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Clinical Research Article

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Effects of Vitamin D Supplementation on Insulin Sensitivity and Secretion in Prediabetes

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Abbreviations: CPI, C-peptide index; D2d, Vitamin D and Type 2 Diabetes study; DI_{cpep}, disposition index using c-peptide-based indices; D_{Ins}, disposition index using insulin-based indices; HbA1c, hemoglobin A1c; $HOMA2\%B_{cpep}$, Homeostasis Model Assessment of β-cell function using C-peptide values; $HOMA2\%B_{ins}$, Homeostasis Model Assessment of β-cell function using insulin values; $HOMA2\%S_{cpep}$, Homeostasis Model Assessment of steady-state insulin sensitivity derived from C-peptide values; $HOMA2\%S_{ins}$, Homeostasis Model Assessment of β-cell function derived from insulin values; IGI, insulinogenic index; OGTT, 75-gram oral glucose tolerance test; VDR, vitamin D receptor.

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Abstract

Context: Vitamin D regulates glucose homeostasis pathways, but effects of vitamin D supplementation on β-cell function remain unclear.

Objective: To investigate the effects of vitamin D_3 supplementation on insulin sensitivity and β-cell function.

Methods: This is a prespecified secondary analysis of the Vitamin D and Type 2 Diabetes study. Overweight/obese adults at high risk for type 2 diabetes (prediabetes) were randomly treated with vitamin D_3 4000 IU or matching placebo daily for 24 months.

Main Outcome: Disposition index (DI), as an estimate of β-cell function, was calculated as the product of Homeostasis Model Assessment 2 indices derived from C-peptide values ($HOMA2\%S_{cpep}$) and C-peptide response during the first 30 minutes of a 75-g oral glucose tolerance test (OGTT).

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Results: Mean age was 60.5 ± 9.8 years and body mass index was 31.9 ± 4.4 kg/m^2. Mean serum 25(OH)D level increased from 27.9 ± 10.3 ng/mL at baseline to 54.9 ng/mL at 2 years in the vitamin D group and was unchanged (28.5 ± 10.0 ng/mL) in the placebo group. The baseline DI predicted incident diabetes independent of the intervention. In the entire cohort, there were no significant differences in changes in DI, HOMA2%S_{cpep}, or C-peptide response between the 2 groups. Among participants with baseline 25(OH)D level <12 ng/mL, the mean percent differences for DI between the vitamin D and placebo groups was 8.5 (95% CI, 0.2-16.8).

Conclusions: Supplementation with vitamin D_3 for 24 months did not improve an OGTT-derived index of β-cell function in people with prediabetes not selected based on baseline vitamin D status; however, there was benefit among those with very low baseline vitamin D status.

Key Words: insulin sensitivity, beta-cell function, prediabetes, vitamin D

Observational studies have reported strong and consistent associations between low blood vitamin D levels and increased risk of type 2 diabetes. Randomized controlled trials of vitamin D supplementation in people with prediabetes have reported nonstatistically significant reductions in diabetes risk (1-3). Recent meta-analyses aggregating data from these trials have reported beneficial effects of vitamin D supplementation in delaying the progression to diabetes (11%-12% relative risk reduction compared with placebo) and also in improving regression to normal glucose regulation (48% relative benefit compared with placebo) (4, 5). Therefore, investigating mechanisms by which vitamin D may influence diabetes risk is important.

Vitamin D has been hypothesized to play a role in glucose homeostasis, but pathways are not clear. The pancreatic β cells express the vitamin D receptor (VDR) and mice lacking functional VDR exhibit impaired insulin secretion in response to a glucose load (6). In addition, transgenic mice overexpressing VDR in β cells were protected against streptozotocin-induced diabetes and showed preserved β-cell mass and a reduction in islet inflammation (7). Preclinical studies suggest that vitamin D may regulate insulin secretion through genomic and nongenomic pathways. Vitamin D increases the expression of the insulin gene as well as other genes involved in cytoskeletal organization and cellular growth of β cells (8, 9). Through nongenomic pathways, vitamin D is engaged in regulation of calcium flux and cell depolarization in the pancreatic β cells, stimulating exocytosis of insulin molecules (10). Vitamin D also increases insulin sensitivity through its effect on muscle cells by increasing insulin receptor expression or increasing the sensitivity of insulin receptor to insulin (10). However, intervention studies with vitamin D supplementation have reported inconsistent effects on measures of insulin sensitivity, insulin secretion, and β-cell function (11-13), at least, in part, because of small sample size, short duration, and heterogeneity in populations and interventions.

The Vitamin D and Type 2 Diabetes (D2d) study is a randomized, placebo-controlled clinical trial of US adults with prediabetes and overweight/obesity designed and conducted to test the effect of vitamin D_3 supplementation for prevention of type 2 diabetes. In a prespecified secondary analysis, we examined the effects of vitamin D supplementation on insulin sensitivity and β-cell function in this cohort of people with prediabetes.

Methods

Overview of the D2d Study

The D2d study (clinicaltrials.gov NCT01942694) was a randomized, double-blind, placebo-controlled clinical trial conducted at 22 sites in the United States and compared vitamin D with placebo for diabetes prevention in adults at high risk for type 2 diabetes. The design of the D2d study has been published and is summarized below (14). The study was approved by the institutional review board of each collaborating site and monitored by an independent Data and Safety Monitoring Board, and all participants provided written informed consent.

Study Population

Eligible participants met 2 of 3 glycemic criteria for prediabetes as defined by the 2010 American Diabetes Association guidelines: fasting plasma glucose 100 to 125 mg/dL (5.6-6.9 mmol/L); plasma glucose 2 hours after a 75-g oral glucose load 140 to 199 mg/dL (7.8-11.0 mmol/L); and hemoglobin A1c (HbA1c) 5.7% to 6.4% (39-47 mmol/mol) (15). Other inclusion criteria were age ≥ 30 years (25 years for American Indians, Alaska

Natives, Native Hawaiians, or other Pacific Islanders) and body mass index of 24 to 42 kg/m^2 (22.5-42 kg/m^2 for Asian Americans). A low blood 25(OH)D level was not an inclusion criterion. Key exclusion criteria included: any glycemic criterion in the diabetes range, use of diabetes or weight loss medications, conditions (other than hyperglycemia and race) affecting HbA1c assessment, recent history of hyperparathyroidism or nephrolithiasis, hypercalcemia, and bariatric surgery (14). The complete list of eligibility criteria and the recruitment and screening process have been described previously (16).

Intervention and Procedures

Participants were randomized to take, once daily, either a single soft gel that contained 4000 IU of vitamin D_3 (cholecalciferol) or a matching placebo. Randomization was block-stratified by site, body mass index (< 30 or ≥ 30 kg/m^2), and race (White or non-White). To maximize the study's ability to observe a treatment effect, participants were asked to refrain from using diabetes-specific or weight loss medications during the study and to limit the use of outside-of-study vitamin D to 1000 IU per day from all supplements, including multivitamins. During the study, participants were provided with information on diabetes prevention through information sheets and twice-yearly group meetings.

Follow-up and Measurements

At baseline and yearly thereafter, a 75-g oral glucose tolerance test (OGTT) was performed after an 8-hour overnight fast in all participants who had not reached the primary outcome of diabetes. Blood was obtained while fasting and at 30 and 120 minutes after ingestion of the glucose load. Plasma for glucose was processed locally, frozen, and shipped to the central laboratory for immediate measurement. Serum for 25(OH)D, C-peptide, and insulin was processed locally and shipped to the central laboratory for long-term storage at -80°C until analyses. All before- and after-intervention samples for each participant were included in the same analytical run to reduce systematic error and inter-assay variability. Plasma glucose was measured using a hexokinase method, and serum 25(OH)D was measured by liquid chromatography–tandem mass spectrometry, as previously described (1). Stored serum samples were used to measure C-peptide and insulin at baseline, month 12, and month 24 visits. C-peptide was measured by a 2-site immunoenzymatic assay using a Tosoh 2000 autoanalyzer calibrated against the World Health Organization IS 84/510 standard. The assay has a sensitivity level of 0.02 ng/mL. The interassay coefficients of variation for the low, medium, and high C-peptide controls are 3.2%, 1.6%, and 1.8%, respectively. Insulin was measured by a 2-site immunoenzymatic assay on a Tosoh 2000 autoanalyzer calibrated against the World Health Organization IRP 66/304 reference standard. The assay has a sensitivity level of 0.5 μU/mL and is linear up to 330 μU/mL. The interassay coefficients of variation for low, medium, and high insulin controls are 2.8%, 2.5%, and 2.0%, respectively.

Outcomes

Key outcomes included changes in insulin sensitivity, insulin secretion, and β-cell function in response to the trial intervention (vitamin D vs placebo) over the first 2 years of the study among participants with available data. We used the Homeostasis Model Assessment (HOMA) to estimate steady-state insulin sensitivity (%S) and β-cell function (%B) as percentages of a normal reference population. HOMA indices were calculated using HOMA2 calculator version 2.2.3 (Diabetes Trials Unit, University of Oxford, Oxford, UK, www.dtu.ox.ac.uk/homacalculator) (17, 18). HOMA indices derived from C-peptide values were presented as HOMA2%S_{cpep} and HOMA2%B_{cpep}, and indices using insulin values were presented as HOMA2%S_{ins} and HOMA2%B_{ins}.

Early C-peptide and insulin responses to glucose during the OGTT were calculated using the C-peptide index (CPI) and insulinogenic index (IGI), respectively. These indices were calculated as the increment in C-peptide or insulin values, respectively, over the first 30 minutes of the OGTT divided by the increment in glucose over the first 30 minutes as follows: CPI = 100 × (C-peptide$_{30min}$ − C-peptide$_{0min}$)/(Glucose$_{30min}$ − Glucose$_{0min}$) and IGI = 100 × (Insulin$_{30min}$ − Insulin$_{0min}$)/(Glucose$_{30min}$ − Glucose$_{0min}$). Disposition indices were used as markers for β-cell function. The primary endpoint for the present analysis was the change in the disposition index (DI_{cpep}), defined as CPI × HOMA2%S_{cpep} to minimize the effect of hepatic insulin clearance on estimating β-cell function. We also calculated a disposition index (DI_{ins}) using insulin-based indices (IGI × HOMA2%S_{ins}).

Statistical Methods

The analyses were restricted to the baseline, month 12, and month 24 follow-up visits when C-peptide and insulin concentrations were measured. Because the study focuses on the effect of vitamin D on mechanisms related to the pathogenesis of type 2 diabetes, all analyses censored follow-up data

when a participant developed diabetes, stopped trial pills, started a diabetes or weight loss medication, or took out-of-study vitamin D from supplements above the study limit of 1000 IU per day as described previously (1). This analysis, known as the "per-protocol" analysis, aims to better capture the effects of the active intervention (vitamin D_3 supplementation) on diabetes pathophysiology compared to placebo without the confounding effects of trial product discontinuation, personal use of vitamin D supplements, or use of medications that affect glucose homeostasis.

To allow estimation of the disposition index and its components (CPI, IGI, HOMA2%S_{cpep}, HOMA2%S_{ins}), the analysis population included all participants who had data for glucose, C-peptide, and insulin while fasting and at 30-minutes at the baseline visit and at least 1 follow-up visit (month 12 or month 24). Participants who did not consent to the repository and did not have stored samples for insulin and C-peptide measurements (n = 247), who never took the trial pills (n = 1), were taking a glucose-lowering medication at baseline (n = 3), or had incomplete glucose, C-peptide, or insulin data at baseline (n = 84) were excluded. During follow-up, participants with incomplete glucose, C-peptide, or insulin data (n = 254), or those who had a censoring event (eg, developed diabetes, started a glucose-lowering medication; n = 51) did not contribute to the analysis. We also excluded participants (n = 9) who were outliers in CPI and IGI values, defined as extreme values because of a small glucose increment in the first 30 minutes of the OGTT. The CPI and IGI calculations are based on the assumption that glucose level increases meaningfully after oral glucose ingestion; therefore, we excluded those who had $Glucose_{30min} - Glucose_{0min} < 5$ mg/dL at any time point. In addition to analyses of data in the entire cohort, we also conducted an analysis to evaluate the effect of vitamin D supplementation on β-cell function in participants with low baseline vitamin D status defined as 25(OH)D of < 12 ng/mL and < 20 ng/mL.

Descriptive statistics included percentages, means ± SD, or median and interquartile range (25th to 75th percentile) for nonnormally distributed data. Tests were pooled variance *t* tests for continuous variables and χ^2 for categorical variables. For glucose, C-peptide, and insulin, Wilcoxon rank-sum tests were used to compare values at each visit, and general linear mixed models (for repeated measures data) were used to compare for changes over time. We used natural log transformation of change variables (HOMA2%$S_{cpep,}$ CPI, $DI_{cpep,}$ HOMA2%$S_{Ins,}$ IGI, DI_{Ins}) to limit the impact of extreme values in group comparisons. To account for declines from baseline (ie, negative values), a constant was added to all observations making the log value a real number. Between-group differences were described on a relative scale after back-transformation to the original scale. Between-group differences for the changes in continuous variables were determined using a linear mixed-effects model approach to account for within-participant correlation across the timepoints. An interaction term between treatment assignment and time from baseline, also included as a covariate, was used to assess if the change trajectories in variable levels differed between randomization groups. Sensitivity analyses explored potential nonlinear changes over the entire follow-up. According to the study design, OGTTs were not performed after the diagnosis of diabetes, but fasting insulin, C-peptide, and insulin concentrations were available. To explore whether unavailable data after development of diabetes influenced the results, we reanalyzed HOMA2%B and HOMA2%S regardless of whether participants developed diabetes during follow-up. As typically done in analyses of secondary outcomes, we did not perform power calculations and results were not adjusted for multiple comparisons.

Before outcomes data were analyzed and presented by treatment group, the statistical analysis plan was reviewed and approved by all coauthors and the study's publication and presentations committee. Statistical analyses were performed using SAS version 9.4 (SAS Institute Inc).

Results

A total of 1774 participants with available data were included in the present analysis (Fig. 1, Table 1). The mean age was 60.5 ± 9.8 years and BMI was 31.9 ± 4.4 kg/m^2, 44% were women, and 69% were White. Mean HbA1c was 5.9%, fasting plasma glucose was 107.7 mg/dL, and 2-hour plasma glucose after the glucose challenge was 137.1 mg/dL. About one-third of participants met all 3 prediabetes criteria (fasting plasma glucose, 2-hour plasma glucose after the 75-g oral glucose load and HbA1c). The baseline characteristics were similar between the 2 groups and did not differ from the entire D2d cohort (1). The mean baseline vitamin D level was 27.9 ng/mL, and it increased to 52.8 and 54.9 mg/dL at 12 months and 24 months, respectively, in the vitamin D group, whereas it remained unchanged in the placebo group (28.5 ng/mL at baseline, 27.9 ng/mL at 12 months, and 28.4 ng/mL at 24 months).

In the D2d subcohort used in the present analysis (n = 1774), 275 (15.5%) participants met the diabetes outcome between the month 12 and month 24 follow-up visits (inclusive), 116 (13.1%) in the vitamin D vs 159 (18.0%) in the placebo group (hazard ratio 0.70; 95% CI, 0.54-0.91). Thus, rate of incident diabetes was significantly lower in the vitamin D group during the first 24 months. Both DI_{cpep} and DI_{ins} were significant predictors of incident diabetes during follow-up. The area under the curve of

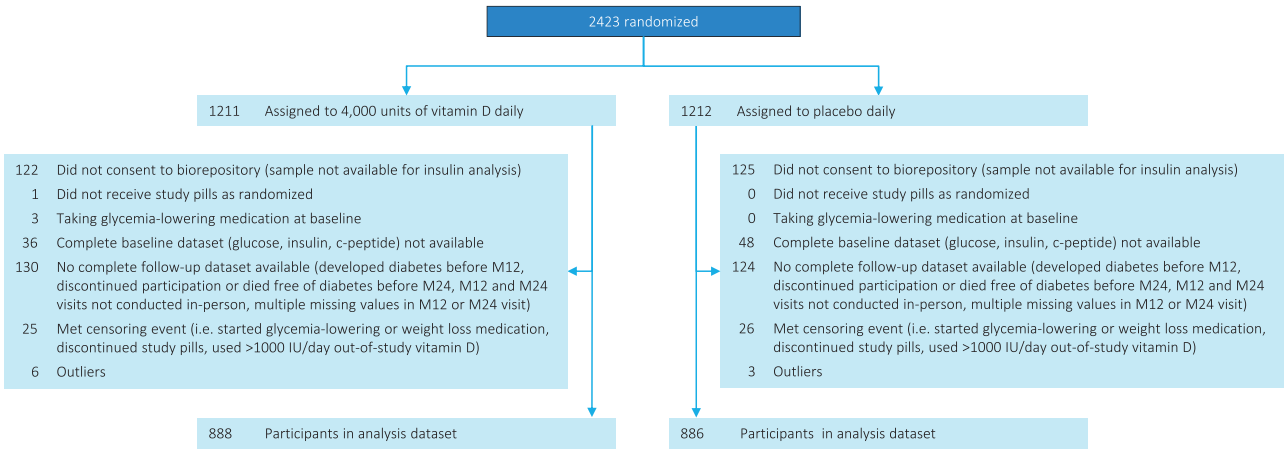

Figure 1. Flow diagram of study cohort.

receiver operating characteristic for DI_{cpep} was 0.69 (95% CI, 0.66-0.72) for the entire cohort, 0.70 (95% CI, 0.66-0.74) for the vitamin D group and 0.69 (95% CI, 0.66-0.72) for the placebo group. For DI_{ins}, the area under the curve of receiver operating characteristic was 0.68 (95% CI, 0.65-0.71) for the entire cohort, 0.69 (95% CI, 0.65-0.73) for the vitamin D group and 0.68 (95% CI, 0.65-0.71) for the placebo group.

At baseline, $HOMA2\%S_{cpep}$ medians (lower quartile to upper quartile) were similar between the 2 groups, 17.1 (13.2-22.4) and 16.9 (13.3-21.4) in vitamin D and placebo groups, respectively (P = 0.32), but the vitamin D group had slightly higher $HOMA2\%S_{ins}$, 62.0 (43.1-91.1), compared with 59.2 (40.8-87.7) in the placebo group (P = 0.03) (Table 2).

$HOMA2\%S_{cpep}$ declined over time in both the vitamin D and placebo groups, whereas $HOMA2\%S_{ins}$ did not change significantly over time (Table 2). The mean difference in change over time was not different between the 2 groups in either $HOMA2\%S_{cpep}$ (0.1%; 95% CI, -1.4 to 1.5) or $HOMA2\%S_{ins}$ (-0.6%; 95% CI, -3.8 to 2.6).

C-peptide index declined over time in the vitamin D group and was unchanged in the placebo group, whereas the IGI declined over time in both groups (Table 2). However, the mean difference in change over time was not significantly different between the 2 groups in either CPI (-0.8%; 95% CI, -2.4 to 0.8) or IGI (0.9%; 95% CI, -2.7 to 0.9).

Both DI_{cpep} and DI_{ins} declined over time in the vitamin D group and were unchanged in the placebo group (Table 2). The mean percent differences between the vitamin D and placebo groups were not different for both DI_{Cpep} (-0.8%; 95% CI, -2.4 to 0.8) and DI_{ins} (-1.5%; 95% CI, -5.1 to 2.1) (Table 2). Similarly, the mean changes in $HOMA2\%B_{cpep}$ and $HOMA2\%B_{ins}$ in the vitamin D and placebo groups did not differ.

Among participants with baseline 25(OH)D < 12 ng/mL, DI_{cpep} and DI_{ins} declined over time in the placebo group, whereas they increased in the vitamin D group (Table 3). The mean percent differences between the vitamin D and placebo groups for DI_{Cpep} was 8.5%; (95% CI, 0.2-16.8) and DI_{ins} was 18.5% (95% CI, 1.1-35.9), indicating a benefit for vitamin D in β-cell function among those with very low 25(OH)D levels to begin with. Changes were in the same direction among participants with baseline 25(OH)D < 20 ng/mL, although the differences were not statistically significant (Table 4).

Participants who developed diabetes before the month 12 visit (42 in the vitamin D and 36 in the placebo group) did not contribute data to the present analysis because no follow-up OGTT was done in these participants (per study design). When we examined changes in HOMA2%B and HOMA2%S without censoring data after the diagnosis of diabetes, we found no differences in the changes in $HOMA2\%B_{cpep}$ or $HOMA2\%B_{ins}$ between the vitamin D and placebo groups. Similarly, we found no differences between groups in the changes in $HOMA2\%S_{cpep}$ or $HOMA2\%S_{ins}$ (data not shown).

Discussion

In this double-blind, placebo-controlled, randomized clinical trial, we report the effect of oral, daily vitamin D_3 supplementation on β-cell function in overweight or obese participants at high risk for diabetes who were not selected for vitamin D deficiency. Our results showed no difference with vitamin D supplementation vs placebo on change in β-cell function assessed by indices derived from OGTT data. However, vitamin D improved β-cell function among those with baseline 25(OH)D levels less than 12 ng/mL.

Prior studies of vitamin D supplementation on β-cell function and insulin sensitivity have been inconclusive.

Table 1. Baseline characteristics

	Overall (n = 1774)	Vitamin D (n = 888)	Placebo (n = 886)
Age, y	60.5 ± 9.8	60.1 ± 9.6	61.0 ± 9.9
Women, no. (%)	773 (43.6)	385 (43.4)	388 (43.8)
Race, no. (%)[a]			
Asian	98 (5.5)	50 (5.6)	48 (5.4)
Black or African-American	403 (22.7)	200 (22.5)	203 (22.9)
White	1225 (69.1)	616 (69.4)	609 (68.7)
Other	48 (2.7)	22 (2.5)	26 (2.9)
Hispanic or Latino Ethnicity, no. (%)[a]	159 (9.0)	89 (10.0)	70 (7.9)
Family history of diabetes (first-degree relative), no. (%)	1110 (62.6)	556 (62.6)	554 (62.5)
Smoking, no. (%)			
Never	1015 (57.2)	508 (57.2)	507 (57.2)
Former	642 (36.2)	327 (36.8)	315 (35.6)
Current	103 (5.8)	46 (5.2)	57 (6.4)
Unknown or not reported	14 (0.8)	7 (0.8)	7 (0.8)
Dietary supplement use[b]			
Participants taking vitamin D supplements, no. (%)	784 (44.2)	377 (42.5)	407 (45.9)
Vitamin D intake among all participants, IU/d[c]	417 ± 798	357 ± 587	477 ± 960
Vitamin D intake among participants using supplements, IU/d[b]	725 ± 250	742 ± 248	710 ± 251
Participants taking calcium supplements, no. (%)	618 (34.8)	289 (32.5)	329 (37.1)
Calcium intake among all participants, mg/d[c]	110 ± 179	105 ± 178	115 ± 181
Calcium intake among participants using supplements, mg/d[b]	315 ± 167	321 ± 167	309 ± 167
Physical activity, total MET hour/wk, median (IQR)[d]	56.3 (25.8-126)	59.6 (24.5-129.9)	55.4 (26.6-120)
Body mass index, kg/m^2	31.9 ± 4.4	31.9 ± 4.5	32.0 ± 4.4
Prediabetes category, no. (%)[e]			
Met all 3 glycemic criteria (IGT + iA1c + IFG)	615 (34.7)	299 (33.7)	316 (35.7)
Met 2 glycemic criteria only			
IGT + IFG	116 (6.5)	61 (6.9)	55 (6.2)
IGT + iA1c	175 (9.9)	81 (9.1)	94 (10.6)
IFG + iA1c	868 (48.9)	447 (50.3)	421 (47.5)
Laboratory			
Hemoglobin A1c, %	5.9 ± 0.2	5.9 ± 0.2	5.9 ± 0.2
FPG, mg/dL	107.7 ± 7.2	107.7 ± 7.3	107.7 ± 7.2
2-h plasma glucose after 75-g glucose load, mg/dL	137.1 ± 34.1	136.9 ± 34.4	137.3 ± 33.9
Fasting serum insulin, µU/mL	12.3 (8.2-18)	12.0 (8.1-17.3)	12.7 (8.5-18.5)
Fasting serum C-peptide, ng/mL, median (IQR)	2.6 (2-3.3)	2.5 (1.9-3.3)	2.6 (2-3.3)
Serum 25-hydroxyvitamin D, ng/mL	28.2 ± 10.2	27.9 ± 10.3	28.5 ± 10.0
Serum 25-hydroxyvitamin D category, no. (%)[f]			
< 12 ng/mL	72 (4.1)	42 (4.7)	30 (3.4)
12-19 ng/mL	303 (17.1)	162 (18.2)	141 (15.9)
20-29 ng/mL	625 (35.2)	318 (35.8)	307 (34.7)
≥ 30 ng/mL	774 (43.6)	366 (41.2)	408 (46.0)

Data are presented as mean ± SD or median (IQR). Percentages may not add up to 100 because of rounding. To convert 25-hydroxyvitamin D from ng/mL to nmol/L, multiply by 2.496. To convert glucose from mg/dL to mmol/L, multiply by 0.055. To convert vitamin D intake from IU to mcg, divide by 40.

Abbreviations: FPG, fasting plasma glucose; iA1c, impaired A1c; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; IQR, interquartile range; MET, metabolic equivalent.

[a]Race and ethnicity were reported by the participant. The category "other" includes Asian, American Indian or Alaska Native, Native Hawaiian or other Pacific Islander, or other race. Ethnicity includes any race.

[b]Data on vitamin D and calcium intake are derived from a question about dietary supplements, including multivitamins and high-dose prescribed doses. Participants were allowed to take from supplements up to 1000 IU/d of vitamin D and 600 mg/d of calcium. Dietary intake of vitamin D and calcium was not limited.

[c]Value shown is among all participants regardless of whether they reported use of supplements or not.

[d]Based on International Physical Activity Questionnaire.

[e]IFG defined as fasting plasma glucose 100 to 125 mg/dL (5.6-6.9 mmol/L); IGT defined as 2-hour plasma glucose after a 75-g glucose load 140 to 199 mg/dL (7.8-11.0 mmol/L); iA1c defined as HbA1c 5.7% to 6.4% (39-47 mmol per mol).

[f]Categories based on 2010 Dietary Reference Intakes for Calcium and Vitamin D (23).

Table 2. Changes in insulin sensitivity and β-cell function over time

	Baseline	Month 12	Month 24	Average percent difference compared with baseline (95% CI)[a]	Overall (unadjusted) least-squares mean percent difference for vitamin D vs placebo (95% CI)[b]
$HOMA2\%S_{cpep}$					
Vitamin D	17.1 (13.2, 22.4)	16.8 (13, 22.3)	16.9 (13.1, 22.4)	-1.0% (-2.1 to 0.0)	0.1% (-1.4 to 1.5)
n	888	886	696		
Placebo	16.9 (13.3, 21.4)	16.8 (12.6, 22.5)	16.5 (12.8, 21.4)	-1.0% (-2.1 to 0.0)	
n	886	877	665		
P value	0.32	0.56	0.16		
$HOMA2\%S_{ins}$					
Vitamin D	62.0 (43.1, 91.1)	63.1 (41.8, 95.4)	65.2 (42.4, 93.9)	-0.1% (-2.3 to 2.2)	-0.6% (-3.8 to 2.6)
n	888	886	696		
Placebo	59.2 (40.8, 87.7)	61.5 (39.1, 91.1)	60.1 (41.4, 88.2)	0.5% (-1.7 to 2.8)	
n	886	877	665		
P value	0.03	0.12	0.07		
CPI					
Vitamin D	5.9 (3.9, 8.7)	5.86 (3.8, 8.5)	5.84 (3.7, 8.4)	-1.6% (-2.7 to -0.5)	-0.8% (-2.4 to 0.8)
n	888	864	670		
Placebo	5.8 (4.1, 8.3)	5.9 (3.9, 8.5)	5.8 (3.9, 8.2)	-0.8% (-2.0 to 0.3)	
n	886	861	642		
P value	0.64	0.61	0.995		
IGI					
Vitamin D	91.2 (55.5, 152.4)	90.2 (52.2, 145.6)	87.8 (50.2, 144.9)	-2.6% (-3.9 to -1.3)	-0.9% (-2.7 to 0.9)
n	888	864	670		
Placebo	95.7 (60.3, 148.7)	90.6 (56.6, 149.5)	89.19 (53.9, 147.2)	-1.7% (-3.0 to -0.5)	
n	886	861	642		
P value	0.32	0.44	0.59		
DI_{cpep}					
Vitamin D	100.2 (66.4, 146.3)	94.6 (63.5, 147.6)	96.5 (62.3, 141.2)	-1.2% (-2.3 to -0.1)	-0.8% (-2.4 to 0.8)
n	888	864	670		
Placebo	98.4 (67.9, 144.1)	96.1 (62.3, 148.3)	93.0 (59.9, 143.6)	-0.4% (-1.5 to 0.7)	
n	886	861	642		
P value	0.51	0.87	0.61		
DI_{ins}					
Vitamin D	5641.4 (3741.9, 8662.1)	5365.4 (3488.1, 8922.3)	5401.1 (3369.5, 8422.7)	-3.7% (-6.2 to -1.2)	-1.5% (-5.1 to 2.1)
n	888	864	670		
Placebo	5571.9 (3615.9, 8191.7)	5383.1 (3346.4, 8859.2)	5293.2 (3430.2, 8505.8)	-2.2% (-4.8 to 0.3)	
n	886	861	642		
P value	0.30	0.69	0.45		
$HOMA2\%B_{cpep}$					
Vitamin D	235.5 (190.0, 285.9)	235.7 (192.1, 287.5)	229.7 (186.3, 275.3)	-0.8% (-1.8 to 0.2)	0.7% (-0.7 to 2.0)
n	888	886	696		
Placebo	236.45 (194.1, 286.5)	237 (194.6, 289.1)	233 (191.8, 276.5)	-1.5% (-2.4 to -0.5)	
n	886	877	665		
P value	0.28	0.55	0.26		
$HOMA2\%B_{ins}$					
Vitamin D	90.6 (68.3, 119.9)	90.5 (69.5, 116.6)	86.4 (66, 113.6)	-2.1% (-3.8 to -0.5)	1.8% (-0.5 to 4.2)
n	888	886	696		
Placebo	94.6 (71.7, 125.3)	91.8 (70.3, 123.3)	90.3 (67.7, 113.5)	-4.0% (-5.6 to -2.3)	
n	886	877	665		
P value	0.02	0.13	0.08		

Data are presented as median (IQR). *P* values for each visit are from Wilcoxon rank-sum test.

Abbreviations: CPI, C-peptide index; DI_{ins}, disposition index using insulin-based indices; $HOMA2\%B_{cpep}$, Homeostasis Model Assessment of β-cell function using C-peptide values; $HOMA2\%B_{ins}$, Homeostasis Model Assessment of β-cell function using insulin values; $HOMA2\%S_{cpep}$, Homeostasis Model Assessment of steady-state insulin sensitivity derived from C-peptide values; $HOMA2\%S_{ins}$, Homeostasis Model Assessment of β-cell function derived from insulin values; IGI, insulinogenic index; IQR, interquartile range.

[a]Average percent difference compared with baseline is based on linear mixed-model for repeated measures data.

[b]Unadjusted between group difference in least square means using all available visits.

Table 3. Changes in β-cell function over time among those with 25(OH)D < 12 ng/mL at baseline

	Baseline	Month 12	Month 24	Average percent difference compared with baseline (95% CI)[a]	Overall (unadjusted) least-squares mean percent difference for vitamin D vs placebo (95% CI)[b]
DI_{cpep}					
Vitamin D	107.9 (92.0, 152.0)	122.7 (78.6, 195.8)	111.0 (82.2, 161.4)	3.6% (-1.5 to 8.8)	8.5% (0.2 to 16.8)
n	42	42	32		
Placebo	121.9 (74.0, 170.3)	83.1 (54.9, 170.5)	137.9 (49.1, 180.3)	-4.9% (-11.4 to 1.6)	
n	30	29	18		
P value	0.74	0.16	0.90		
DI_{ins}					
Vitamin D	6579.9 (4333.0, 8807.0)	5552.0 (3971.7, 10 336.0)	6730.8 (3815.1, 10 385.5	4.9% (-5.9 to 15.7)	18.5% (1.1 to 35.9)
n	42	42	32		
Placebo	7784.8 (4124.6, 9680.0)	4852.5 (2710.5, 9180.4)	6841.6 (3387.3, 10 101.7	-13.6% (-27.2 to 0)	
n	30	29	18		
P value	0.74	0.19	0.72		

Data are presented as median (IQR). *P* values for each visit are from Wilcoxon rank-sum test.
Abbreviations: DI_{cpep}, change in the disposition index for C peptide; DI_{ins}, disposition index using insulin-based indices; IQR, interquartile range.
[a]Average percent difference compared with baseline is based on linear mixed model for repeated measures data.
[b]Unadjusted between-group difference in least square means using all available visits.

Two trials have reported significant improvements (12, 19). In the first trial, 92 adults at risk for type 2 diabetes were randomized to short-term supplementation with 2000 IU/d of vitamin D_3 and had improvements in DI derived from a frequently sampled IV glucose tolerance test (20). In the second trial of 95 adults with newly diagnosed diabetes or at risk for diabetes, 5000 IU/d of vitamin D_3 and calcium supplementation for 6 months showed improvements in insulin sensitivity based on the M-value derived from a 2-hour hyperinsulinemic-euglycemic clamp, but—similar to our results—there were no changes in OGTT-derived measures of insulin sensitivity or β-cell function (12). Other trials have also reported no effect on OGTT-based measures. For example, supplementation with 28 000 IU/week of vitamin D_3 in 72 adults at risk for type 2 diabetes with suboptimal vitamin D levels did not improve insulin sensitivity or β-cell function based on OGTT-derived indices (20). Similarly, high-dose vitamin D (120 000 IU once per month) in about 200 children and adolescents with vitamin D deficiency did not result in significant changes in DI derived from OGTT values (11). The divergent results may be due to differences in populations (normal glucose tolerance, prediabetes, or established diabetes), methods of measuring insulin sensitivity, insulin secretion, and β-cell function, or variation in the dose and duration of treatment with vitamin D (eg, intermittent, very high-doses of vitamin D are considered nonphysiologic compared with daily doses). Most of these studies also had a small sample size. A recent meta-analysis of 18 trials comparing vitamin D supplementation with placebo (13) included 1220 individuals, which is fewer than our study sample. The meta-analysis reported no effect of vitamin D supplementation on insulin sensitivity, but there were major limitations because the authors combined data from trials that varied in study design and quality and included a variety of populations and different indices of insulin sensitivity (21).

Although our analyses found no difference in insulin sensitivity and secretion between the vitamin D and placebo groups, the rate of incident diabetes in year 2 of the study was significantly lower in the vitamin D group compared with the placebo group. There are several potential explanations for this discrepancy. First, we used OGTT-derived measures to estimate β-cell function; these measures have a large variability resulting in large CIs, making it difficult to detect statistically significant differences. Second, according to the study design, we did not perform OGTTs in participants after they met the primary outcome of diabetes; therefore, data after these participants developed diabetes were not included in the analytical cohort. The participants who developed diabetes had lower

Table 4. Changes in β-cell function over time among those with 25(OH)D < 20 ng/mL at baseline

	Baseline	Month 12	Month 24	Average percent difference compared with baseline (95% CI)[a]	Overall (unadjusted) least-squares mean percent difference for vitamin D vs placebo (95% CI)[b]
DI_{cpep}					
Vitamin D	102.2 (70.3, 147.7)	95.4 (66.3, 153.3)	101.5 (64.8, 137.4)	-0.3% (-2.8 to 2.2)	1.6% (-2.2 to 5.3)
n	204	201	145		
Placebo	107.4 (69.4, 155.4)	94.4 (59.9, 151.0)	100.1 (60.4, 146.2)	-1.9% (-4.7 to 0.9)	
n	171	168	112		
P value	0.75	0.71	0.72		
DI_{ins}					
Vitamin D	5695.7 (3763.2, 8420.3)	5189.8 (3698.4, 8929.8)	5421.2 (3467.8, 8847.0)	-0.3% (-6.1 to 5.5)	7.5% (-1.2 to 16.2)
n	204	201	145		
Placebo	5660.6 (3745.2, 8636.4)	4882.5 (2919.9, 8664.8)	5217.8 (3298.7, 8757.4)	-7.8% (-14.3 to -1.3)	
n	171	168	112		
P value	0.97	0.22	0.48		

Data are presented as median (IQR). *P* values for each visit are from Wilcoxon rank sum test.
Abbreviations: DI_{cpep}, change in the disposition index for C peptide; DI_{ins}, disposition index using insulin-based indices; IQR, interquartile range.
[a]Average percent difference compared with baseline is based on linear mixed model for repeated measures data.
[b]Unadjusted between-group difference in least square means using all available visits.

baseline DI values and higher diabetes risk than those who remained free of diabetes. The unavailable OGTT data of higher risk participants enriches the follow-up cohort with lower risk participants, making it difficult to detect a significant effect of vitamin D supplementation, if there is one, potentially introducing a selection bias. We attempted to overcome this limitation by reanalyzing HOMA indices without censoring data after the diagnosis of diabetes, but HOMA indices may be insufficient to identify differences in β-cell function or insulin sensitivity, especially in diabetes. Third, it may be that the effects of vitamin D supplementation on insulin sensitivity and β-cell function are modest and may require use of more labor-intensive or expensive methods such as multisample OGTT-based modeling, clamps, or frequently sampled IV glucose tolerance tests—difficult to implement in a large study such as D2d—to detect small differences. For example, as noted previously, in a small trial of adults at risk of type 2 diabetes, short-term supplementation with cholecalciferol improved β-cell function as assessed by frequently sampled IV glucose tolerance tests (19). The high proportion of participants with adequate vitamin D status at baseline may have limited the ability of the study to detect a significant effect of vitamin D supplementation on β-cell function. Indeed, in the small group of participants with very low 25(OH)D at baseline (<12 ng/mL), vitamin D supplementation significantly improved β-cell function. As previously reported, among these same participants with very low baseline 25(OH)D levels, vitamin D supplementation decreased the risk of diabetes with 62% (1). Alternatively, it is conceivable that vitamin D does not affect insulin sensitivity or β-cell function, and its potential effect on risk of type 2 diabetes is mediated through other pathways.

Strengths and Limitations

Our study has several strengths, including the large sample size and long duration of treatment. The median follow-up for the parent D2d study was 2.5 years; therefore, OGTT data from baseline, month 12, and month 24 closely reflect the parent study. Given the multiple factors that can influence glycemia and confound results (eg, use of diabetes medications, outside-of-study high-dose vitamin D), we followed a prespecified per-protocol analysis, which minimizes postrandomization confounding. In this analysis, follow-up data were censored when a participant developed diabetes, stopped trial pills, started a diabetes or weight loss medication, or took out-of-study vitamin D from supplements above the study limit of 1000 IU per day. The study has additional strengths including: randomized, double-blind, placebo-controlled study design; use of a high-dose (4000 IU), daily vitamin D supplementation; baseline 25(OH)D that is representative of the US adult population (22); and excellent retention of participants and adherence to study medications. The main limitation was that the population was mostly sufficient in vitamin D. Other limitations (as noted earlier) include use of OGTT-derived parameters to

estimate insulin sensitivity and β-cell function compared with more accurate estimations from clamp procedures.

In conclusion, in this prespecified secondary analysis from the D2d study, we did not find evidence that supplementation with vitamin D_3 (4000 IU daily) for 24 months in participants with prediabetes improved β-cell function when the entire cohort was examined; however, vitamin D supplementation improved β-cell function among those with very low baseline 25(OH)D levels (<12 ng/mL). Given the limitations of OGTT-based measures, studies using more rigorous methods of estimating β-cell function, especially among people with low vitamin D levels, may be required to better understand the effect of vitamin D supplementation on the pathophysiology of type 2 diabetes.

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