

# Plasma 25-Hydroxyvitamin D Concentration and Risk of Incident Type 2 Diabetes in Women

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**OBJECTIVE** — To determine the association between 25-hydroxyvitamin D (25-OHD) concentration and risk of incident type 2 diabetes.

**RESEARCH DESIGN AND METHODS** — In a nested case-control study conducted among 608 women with newly diagnosed type 2 diabetes and 559 control subjects in the Nurses' Health Study, we measured the association between baseline plasma 25-OHD concentration and risk of incident diabetes.

**RESULTS** — After adjusting for matching factors and diabetes risk factors, including BMI, higher levels of plasma 25-OHD were associated with a lower risk for type 2 diabetes. The odds ratio for incident type 2 diabetes in the top (median 25-OHD, 33.4 ng/ml) versus the bottom (median 25-OHD, 14.4 ng/ml) quartile was 0.52 (95% CI 0.33–0.83). The associations were consistent across subgroups of baseline BMI, age, and calcium intake.

**CONCLUSIONS** — Plasma 25-OHD concentration was associated with lower risk of incident type 2 diabetes in women.

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**G**rowing evidence indicates that sub-optimal vitamin D status may play a role in the development of type 2 diabetes (1). Results from longitudinal observational studies support the hypothesis that low vitamin D status is associated with development of type 2 diabetes; however, only one study has examined the association between blood 25-hydroxyvitamin D (25-OHD) concentration and incident type 2 diabetes, and there was no significant association among women (2,3). We examined prospectively the association between plasma 25-OHD concentration and risk of incident type 2 diabetes among women in a case-control study nested within the Nurses' Health Study (NHS).

## RESEARCH DESIGN AND METHODS

The NHS is a large, longitudinal ongoing cohort of U.S. female nurses who respond to questionnaires mailed every 2 years to update information on health-related behavior and to identify incident disease (4). During 1989–1990, 32,826 women aged 43–70 years, who were free of diagnosed diabetes, coronary heart disease, stroke, or cancer, provided blood samples. Through June 2004, 1,106 of these women had a confirmed diagnosis of type 2 diabetes and were classified as case participants. For each case, control participants providing blood samples during the same period were selected and matched by age, race, fasting status at blood draw, and

date of blood draw. After excluding women with unavailable information on 25-OHD, the final analytical sample consisted of 608 case and 569 control subjects. Plasma 25-OHD was measured by the <sup>125</sup>I radioimmunoassay procedure (DiaSorin, Stillwater, MN), with mean coefficient of variation of 8.5% (intra-assay) and 8.7% (interassay).

For external validation, the laboratory participated in the vitamin D External Quality Assessment Scheme (<http://deqas.org>). Matched case-control pairs were handled identically and assayed in the same analytical run by personnel blinded to the case-control status of the samples. Measurements were done in random order and in duplicate to reduce systematic error and interassay variability.

Incident cases of type 2 diabetes were identified by self-report and confirmed by a supplementary questionnaire, as previously reported (5). BMI, physical activity, and smoking status; family history of diabetes in a first-degree relative; and physician-diagnosed hypertension and hypercholesterolemia were self-reported. Physical activity was computed as metabolic equivalents (METs) per week based on average time spent per week on various leisure-time activities, weighted by their intensity level (5). Information on dietary intake was obtained from the semiquantitative validated food frequency questionnaire (6). Total nutrient intakes were calculated by adding intake from different food sources to intake from multivitamins and supplements. Intake of nutrients was adjusted for total energy intake with regression analysis (5).

We calculated odds ratios (ORs) for type 2 diabetes using unconditional logistic regression analysis adjusted for the matching factors, latitude of participants' residence, and laboratory batch for 25-OHD assay. We also adjusted for known and suggested risk factors for type 2 diabetes. We used restricted cubic spline regression with three knots to examine for possible nonlinear relation of 25-OHD with incident type 2 diabetes (7).

**RESULTS** — The mean age of the cohort was 56.4 years, BMI was 27.8 kg/m<sup>2</sup>,

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Table 1—OR for incident type 2 diabetes in women, according to plasma 25-OHD concentration

	25-OHD quartile				P for trend*
	1 (lowest)	2	3	4 (highest)	
Plasma 25-OHD concentration (ng/ml) [median (range)]†	14.4 (6.7–17.8)	20.8 (17.9–23.1)	25.9 (23.2–28.9)	33.4 (29.1–87.6)	
n (case/control subjects)	201/140	193/145	139/144	75/140	
OR (95% CI)					
Crude‡	1.00 (reference)	0.91 (0.67–1.24)	0.65 (0.47–0.90)	0.35 (0.25–0.51)	<0.001
Multivariate model§	1.00 (reference)	1.02 (0.71–1.47)	0.79 (0.54–1.16)	0.40 (0.26–0.62)	<0.001
Multivariate model plus BMI	1.00 (reference)	1.09 (0.74–1.61)	0.95 (0.63–1.45)	0.52 (0.33–0.83)	0.008

\*Statistical tests for trend were conducted using the median value of each quartile of plasma 25-OHD concentration as a continuous variable. †To convert 25-OHD concentration from ng/ml to nmol/l multiplied by 2.459. ‡Adjusted for matching variables (age, race, fasting status, month of blood draw, and laboratory batch for plasma 25-OHD). §Adjusted for everything in ‡ plus latitude (residence in southern states [ $<40^{\circ}\text{N}$ ; California, Florida, and Texas] or northern states [ $\geq 40^{\circ}\text{N}$ ; Connecticut, Maryland, Massachusetts, Michigan, New Jersey, New York, Ohio, and Pennsylvania]), history of hypercholesterolemia (yes or no), history of hypertension (yes or no), family history of diabetes (yes or no), smoking status (never, past, or currently smoking), physical activity (METs/week, in quartiles), alcohol consumption (grams/day, in quartiles), multivitamin use (yes or no), and dietary variables in quartiles (caffeine [mg/day], *trans* fat [g/day], cereal fiber [g/day], heme iron [mg/day], magnesium [mg/day], fish [servings/day], and calcium intake [mg/day]).

and 25-OHD was 22.7 ng/ml. Average 25-OHD was higher among white than nonwhite subjects (23.0 vs. 21.4 ng/ml, respectively,  $P = 0.016$ ) and among normal-weight than overweight/obese women (24.4 vs. 21.7 ng/ml, respectively,  $P < 0.001$ ). Total vitamin D intake was 321 IU/day without difference between case and control subjects. After multivariate adjustment, the OR for incident diabetes in the top versus the bottom quartile for 25-OHD concentration was 0.52 (95% CI 0.33–0.83;  $P$  for trend = 0.008) (Table 1). Spline regression models showed no apparent threshold and no deviation from linearity for the relation between 25-OHD and risk of incident type 2 diabetes ( $P$  for linearity = 0.015), although the shape of the figure suggested a stronger decrease in risk within the higher range of 25-OHD concentration (online appendix figure, available at <http://care.diabetesjournals.org/cgi/content/full/dc10-0790/DC1>). The associations were consistent across all subgroup analyses, with the exception of the subgroup analyses by BMI. The association was stronger among overweight/obese women (0.46 [0.25–0.83];  $P$  for trend = 0.016) compared with normal-weight women, where the association was in the same direction but was not statistically significant (BMI  $<25$  kg/m<sup>2</sup>; 0.63 [0.25–1.56];  $P$  for trend = 0.24) (online appendix Table).

**CONCLUSIONS**— In this nested case-control study of middle-aged women, plasma 25-OHD concentration was inversely associated with development of type 2 diabetes, independent of known diabetes risk factors, including

age, BMI, and race. To our knowledge, this study is the largest observational longitudinal study that used blood 25-OHD concentration to assess vitamin D status prior to development of type 2 diabetes and the first one that reported such an association in a U.S. cohort.

Four other longitudinal observational studies (2,3,5,8,9) have reported data on the association between vitamin D status and risk of developing type 2 diabetes. Vitamin D was assessed by vitamin D intake in two studies (5,8) and by predicted 25-OHD score in another study (9). All three studies reported an inverse association between vitamin D status and incident type 2 diabetes. However, only one study, using pooled data from two Finnish cohorts, has reported the association between blood 25-OHD concentration and incident type 2 diabetes (2,3). Among men, those in the highest quartile had a 72% lower risk of developing type 2 diabetes compared with men in the lowest quartile, but there was no association among women. In the Finnish cohorts, men had higher baseline 25-OHD than women (19 vs. 15 ng/ml, respectively), while among women in the NHS, 25-OHD was higher (23 ng/ml), which may explain the lack of association among women in the Finnish study.

There are no published clinical trials designed to test the hypothesis that increasing 25-OHD concentration prevents new-onset type 2 diabetes. Only one trial (Women's Health Initiative) has examined, in a post hoc analysis, the effect of 400 IU of vitamin D versus placebo on incident type 2 diabetes, and there was no difference (1,10). However, that trial used 400 IU/day vitamin D, which is

inadequate to raise 25-OHD concentration enough to see any effect.

The strengths of our study include its longitudinal study design, large size, long-term follow-up, the validated measurements of the exposure and outcome, and the availability of detailed information on risk factors for type 2 diabetes and other covariates, including seasonality and latitude. The major limitation of our study is its observational nature; therefore, residual confounding cannot be excluded. Also, our results cannot be directly extrapolated to men or nonwhite women.

In conclusion, our findings suggest that raising 25-OHD concentration may be an effective strategy at reducing risk of incident type 2 diabetes in women. Because observational studies of vitamin D have a high potential for confounding, our results need to be confirmed in randomized controlled trials specifically designed to test such a hypothesis.

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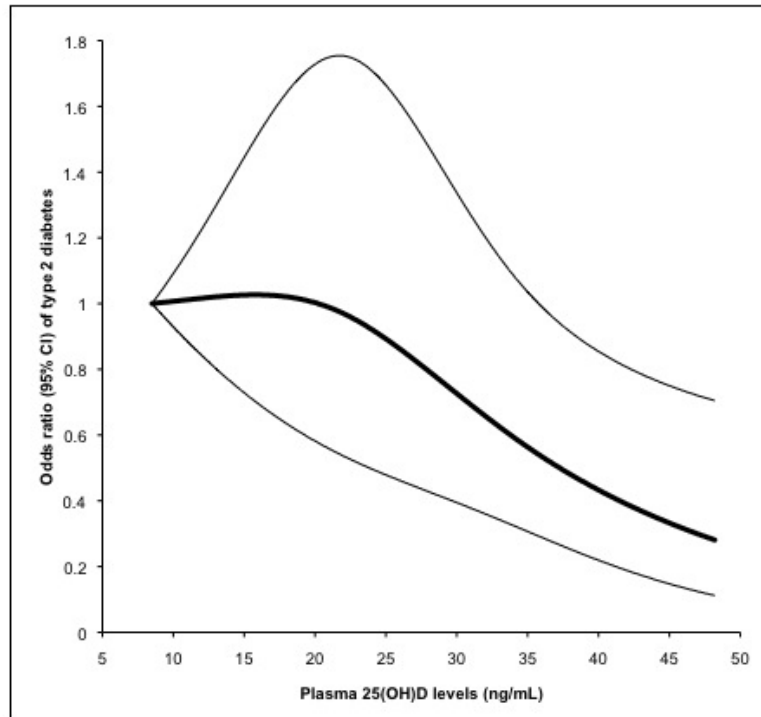
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**ONLINE APPENDIX FIGURE.** Spline regression models examining the possible non-linear relation between plasma 25-OHD concentration and incident type 2 diabetes. Odds ratios are adjusted for the same variables as in the multivariate model of the Table, including body mass index. Women with extremely low or high plasma 25-OHD concentrations (>1<sup>st</sup> or 99<sup>th</sup> percentile) were excluded from the analyses. Solid lines represent odds ratios, and dotted lines represent 95% CI. To convert 25-OHD concentration from ng/mL to nmol/L multiply by 2.459.



**ONLINE APPENDIX TABLE.** Odds ratio for incident type 2 diabetes in women, according to plasma 25-hydroxyvitamin D (25-OHD) concentration by baseline body mass index, age and calcium intake

	25-OHD Quartile				P for trend	P for interaction
	1 (Lowest)	2	3	4 (Highest)		
Plasma 25-OHD concentration, Median (range), ng/ml <sup>2</sup>	14.4 (6.7 – 17.8)	20.8 (17.9 – 23.1)	25.9 (23.2 – 28.9)	33.4 (29.1 – 87.6)		
Body Mass Index (kg/m <sup>2</sup> ) <sup>3</sup>						
BMI < 25	1.00 (reference)	1.35 (0.60, 3.05)	1.07 (0.47, 2.49)	0.63 (0.25, 1.56)	0.24	0.20
BMI ≥ 25	1.00 (reference)	0.99 (0.61, 1.59)	0.88 (0.51, 1.49)	0.46 (0.25, 0.83)	0.016	
Age (median, years) <sup>4</sup>						
< 57	1.00 (reference)	1.00 (0.56, 1.80)	0.89 (0.48, 1.65)	0.46 (0.23, 0.93)	0.042	0.21
≥ 57	1.00 (reference)	1.22 (0.69, 2.16)	1.05 (0.56, 1.94)	0.53 (0.27, 1.04)	0.066	
Calcium intake (median, mg/day) <sup>5</sup>						
< 700	1.00 (reference)	1.01 (0.58, 1.74)	0.83 (0.46, 1.51)	0.50 (0.25, 0.98)	0.043	
≥ 700	1.00 (reference)	1.07 (0.56, 2.04)	1.09 (0.55, 2.20)	0.39 (0.18, 0.83)	0.022	

<sup>1</sup> All data presented as Odds ratio (95% confidence interval). All models adjusted for matching variables (age, race, fasting status, month of blood draw and laboratory batch for plasma 25-hydroxyvitamin D) plus latitude (residence in southern states [ $<40^{\circ}\text{N}$ ; California, Florida and Texas] or northern states [ $\geq 40^{\circ}\text{N}$ ; Connecticut, Maryland, Massachusetts, Michigan, New Jersey, New York, Ohio and Pennsylvania]), history of hypercholesterolemia (yes or no), history of hypertension (yes or no), family history of diabetes (yes or no), smoking status (never, past or currently smoking), physical activity (metabolic equivalent/week, in quartiles), alcohol consumption (grams/day, in quartiles) and multivitamin use (yes or no), plus dietary variables in quartiles (caffeine [mg/day], trans fat [g/day], cereal fiber [g/day], heme iron [mg/day], magnesium [mg/day], fish [servings/day] and calcium intake [mg/day]) and body mass index.

<sup>2</sup> To convert 25-OHD concentration from ng/mL to nmol/L multiply by 2.459.

<sup>3</sup> Model for BMI < 25 kg/m<sup>2</sup> includes 97 cases and 339 controls; model for BMI  $\geq 25$  kg/m<sup>2</sup> includes 510 cases and 230 controls

<sup>4</sup> Model for age < 57 years includes 312 cases and 288 controls; model for age  $\geq 57$  years includes 296 cases and 281 controls

<sup>5</sup> Model for calcium intake < 700 includes 343 cases and 310 controls; model for calcium intake  $\geq 700$  includes 265 cases and 259 controls