

# Serum 25-Hydroxyvitamin D Concentration and Subsequent Risk of Type 2 Diabetes

CATHARINA MATTILA, MSC  
PAUL KNEKT, PHD  
SATU MÄNNISTÖ, PHD  
HARRI RISSANEN

MAARIT A. LAAKSONEN, MSC  
JUKKA MONTONEN, PHD  
ANTTI REUNANEN, PHD, MD

It has been suggested that vitamin D reduces the risk of type 2 diabetes. The finding that vitamin D deficiency is associated with impaired  $\beta$ -cell function and insulin resistance in animals (1,2) and humans (3,4) is in line with that hypothesis. In the only cohort study published, the intake of vitamin D supplements was inversely associated with the development of type 2 diabetes (5). Since vitamin D intake covers only a part of the total vitamin D available, the purpose of this study was to evaluate the prediction of serum 25-hydroxyvitamin D (25OHD) on subsequent type 2 diabetes incidence.

## RESEARCH DESIGN AND METHODS

The study population, collected from 1978 to 1980 as part of the Mini-Finland Health Survey (6), consisted of 4,423 men and women aged 40–69 years. After exclusion of 247 individuals with type 2 diabetes at baseline (i.e., fasting plasma glucose  $>7.0$  mmol/l on two occasions or  $>11.0$  mmol/l on a single occasion) and 79 individuals with missing values of serum 25OHD, the final sample size was 4,097. Data on education, smoking, leisure-time exercise, and hypertension medication were collected in a health examination (6). Height, weight, and blood pressure were measured, and blood samples were collected and stored at  $-20^{\circ}\text{C}$ . In 2003–2004, radioimmunoassay ( $^{125}\text{I}$  RIA kit; DiaSorin, Stillwater, MN) was used to assess serum 25OHD in thawed samples. The coefficient of variation for serum 25OHD varied from 8 to 13%. During 17 years of

follow-up, 187 incident type 2 diabetes cases were identified from a nationwide registry of patients receiving medication reimbursement (7), and these cases were linked to the study population by the unique social security numbers assigned to Finnish citizens.

Relative risks of type 2 diabetes between quartiles of serum 25OHD, adjusted for confounding factors, were estimated using Cox's model. The follow-up time was defined as the time from the baseline examination to the date of type 2 diabetes occurrence, death, or end of follow-up—whichever came first. Four models were defined (Table 1). The calculations were performed with SAS (version 9.1.3; SAS Institute, Cary, NC).

**RESULTS**— The proportion of men was 47%, the mean age 53 years, and the mean  $\pm$  SD serum 25OHD concentration  $43.6 \pm 19.5$  nmol/l. The correlation coefficients between serum 25OHD and type 2 diabetes risk factors were low, varying from  $-0.13$  to  $0.21$ . Participants in the highest serum 25OHD quartile had lower BMIs than those in the lowest quartile ( $26.0$  vs.  $26.4$  kg/m $^2$ ;  $P_{\text{heterogeneity}} < 0.001$ ). After adjustment for age, sex, and the month when the blood samples were collected, a statistically significant inverse association was observed between serum 25OHD concentration and incidence of type 2 diabetes. The relative risk (RR) between the highest and lowest serum 25OHD quartile was  $0.60$  (95% CI  $0.36$ – $0.98$ ;  $P_{\text{trend}} = 0.01$ ) (Table 1). This association was attenuated after further adjustments for BMI, leisure-time exer-

cise, smoking, and education (RR  $0.70$  [95% CI  $0.42$ – $1.16$ ];  $P_{\text{trend}} = 0.07$ ). The inclusion of blood pressure or exclusion of the first 5 years of follow-up did not notably alter the results.

**CONCLUSIONS**— We found a significant inverse association between serum 25OHD and risk of type 2 diabetes in the simple model. However, the association was attenuated in the multivariate analysis, adjusting for potential risk factors of type 2 diabetes. To our knowledge, this is the first cohort study investigating the association between serum 25OHD and incidence of type 2 diabetes. Our results are in line with those from the Nurses' Health Study (5), where an inverse association was observed for the intake of vitamin D supplements. We could not differentiate whether the results depended on the effect of vitamin D deficiency on  $\beta$ -cell function or on insulin resistance.

The strengths of this study include its longitudinal design and the use of serum 25OHD concentration as an indicator of vitamin D status, reflecting vitamin D obtained from diet, supplements, and cutaneous synthesis (8). Also, several limitations are related to the use of serum 25OHD. First, 25OHD levels in one individual fluctuate from one season to another. Blood samples were collected only once, and this took place throughout the year except for high summer, which means that the 25OHD levels for individuals might not be fully comparable. However, adjustment for time when blood samples were collected did not notably change the results. Second, the serum samples were stored at  $-20^{\circ}\text{C}$  before assessing 25OHD. It cannot be excluded that the 25OHD concentrations might have changed during storage (9). In one study (10), the average plasma 25OHD concentration was significantly lowered during storage at  $-18^{\circ}\text{C}$ , whereas another study (11) did not observe any notable changes. Our earlier study indicated good stability for selenium and  $\alpha$ -tocopherol (12), which gives indirect evidence about the stability of 25OHD in our serum samples. Third, we observed low average serum 25OHD concentrations compared with those of other cohorts

From the National Public Health Institute, Helsinki, Finland.

Address correspondence and reprint requests to Paul Knekt, National Public Health Institute, Mannerheimintie 166, 00300 Helsinki, Finland. E-mail: paul.knekt@ktl.fi.

Received for publication 12 February 2007 and accepted in revised form 4 July 2007.

Published ahead of print at <http://care.diabetesjournals.org> on 12 July 2007. DOI: 10.2337/dc07-0292.

**Abbreviations:** 25OHD, 25-hydroxyvitamin D.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

© 2007 by the American Diabetes Association.

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—RR of type 2 diabetes between quartiles of serum 25OHD concentration (n = 4,097)

	Serum 25OHD quartile*				P for trend
	1	2	3	4	
N	1,051	1,079	986	981	—
n	52	62	39	30	—
Mean serum 25OHD (nmol/l)	22.4	35.5	47.9	70.9	—
Model†					
1	1.00	1.18 (0.81–1.72)	0.79 (0.51–1.22)	0.60 (0.36–0.98)	0.01
2	1.00	1.15 (0.79–1.69)	0.83 (0.53–1.29)	0.70 (0.42–1.16)	0.07
3	1.00	1.10 (0.75–1.61)	0.80 (0.51–1.25)	0.67 (0.41–1.11)	0.05
4	1.00	1.23 (0.80–1.89)	0.97 (0.59–1.58)	0.58 (0.32–1.06)	0.06

Data are RR or OR (95% CI) unless otherwise indicated. \*Quartile 1, <30 nmol/l; 2, 30–41 nmol/l; 3, 42–55 nmol/l; and 4, >55 nmol/l. †Model 1: RRs adjusted for age (continuous variable), sex, and month of collecting blood samples; N (all data) = 4,097 and n (type 2 diabetes cases) = 183. Model 2: RRs were adjusted for all of the factors in model 1 plus BMI (<23, 23–24.9, 25–27.4, 27.5–29.9, or ≥30 kg/m<sup>2</sup>), leisure-time exercise (little, occasionally, or regularly), smoking (never smokers, ex-smokers, a cigar or pipe or <20, 20–29, or >29 cigarettes/day), and education (<7, 7–9, 10–12, or >12 years); N = 4,083 and n = 183. Model 3: RRs were adjusted for all of the factors in model 2 plus blood pressure (normotension, borderline hypertension, mild hypertension, or definite hypertension); N = 4,030 and n = 181. Model 4: RRs were adjusted for all of the factors in model 2, and the first 5 years of follow-up were excluded; N = 4,044 and n = 144.

(13,14). This may be due to limited sun exposure in northern latitudes and the fact that serum 25OHD concentrations may be lower when measured by radioimmunoassay rather than competitive protein binding assay (9). Finally, the incident type 2 diabetes cases were identified from a nationwide registry of patients receiving diabetes medication reimbursement (7). The register is valid, but it does not include diabetic patients undergoing dietary therapy only. Thus, the identified diabetic patients had more severe disease than diabetic patients on average.

In summary, the results are in line with the hypothesis that a high serum 25OHD concentration may reduce the risk of type 2 diabetes. Further research is needed to confirm the association and to distinguish between the independent role of vitamin D and the role of healthy dietary and lifestyle patterns in reducing the risk of type 2 diabetes.

## References

- Norman AW, Frankel JB, Heldt AM, Grodsky GM: Vitamin D deficiency inhibits pancreatic secretion of insulin. *Science* 209:823–825, 1980
- Cade C, Norman AW: Vitamin D3 improves impaired glucose tolerance and insulin secretion in the vitamin D-deficient rat in vivo. *Endocrinology* 119:84–90, 1986
- Chiu KC, Chu A, Go VL, Saad MF: Hypovitaminosis D is associated with insulin resistance and beta cell dysfunction. *Am J Clin Nutr* 79:820–825, 2004
- Scragg R, Sowers M, Bell C: Serum 25-hydroxyvitamin D, diabetes, and ethnicity in the Third National Health and Nutrition Examination Survey. *Diabetes Care* 27:2813–2818, 2004
- 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* 29:650–656, 2006
- Aromaa A, Heliövaara M, Impivaara O, Knekt P, Maatela J: *The Execution of the Mini-Finland Health Survey: Aims, Methods, and Study Population*. Aromaa A, Heliövaara M, Impivaara O, Knekt P, Maatela J, Eds. Helsinki and Turku, Finland, Publications of the Social Insurance Institution, Finland, ML:88, 1989 [in Finnish, with English summary]
- Reunanen A, Kangas T, Martikainen J, Klaukka T: Nationwide survey of comorbidity, use, and costs of all medications in Finnish diabetic individuals. *Diabetes Care* 23:1265–1271, 2000
- Standing Committee on the Scientific Evaluation of Dietary Reference Intakes FaNB. Institute of Medicine: *Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D and Fluoride*. Washington DC, National Academy Press, 2003
- Lips P, Chapuy MC, Dawson-Hughes B, Pols HA, Holick MF: An international comparison of serum 25-hydroxyvitamin D measurements. *Osteoporos Int* 9:394–397, 1999
- Norris RL, Thomas MJ, Craswell PW: Assessment of a two-step high-performance liquid chromatographic assay using dual-wavelength ultraviolet monitoring for 25-hydroxyergocalciferol and 25-hydroxycholecalciferol in human serum or plasma. *J Chromatogr* 381:53–61, 1986
- Stamp TC, Round JM: Seasonal changes in human plasma levels of 25-hydroxyvitamin D. *Nature* 247:563–565, 1974
- Knekt P, Marniemi J, Teppo L, Heliövaara M, Aromaa A: Is low selenium status a risk factor for lung cancer? *Am J Epidemiol* 148:975–982, 1998
- Looker AC, Dawson-Hughes B, Calvo MS, Gunter EW, Sahyoun NR: Serum 25-hydroxyvitamin D status of adolescents and adults in two seasonal subpopulations from NHANES III. *Bone* 30:771–777, 2002
- Rockell JE, Skeaff CM, Williams SM, Green TJ: Serum 25-hydroxyvitamin D concentrations of New Zealanders aged 15 years and older. *Osteoporos Int* 17:1382–1389, 2006