Plasma 25-Hydroxyvitamin D and Progression to Diabetes in Patients at Risk for Diabetes

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An ancillary analysis in the Diabetes Prevention Program

Anastassios G. Pittas, MD, MS[1]
Jason Nelson, MPH[2]
Joanna Mitri, MD[1]
William Hillmann, MS[3]
Cheryl Garganta, MD, PhD[4]
David M. Nathan, MD[5,6]
Frank B. Hu, MD, PhD[7,8]
Bess Dawson-Hughes, MD[1,9]
the Diabetes Prevention Program Research Group

OBJECTIVE—To investigate the association between vitamin D status, assessed by plasma 25-hydroxyvitamin D, and risk of incident diabetes.

RESEARCH DESIGN AND METHODS—Prospective observational study with a mean follow-up of 2.7 years in the Diabetes Prevention Program (DPP), a multicenter trial comparing different strategies for prevention of diabetes in patients with prediabetes. We assessed the association between plasma 25-hydroxyvitamin D, measured repeatedly during follow-up, and incident diabetes in the combined placebo (n = 1,022) and intensive lifestyle (n = 1,017) randomized arms of the DPP. Variables measured at multiple study time points (25-hydroxyvitamin D, BMI, and physical activity) entered the analyses as time-varying "lagged" covariates, as the mean of the previous and current visits at which diabetes status was assessed.

RESULTS—After multivariate adjustment, including for the DPP intervention, participants in the highest tertile of 25-hydroxyvitamin D (median concentration, 30.1 ng/mL) had a hazard ratio of 0.72 (95% CI 0.56–0.90) for developing diabetes compared with participants in the lowest tertile (median concentration, 12.8 ng/mL). The association was in the same direction in placebo (0.70; 0.52–0.94) versus lifestyle arm (0.80; 0.54–1.17).

CONCLUSIONS—Higher plasma 25-hydroxyvitamin D, assessed repeatedly, was associated with lower risk of incident diabetes in high-risk patients, after adjusting for lifestyle interventions (dietary changes, increased physical activity, and weight loss) known to decrease diabetes risk. Because of the observational nature of the study, the potential association between vitamin D and diabetes needs to be confirmed in intervention studies.

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The incidence of diabetes is increasing at an alarming rate both nationally and worldwide, with 1.9 million new cases diagnosed in 2010 in the U.S. alone (1). Nearly 9 out of 10 new cases are due to type 2 diabetes. In clinical trials, lifestyle changes have proved successful at lowering risk of the disease (2,3). In the Diabetes Prevention Program (DPP), intensive lifestyle intervention resulting in weight loss reduced the risk of incident type 2 diabetes in adults at increased risk by 58% (2). However, long-term weight maintenance in the clinical setting has proved elusive. Moreover, even after successful weight loss, there is still significant residual risk (2,3).

Recently, evidence has emerged to support the hypothesis that altered vitamin D homeostasis may play a role in the development of type 2 diabetes (4). A potential role of vitamin D in type 2 diabetes is suggested by cross-sectional studies showing that low blood concentration of 25-hydroxyvitamin D is associated with impaired glucose tolerance and diabetes (5,6). Results from longitudinal observational studies on the association between vitamin D status and type 2 diabetes support a potential link; however, these studies have assessed blood 25-hydroxyvitamin D only once at baseline, which may introduce bias (7–15). The results from small clinical trials and post hoc analyses of larger trials on the effect of vitamin D supplementation on glycemic outcomes have been inconsistent (16–23). The 2011 Institute of Medicine (IOM) Dietary Reference Intakes for Calcium and Vitamin D report concluded that the evidence for a potential role of vitamin D in extraskeletal outcomes, including type 2 diabetes, is inconclusive, and the report recommended a blood 25-hydroxyvitamin D level >20 ng/mL as sufficient for the majority of the population in North America (24).

The DPP cohort, representing a large multiracial sample of U.S. adults at high risk for development of diabetes, provides a unique opportunity to study the association between vitamin D status and risk of incident diabetes. The present ancillary study consisted of assessing plasma 25-hydroxyvitamin D concentrations in stored samples from the DPP cohort at multiple study time points, with the purpose of determining the association between vitamin D status, as assessed by plasma 25-hydroxyvitamin D, and the risk of incident diabetes. The large size of the cohort also allows testing of whether higher concentrations of 25-hydroxyvitamin D than those recommended in the 2011 IOM report may provide additional benefit.

From the [1]Division of Endocrinology, Diabetes, and Metabolism, Tufts Medical Center, Boston, Massachusetts; the [2]Institute for Clinical Research and Health Policy Studies, Tufts Medical Center, Boston, Massachusetts; the [3]Tufts University School of Medicine, Boston, Massachusetts; [4]Clinical Genetics, Floating Hospital for Children at Tufts Medical Center, Boston, Massachusetts; the [5]Diabetes Center, Massachusetts General Hospital, Boston, Massachusetts; the [6]Harvard Medical School, Boston, Massachusetts; the [7]Departments of Nutrition and Epidemiology, Harvard School of Public Health, Boston, Massachusetts; the [8]Channing Laboratory, Brigham and Women's Hospital, Boston, Massachusetts; and the [9]Bone Metabolism Laboratory, Jean Mayer U.S. Department of Agriculture Human Nutrition Research Center on Aging, Tufts University, Boston, Massachusetts.
Corresponding author: Anastassios G. Pittas, apittas@tuftsmedicalcenter.org.
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RESEARCH DESIGN AND METHODS

Study participants

The DPP was a randomized, controlled clinical trial from 1996 to 2001 at 27 sites in the U.S., comparing the effects of intensive lifestyle intervention, metformin, or placebo on the development of diabetes in adults at high risk for the disease. The eligibility criteria, design, and methods of the DPP have been described in detail elsewhere (2). In brief, inclusion criteria included age ≥25 years, BMI ≥24 kg/m^2 (≥22 kg/m^2 in Asian Americans), fasting plasma glucose between 5.3 and 6.9 mmol/L (95–125 mg/dL) (≤6.9 mmol/L for American Indian sites), and plasma glucose between 7.8 and 11 mmol/L (140–199 mg/dL) after a 75-g oral glucose tolerance test. The primary exclusion was any medication known to alter glucose tolerance. The Institutional Review Board at each site approved the protocol, and all participants gave written informed consent. The Tufts University Institutional Review Board approved the present ancillary observational study.

All participants were given standard advice on healthy diet and physical activity before randomization to one of three arms: intensive program of lifestyle modification (aiming to achieve a weight reduction of at least 7% of initial body weight), standard lifestyle recommendations plus metformin, or standard lifestyle recommendations plus placebo (2). The present observational study was conducted among participants randomized to two arms, the intensive lifestyle (n = 1,079) and placebo (standard lifestyle, n = 1,082). The metformin arm was excluded to minimize the cost associated with measurement of 25-hydroxyvitamin D. Also, 122 participants were excluded because of lack of consent for ancillary studies (n = 120), or no available specimen for measurement of 25-hydroxyvitamin D at baseline or 6-month follow-up visits (n = 2). After exclusions, 2,039 participants were included in the initial multivariate analyses that included age, sex, and BMI as covariates, and 2,002 participants with complete data for all covariates were included in the additional multivariate analyses.

Measurement of plasma 25-hydroxyvitamin D concentration

Plasma 25-hydroxyvitamin D concentration was measured in samples stored at −70°C from the baseline, 6-month, 1-year, 2-year, 3-year, and 4-year follow-up visits. Stability of vitamin D metabolites during transport and long-term freezing has been documented previously (25,26). Plasma 25-hydroxyvitamin D was measured at the Metabolic Laboratory at Tufts Medical Center by liquid chromatography, tandem mass spectrometry (LC/MS/MS) (Waters ACQUITY UPLC with TQD triple quadrupole mass spectrometer), certified through the National Institute of Standards and Technology (NIST) vitamin D quality assurance program (27). In the most recent testing, correlation with the NIST external standard for total 25-hydroxyvitamin D was 0.994.

Ascertainment of incident diabetes

The primary DPP outcome, development of diabetes, was assessed using strict laboratory criteria, according to the protocol based on oral glucose (75-g) tolerance testing performed annually and fasting plasma glucose performed semiannually or when symptoms consistent with hyperglycemia occurred (2,28). The diagnosis required confirmation by repeat testing. If a participant was started on a diabetes medication by their physician, they were asked to stop the medication and return for glucose testing to confirm the diagnosis of diabetes.

Assessment of potential confounders and laboratory assessment

The self-reported level of leisure physical activity was assessed annually with the modifiable activity questionnaire and expressed as the average metabolic equivalent (MET-hours) per week for the previous year (2). Usual daily nutrient intake during the previous year was assessed at baseline and at the 1-year follow-up visits with the use of a modified version of the Block food-frequency questionnaire, and calcium intake was estimated as described previously (29). Data on vitamin D intake were not available. Standardized interviewer-administered questionnaires were used annually to obtain self-reported data on personal medical history, smoking, medications, alcohol use, and family medical history. Self-reported race/ethnicity was classified according to the 1990 U.S. Census questionnaire. Weight was measured using a standard calibrated scale, height was measured with a standard stadiometer, and BMI was calculated (kg/m^2). Hypertension was defined as blood pressure >140/90 mmHg or use of antihypertensive medication. Fasting blood was obtained and processed according to standardized procedures. Measurement methods for hemoglobin A_{1c}, glucose, C-reactive protein, and creatinine have been described previously (2). To adjust for sun exposure variability at each DPP study site, we constructed an ultraviolet index for each site based on the National Weather Service data on the monthly means of ultraviolet index for each geographic location in 1997 (30).

Statistical analysis

Discrete-time proportional hazards models were used to assess the association between plasma 25-hydroxvitamin D (in tertiles) and incident diabetes to account for interval-censored data (data for diabetes status and other variables were available at 6-month intervals) and time-dependent covariates (25-hydroxyvitamin D, BMI, and physical activity). In multivariate models, we adjusted for potential confounders including DPP clinical site location (categorical variable, 1–27), age (years), sex (male or female), BMI (kg/m^2), race (white, black, or other), family history of diabetes (yes or no), history of hypertension at baseline (yes or no), smoking status at baseline (never, past, or currently smoking), alcohol and calcium consumption (average of values self-reported at baseline and 1-year follow-up visits [g/day and mg/day, respectively]), plasma C-reactive protein (average of values at baseline, 6-month, and 1-year follow-up visits [mg/dL]), glomerular filtration rate using the modification of diet in renal disease estimation (31) (average of values at baseline, 6-month, and 1-year follow-up visits [mL/min/1.73 m^2]), and physical activity (MET-hours per week). We also adjusted for ultraviolet radiation index at the participant's study site (mean annual in 1997, 90 J/m^2/h). Although adjustment for BMI and physical activity reflect the main effects of the intensive lifestyle intervention, to account for additional unmeasured effects of intervention, all analyses were adjusted for treatment assignment (lifestyle intervention, yes or no). The predictor (25-hydroxyvitamin D) and other variables (BMI and physical activity), whose value was measured at multiple time points, entered the analyses as time-varying "lagged" covariates. At each successive 6-month time point when the outcome (diabetes) was assessed, the value of these variables was calculated as the mean of the current and most recent available value prior to that visit. For the time-varying variables, if either the current or most recent

value was missing, we imputed values using the nonmissing observation (current or most recent). If both current and most recent values were missing, then no value was imputed. Plasma 25-hydroxyvitamin D exhibited a normal distribution and entered the multivariate models on a continuous scale. For ease of interpretation, we present results after participants were categorized into three groups using tertiles (33.3rd and 66.7th percentiles) of plasma 25-hydroxyvitamin D concentration, as the mean of the values obtained at baseline and 6-month visits, to account for season differences. The hazard ratio (HR) of diabetes in each of the two highest tertiles was compared with the lowest tertile by extrapolating the per-unit change in estimated hazard from the multivariate model.

We repeated analyses using the cut points for plasma 25-hydroxyvitamin D (as the mean of the values obtained at baseline and 6-month visits), recommended for skeletal outcomes by the 2011 IOM report, as follows: <12 ng/mL (at risk for deficiency, reference group), 12–19.9 ng/mL (at risk for inadequacy), 20–29.9 ng/mL (adequacy), 30–49.9 ng/mL (potentially beneficial), and ≥50 ng/mL (potentially harmful) (32). Results are presented for categories of plasma 25-hydroxyvitamin D concentration as the mean of the values obtained at baseline and 6-month visits, based on cut points suggested by the IOM Dietary Reference Intakes for Calcium and Vitamin D report (24). The results of the HR of diabetes in each of the higher groups were compared with the lowest group by extrapolating the per-unit change in estimated hazard from the multivariate model. We also conducted secondary analyses by tertiles and IOM cut points using data only from the baseline visit to compare with the repeated measures analyses (33).

We performed subgroup analyses to examine potential effect modification by the following covariates: treatment arm (intensive lifestyle or placebo), baseline age (median cohort value, <50 or ≥50 years), sex (male or female), race/ethnicity (white or nonwhite), baseline BMI (overweight, <30 k/m^2, or obese, ≥30 k/m^2), and baseline total calcium intake (median cohort value, <921 or ≥921 mg/day). We checked for the statistical significance of the interaction by using Wald χ^2 tests (34). Regression assumptions of proportional hazards and linearity were assessed for violations, and all *P* values were based on two-sided tests. Statistical analyses were performed using SAS version 9.2 (SAS, Cary, NC).

RESULTS

Participant characteristics

At baseline, the average age of the cohort was 51 years and average BMI was 34 kg/m^2 (Table 1). The racial distribution was diverse: ~57% Caucasian and 21% African American, and the remaining participants were Hispanic American, Asian American or Pacific Islander, or Native American. The mean self-reported calcium intake was 1,106 mg/dL and plasma 25-hydroxyvitamin D concentration was 21.6 ng/mL. Based on the 2011 IOM report (24), 45% of the cohort had inadequate calcium intake at baseline and approximately half (49%) were at risk for deficiency or inadequacy for vitamin D (defined as 25-hydroxyvitamin D <20 ng/mL). Participants in the highest tertile of plasma 25-hydroxyvitamin D concentration were more likely to be older, male, Caucasian, and less overweight. They also had lower fasting plasma glucose, hemoglobin A_{1c}, C-reactive protein, and kidney function; reported higher physical activity and alcohol and calcium intake; and were more likely to reside in areas with higher annual ultraviolet index.

Plasma 25-hydroxyvitamin D concentration and risk of incident diabetes

Participants were followed for an average of 2.7 years (range 0.5–4.5). On a continuous scale, for every 5 ng/mL increase in 25-hydroxyvitamin D, there was a 13% decrease in the risk of progression to diabetes (HR 0.87 [95% CI 0.82–0.92]; *P* value <0.0001 for every 5-unit increase in 25-hydroxyvitamin D). When we categorized participants into tertiles, there was a 39% lower risk among those in the highest tertile (median [IQR] 25-hydroxyvitamin D, 30.1 [27.0–34.5] ng/mL) compared with those in the lowest tertile (12.8 [10.4–14.9] ng/mL) after adjustment for age and sex (Table 2). Further multivariate adjustment for a variety of diabetes risk factors, including change in physical activity and BMI during the study duration, and randomization to the DPP intervention (lifestyle or placebo) attenuated the association, but it remained statistically significant (HR 0.72 [95% CI 0.56–0.90], comparing the third and first tertile). Additionally adjusting for ultraviolet radiation index did not change the association (data not shown). Using data from the baseline visit only, the association between 25-hydroxyvitamin D and diabetes was in the same direction but was not statistically significant (0.94 [0.75–1.17], comparing the third and first tertile).

In analyses by categories of plasma 25-hydroxyvitamin D using cut points recommended for skeletal outcomes by the 2011 IOM report (24), participants with 25-hydroxyvitamin D concentration >30 ng/mL had a lower risk compared with those <30 ng/mL, whereas those in the highest category had a 60% lower risk of developing diabetes compared with participants in the lowest category (HR 0.40 [95% CI 0.20–0.81], for 25-hydroxyvitamin D ≥50 mg/mL vs. <12 ng/mL) (Table 3). However, there were very few participants and diabetes cases in the highest category. Additionally adjusting for ultraviolet radiation index did not change the association (data not shown). Using data from the baseline visit only, the association between 25-hydroxyvitamin D by IOM cut points and diabetes was also the same direction but was not statistically significant (0.89 [0.47–1.65], for 25-hydroxyvitamin D ≥50 mg/mL vs. <12 ng/mL).

Subgroup analyses

The inverse associations between plasma 25-hydroxyvitamin D and incident diabetes were generally consistent across all subgroups (Table 4). The association between 25-hydroxyvitamin D and diabetes appeared to be stronger among participants randomized to placebo compared with lifestyle (HR 0.70 [95% CI 0.52–0.94] vs. 0.80 [0.54–1.17], respectively); among those older than 50 years compared with those younger than 50 years (0.64 [0.46–0.90] vs. 0.81 [0.57–1.12]); among women versus men (0.68 [0.50–0.91] vs. 0.82 [0.55–1.19]); among obese versus nonobese (0.70 [0.52–0.93] vs. 0.78 [0.50–1.18], and among those with self-reported calcium intake ≥921 mg/dL versus <921 mg/dL (0.59 [0.42–0.82] vs. 0.84 [0.59–1.16]). However, the study had inadequate power to assess the significance of the association within subgroups, and the tests for interaction were not statistically significant in any of the strata analyzed (*P* for interactions >0.40) (Table 4).

CONCLUSIONS—In this unique cohort of U.S. adults at high risk for diabetes, higher plasma 25-hydroxyvitamin D concentrations, assessed repeatedly during the follow-up period, were associated with

Table 1—*Baseline characteristics of the DPP cohort included in the current study*

Characteristic	Overall cohort	Lifestyle	Placebo	*P* value[a]	Cohort by tertile of plasma 25-hydroxyvitamin D concentration[b]			*P* value
					1	2	3	
Number of participants	2,040	1,017	1,023	—	666	667	667	—
Age, mean (SD), years	51 (11)	51 (11)	51 (10)	0.6466	49 (10)	52 (11)	52 (11)	<0.0001
Sex, no. (%) women	1,371 (67.2)	678 (66.7)	693 (67.7)	0.6383	499 (74.9)	419 (62.8)	419 (62.8)	<0.0001
Race, no. (%)								<0.0001
Caucasian	1,159 (56.8)	576 (56.6)	583 (57.0)	0.6530	245 (36.8)	412 (61.8)	485 (72.7)	
African American	419 (20.5)	203 (20.0)	216 (21.1)		280 (42.0)	100 (15.0)	25 (3.8)	
Other (Hispanic, Asian, American Indian)	462 (22.6)	238 (23.4)	224 (21.9)		141 (21.2)	155 (23.2)	157 (23.5)	
Weight, mean (SD), kg	94.6 (20.5)	94.5 (20.8)	94.7 (20.2)	0.8873	99.4 (22.4)	94.1 (19.7)	90.1 (18.2)	<0.0001
BMI, mean (SD), kg/m^2	34.0 (6.7)	33.9 (6.7)	34.1 (6.7)	0.3354	35.9 (7.3)	33.7 (6.4)	32.3 (5.9)	<0.0001
Annual ultraviolet index[c]	4.6 (1.4)	4.6 (1.4)	4.6 (1.3)	0.7105	4.4 (1.1)	4.7 (1.3)	4.9 (1.6)	<0.0001
Family history of diabetes, no. (%)	1,416 (69.4)	708 (69.7)	708 (69.3)	0.8788	476 (71.5)	470 (70.5)	440 (66.0)	0.0722
Hypertension, no. (%)[d]	563 (27.6)	282 (27.7)	281 (27.5)	0.9346	170 (25.5)	202 (30.3)	182 (27.3)	0.1455
Physical activity MET-hours, mean (SD), h[e]	15.9 (25.6)	15.3 (21.7)	16.5 (29.0)	0.4648	14.9 (33.9)	14.2 (19.1)	18.5 (21.2)	<0.0001
Smoking status, no. (%)								0.0006
Never	1,174 (57.6)	579 (56.9)	595 (58.2)	0.3500	400 (60.1)	401 (60.1)	344 (51.6)	
Past	721 (35.3)	372 (36.6)	349 (34.1)		212 (31.8)	221 (33.1)	282 (42.3)	
Current	145 (7.1)	66 (6.5)	79 (7.7)		54 (8.1)	45 (6.8)	41 (6.2)	
Alcohol consumption, mean (SD), g/day	2.2 (5.6)	2.3 (6.2)	2.1 (4.9)	0.8437	1.5 (3.5)	2.2 (6.1)	3.0 (6.6)	<0.0001
Fasting plasma glucose, mean (SD), mg/dL	107.0 (8.1)	106.6 (7.9)	107.0 (8.3)	0.3828	107.6 (8.0)	106.8 (7.9)	106.0 (8.3)	0.0008
Glucose 120 min after 75 g of oral glucose, mean (SD), mg/dL	164.0 (17.0)	164.4 (16.8)	164.6 (17.2)	0.8577	164.1 (16.8)	164.3 (17.1)	164.8 (17.0)	0.7302
Hemoglobin A_{1c}, mean (SD), %	5.9 (0.5)	5.9 (0.5)	5.9 (0.5)	0.9489	6.0 (0.5)	5.9 (0.5)	5.8 (0.5)	<0.0001
C-reactive protein, mean (SD), mg/L	5.8 (7.0)	5.8 (6.4)	5.9 (7.5)	0.8195	6.6 (6.9)	5.3 (6.8)	5.5 (6.9)	<0.0001
Glomerular filtration rate, mL/min/1.73 m^2	101 (30)	100 (25)	101 (34)	0.8717	105 (25)	99 (25)	98 (38)	<0.0001
Calcium intake, mean (SD), mg/day	1,106 (727)	1,098 (698)	1,115 (756)	0.7718	946 (678)	1,148 (732)	1,211 (736)	<0.0001
Meeting EAR for calcium, no. (%)[f]	1,110 (55.4)	556 (55.8)	554 (55.0)	0.7497	286 (42.9)	388 (58.2)	409 (61.3)	<0.0001
25(OH)D, mean (SD), ng/mL	21.6 (9.7)	21.8 (9.9)	21.3 (9.5)	0.2029	11.8 (3.0)	20.5 (2.5)	32.4 (7.2)	—

Values are means (SD) for continuous variables or number (%) for categorical variables. Percentages may not add up to 100 because of rounding. To convert 25-hydroxyvitamin D concentration from ng/mL to nmol/L multiply by 2.459; to convert glucose from mg/dL to mmol/L, multiply by 0.0555. 25(OH)D, 25-hydroxyvitamin D. [a]*P* values for differences between placebo and lifestyle or between tertiles of plasma 25-hydroxyvitamin D for continuous variables are based on ANOVA or Kruskal-Wallis tests where appropriate. For categorical variables, the between-group differences are based on χ^2 tests. [b]Plasma 25-hydroxyvitamin D concentration at baseline visit. [c]Average ultraviolet index at participants' DPP clinical site in 1997. [d]Hypertension defined as blood pressure ≥140/90 mmHg or the use of antihypertensive medication. [e]MET denotes metabolic equivalent. MET-hours represent the average amount of time engaged in specified physical activities multiplied by the MET value of each activity. [f]Based on age-appropriate estimated average requirement (EAR) for calcium defined by the 2011 IOM dietary reference intake report (24) as follows: males 19–70 years, 800 mg/day; males >70 years, 1,000 mg/day; females 19–50 years, 800 mg/day; females >50 years, 1,000 mg/day.

Table 2—*Risk for incident diabetes by tertiles of continuous plasma 25-hydroxyvitamin D concentration in the lifestyle and placebo arms of the DPP*

	No. of person-years	No. of participants	Tertile of 25-hydroxyvitamin D concentration			
			1 (Lowest)	2	3 (Highest)	*P* value
Number of participants (events)			680 (162)	678 (141)	681 (123)	
Plasma 25-hydroxyvitamin D concentration, median (IQR), ng/mL	5,536.0	2,039	12.8 (10.4–14.9)	20.9 (18.9–22.6)	30.1 (27.0–34.5)	—
Model adjusted for age and sex	5,374.0	2,039	1.00 (reference)	0.80 (0.72–0.88)	0.61 (0.50–0.75)	<0.0001
Model adjusted for age, sex, and BMI	5,373.5	2,039	1.00 (reference)	0.86 (0.78–0.95)	0.72 (0.58–0.89)	0.0023
Multivariate model[a]	5,272.5	2,002	1.00 (reference)	0.85 (0.76–0.94)	0.70 (0.55–0.88)	0.0024
Multivariate model and calcium intake[b]	5,272.0	2,002	1.00 (reference)	0.84 (0.76–0.94)	0.70 (0.55–0.88)	0.0024
Multivariate model and treatment arm[c]	5,272.0	2,002	1.00 (reference)	0.86 (0.77–0.95)	0.72 (0.56–0.90)	0.0054

Data are HR (95% CI) unless otherwise indicated. Results are presented for tertiles of plasma 25-hydroxyvitamin D concentration as the mean of the values obtained at baseline and 6-month visits. HR of diabetes in each of the two highest tertiles was compared with the lowest tertile by extrapolating the per-unit change in estimated hazard from the multivariate model. Variables measured at multiple time points throughout the study (25-hydroxyvitamin D, BMI, and physical activity) entered the analyses as time-varying "lagged" covariates, as the mean of the previous and current visits at which diabetes status was assessed. To convert plasma 25-hydroxyvitamin D concentration from ng/mL to nmol/L multiply by 2.459. [a]Adjusted for recruitment location, age (years), sex (male or female), BMI (kg/m^2), race (black, white, or other), family history of diabetes (yes or no), personal history of hypertension at baseline (yes or no), smoking status at baseline (never, past, or currently smoking), alcohol consumption (average of values self-reported at baseline and 1-year follow-up visits, g/day), C-reactive protein (average of values at baseline and 6-month and 1-year follow-up visits, mg/L), kidney function (average of estimated glomerular filtration rate at baseline and 6-month and 1-year follow-up visits, mL/min/1.73 m^2), and self-reported physical activity (MET-hours per week). [b]Adjusted for everything in first footnote plus calcium intake (average of values self-reported at baseline and 1-year follow-up visits, mg/day). [c]Adjusted for everything in second footnote plus treatment arm (intensive lifestyle or placebo).

lower risk of diabetes, even after adjusting for weight loss and lifestyle interventions (dietary changes and increased physical activity) known to decrease diabetes risk.

Our results are consistent with those from other observational longitudinal studies that have reported on the association between vitamin D status and risk of developing type 2 diabetes (7–9,11–15,35,36). The association between higher vitamin D levels and lower risk of type 2 diabetes was statistically significant in seven published cohorts (7,9,11–13,15,36) and was in the same direction, albeit not statistically significant, in three other cohorts (8,9,35). In one cohort among older postmenopausal women, lower serum 25-hydroxyvitamin D was not associated with increased risk of developing type 2 diabetes (14).

Our study offers a methodological advantage over previous studies, which assessed vitamin D status (either by self-reported total vitamin D intake [7,35], predicted 25-hydroxyvitamin D score [11], or blood 25-hydroxyvitamin D concentration [9,12–14,36]) based on a single baseline measurement, which may not reflect long-term vitamin D status. Use of repeated measurements and cumulative averages of dietary variables has been shown to yield stronger associations than use of a single baseline measurement (33), and this was the case in our study. The need for repeated measurements is especially true with vitamin D, as single measurements of 25-hydroxyvitamin D may not reflect vitamin D status over time, owing to changes in dietary and other lifestyle changes, weight changes, sun exposure, and other relevant factors that may change during follow-up. Measuring 25-hydroxyvitamin D at multiple time points during follow-up allowed us to reduce measurement error and obtain an integrated measure that reflects long-term vitamin D status for each participant, which may be more relevant etiologically than the most remote (baseline) value (33). An alternative explanation of our results may be that plasma 25-hydroxyvitamin D is a marker of the DPP treatment effects from increased physical activity and weight loss; however, the association remained after we adjusted for the main effects of the DPP intervention (changes in physical activity and weight) during the study period. Although the analyses adjusted for these factors, residual confounding cannot be excluded. Our study has additional strengths, such as the inclusion of a large clinically relevant population at high risk for diabetes with a substantial proportion of nonwhite participants, which improves the external validity of our results because they are directly applicable to people at risk for diabetes who are in need for effective interventions to delay progression to diabetes. Also, the diagnosis of diabetes was rigorously evaluated and confirmed based on repeated laboratory measures, which is in contrast with other observational studies that ascertained diabetes by validated self-report (7,8,12,14,35), combination of self-report and laboratory measurement (11), or registry-based data (9,36). Furthermore, the method we used to measure 25-hydroxyvitamin D has been validated through the NIST vitamin D quality assurance program (27).

The results from small clinical trials and post hoc analyses of larger trials on the effect of vitamin D supplementation on glycemia or incident diabetes have been inconsistent (16–23,37–39). In these studies, vitamin D appears to have beneficial effects only in people at risk for diabetes (17,20,23), which is consistent with the findings of the current study. In contrast, vitamin D supplementation appears to have no effect among those with normal glucose tolerance, or a very small effect that would require a very large sample population to detect (17,18,22,37,39). The studies that have reported on the effect of vitamin D supplementation on glycemia in patients with established type 2 diabetes were underpowered to draw any firm conclusions.

The hypothesis that vitamin D may be relevant to prevention of type 2 diabetes is biologically plausible. Both insulin resistance and impaired pancreatic β-cell function have been reported with vitamin D insufficiency (4). Vitamin D may have a direct effect mediated by binding of the active form, $1,25(OH)_2D$, to the vitamin D

Table 3—*Risk for incident diabetes by categorical cut points of plasma 25-hydroxyvitamin D concentration in the lifestyle and placebo arms of the DPP*

	No. of person-years	No. of participants	Category of 25-hydroxyvitamin D concentration					*P* value
			<12	[12–19.9]	[20–29.9]	[30–49.9]	≥50	
Number of participants (events)			287 (81)	656 (140)	751 (147)	333 (56)	12 (2)	
Plasma 25-hydroxyvitamin D concentration, median (IQR), ng/mL	5,536.0	2,039	9.9 (8.5–11.1)	16.2 (14.1–18.2)	24.4 (21.9–26.7)	34.2 (31.8–37.8)	57.4 (56.4–67.7)	—
Model adjusted for age and sex	5,374.0	2,039	1.00 (reference)	0.83 (0.77–0.90)	0.66 (0.54–0.79)	0.49 (0.36–0.68)	0.25 (0.13–0.47)	<0.0001
Model adjusted for age, sex, and BMI	5,373.5	2,039	1.00 (reference)	0.89 (0.81–0.96)	0.76 (0.62–0.92)	0.63 (0.45–0.86)	0.40 (0.21–0.75)	0.0046
Multivariate model[a]	5,272.5	2,002	1.00 (reference)	0.88 (0.80–0.96)	0.74 (0.60–0.92)	0.61 (0.43–0.86)	0.38 (0.19–0.75)	0.0059
Multivariate model and calcium intake[b]	5,272.0	2,002	1.00 (reference)	0.88 (0.80–0.96)	0.74 (0.60–0.92)	0.61 (0.42–0.86)	0.37 (0.18–0.75)	0.0059
Multivariate model and treatment arm[c]	5,272.0	2,002	1.00 (reference)	0.89 (0.81–0.97)	0.76 (0.61–0.94)	0.63 (0.44–0.90)	0.40 (0.20–0.81)	0.0113

Data are HR (95% CI) unless otherwise indicated. Results are presented for categories of plasma 25-hydroxyvitamin D concentration as the mean of the values obtained at baseline and 6-month visits, based on cut points suggested by the IOM Dietary Reference Intakes for Calcium and Vitamin D report (32). HR of diabetes in each of the four highest categories was compared with the lowest category by extrapolating the per-unit change in estimated hazard from the multivariate model. Variables measured at multiple time points throughout the study (25-hydroxyvitamin D, BMI, and physical activity) entered the analyses as time-varying "lagged" covariates, as the mean of the previous and current visits at which diabetes status was assessed. To convert plasma 25-hydroxyvitamin D concentration from ng/mL to nmol/L, multiply by 2.459. [a]Adjusted for recruitment location, age (years), sex (male or female), BMI (kg/m^2), race (black, white, or other), family history of diabetes (yes or no), personal history of hypertension at baseline (yes or no), smoking status at baseline (never, past, or currently smoking), alcohol consumption (average of values self-reported at baseline and 1-year follow-up visits, g/day), C-reactive protein (average of values at baseline and 6-month and 1-year follow-up visits, mg/L), kidney function (average of estimated glomerular filtration rate at baseline and 6-month and 1-year follow-up visits, mL/min/1.73 m^2), and self-reported physical activity (MET-hours per week). [b]Adjusted for everything in first footnote plus calcium intake (average of values self-reported at baseline and 1-year follow-up visits, mg/day). [c]Adjusted for everything in second footnote plus treatment arm (intensive lifestyle or placebo).

receptor expressed in pancreatic β-cells. The presence of the vitamin D response element in the human insulin gene promoter and transcriptional activation of the human insulin gene caused by $1,25(OH)_2D$ further support a direct effect of vitamin D on insulin synthesis and secretion. Finally, activation of vitamin D may occur within the β-cell by 25-OHD-1α-hydroxylase (CYP27B1), which is expressed in β-cells.

Although 25-hydroxyvitamin D is a well-established biomarker of exposure for vitamin D due to intake or biosynthesis, there is a lack of clarity regarding the validity of 25-hydroxyvitamin D as a marker of biological effects (24). The 2011 IOM Dietary Reference Intakes for Calcium and Vitamin D report concluded that adequate evidence for setting dietary reference intakes for vitamin D exists only in relation to skeletal outcomes and recommended a 25-hydroxyvitamin D level >20 ng/mL as sufficient. For nonskeletal outcomes, including diabetes, the IOM report concluded that available data are inconclusive and therefore not sufficient for dietary reference intakes development. The report also noted that levels >30 ng/mL are not consistently associated with increased benefit, whereas a level >50 ng/mL may be a "reason for concern." Our results support the hypothesis that higher blood 25-hydroxyvitamin D (>30 ng/mL) is associated with lower risk of diabetes, and the observed inverse association was linear at all levels of 25-hydroxyvitamin D. Although our study included very few participants with very high (≥50 ng/mL) 25-hydroxyvitamin D levels, potentially limiting interpretation, this group appeared to have the lowest risk of progressing to diabetes, and our modeling of vitamin D levels as a continuous linear variable supports a benefit for this upper tail of the distribution.

In subgroup analyses, the inverse associations between plasma 25-hydroxyvitamin D and incident diabetes were consistent, across all subgroups. The association appeared to be stronger among participants randomized to placebo, those older than 50 years, obese people, women, and those with higher calcium intake. It is important to note that the tests for interaction were not statistically significant; therefore, no firm conclusions can be drawn from these subgroup analyses.

Of note, the observed inverse association between vitamin D status and incident diabetes did not appear to be

Table 4—*Risk for incident diabetes by tertiles of plasma 25-hydroxyvitamin D concentration in the lifestyle and placebo arms of the DPP by subgroups*

	No. of person-years	No. of participants	Tertiles of 25-hydroxyvitamin D concentration 1 (Lowest)	2	3 (Highest)	*P* value for HR	*P* value for interaction
Plasma 25-hydroxyvitamin D concentration, median (IQR), ng/mL	5,272.0	2,002	12.8 (10.4–14.9)	20.9 (18.9–22.6)	30.1 (27.0–34.5)		
By DPP treatment arm							0.6721
Lifestyle only	2,751.0	1,006	1.00 (reference)	0.90 (0.75–1.08)	0.80 (0.54–1.17)	0.2713	
Number of participants (events)			304 (46)	349 (49)	353 (43)		
Placebo only	2,521.0	996	1.00 (reference)	0.85 (0.74–0.97)	0.70 (0.52–0.94)	0.0201	
Number of participants (events)			364 (106)	310 (88)	322 (75)		
Baseline age (median, years)							0.6910
<50	2,462.5	956	1.00 (reference)	0.91 (0.77–1.06)	0.81 (0.57–1.12)	0.2190	
Number of participants (events)			361 (83)	312 (66)	283 (53)		
≥50	2,809.5	1,046	1.00 (reference)	0.81 (0.69–0.95)	0.64 (0.46–0.90)	0.0103	
Number of participants (events)			307 (69)	347 (71)	392 (65)		
Sex							0.5933
Female	3,510.5	1,339	1.00 (reference)	0.83 (0.72–0.96)	0.68 (0.50–0.91)	0.0118	
Number of participants (events)			513 (108)	416 (93)	410 (61)		
Male	1,761.5	663	1.00 (reference)	0.91 (0.76–1.09)	0.82 (0.55–1.19)	0.3144	
Number of participants (events)			155 (44)	243 (44)	265 (57)		
Race							0.6266
White	3,044.0	1,143	1.00 (reference)	0.88 (0.76–1.01)	0.76 (0.56–1.02)	0.0737	
Number of participants (events)			237 (58)	410 (85)	496 (86)		
Nonwhite	2,228.0	859	1.00 (reference)	0.85 (0.70–1.02)	0.71 (0.47–1.04)	0.0892	
Number of participants (events)			431 (94)	249 (52)	179 (32)		
Baseline BMI (kg/m^2)							0.7666
BMI <30	1,994.0	735	1.00 (reference)	0.89 (0.72–1.08)	0.78 (0.50–1.18)	0.2475	
Number of participants (events)			156 (31)	238 (32)	341 (49)		
BMI ≥30	3,278.0	1,267	1.00 (reference)	0.85 (0.74–0.97)	0.70 (0.52–0.93)	0.0160	
Number of participants (events)			512 (121)	421 (105)	334 (69)		
Baseline calcium (median, mg/day)							0.4102
<921	2,667.5	1,008	1.00 (reference)	0.92 (0.78–1.07)	0.84 (0.59–1.16)	0.3005	
Number of participants (events)			419 (94)	300 (59)	289 (56)		
≥921	2,604.5	994	1.00 (reference)	0.78 (0.66–0.91)	0.59 (0.42–0.82)	0.0020	
Number of participants (events)			249 (58)	359 (78)	386 (62)		

Data are HR (95% CI) unless otherwise indicated. Results are presented for tertiles of plasma 25-hydroxyvitamin D concentration, as the mean of the values obtained at baseline and 6-month visits. HR of diabetes in each of the two highest tertiles was compared with the lowest tertile by extrapolating the per-unit change in estimated hazard from the multivariate model. Variables measured at multiple time points throughout the study (25-hydroxyvitamin D, BMI, and physical activity) entered the analyses as time-varying "lagged" covariates, as the mean of the previous and current visits at which diabetes status was assessed. To convert plasma 25-hydroxyvitamin D concentration from ng/mL to nmol/L, multiply by 2.459. Models adjusted for recruitment location, age (years), sex (male or female), BMI (kg/m^2), race (black, white, or other), family history of diabetes (yes or no), hypertension at baseline (yes or no), smoking status at baseline (never, past, or currently smoking), alcohol consumption (average of values self-reported at baseline and 1-year follow-up visits, g/day), C-reactive protein (average of values at baseline and 6-month and 1-year follow-up visits, mg/L), kidney function (average of estimated glomerular filtration rate at baseline and 6-month and 1-year follow-up visits, mL/min/1.73 m^2), self-reported physical activity (MET-hours per week), calcium intake (average of values self-reported at baseline and 1-year follow-up visits, mg/day). All analyses also adjusted for DPP treatment arm (intensive lifestyle or placebo), except for analyses by treatment arm.

affected by race (as a proxy for the altered vitamin D homeostasis in people with dark skin). These data suggest that although there are well-recognized differences in vitamin D metabolism among different race/ethnic groups (40), higher vitamin D status is associated with lower risk of diabetes among all people regardless of skin color.

In conclusion, higher vitamin D status, assessed by plasma 25-hydroxyvitamin D concentration measured repeatedly during follow-up, was associated with a lower risk of incident diabetes among people at high risk for the disease. If these results are confirmed in ongoing and planned randomized trials of vitamin D, they will have important public health implications because both of these interventions can be implemented easily and inexpensively to prevent progression of type 2 diabetes among those at high risk.

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