

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

ANASTASSIOS G. PITTAS, MS, MD^{1,2}
 QI SUN, MD, SCD³
 JOANN E. MANSON, MD, DRPH^{3,4}

BESS DAWSON-HUGHES, MD^{1,5}
 FRANK B. HU, MD, PHD³

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.

Diabetes Care 33:2021–2023, 2010

Growing 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 ¹²⁵I 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/m²,

From the ¹Division of Endocrinology, Diabetes and Metabolism, Tufts Medical Center, Boston, Massachusetts; the ²Friedman School of Nutrition Science and Policy, Tufts University, Boston, Massachusetts; the ³Harvard School of Public Health and Channing Laboratory, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts; the ⁴Division of Preventive Medicine, Brigham and Women's Hospital, Boston, Massachusetts; and the ⁵Bone 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.

Received 28 April 2010 and accepted 14 June 2010.

DOI: 10.2337/dc10-0790

© 2010 by the American Diabetes Association. Readers may use this article as long as the work is properly cited, the use is educational and not for profit, and the work is not altered. See <http://creativecommons.org/licenses/by-nc-nd/3.0/> for details.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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/m²; 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.

Acknowledgments— This work was supported by National Institutes of Health Research Grants R01-DK-76092 and R01-DK-79003 both to A.G.P. (funded by the National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health Office of the Director, and the National Institutes of Health Office of Dietary Supplements), R21-DK-78867 to A.G.P., and R01-DK-58845 to J.E.M. and F.B.H. (funded by the National Institute of Diabetes and Digestive and Kidney Diseases), and U.S. Department of Agriculture Agreement 58-1950-9001 to B.D.-H.

No potential conflicts of interest relevant to this article were reported.

A.G.P. researched data and wrote the manuscript. Q.S. researched data, contributed to the discussion, and reviewed/edited the manuscript. J.E.M. contributed to discussion and reviewed/edited the manuscript. B.D.-H. contributed to the discussion and reviewed/edited the manuscript. F.B.H. contributed to the discussion and reviewed/edited the manuscript.

This work was presented as an oral presentation at the 70th Scientific Sessions of the American Diabetes Association, 25–29 June 2010, Orlando, Florida.

The authors thank Gayle Petty in the Nutrition Evaluation Laboratory at the Human Nutrition Research Center on Aging at Tufts University for assistance with the measurement of plasma 25-OHD.

References

1. Pittas AG, Chung M, Trikalinos T, Mitri J, Brendel M, Patel K, Lichtenstein AH, Lau J, Balk EM. Systematic review: vitamin d and cardiometabolic outcomes. *Ann Intern Med* 2010;152:307–314
2. Mattila C, Knekt P, Mannisto S, Rissanen H, Laaksonen MA, Montonen J, Reunanen A. Serum 25-hydroxyvitamin D concentration and subsequent risk of type 2 diabetes. *Diabetes Care* 2007;30:2569–2570
3. Knekt P, Laaksonen M, Mattila C, Harkanen T, Marniemi J, Heliövaara M, Rissanen H, Montonen J, Reunanen A. Serum vitamin D and subsequent occurrence of type 2 diabetes. *Epidemiology* 2008;19:666–671
4. Colditz GA, Hankinson SE. The Nurses' Health Study: lifestyle and health among women. *Nat Rev Cancer* 2005;5:388–396
5. Pittas AG, Dawson-Hughes B, Li T, Van Dam RM, Willett WC, Manson JE, Hu FB. Vitamin D and calcium intake in relation to type 2 diabetes in women. *Diabetes Care* 2006;29:650–656
6. Salvini S, Hunter DJ, Sampson L, Stampfer MJ, Colditz GA, Rosner B, Willett WC. Food-based validation of a dietary questionnaire: the effects of week-to-week variation in food consumption. *Int J Epidemiol* 1989;18:858–867
7. Greenland S. Dose-response and trend analysis in epidemiology: alternatives to categorical analysis. *Epidemiology* 1995;6:356–365
8. Liu S, Song Y, Ford ES, Manson JE, Buring JE, Ridker PM. Dietary calcium, vitamin D, and the prevalence of metabolic syndrome in middle-aged and older U.S. women. *Diabetes Care* 2005;28:2926–2932
9. Liu E, Meigs JB, Pittas AG, Economos CD, McKeown NM, Booth SL, Jacques PF. Predicted 25-hydroxyvitamin D score and incident type 2 diabetes in the Framingham Offspring Study. *Am J Clin Nutr* 2010;91:1627–1633
10. de Boer IH, Tinker LF, Connelly S, Curb JD, Howard BV, Kestenbaum B, Larson JC, Manson JE, Margolis KL, Siscovick DS, Weiss NS. Calcium plus vitamin D supplementation and the risk of incident diabetes in the Women's Health Initiative. *Diabetes Care* 2008;31:701–707