

Serum 25(OH)D and Type 2 Diabetes Association in a General Population

A prospective study

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OBJECTIVE—This study aimed to examine vitamin D status as a determinant for development of type 2 diabetes and deterioration of glucose homeostasis.

RESEARCH DESIGN AND METHODS—A random sample of the general population of Copenhagen, Denmark, was taken as part of the Inter99 study. Included were 6,405 men and women aged 30–65 years at baseline (1999–2001), with 4,296 participating in the follow-up examination 5 years later (2004–2006). Vitamin D was determined at baseline as serum 25-hydroxyvitamin D [25(OH)D]. Diabetes was defined based on an oral glucose tolerance test and a glycosylated hemoglobin (HbA_{1c}) test. Secondary outcomes included continuous markers of glucose homeostasis.

RESULTS—The risk of incident diabetes associated with a 10 nmol/L increase in 25(OH)D was odds ratio (OR) 0.91 (95% CI 0.84–0.97) in crude analyses. The association became statistically nonsignificant after adjustment for confounders, with an OR per 10 nmol/L of 0.94 (0.86–1.03). Low 25(OH)D status was significantly associated with unfavorable longitudinal changes in continuous markers of glucose homeostasis after adjustment for confounders. Fasting and 2-h glucose and insulin as well as the degree of insulin resistance increased significantly more during follow-up among those with low 25(OH)D levels compared with those with higher levels.

CONCLUSIONS—Low 25(OH)D status was not significantly associated with incident diabetes after adjustment for confounders. However, it was significantly associated with unfavorable longitudinal changes in continuous markers of glucose homeostasis, indicating that low vitamin D status could be related to deterioration of glucose homeostasis.

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Vitamin D is vital to calcium homeostasis. Deficiency may lead to bone-deforming disease in children and development of osteoporosis and osteomalacia in adults. In addition, low vitamin D status has recently been proposed as a risk factor for common chronic diseases, such as cancer, cardiovascular disease (CVD), autoimmune diseases, asthma, and allergy (1). The many roles of vitamin D

are supported by the widespread distribution of the vitamin D receptor in >36 cell types, including pancreatic β -cells, skeletal muscle, and adipose tissue (2).

For most people, the main source of vitamin D is synthesis in the skin after exposure to sunlight (1). Consequently, during the winter, inhabitants of countries at latitudes above 40°N (e.g., Denmark, 56°N) cannot produce sufficient vitamin D.

Thus, low vitamin D status is likely to be prevalent in Denmark and has been found in a general population sample (3).

Vitamin D deficiency has been linked to increased risk of type 2 diabetes, but the available epidemiological evidence is limited because most studies are cross-sectional (4). The few prospective studies measure dietary intake of vitamin D, which does not account for the large proportion of vitamin D produced in the skin, or they ascertain diabetes by self-report or medication registry data, which may result in misclassification of undiagnosed individuals and individuals who treat their disease with dietary changes only (5–11). The aim of this study was to examine the association between serum 25-hydroxyvitamin D [25(OH)D] status and the risk of incident type 2 diabetes and longitudinal changes in markers of glucose homeostasis in a large prospective study of the general population.

RESEARCH DESIGN AND METHODS

Study population

We used data from the Inter99 study, a population-based randomized controlled trial (CT00289237, ClinicalTrials.gov) on residents ($N = 61,301$) in the southern part of former Copenhagen County, which is investigating the effects of lifestyle intervention (smoking cessation, increased physical activity, and healthier dietary habits) on CVD (12). The study population was drawn from the Danish Civil Registration System and prerandomized into four groups: 1) high-intensity intervention group ($n = 11,708$ invited), 2) low-intensity intervention group ($n = 1,308$ invited), 3) control group receiving questionnaires ($n = 5,264$ invited), and 4) control group followed only in central registries ($n = 43,021$). Subjects in groups 1 and 2 were invited to participate in a health examination in 1999–2001 and included in the current observational study. The health examination included self-administered questionnaires, a physical examination, a 2-h oral glucose tolerance test (OGTT), and various blood tests, as well as a lifestyle

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consultation with personal health advice. Furthermore, participants at high risk of ischemic heart disease were included for lifestyle intervention (60%). Group 1 was offered lifestyle counseling in groups on smoking cessation and physical activity/diet during 6-month periods, and group 2 was referred to their general practitioner (12).

More specifically, groups 1 and 2 consisted of an age- and sex-stratified random sample of 13,016 men and women aged 30–65 years with 12,934 eligible for invitation. The baseline participation rate was 52.5% ($n = 6,784$). Participation at baseline was associated with obesity, nonsmoking, and fewer admissions for ischemic heart disease, CVD, and diabetes (12). After ~5 years (median: 5.5; range: 5.0–6.0) of follow-up, all participants were invited in 2004–2006 to a reexamination with essentially the same questionnaires and examinations, with 4,513 participating. Nonparticipation at follow-up was associated with the following baseline characteristics: female sex, younger age, a less healthful lifestyle, and a less favorable risk factor profile, including lower 25(OH)D levels and higher diabetes prevalence. Only participants with a northern European origin (Denmark, Norway, Sweden, Iceland, and Faroe Islands) were included ($n = 6,405$ at baseline; $n = 4,296$ at follow-up). Prior informed written consent was obtained from all participants. The study was approved by the ethical committees of Copenhagen (KA 98155) and was in accordance with Helsinki Declaration II principles.

Diabetes and markers of glucose homeostasis

Both at baseline and follow-up, all participants without a history of diabetes underwent a 2-h standardized 75-g OGTT in the morning after an overnight fast. Plasma glucose and serum insulin were measured at 0, 30, and 120 min. Glucose concentrations were analyzed by the hexokinase/glucose-6-phosphate dehydrogenase assay (Boehringer, Mannheim, Germany). Insulin levels were measured by a fluoroimmunoassay technique (Dako Diagnostics Ltd., Cambridgeshire, U.K.). Diabetes was diagnosed based on OGTT results as fasting plasma glucose ≥ 7.0 or 2-h plasma glucose ≥ 11.1 mmol/L (13). Hemoglobin A_{1c} (HbA_{1c}) also was measured for all participants for a definition of diabetes as HbA_{1c} $\geq 6.5\%$ (14). Prevalent diabetes at baseline was defined as having

diabetes according to OGTT criteria, HbA_{1c} criteria, a known history of diabetes, and/or use of diabetes medication. Incident diabetes was defined as diabetes at follow-up among those without diabetes at baseline according to the criteria given above.

Insulin resistance and pancreatic β -cell function were estimated using both the widely used homeostasis model assessment (HOMA), which is based on fasting glucose and insulin levels only (15), and the BIGTT (β -Cell Function, Insulin Sensitivity, and Glucose Tolerance Test) (16), which is based on serum insulin and plasma glucose for the entire OGTT at 0, 30, and 120 min, as well as BMI and sex. Thus, HOMA gives estimates of basal insulin secretion (HOMA-%B) and hepatic insulin resistance (HOMA-IR). The BIGTT model, on the other hand, gives estimates of the acute glucose-stimulated insulin response (BIGTT-AIR) and stimulated insulin resistance (BIGTT-IR) that reflect both hepatic and muscle insulin resistance.

Vitamin D

Fasting blood was collected beginning with initial examinations in 1999, and serum samples were stored at -20°C until analysis in 2009. Vitamin D status was measured at baseline as serum 25(OH)D by high-performance liquid chromatography. The assay was standardized using the DEQUAS external standard. Stability of 25(OH)D in serum samples under different conditions has been demonstrated, so we assumed our measurements were unaffected by storage time (17). The detection limit was 10 nmol/L, and observations below this limit were assigned 10 nmol/L ($n = 74$). 25(OH)D was categorized into <25 , ≥ 25 –50, ≥ 50 –75, and ≥ 75 nmol/L.

Self-administered questionnaire

For physical activity during leisure time, participants scored themselves as mostly sedentary, moderate activity, regular exercise, or heavy training. Smoking status was never, ex-smoker, or current smoker at <15 g/day, ≥ 15 –25 g/day, or ≥ 25 g/day. On the basis of responses to qualitative questions about intake of fruit, vegetables, fish, and saturated fat, a dietary quality score was calculated (18). The average amount and type of alcoholic beverages per week during the past 12 months was used to estimate a total alcohol consumption of 0, 1–7, >7 –14, >14 –21, or >21 standard drinks (1.5 cL or 12 g ethanol) per week. Social class

was defined based on questions regarding years of vocational training and employment status and was categorized into five classes. Family history of diabetes was defined as having a first-degree relative with diabetes. An extensive food frequency questionnaire provided information on total energy intake and specific nutrients (19). No information on supplement use was available. Drug information included insulin and other diabetes medications. Self-reported changes in dietary habits, physical activity, smoking status, and alcohol consumption were recorded at follow-up.

Physical examinations

Height and weight were measured wearing light clothes and no shoes. BMI was calculated as weight divided by height squared and categorized as underweight (<18.5 kg/m²), normal (≥ 18.5 – 25 kg/m²), overweight (≥ 25 – 30 kg/m²), or obese (≥ 30 kg/m²). Changes in weight during follow-up were calculated by subtraction and categorized as increased, unchanged, or decreased.

Statistical analyses

Data were considered observational. Statistics were performed with SAS, version 9.1 (SAS Institute Inc, Cary, NC). All reported *P* values are two-tailed, and statistical significance was defined as $P < 0.05$. Crude and adjusted associations were evaluated in a series of linear and logistic regression models. Effects from logistic regression models were reported as odds ratios (OR) with 95% CIs. Continuous outcome measures were log transformed to achieve normal distributions. β -Coefficients from linear regression models with log-transformed outcomes were back transformed and reported as percent with 95% CI. To adjust for regression to the mean and allow effect estimates to be interpreted as outcome changes from baseline to follow-up, linear regression models with outcomes measured at follow-up were adjusted for the baseline value of the outcome. Continuous 25(OH)D was tested for linear associations by including the squared term of 25(OH)D in the models. Statistical interaction effects were evaluated by including a product term between 25(OH)D and relevant covariates. *F* tests and Wald tests for single parameters were used to determine significance in regression analyses. The number of participants included in the analyses differed since complete case analyses were performed and not all

participants had complete information on all variables examined.

RESULTS—The baseline study population was 48.4% ($n = 3,099$) men and 51.6% ($n = 3,306$) women with a mean age of 46.3 years (range: 29.7–61.3). The median 25(OH)D concentration was 48.0 nmol/L, and percentiles 2.5–97.5 were 12.0–118.0 nmol/L.

25(OH)D levels strongly correlated to the season in which blood was collected, whereas dietary vitamin D intake showed only a weak and nonsignificant association with circulating 25(OH)D (Table 1). Furthermore, male sex, current smoking, low physical activity, less healthful dietary habits, obesity, low social class, and prevalent diabetes were associated with lower 25(OH)D concentrations in crude analyses (Table 1). No association with 25(OH)D concentration was observed for age, alcohol intake, total energy intake, or family history of diabetes.

The prevalence of diabetes at baseline was 10.6% ($n = 677$). During follow-up, 3.6% ($n = 141$) of participants without diabetes at baseline developed diabetes.

Low 25(OH)D status was significantly associated with a higher prevalence of diabetes at baseline both in crude analyses and after adjustment for confounders (Supplementary Table 1). Low 25(OH)D status also was significantly associated with a higher risk of incident diabetes in crude analyses (Table 2). However, the association became statistically nonsignificant after adjustment for confounders. No statistical significant interaction effects

were observed (Supplementary Table 2). Supplemental analyses of incident diabetes defined according to either OGTT or HbA_{1c} criteria showed a borderline significant association between 25(OH)D and OGTT-based diabetes (OR per 10 nmol/L was 0.92 [95% CI 0.84–1.01]; $P = 0.080$), whereas no association was observed with HbA_{1c}-based diabetes (Supplementary Table 3). Table 3 shows the associations between 25(OH)D status and longitudinal changes in continuous outcome variables adjusted for confounders. The degree of insulin resistance as assessed by the HOMA-IR and BIGTT-IR indexes increased significantly more during follow-up among those with low 25(OH)D levels compared with those with higher levels. In addition, low 25(OH)D status was significantly associated with an increase in fasting and 2-h glucose as well as in fasting and 2-h insulin during follow-up. No association was observed with HbA_{1c} or with surrogate measures of pancreatic β -cell function (Table 3).

CONCLUSIONS—Low 25(OH)D status was not significantly associated with incident diabetes after adjustment for confounders. However, it was significantly associated with unfavorable longitudinal changes in continuous markers of glucose homeostasis. Our results indicate that low vitamin D status could be related to deterioration of glucose homeostasis. The major strengths of this study include its prospective study design, which allowed for assessment of temporal relationships, and the use of a large random sample of the

general population of Denmark, a country with a high prevalence of low vitamin D status. The use of serum 25(OH)D as a measure of vitamin D status is an important strength because 25(OH)D measures total, rather than solely dietary, vitamin D. The objective definitions of diabetes by OGTT and HbA_{1c} are also important strengths. In addition, the availability of detailed information on important covariates reduced potential confounding. On the other hand, the many covariates, together with the relatively low number of incident cases, may have diminished the power and contributed to the nonsignificant results in the fully adjusted models with incident diabetes. The major limitation of the study is its observational design, which despite the detailed covariate information, cannot exclude residual confounding. Another weakness was the single serum 25(OH)D measurement, which did not account for individual seasonal variation. This potential random misclassification could contribute to the low power and cause an underestimation of the true association between 25(OH)D and diabetes risk. The offer of intervention may also have influenced the results, and again, any beneficial effects of the lifestyle intervention program would cause an underestimation of the true association. Finally, loss to follow-up may have introduced bias, and the fact that participants were only of northern European origin limits the generalization of the results.

Previous cross-sectional and case-control studies find an inverse association between serum 25(OH)D and type 2

Table 1—Baseline characteristics of the study population by 25(OH)D status

Characteristic	Participant			P value‡
	All ($N = 6,405$)†	25(OH)D <50 nmol/L (52.2%, $n = 3,207$)	25(OH)D \geq 50 nmol/L (47.8%, $n = 2,939$)	
Men	48.4 (3,099)	50.5 (1,620)	47.2 (1,386)	0.009
Current smoker	35.7 (2,274)	38.5 (1,229)	32.5 (950)	<0.001
Mainly sedentary physical activity	20.7 (1,300)	22.4 (705)	18.6 (538)	<0.001
Less healthful dietary habits	16.0 (990)	16.7 (518)	15.0 (428)	0.03
Low social class (class 1+2)	10.8 (639)	11.5 (340)	9.8 (267)	0.09
Family history of diabetes	17.9 (1,143)	18.3 (588)	17.5 (514)	0.39
Blood collection in January to March	21.7 (1,388)	29.4 (942)	13.6 (400)	<0.001
Diabetes	10.6 (677)	12.3 (394)	8.6 (253)	<0.001
Age (years), mean (SD)	46.26 (7.91)	46.37 (7.85)	46.12 (7.99)	0.40
BMI (kg/m ²), mean (SD)	26.31 (4.65)	26.65 (4.90)	25.87 (4.17)	<0.001
Alcohol (drinks/week), mean (SD)	10.46 (13.37)	10.55 (13.62)	10.44 (13.19)	0.45
Total energy intake (kJ/day), mean (SD)	9,764.13 (3,415.10)	9,780.03 (3,312.44)	9,765.70 (3,463.01)	0.59
Dietary vitamin D intake (μ g/day), mean (SD)	3.60 (2.42)	3.58 (2.35)	3.65 (2.49)	0.98

Data are % (n) unless otherwise indicated. † N_{total} may differ because of missing values. ‡P value for differences between 25(OH)D groups below and above 50 nmol/L were tested by χ^2 test and Kruskal-Wallis test.

Table 2—Multiple logistic regression analyses of the prospective association between baseline 25(OH)D status and 5-year diabetes incidence

25(OH)D status (nmol/L)	Diabetes incidence % (n/n _{total}) [†]	Risk of incident diabetes, OR (95% CI)			
		Crude	Adjusted (1) [‡]	Adjusted (2)	Adjusted (3)
<25	4.6 (22/477)	1.96 (1.02–3.78)	1.65 (0.83–3.27)	1.80 (0.83–3.88)	1.65 (0.75–3.63)
≥25–50	4.2 (60/1,415)	1.80 (1.03–3.15)	1.59 (0.89–2.86)	1.55 (0.80–3.00)	1.43 (0.73–2.80)
≥50–75	2.9 (35/1,201)	1.22 (0.67–2.22)	1.20 (0.69–2.22)	1.40 (0.70–2.77)	1.25 (0.62–2.52)
≥75	2.4 (16/666)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
<i>P</i> _{trend} value		0.008	0.063	0.13	0.18
Per 10 nmol/L increase		0.91 (0.84–0.97)	0.93 (0.86–1.00)	0.93 (0.86–1.02)	0.94 (0.86–1.03)
<i>P</i> value		0.006	0.059	0.11	0.18

[†]Incident diabetes at follow-up was defined as having diabetes according to OGTT criteria, HbA_{1c} criteria, a known history of diabetes, and/or use of diabetes medication among those without diabetes at baseline. [‡]Models were adjusted for (1) season of blood collection, sex, age, family history of diabetes, BMI, and change in weight during follow-up; (2) plus leisure time physical activity, dietary habits, alcohol consumption, smoking status, total energy intake, and social class; (3) plus randomization group and self-reported changes in dietary habits, physical activity, smoking status, and alcohol consumption during follow-up.

diabetes as well as markers of adverse glucose homeostasis in many, although not all, studies (4,20). The major drawback of these studies is the potential for reverse causation (i.e., diabetes or its early determinants causing low vitamin D status rather than vice versa). Moreover, many studies do not adjust for important confounders. Previous prospective studies are inconsistent. The Nurses' Health Study and Women's Health Study both reported association of low supplemental and dietary vitamin D intake with increased incident diabetes risk (5,11), whereas no association was observed in a Japanese cohort (8). However, those studies are limited by self-reports of

dietary vitamin D intake, which do not consider the large proportion of vitamin D produced in the skin. A prospective study based on the Framingham Offspring cohort associates a predicted 25(OH)D score with incident type 2 diabetes (21). However, that study is limited because 25(OH)D was not actually measured.

Four studies previously examined the prospective association of serum 25(OH)D and diabetes incidence (6,7,9,10). One used pooled data from two Finnish cohorts, the Finnish Mobile Clinic Health Examination Survey and the Mini-Finland Health Survey, and finds an inverse association in men, but not in women (6).

A major limitation of the study is the ascertainment of diabetes from registry data of patients receiving reimbursements for type 2 diabetes medication, resulting in misclassification of undiagnosed individuals or those treated with dietary changes only. The second study also used registry data and finds a significant inverse association between 25(OH)D and incidence of diabetes (9). That study had a relatively short follow-up time (average 1.3 years) and was restricted to patients with measured 25(OH)D. The third study was nested within the Nurses' Health Study and, thus, restricted to women; it finds that higher levels of plasma 25(OH)D were associated with lower risk of self-reported incident type 2 diabetes (7). In contrast, a fourth study restricted to older women does not find an association of 25(OH)D with self-reported diabetes in a post hoc analysis of three nested case-control studies within the Women's Health Initiative Clinical Trials and Observational Study (10).

Finally, in agreement with our results, a previous study reports that baseline serum 25(OH)D was predictive of future continuous distribution of glycemic status and insulin resistance in a nondiabetic population after 10 years of follow-up. That study lacked statistical power to examine clinical outcomes and was restricted by sample size to quantitative traits (22).

Results from randomized trials of the effect of vitamin D supplementation on glucose homeostasis are inconsistent, but several studies had other primary end points and, therefore, were underpowered for our outcomes of interest (23–25). In addition, many of the studies were performed in patients with diabetes. As yet, no randomized intervention trials specifically designed to

Table 3—Multiple linear regression analyses of association between baseline serum 25(OH)D and longitudinal changes in continuous markers of glucose homeostasis during 5 years of follow-up

Outcome	Change in outcome during follow-up in % per 10 nmol/L increase in baseline 25(OH)D, % (95% CI)	<i>P</i> value
HbA _{1c}	−0.04 (−0.09 to 0.02)	0.18
HOMA-IR	−0.91 (−1.57 to −0.24)	0.008
BIGTT-IR	−1.04 (−1.57 to −0.50)	<0.001
HOMA-%B	0.21 (−0.37 to 0.80)	0.47
BIGTT-AIR	−0.27 (−0.19 to −0.72)	0.25
Fasting glucose	−0.20 (−0.41 to −0.19)	<0.001
2-h glucose	−0.80 (−1.14 to −0.46)	<0.001
Fasting insulin	−0.63 (−1.24 to −0.01)	0.045
2-h insulin	−1.66 (−2.56 to −0.75)	<0.001

β -Coefficients from linear regression models were back transformed and reported as percent because of log transformation of the outcome variables. Regression models with outcomes measured at follow-up were adjusted for baseline outcome values to adjust for regression to the mean and to allow effect estimates to be interpreted as outcome changes from baseline to follow-up. Regression models were adjusted for sex, age, BMI, season of blood collection, leisure time physical activity, dietary habits, alcohol consumption, smoking status, total energy intake, social class, family history of diabetes, randomization group, change in weight, and self-reported changes in dietary habits, physical activity, smoking status, and alcohol consumption during follow-up. Subjects with a known history of diabetes did not undergo an OGTT and, therefore, were not included in the analyses.

test the effect of vitamin D supplementation on incident diabetes in healthy individuals have been published. However, one study reports no effect of a relatively low dose (400 IU) of vitamin D supplementation on incident diabetes after 7 years of follow-up, in post hoc analysis (24).

The mechanism by which vitamin D deficiency and type 2 diabetes may be related is not well understood. Experimental evidence from animal and in vitro studies suggests that vitamin D may preserve glucose tolerance through effects on insulin secretion and sensitivity. These effects may be caused by effects on intracellular calcium, regulation of insulin receptor expression in peripheral tissues, or increased resilience of β -cells to the systemic inflammation seen in type 2 diabetes (23).

In conclusion, low 25(OH)D status was not significantly associated with incident diabetes after adjustment for confounders. However, it was significantly associated with unfavorable longitudinal changes in continuous markers of glucose homeostasis, indicating that low vitamin D status could be related to deterioration of glucose homeostasis. This study emphasizes the need for randomized intervention trials specifically designed to assess whether vitamin D supplementation effectively decreases the risk of developing diabetes in high-risk individuals.

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L.L.N.H. contributed to development of the hypothesis and study design, was responsible for statistical analyses, wrote the first draft of the manuscript, and coordinated the completion of the manuscript. B.H.T. and M.F. were responsible for the measurement of vitamin D, contributed to the interpretation of results, and revised the manuscript. T.J. contributed to development of the hypothesis and study design, was the principal investigator of the Inter99 study and responsible for data collection, contributed to the interpretation of results, and revised the manuscript. C.G., J.S., and D.R.W. provided expertise within the field of diabetes, contributed to the interpretation of results, and revised the manuscript. L.O. provided expertise within the field of vitamin D, contributed to the interpretation of results, and revised the manuscript. A.L. contributed to development of the hypothesis and study design, was responsible for the statistical analyses, contributed to the interpretation of results, and revised the manuscript. All authors approved the final version of the manuscript. L.L.N.H. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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