning at baseline and at 6 months. The OGTT was scheduled in a period of 1 to 7 days prior to or after the hyperinsulinemic-euglycemic clamp. Blood samples were collected through a venous catheter in serum separating tubes (SST) at 0, 15, 30, 60, 90 and 120 minutes for measurement of insulin, glucose and C-peptide concentrations. Hepatic insulin sensitivity was assessed by HOMA2%S using fasting glucose and insulin concentrations (HOMA2 calculator – www.dtu.ox.ac.uk/homa) and whole-body insulin sensitivity was assessed by Matsuda index (10,000 divided by the squared root of [(fasting glucose x fasting insulin) x (mean glucose$^{30–120\ min}$ x mean insulin$^{30–120\ min}$)]. Matsuda index was added *a posteriori* for

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comparison with previously published studies. Insulin secretion was assessed by HOMA2-B using fasting C-peptide and glucose (HOMA2 calculator), the insulinogenic index (delta C-peptide$^{0\text{-}30\ min}$ divided by delta glucose$^{0\text{-}30\ min}$) and the area under the curve (AUC) for C-peptide. Disposition index, calculated by multiplying M-value by insulinogenic index, was used to estimate β-cell function.

Anthropometry and blood pressure

Blood pressure and anthropometric measurements including weight, height, waist and hip circumference were assessed at baseline and every 3 months. Participants were weighed without shoes in light clothing on a calibrated scale to the nearest 0.1 kg. Height was measured at the nearest millimeter, using a wall-mounted stadiometer. BMI was calculated in kg/m^2. Blood pressure was measured three times in a sitting position after a 15-minute rest; the mean of the last two measurements was calculated. Waist circumference was measured twice, at the nearest 0.5 cm, using a tape placed on top of the upper iliac crest, at the end of a normal expiration. Hip circumference was measured in duplicate, at the nearest millimeter, using a tape placed at the maximum width of the buttocks. Percent body fat was calculated by bioimpedance, using the InBody520 device.

Serum 25(OH)D, parathyroid hormone and metabolic markers

Serum was stored at -80ºC until the end of the study, where samples were analysed in a single batch for all biomarkers. For screening of the participants, serum 25-hydroxyvitamin D was analysed by an electrochemiluminescence immunoassay (Roche, Cobas 6000, CV 10%), which was more readily available. However, for all statistical analyses, serum

25(OH)D was analysed in a single batch at the study end for assessment of baseline, 3-month and 6-month frozen samples by ultra-performance liquid-chromatography tandem mass spectrometry (Waters ACQUITY UPLC System, CV 7.3%). The Department of biochemistry measuring serum 25(OH)D met the performance target set by the DEQAS Advisory Panel. PTH was assessed by a chemiluminescence immunoassay (Beckman Coulter, Access 2, CV 4%) while HbA_{1c} was measured by a second-generation colorimetric method (Roche, Integra 800, CV 4%). Plasma glucose was measured enzymatically by the hexokinase method (Roche, Modular E170, CV 1.8%) whereas insulin and C-peptide were measured with an electrochemiluminescence immunoassay (Roche, Modular E170, CV 3% and 2.5%, respectively). Calcium was measured via the o-cresolphthalein complexone method (Roche, Modular E170, CV 3.6%) every 3 months for safety purposes.

Diet, physical activity and sun exposure

Weekly sun exposure was calculated (h/week) via a non-validated questionnaire, based on the time that participants reported spending outdoors between 11am and 2pm over the last 3 months(14), excluding days between November 1st and March 31th, as there is no skin production of vitamin D_3 during that period in Québec City. Participants were also asked about their sunscreen use (sun protection factor, frequency), clothes worn (hat, shirt, pants, long sleeves, etc.) and localisation (shadow, sun) while performing outdoor activities. Physical activity habits during the last week before the baseline and 6-month study visits were estimated using the short International Physical Activity Questionnaire (IPAQ, 2005). The volume of activity per week (walking, moderate and vigorous physical activity) was computed by weighting each type of activity by its energy requirements (METs) and by multiplying by the number of corresponding days per week. Results were summed to yield a

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score in MET–minutes per week. Finally, a web-based, food frequency questionnaire, validated in the Québec City metropolitan area, was administered to evaluate daily energy, vitamin D and calcium intakes both at the beginning and at the end of the study (15).

Statistical methods

As there was no available similar study to estimate the mean M-value of our study population when the study was planned, our sample size was based on the mean M-value (5.49 ± 2.33) of 65 vitamin D-deficient postmenopausal women aged 50-68 years with a waist circumference measurement of >88 cm who had taken part in a cross-sectional study conducted in the Québec City area (unpublished result). We hypothesized that the M-value would remain stable between baseline and 6 months in the control group while it would increase by 22% in the treatment group. This was based on the study by von Hurst et al., who showed that vitamin D-deficient South Asian women with insulin resistance who reached serum 25(OH)D concentrations >80 nmol/L with vitamin D supplementation experienced an increase in insulin sensitivity (HOMA-IS) by 22% versus placebo at 6 months (16). This difference corresponds to a medium effect with a Cohen's d of 0.52 (17). The trial was designed to recruit a total of 120 participants, providing a statistical power of 80% to detect this clinically significant difference between groups at 6 months with a Student’s t-test and with an alpha error of 0.05. To account for a dropout rate of 10%, sample size was increased at 130 participants.

Statistical analyses were performed by an independent statistician using SAS version 9.4 (SAS Institute Inc., Cary, NC). Group comparability at baseline was assessed using Student’s t-test and Chi-squared tests for continuous and categorical variables, respectively.

Baseline comparison between participants who completed the study and those who dropped out was performed using the same tests. Changes from baseline to endpoint measures of primary and secondary outcomes between and within groups were assessed using repeated measures two-way analyses of variance (rmANOVA). Treatment and time effects and their interaction on outcome measures were tested. The interaction term (time by treatment effect) is the term of interest as it tests whether the temporal change differs between groups. The treatment term tests if a difference exists between groups, for all visits combined, while the time term tests if a difference exists between visits, for all groups. rmANOVA were estimated using a linear mixed model. These models were adjusted for variables that differed at baseline between groups. Squared-root transformation was used when needed to meet model assumptions, such as normality and homoscedasticity of the residuals. According to the intention-to-treat principle, data was analysed for all randomized participants regardless of compliance to treatment and dropout. Linear mixed model enables to use all the available data, even if no values are present after baseline for an individual; thus, no participant was excluded from the analysis. A sensitivity analysis was also conducted for the primary outcome (M-value by clamp), excluding participants with hypoglycaemia (capillary blood glucose < 4.0 mmol/L) at any timepoint during the clamp and/or with a difference of ≥0.5 mmol/L between mean capillary blood glucose in the last 30 minutes of the clamp at 0 and 6 months. A pre-specified subgroup analysis was performed per-protocol, including participants who took ≥80% of their study medication. Moreover, post-hoc exploratory subgroup analyses were done according to baseline glucose tolerance status (normal glucose tolerance versus prediabetes versus diabetes) and baseline 25(OH)D (<50 nmol/L).

Results

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Due to difficulties enrolling participants in the trial, recruitment was stopped after randomizing the 96th participant. Out of the 796 screened participants, 96 met the inclusion criteria and were randomized, 48 in each group (**Figure 1**). Main reasons for exclusion were: absence of serum 25(OH)D criterion (n=185), declined participation (n=154), waist circumference below defined inclusion criteria (n=104), consumption of vitamin D supplements (n=65) or diabetes medication (n=42), and absence of risk factors associated with insulin resistance (n=38). One participant in the treatment group withdrew after the first part of the baseline study visit (did not undergo the OGTT nor the clamp) for personal reasons and did not receive the allocated intervention. Four participants in the placebo group withdrew during the trial and did not complete the follow-up visits. Reasons for withdrawal were personal reasons (n=2) or experiencing side effects (n=2). Moreover, three more participants stopped taking the study supplementation during the trial because of adverse effects (treatment n=2, placebo n=1) but completed the follow-up visits. Finally, one participant in the placebo group had no data for the primary outcome due to impossibility to insert an intravenous catheter to perform the hyperinsulinemic-euglycemic clamp. Compared to the participants who did not complete the study, those who completed had a worse insulin sensitivity profile, as determined by significantly lower HOMA2%S (40.6 versus 62.4, p=0.008) and Matsuda (2.14 versus 3.51, p=0.015) indices. There was also a trend for the inclusion of more men (63.7% vs 20.0%, p=0.071) with higher fasting glucose (6.0 versus 5.2 mmol/L, p=0.079) and insulin (130 versus 81 pmol/L, p=0.091) concentrations.

Baseline characteristics of the 96 randomized participants are shown in **Table 1**. Participants were a majority of men in their 50's with obesity. Over half of the participants had either prediabetes (35.8%) or newly diagnosed type 2 diabetes (20%). Mean baseline serum

25(OH)D concentration by mass spectrometry was 51.1 nmol/L, with 45.8% of the participants having a 25(OH)D concentration ≤50 nmol/L (min-max: treatment, 19-81 nmol/L; placebo, 25-97 nmol/L). Baseline characteristics were similar for both groups, except for significantly lower serum triglycerides in the treatment group, a trend for a higher dietary vitamin D intake and for a lower serum 25(OH)D concentration in the treatment group. Difference in 25(OH)D between groups was thought to be potentially clinically significant and therefore, results were adjusted for baseline 25(OH)D. As further adjustment for serum triglycerides did not change results, these data are not presented.

Change in serum 25(OH)D concentrations and compliance with study medication

Serum 25(OH)D was significantly higher in the treatment group at 3 months (122.9 versus 52.2 nmol/L, p<0.001) and 6 months (127.6 versus 51.8 nmol/L, p<0.001) compared with the control group. After 6 months, serum 25(OH)D increased by a mean of 79.1 nmol/L (95% CI 73.1, 85.2) in the treatment group and did not change in the placebo group (1.87 nmol/L (95% CI -4.33, 8.07)) (**Figure 2**). Moreover, 95.7% of the participants in the treatment group reached a serum 25(OH)D value of >75 nmol/L at 6 months. Compliance was similar in both groups, with 93.2% and 88.6% of the participants in the treatment and control groups who took ≥80% of their study medication, respectively (p=0.713). Compliance data was missing for 8 participants.

Change in primary outcome (M-value)

At baseline, 80.9% (n=38/47) of participants in both groups reached the target capillary blood glucose during the last 30 minutes of the clamp while at 6 months, 85.1% (n=40/47) and 83.7% (n=36/43) of participants in treatment and placebo groups, respectively met this

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criterion. There was a significant time-by-treatment interaction for insulin sensitivity, as assessed by M-value and M/I ratio, in favour of the vitamin D group **(Table 2).** Indeed, M-value increased by a mean of 22.9% in the treatment group while it remained stable in the placebo group. Results were similar in the sensitivity analysis including participants who had no hypoglycaemia and/or a difference <0.5 mmol/L between mean capillary blood glucose in the last 30 minutes of the clamp at 0 and 6 months (n=72). A post-hoc exploratory subgroup analysis revealed that between-group differences in M-value appeared to be more important in participants with diabetes or prediabetes at baseline **(Table 3).** A second post-hoc subgroup analysis restricted to participants with baseline serum 25(OH)D <50 nmol/L (n=44) no longer demonstrated a significant between-group difference for change in M-value.

Changes in secondary outcomes (insulin sensitivity, insulin secretion, ß-cell function, metabolic markers, blood pressure and anthropometric measures)

No between group differences were observed for changes in insulin sensitivity and insulin secretion indices derived from fasting values or the OGTT **(Table 4).** However, there was a significant time-by-treatment interaction (p=0.039) for disposition index, calculated as M-value x insulinogenic index, with stability in the control group and improvement in the treatment group at 6 months. Changes in fasting glucose, 2-h glucose post OGTT and HbA_{1c} were similar between groups. Moreover, changes in weight, BMI, waist and hip circumference, body fat mass and blood pressure did not differ between the intervention and placebo groups **(Table 5).** Subgroup analyses restricted to participants who took ≥80% of

their study medication or to those with abnormal baseline glucose tolerance status (prediabetes or diabetes) gave similar results.

Safety and adverse events

Five participants discontinued the study because of side effects, two in the treatment group (gastrointestinal complaints and dizziness) and three in the placebo group (gastrointestinal complaints, anxiety and hypoglycaemia). Moreover, participants who completed the study reported gastrointestinal issues (treatment, n=6, placebo, n=5), dizziness (placebo, n=1), polyuria (treatment, n=1) and musculoskeletal symptoms (placebo, n=1). None of the participants developed hypercalcemia, hypercalciuria or nephrolithiasis during the trial.

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Discussion

In this randomized, placebo-controlled trial conducted in individuals at high risk of type 2 diabetes or with newly diagnosed type 2 diabetes, vitamin D_3 supplementation at a dosage of 5,000 IU once daily for 6 months significantly increased peripheral insulin sensitivity, as measured by the gold-standard hyperinsulinemic-euglycemic clamp. A subgroup analysis revealed that this effect appeared to be more important in subjects with prediabetes or newly diabetes at baseline. Moreover, although there were no between group differences in indices of insulin secretion, there was a significant beneficial effect of vitamin D on the disposition index, suggesting that vitamin D may improve β-cell function. However, there was no effect of vitamin D supplementation on any measures of insulin sensitivity derived from fasting values and from the OGTT, anthropometric measures, blood pressure, fasting and 2-h glucose and HbA_{1C}.

All five RCTs that used the hyperinsulinemic-euglycemic clamp failed to demonstrate a beneficial effect of vitamin D on insulin sensitivity. However, three of them were limited by small sample size and short duration of treatment. Indeed, one study treated 18 healthy subjects with 1.5 mcg of calcitriol daily for 7 days while in another study, 12 healthy subjects received 50,000 IU of vitamin D_2 weekly for 8 weeks (6, 7). The third trial included 16 patients with type 2 diabetes who were supplemented with vitamin D_3 11,200 IU daily for 2 weeks and then 5,600 IU daily for 10 weeks (8). While insulin sensitivity did not differ between groups, borderline improvements in insulin secretion were noted in the treatment group. In the fourth trial, the effect of a bolus dose of 100,000 IU of vitamin D_3 followed by 4,000 IU daily for 16 weeks in 65 overweight and vitamin D-deficient participants was

evaluated (10). Compared with our trial, participants in this study were healthier and had a better insulin sensitivity profile, with a higher baseline M-value (6.7 ± 2.9 versus 4.1 ± 2.3 mg/kg x min). Results of this study are concordant with our subgroup analysis showing that vitamin D supplementation did not change M-value in participants with normal glucose tolerance. Finally, in a 6-month trial, 62 participants of Nordic or South Asian ethnicity with type 2 diabetes and vitamin D deficiency were treated with a single high dose of vitamin D_3 400,000 IU, with an additional dose of 200,000 IU at 4 weeks if serum 25(OH)D was below 100 nmol/L (9). Divergence in results with our study may be explained by the lack of sustained concentrations of serum 25(OH)D in the treatment group, with a mean of 53.7 ± 9.2 nmol/L at 6 months and by the inclusion of participants with long standing type 2 diabetes (mean diabetes duration of 11.0 ± 6.6 years and 7.9 ± 5.7 years in the vitamin D and placebo group, respectively), compared to our trial, which included participants at high risk for diabetes or with newly diagnosed type 2 diabetes. However, it remains unclear how the diabetes duration could have affected the results. Furthermore, no effect of vitamin D was found in two RCTs that used the hyperglycemic clamp to evaluate insulin sensitivity. One was performed in 104 healthy subjects who received 20,000 IU of vitamin D_3 twice weekly for 6 months and the other one in 44 participants with prediabetes or type 2 diabetes who were treated with 30,000 IU of vitamin D_3 once weekly for 8 weeks (18, 19). Nevertheless, the hyperglycemic clamp is less accurate than the gold standard hyperinsulinemic-euglycemic clamp to estimate insulin sensitivity (20).

In concordance with three recent meta-analyses, one including 23 RCTs in patients with type 2 diabetes, another including 35 RCTs performed in various populations and the third one

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including 10 RCTs in prediabetes patients (4, 5, 21), we did not find any effect of vitamin D supplementation on measures of insulin sensitivity and insulin secretion based on fasting indices and the OGTT. The vast majority of RCTs, and therefore those included in the meta-analyses, used indirect insulin sensitivity markers such as HOMA and Matsuda instead of the hyperinsulinemic-euglycemic clamp. Just like the population included in our study, many previous trials were performed in insulin-resistant participants, and most of them have shown negative results with the use of surrogate markers of insulin sensitivity (13, 22-27). One trial demonstrated a significant improvement in insulin sensitivity assessed by HOMA2%S in 81 vitamin D-deficient South Asian women with insulin resistance treated with vitamin D_3 4,000 IU daily for 6 months, and a second one showed a significant decrease in HOMA-IR in Iranian women with polycystic ovary syndrome (16, 28). However, these results may not be generalizable to Caucasian populations.

The discordant effects of vitamin D supplementation that we observed on insulin sensitivity measures, with a significant beneficial effect on M-value and no difference on HOMA2%S and Matsuda indices, suggests that vitamin D acts mainly on peripheral insulin sensitivity. Indeed, HOMA2%S primarily reflects hepatic insulin sensitivity, and Matsuda whole-body insulin sensitivity with a major contribution of hepatic insulin sensitivity (29) whereas the hyperinsulinemic-euglycemic clamp (M-value), at the insulin dose that we used, evaluates predominantly muscle insulin sensitivity (30, 31). Although the exact mechanisms by which vitamin D influences muscle insulin sensitivity in humans remain to be elucidated, preclinical evidence suggests that vitamin D may improve diabetes-induced muscle dysfunction and atrophy (32) as well as muscle fat infiltration (33). Also, vitamin D could

increase glucose uptake in adipose tissue and muscle by stimulating GLUT-4 expression (34, 35) and reduce low-grade chronic systemic inflammation (36, 37).

Another notable finding of our study is the significant beneficial effect of vitamin D supplementation on disposition index, a measure of ß-cell compensatory capacity, with stability in the control group and improvement in the treatment group. Preservation of ß-cell function is important, as it was shown to predict reduced diabetes risk (38). Indeed, DeFronzo *et al.* demonstrated that deterioration of ß-cell function, assessed by the disposition index, is the strongest predictor of conversion to diabetes in subjects with impaired glucose tolerance (39). Even though we observed no differences in indices of insulin secretion following OGTT between groups, a beneficial effect of vitamin D on insulin secretion relative to insulin sensitivity was seen, reflected by the disposition index. Moreover, we could have missed positive effects of vitamin D on insulin secretion *per se*, as we did not use the hyperglycemic clamp, the gold standard method to assess insulin secretion (20). Mechanisms by which vitamin D plays a role on preservation of ß-cell function remain largely unknown. However, previous studies have shown that pancreatic ß-cells contain high levels of vitamin D receptor and that vitamin D enhances ß-cell intracellular calcium concentration and influx (40, 41).

Our study has notable strengths including the randomized, double-blind design and the use of the gold-standard hyperinsulinemic-euglycemic clamp to evaluate insulin sensitivity. Furthermore, we selected participants at high risk for type 2 diabetes or with newly type 2 diabetes, a group that had not been specifically studied using the clamp. Another strength is the use of high-dose vitamin D supplementation for 6 months and the high rate of

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compliance, with a large difference in serum 25(OH)D during the study between the treatment and control groups. However, limitations include the selection of a Caucasian population, restricting generalizability to other ethnic groups. Moreover, we did not reach our target sample size. However, our sample of 96 participants still had the power to detect a medium to large effect. Indeed, using the same criteria as mentioned in the methods section, it was still possible to detect an effect size of d=0.58 with a sample size of 96 participants (where d of 0.5 is considered a medium effect and 0.80 a large effect), compared to our planned sample of 120 participants that could detect a medium effect (d=0.52)(17). Since effect sizes in the present study were mostly small, it is probable that reaching the target sample size would not have changed the conclusions. Furthermore, the sample size calculation was based on postmenopausal women, even though the present study included men and women, due to lack of available data at the time of the study. Another limitation is that the mean baseline serum 25(OH)D was higher than expected and only about half of the participants had vitamin D deficiency at study entry due to different methods for serum 25(OH)D measurement and to time difference of up to 3 months between screening and baseline visits. Although subgroup analysis restricted to participants with baseline 25(OH)D <50 nmol/L did not show any significant differences between groups, these results should be interpreted with caution due to reduced study power and the post-hoc nature of the analysis. Additionally, although both groups were comparable in terms of confounding factors such as sun exposure, dietary intake of calcium and vitamin D and physical activity, the assessment methods employed, such as the use of a non-validated questionnaire for sun exposure, may have failed to capture differences. Furthermore, although a minority of patients dropped out of the study, excluded participants had a better insulin sensitivity profile. These selection and attrition bias could have overestimated the effect in the mixed models. Finally, as we did

not use tracer infusion to estimate glucose rate of appearance and disappearance and as we used an insulin dose that may not have suppressed completely endogenous glucose production in the most highly insulin-resistant participants, we may have underestimated peripheral insulin sensitivity as assessed by M-value or M/I.

In conclusion, this study showed that high-dose vitamin D supplementation for 6 months significantly improved peripheral insulin sensitivity, as assessed by the hyperinsulinemic-euglycemic clamp, and ß-cell function in individuals at high risk of diabetes or with newly diagnosed type 2 diabetes. Of interest, two large RCTs evaluating the effect of high-dose vitamin D_3 (20 000 IU/week for 5 years and 4 000 IU/day for a median of 2.5 years) in patients with prediabetes who were mostly vitamin D sufficient at baseline did not show any effect on progression to type 2 diabetes (42, 43). Larger and longer-term RCTs are required to evaluate whether subgroups of patients including those with low vitamin D status may benefit from vitamin D supplementation.

Disclosure

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Authorship

CG formulated the research question, CG and SJW designed research; CG conducted research; ASJ analyzed data; PL wrote the first draft of the paper; CG had primary responsibility for final content; and all authors critically revised the protocol, read and approved the final version of the manuscript prior to submission.

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Table 1. Baseline characteristics of the participants by treatment group

Table 2. Mean baseline and 6-month values and the mean absolute changes in M-value and M/I ratio in the treatment and placebo groups.

Table 3. Mean baseline and 6-month values and the mean absolute changes in M-value in the subgroups of participants with normal glucose tolerance, prediabetes or diabetes at baseline.

Table 4. Mean baseline and 6-month values and the mean absolute changes in insulin sensitivity, insulin secretion, ß-cell function and metabolic markers in the treatment and placebo groups.

Table 5. Mean baseline and 6-month values and the mean absolute changes in anthropometry and blood pressure in the treatment and placebo groups.

Figure 1. Flowchart showing participant enrolment, allocation and analysis

Figure 2. Mean (SD) serum 25-hydroxyvitamin D concentrations at baseline and after 3 and 6 months in the vitamin D and placebo groups. *P<0.001 for the difference between groups.

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Table 1. Baseline characteristics of the participants by treatment group[1]

	Treatment, n = 48	Placebo, n = 48	*P* value[2]
Age, years	58.3 (8.8)	59.1 (9.5)	0.674
Women	41.7	35.4	0.675
Menopausal women	85.0	82.4	1.000
Season of recruitment			0.782
Winter	29.2	29.2	
Spring	35.4	29.2	
Summer	16.7	25.0	
Fall	18.8	16.7	
Education			0.127
Primary school	0.0	4.2	
High school	16.7	22.9	
College	14.6	25.0	
University	68.8	47.9	
Family income			0.489
0 – 19,000$	10.4	2.1	
20,000 – 39,000$	10.4	14.6	
40,000 – 59,000$	16.7	22.9	
60,000 – 79,000$	14.6	16.7	
80,000 – 99,000$	14.6	16.7	
>100,000$	29.2	27.1	
Declined to answer	4.2	0.0	
Current smoking	8.3	4.2	0.677
First degree relative with diabetes	59.6	47.9	0.306
BMI, kg/m^2	32.2 (4.3)	32.1 (3.9)	0.889
Waist circumference, cm	107.9 (9.0)	108.5 (9.5)	0.760
Systolic blood pressure, mm Hg	129.2 (13.6)	131.2 (17.3)	0.532
Diastolic blood pressure, mm Hg	76.2 (9.3)	76.5 (9.7)	0.862
HbA_{1c}, %	5.7 (0.3)	5.8 (0.3)	0.678

Fasting glucose, mmol/L	5.9 (0.8)	6.1 (1.1)	0.588
Fasting insulin, pmol/L	130.7 (60.0)	125.4 (54.0)	0.649
2-h glucose post OGTT, mmol/L	8.2 (3.4)	8.4 (3.1)	0.745
Glucose status			0.895
Prediabetes[3]	34.0	37.5	
Diabetes[4]	19.1	20.8	
25-hydroxyvitamin D[5], nmol/L	48.5 (13.0)	53.7 (15.0)	0.073
Parathyroid hormone, ng/L	57.3 (21.2)	57.3 (20.3)	0.992
Corrected calcium, mmol/L	2.25 (0.08)	2.27 (0.07)	0.251
Serum creatinine, µmol/L	74.6 (14.1)	73.9 (13.1)	0.800
Triglycerides, mmol/L	1.66 (0.65)	2.33 (1.90)	0.023
ALT, U/L	26.5 (14.0)	29.6 (14.5)	0.302
Urinary calcium/creatinine ratio, mmol/mmolcr	0.19 (0.15)	0.17 (0.11)	0.623
Time spent outdoor between 11h and 14h, h/week	4.0 (4.9)	3.6 (4.8)	0.738
Physical activity, MET-min/week	1709 (1928)	1371 (1798)	0.377
Percent body fat[6], %	38.5 (8.3)	37.4 (7.8)	0.581
Daily energy intake, kJ	11740 (3879)	11033 (3075)	0.328
Dietary vitamin D intake, IU/day	380 (228)	312 (168)	0.086
Dietary calcium intake, mg/day	1528 (678)	1352 (512)	0.153

MET, metabolic equivalent of task

[1]Data are presented as mean (SD) or %

[2]Student's t-test or exact chi-squared test, as appropriate

[3]Fasting glucose 6.1-6.9 mmol/L or 2-h glucose post OGTT 7.8-11.0 mmol/L

[4]Fasting glucose ≥7.0 mmol/L or 2-h glucose post OGTT ≥11.1 mmol/L

[5]By liquid-chromatography tandem mass spectrometry

[6]By bioimpedance

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Table 2. Mean baseline and 6-month values and the mean absolute changes in M-value and M/I ratio in the treatment and placebo groups.

	Mean (SEM)		Mean change (95% CI)	*P* value[1]		
	Baseline[2]	6 months	Δ 6 months	Time	Treatment	Interaction
M-value[3], mg/kg • min						
Treatment (n=47)	3.97 (0.41)	4.88 (0.41)	0.92 (0.24, 1.59)	0.234	0.628	0.009
Placebo (n=47/43[4])	4.15 (0.41)	4.12 (0.42)	-0.03 (-0.73, 0.67)			
M/I ratio[3], mg/kg • min / pmol/L						
Treatment (n=47)	0.0062 (0.0007)	0.0077 (0.0007)	0.0015 (0.0003, 0.0027)	0.050	0.705	0.031
Placebo (n=45/43)	0.0064 (0.0007)	0.0069 (0.0007)	0.0005 (-0.0007, 0.0017)			

SEM, standard error of the mean

[1]Two-way analysis of variance (ANOVA) with repeated measures was used to assess time and treatment effects as well as their interactions, after adjustment for baseline serum 25-hydroxyvitamin D. The p-value for the interaction (time by treatment effect) is the one of interest as it tests whether the temporal change differs between groups.
[2]Baseline and 6 month data are presented as mean (SEM)
[3]Variables were squared-root-transformed for analysis but original values are presented
[4]For each measure, if the number of participants with available data was different between baseline and 6 months, n is presented as baseline/6 months

Table 3. Mean baseline and 6-month values and the mean absolute changes in M-value in the subgroups of participants with normal glucose tolerance, prediabetes or diabetes at baseline.

	Mean (SEM)		Mean change (95% CI)	*P* value[1]		
M-value[2], mg/kg • min	Baseline[3]	6 months	Δ 6 months	Time	Treatment	Interaction
Normal glucose tolerance						
Treatment (n=22)	5.49 (0.67)	5.94 (0.67)	0.45 (-0.74, 1.64)	0.641	0.788	0.677
Placebo (n=19/17[4])	5.31 (0.72)	5.75 (0.75)	0.43 (-0.91, 1.77)			
Prediabetes						
Treatment (n=16)	3.13 (0.56)	4.38 (0.56)	1.25 (0.12, 2.38)	0.591	0.952	0.015
Placebo (n=18/16)	3.88 (0.52)	3.32 (0.55)	-0.56 (-1.68, 0.56)			
Diabetes						
Treatment (n=9)	1.50 (0.49)	2.96 (0.49)	1.46 (0.58, 2.34)	0.080	0.562	0.030
Placebo (n=10)	2.62 (0.46)	2.70 (0.46)	0.08 (-0.76, 0.91)			

SEM, standard error of the mean

[1]Two-way analysis of variance (ANOVA) with repeated measures was used to assess time and treatment effects as well as their interactions, after adjustment for baseline serum 25-hydroxyvitamin D. The p-value for the interaction (time by treatment effect) is the one of interest as it tests whether the temporal change differs between groups.

[2]Variables were squared-root-transformed for analysis but original values are presented

[3]Baseline and 6 month data are presented as mean (SEM)

[4]For each measure, if the number of participants with available data was different between baseline and 6 months, n is presented as baseline/6 months

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Table 4. Mean baseline and 6-month values and the mean absolute changes in insulin sensitivity, insulin secretion, ß-cell function and metabolic markers in the treatment and placebo groups.

	Mean (SEM)		Mean change (95% CI)	*P* value[1]		
	Baseline[2]	6 months	Δ 6 months	Time	Treatment	Interaction
Insulin sensitivity						
HOMA2%S						
Treatment (n=47)	40.6 (2.4)	41.9 (2.4)	1.3 (-2.6, 5.2)	0.723	0.979	0.201
Placebo (n=48/44[3])	42.4 (2.4)	40.1 (2.4)	-2.3 (-6.3, 1.7)			
Matsuda index						
Treatment (n=47)	2.09 (0.17)	2.19 (0.17)	0.10 (-0.13, 0.33)	0.640	0.496	0.481
Placebo (n=46/44)	2.30 (0.17)	2.28 (0.17)	-0.02 (-0.26, 0.22)			
Insulin secretion						
HOMA2-B						
Treatment (n=47)	132.7 (5.1)	127.5 (5.1)	-5.2 (-13.7, 3.2)	0.044	0.800	0.751
Placebo (n=48/44)	135.4 (5.1)	128.2 (5.2)	-7.2 (-15.8, 1.5)			
Insulinogenic Index, pmol/L / mmol/L						
Treatment (n=47)	487.5 (45.3)	496.4 (45.3)	8.8 (-44.6, 62.2)	0.855	0.293	0.525
Placebo (n=47/44)	434.9 (44.9)	419.0 (45.4)	-15.9 (-71.5, 39.6)			
AUC for C-peptide, pmol/L x 120min						
Treatment (n=47)	410.2 (16.4)	403.3 (16.4)	-6.9 (-25.6, 11.8)	0.968	0.256	0.296
Placebo (n=47/43)	377.4 (16.3)	384.9 (16.5)	7.5 (-12.2, 27.1)			
ß-cell function						
Disposition index[4]						
Treatment (n=47)	2425.5 (421.9)	2692.5 (421.9)	267.0 (-343.4, 877.4)	0.497	0.237	0.039
Placebo (n=47/43)	1768.1 (423.6)	1712.6 (430.5)	-55.5 (-696.3, 585.3)			
Metabolic markers						

HbA_{1c}, %						
Treatment (n=48/47)	5.7 (0.1)	5.6 (0.1)	-0.10 (-0.19, -0.01)	0.017	0.604	0.694
Placebo (n=48/43)	5.8 (0.1)	5.7 (0.1)	-0.07 (-0.17, 0,03)			
Fasting glucose, mmol/L						
Treatment (n=47)	5.9 (0.1)	6.1 (0.1)	0.14 (-0.07, 0.35)	0.520	0.817	0.242
Placebo (n=48/44)	6.1 (0.1)	6.0 (0.2)	-0.04 (-0.26, 0.18)			
2h glucose post-OGTT, mmol/L						
Treatment (n=47)	8.1 (0.5)	8.1 (0.5)	-0.00 (-0.53, 0.53)	0.594	0.552	0.586
Placebo (n=48/44)	8.4 (0.5)	8.6 (0.5)	0.21 (-0.34, 0.75)			

AUC, area under the curve; HOMA2%S, Homeostasis Model Assessment 2 index of insulin sensitivity; HOMA2-B, Homeostasis Model Assessment 2 index of ß-cell function; OGTT, oral glucose tolerance test; SEM, standard error of the mean

[1]Two-way analysis of variance (ANOVA) with repeated measures was used to assess time and treatment effects as well as their interactions, after adjustment for baseline serum 25-hydroxyvitamin D. The p-value for the interaction (time by treatment effect) is the one of interest as it tests whether the temporal change differs between groups.
[2]Baseline and 6 month data are presented as mean (SEM)
[3]For each measure, if the number of participants with available data was different between baseline and 6 months, n is presented as baseline/6 months
[4]Calculated by multiplying M-value by insulinogenic index. Variable was squared-root-transformed for analysis but original values are presented. Mean change for the squared-root values are 4.03 (0.05, 8.01) in the treatment group and -2.05 (-6.23, 2.14) in the control group

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Table 5. Mean baseline and 6-month values and the mean absolute changes in anthropometry and blood pressure in the treatment and placebo groups.

	Mean (SEM)	Mean change from baseline (95% CI)		*P* value[1]		
	Baseline[2]	Δ 3 months	Δ 6 months	Time	Treatment	Interaction
Weight, kg						
Treatment (n=48/47[3])	91.0 (2.1)	0.01 (-0.63, 0.65)	-0.59 (-1.49, 0.31)	0.004	0.911	0.696
Placebo (n=48/44)	90.7 (2.1)	0.14 (-0.51, 0.80)	-0.84 (-1.76, 0.09)			
BMI, kg/m^2						
Treatment (n=48/47)	32.2 (0.6)	0.02 (-0.20, 0.24)	-0.18 (-0.49, 0.13)	0.004	0.868	0.674
Placebo (n=48/44)	32.1 (0.6)	0.06 (-0.17, 0.28)	-0.28 (-0.60, 0.03)			
Fat mass by bioimpedance, %						
Treatment (n=36)	38.4 (1.3)	ND	-0.32 (-1.00, 0.37)	0.026	0.303	0.305
Placebo (n=30)	36.8 (1.3)	ND	-0.84 (-1.59, -0.09)			
Waist circumference, cm						
Treatment (n=48/47)	107.9 (1.4)	0.75 (-0.02, 1.53)	0.18 (-0.90, 1.26)	0.020	0.826	0.814
Placebo (n=48/44)	108.6 (1.4)	0.39 (-0.40, 1.19)	-0.19 (-1.30, 0.92)			
Hip circumference, cm						
Treatment (n=46)	112.5 (1.2)	ND	0.41 (-0.52, 1.35)	0.912	0.205	0.179
Placebo (n=47/44)	112.9 (1.2)	ND	-0.49 (-1.43, 0.45)			
SBP, mmHg						
Treatment (n=48/47)	129.4 (2.2)	1.52 (-1.83, 4.88)	-1.61 (-5.94, 2,72)	0.001	0.756	0.526
Placebo (n=47/44)	131.0 (2.2)	1.85 (-1.57, 5.27)	-4.06 (-8.49, 0.37)			
DBP, mmHg						
Treatment (n=48/47)	76.5 (1.4)	0.66 (-1.33, 2.65)	-2.45 (-5.04, 0.15)	<0.001	0.576	0.487
Placebo (n=48/44)	76.3 (1.4)	2.22 (0.20, 4.25)	-0.37 (-3.03, 2.28)			

DBP, diastolic blood pressure; ND, not documented; SBP, systolic blood pressure; SEM, standard error of the mean

[1]Two-way analysis of variance (ANOVA) with repeated measures was used to assess time and treatment effects as well as their

interaction, after adjustment for baseline serum 25-hydroxyvitamin D. The p-value for the interaction (time by treatment effect) is the one of interest as it tests whether the temporal change differs between groups.
[2]Baseline data are presented as mean (SEM)
[3]For each measure, if the number of participants with available data was different between baseline and 6 months, n is presented as baseline/6 months

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