Maternal Serum Levels of 25-Hydroxy-Vitamin D During Pregnancy and Risk of Type 1 Diabetes in the Offspring

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Previous studies indicate reduced risk of type 1 diabetes after intake of vitamin D supplements during pregnancy or early childhood. We aimed to test whether lower maternal serum concentrations of 25-hydroxy-vitamin D (25-OH D) during pregnancy were associated with an increased risk of childhood-onset type 1 diabetes. In this case-control study nested within a cohort of 29,072 women in Norway, 25-OH D levels were measured using a radioimmunoassay on samples from late pregnancy in 109 women delivering a child who developed type 1 diabetes before 15 years of age (case subjects) and from 219 control women. Dividing the levels of maternal 25-OH D into quartiles, there was a trend toward a higher risk of type 1 diabetes with lower levels of vitamin D during pregnancy. The odds of type 1 diabetes was more than twofold higher for the offspring of women with the lowest levels of 25-OH D compared with the offspring of those with levels above the upper quartile. Given future replication in independent cohorts, our findings provide support for the initiation of a randomized intervention trial to prevent type 1 diabetes in children by enhancing maternal 25-OH D status during pregnancy. ***Diabetes*** **61:175–178, 2012**

Type 1 diabetes is an autoimmune disease that is one of the most common chronic diseases during childhood. With the exception of certain susceptibility genes, the causes of type 1 diabetes are essentially unknown. During recent years, there has been increasing interest in the immunomodulating actions of vitamin D in type 1 diabetes and other autoimmune diseases (1,2).

The classical function of vitamin D is related to calcium and phosphate homeostasis and bone mineralization. Research over the last 30 years has revealed that vitamin D receptors are present in almost all body tissues, and there is increasing evidence that vitamin D is involved in a variety of processes in the body, including immunity (3). Furthermore, immune cells, such as macrophages and dendritic cells as well as activated T cells and B cells, express the enzyme 1α-hydroxylase (CYP27B1), which is responsible for converting the major circulating metabolite of vitamin D, 25-hydroxy-vitamin D (25-OH D), to 1α,25-dihydroxyvitamin D [1,25-$(OH)_2$D], the active form of vitamin D (4). In autoimmune diseases, vitamin D is thought to have a protective effect, probably by enhancing immunologic tolerance.

Development of the immune system starts in early fetal life. Earlier studies in humans based on parental reports of vitamin D supplement use suggest that vitamin D supplementation during the 1st year of life may be associated with a reduced risk of type 1 diabetes (5,6). Exogenous protective factors such as vitamin D may be of importance in utero. Some studies indicate an association between maternal intake of vitamin D during pregnancy and the development of diabetes-associated autoantibodies in the children (7,8). On the other hand, a study from Finland (9) did not find such associations with either autoantibodies or overt diabetes in the offspring. We have previously found that the use of cod liver oil (which is an important source of vitamin D in Norway) during the 1st year of life (10), and by the mother during pregnancy (11), was less frequent in cases of childhood-onset type 1 diabetes than in control subjects. In addition to oral intake of vitamin D, the conversion of 7-dihydrocholesterol into vitamin D_3 in the skin upon exposure to sunlight is an important source of vitamin D. The fact that the highest incidence of type 1 diabetes (with some exceptions) exists in the northern part of the world (12), where sunshine hours are reduced for several months during the year, has been taken as support for the hypothesis that insufficient vitamin D status may increase the risk of type 1 diabetes.

Serum levels of 25-OH D are an accepted measure of the total vitamin D status, regardless of the source of vitamin D. In the current study, we tested whether lower maternal serum 25-OH D concentrations during late pregnancy were associated with an increased risk of childhood-onset type 1 diabetes in the offspring.

RESEARCH DESIGN AND METHODS

We designed a case-control study nested within a cohort of 35,940 women who gave birth in Norway between 1992 and 1994. All pregnant women in 11 of 19 counties in Norway participated. The purpose of the original cohort was to study *Toxoplasma gondii* infection in pregnancy (13). One to four blood samples was collected from each woman throughout pregnancy in their respective primary health care centers at regular maternity check-ups. All samples were sent to the Norwegian Institute of Public Health in Oslo. After testing for *T. gondii* antibodies, in accordance with the original study objective, the sera were stored at −20°C. Subsequent to the toxoplasmosis study, all women were asked by mail to participate in additional research, of whom 29,072 women consented. We obtained information on the pregnancy outcomes by linkage to the Medical Birth Registry of Norway. This registry includes information on all deliveries in Norway after 16 weeks of gestation since 1967 (14).

To identify children born within the cohort who developed type 1 diabetes, linkage to the Norwegian Childhood Diabetes Registry was performed. Linkages were conducted using the unique 11-digit personal identification number

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given to every person in Norway. Children with newly diagnosed type 1 diabetes are reported to the Norwegian Childhood Diabetes Registry after a written consent from their parents, and 92% of all children with type 1 diabetes in Norway younger than 15 years of age are included (15). In total, the offspring of 119 pregnancies were identified to have type 1 diabetes. Serum samples from 109 of these pregnancies were available for analysis (case subjects). Among 280 control subjects, in a computer-generated random sample among the non–case subjects in the cohort, 219 subjects had an available serum sample of sufficient quality for 25-OH D analysis. We analyzed the latest available serum sample taken during pregnancy from each woman in both case and control subjects, mainly from the last trimester (Table 1).

The study was approved by the regional committee for medical and health research ethics and by the data inspectorate.

Laboratory analyses of 25-OH D. Sera were analyzed at the Hormone Laboratory at Oslo University Hospital, Aker, Norway, using a radioimmunoassay (DiaSorin, Stillwater, MN). The serum levels of 25-OH D are expressed as nanomoles per liter, and the reference range for the normal adult population is 37–131 nmol/L. The intra-assay coefficient of variation is 6%. The total coefficient of variation is 13% at low levels (38 nmol/L), 16% at middle levels (75 nmol/L), and 14% at high levels (148 nmol/L). The Hormone Laboratory participates in the following external quality assessment schemes that include 25-OH D determination: the Vitamin D External Quality Assessment Scheme and Labquality.

Statistical analyses. Before initiating this study, we estimated (based on the expected number of cases in the cohort) that we would have at least 80% power to obtain a significant result with a two-tailed test for trend assuming a logit-linear dose response, if the true odds ratio (OR) comparing the upper and lower quartiles of 25-OH D was ≤0.3. Statistical analyses were carried out using SPSS version 15.0 (SPSS, Chicago, IL) and Stata version 11.0 (Stata, College Station, TX). The main analysis was conducted using logistic regression, with the 25-OH D divided into quartiles (derived from the values in the control group), and test for trend was obtained by including 25-OH D as a continuous variable. A 95% CI for the OR excluding 1.00 or a P value <0.05 was considered statistically significant. We adjusted for potential confounders by including these factors in the logistic regression model. As an additional analysis to assess the dose-response relationship, we plotted predicted values from logistic regression models with fractional polynomials with default settings in Stata version 11 (16).

TABLE 1
Description of study population

	Case subjects*	Control subjects
n	109	219
Age of child at diagnosis of type 1 diabetes (years) [means (SD)]	9.0 (3.6)†	
Sex of child, female [n (%)]	63 (57.8)	98 (44.7)
Mother's age at delivery (years) [means (SD)]	28.2 (5.7)	28.3 (5.2)
Previous pregnancies [n (%)]‡		
0	46 (42.2)	91 (41.6)
1	42 (38.5)	92 (42.0)
≥2	20 (18.3)	35 (16.0)
Gestational week of blood sample [median (interquartile range)]	37 (22–38)	37 (24–38)
Caesarean section [n (%)]	13 (11.9)	31 (14.2)
Pregestational diabetes [n (%)]	1 (0.9)	0 (0)
Gestational diabetes [n (%)]	2 (1.8)	1 (0.5)
Season of blood sample [n (%)]§		
January through March	27 (24.8)	49 (22.4)
April through June	32 (29.4)	55 (25.1)
July through September	26 (23.9)	69 (31.5)
October through December	24 (22.0)	46 (21.0)
Region of residence [n (%)]‖		
Northern Norway	9 (8.3)	19 (8.7)
Central Norway	27 (24.8)	66 (30.1)
Western Norway	30 (27.5)	55 (25.1)
Eastern Norway	43 (39.4)	79 (36.1)
Season of birth [n (%)]¶		
January through March	28 (25.7)	51 23.3)
April through June	34 (31.2)	55 (25.1)
July through September	26 (23.9)	60 (27.4)
October through December	21 (19.3)	53 (24.2)

*Women who delivered a child who developed type 1 diabetes before 15 years of age. †Information on age at diagnosis is missing in 11 case subjects. ‡Information on previous pregnancies is missing in one case and one control subject. §Global test for association: P = 0.52. ‖Global test for association: P = 0.41. ¶Global test for association: P = 0.22.

RESULTS

Characteristics of the study population are shown in Table 1. The mean age at diabetes diagnosis in the children was 9.0 years (SD 3.6). No significant differences were observed between case and control subjects regarding age and parity of the women, gestational week of the blood sample, frequency of caesarean section, or maternal diabetes before pregnancy. Unexpectedly, there were significantly more girls among the case children than in the control subjects.

The mean serum levels of 25-OH D were significantly lower in case subjects than in control subjects (65.8 vs. 73.1 nmol/L) (Table 2), and there was an expected seasonal variation in 25-OH D levels (Fig. 1). The relationship between the serum levels of 25-OH D during pregnancy and the risk of childhood-onset type 1 diabetes in offspring is presented in Table 2. The odds of type 1 diabetes was more than twofold higher for children born from women with a 25-OH D level in the first quartile compared with women with a 25-OH D level in the fourth quartile, and the linear trend was significant, even after adjustment for sex and season of blood draw (Table 2). Additional adjustments were done for all variables in Table 1, and the results remained similar and significant (OR for the first vs. fourth quartile 2.39 [95% CI 1.07–5.31]; P (trend) = 0.032, data not shown). Figure 2 shows the distribution of maternal 25-OH D during pregnancy in case mothers and in control mothers. The two subjects with the highest 25-OH D values were case subjects. This resulted in a suggested upward trend in risk at the highest values of 25-OH D, but this should be interpreted with caution because of few observations and wide CIs (Supplementary Fig. 1). We further formally compared the model in which 25-OH D was modeled linearly to models with a square and a cubic term and found that the polynomial regression models did not provide any significantly better fit than the linear one ($P \geq 0.23$, likelihood ratio tests).

DISCUSSION

This study is to our knowledge the first to report an association of lower levels of 25-OH D during pregnancy with a higher risk of type 1 diabetes in the offspring. Our population-based study is nested within a cohort of ~30,000 pregnant women, and selection bias is likely to be a smaller problem than in studies where control subjects are selected from other sources than the case subjects. Nevertheless, we observed a higher than expected proportion of girls among the case subjects and a lower than expected proportion of girls among control subjects. Because the case subjects were identified by linkage to the nearly complete nationwide registry and control subjects were selected by computer-generated random sampling, we do not have any explanation for this other than random chance. At any rate, statistical adjustment for sex did not influence our main results.

As a result of the long follow-up time, we were able to identify almost all children of the original maternal cohort

TABLE 2
Mean values of maternal serum 25-OH D in case and control subjects and the relationship between serum 25-OH D during pregnancy and the risk of childhood-onset type 1 diabetes in offspring

	Case subjects*	Control subjects	Unadjusted OR (95% CI)	Adjusted OR (95% CI)†
n	109	219		
25-OH D (nmol/L) [means (SD)]‡	65.8 (26.5)	73.1 (27.2)		
25-OH D (quartiles) [*n* (%)]				
Quartile 1: ≤54 nmol/L	39 (38.5)	55 (25.1)	2.25 (1.14–4.46)	2.38 (1.12–5.07)
Quartile 2: >54 and ≤69 nmol/L	31 (28.4)	57 (26.0)	1.73 (0.86–3.48)	1.78 (0.85–3.74)
Quartile 3: >69 and ≤89 nmol/L	22 (20.2)	53 (24.2)	1.32 (0.63–2.76)	1.35 (0.63–2.89)
Quartile 4: >89 nmol/L	17 (15.6)	54 (24.7)	1.0 (reference)	1.0 (reference)
Test for trend (continuous)			$P = 0.022$	$P = 0.031$

Quartiles were derived from values in the control group. *Women who delivered a child who developed type 1 diabetes before 15 years of age. †Adjusted for season of blood sample (January through March, April through June, July through September, and October through December) and sex of the child. ‡Independent samples *t* test gives $P = 0.021$.

who developed type 1 diabetes during childhood. The long follow-up gives an equivalent long storage time for the serum samples, but 25-OH D seems to be stable over a long period of time (17,18). Even in the event that some degradation has occurred, this is not likely to differ according to whether the unborn child later develops type 1 diabetes. Furthermore, the expected seasonal pattern is supportive of valid 25-OH D measurements. In a few instances, we could not obtain a 25-OH D measurement, partly because the serum sample had been used in other studies and partly because the serum sample had dried up and was unsuitable for analyses.

Previous studies have been based on the reported intake of vitamin D via food or supplements during pregnancy, which is subject to recall bias and other measurement errors, including the fact that contributions to vitamin D status from sun exposure are not accounted for. Our findings are consistent with small cohort studies in which maternal reported intake of vitamin D via food or dietary supplements during pregnancy was associated with a decreased risk of diabetes-related autoantibodies in offspring (7,8). Another larger study did not find any association between reported dietary intake of vitamin D by pregnant women and the risk of islet autoimmunity or early clinical diabetes in children (9). These previous studies are not directly comparable with ours, because we have not only measured maternal 25-OH D but also followed the children until they were 15 years of age, with respect to the onset of clinical type 1 diabetes. Intake of vitamin D and levels of 25-OH D during childhood were measured in a newly published study from the Diabetes Autoimmunity Study in the Young (DAISY), and they did not find any relationship to the risk of islet autoimmunity or progression to type 1 diabetes (19). This study has no information on fetal or maternal levels of 25-OH D.

We did not have separate information on the intake of vitamin D from food or supplements or on sun exposure, but measuring the serum levels of 25-OH D should be superior to questionnaires in that they reflect the integrated vitamin D status of an individual. Blood sampling in our study was not standardized for season, because this would not be possible in practice, but we adjusted for this in the statistical analysis. Previous literature suggests that the serum levels of 25-OH D vary little over gestational age (20), and our data (not shown) suggested that the season of the blood collection contributed more to between-sample variation than did gestational age.

We did not have access to DNA for genotyping of the participants in our study, but previous studies have not indicated that associations between measures of vitamin D and type 1 diabetes differed by HLA type (8,21). Recent

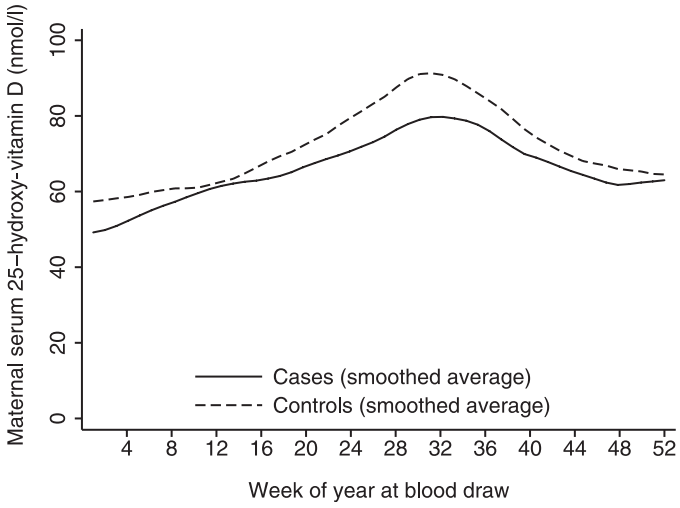

FIG. 1. Smoothed average serum levels of 25-OH D versus season of blood collection in pregnant women whose child later developed type 1 diabetes (case subjects, solid line) and control subjects (dotted line). Smoothing was conducted using kernel-weighted local polynomial regression in Stata version 11.

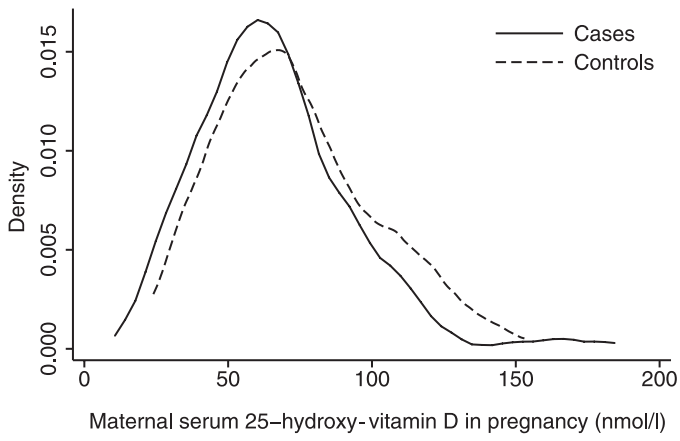

FIG. 2. Distribution of maternal 25-OH D during pregnancy in case mothers whose child later developed type 1 diabetes and in control mothers. Two case subjects and one control subject had a 25-OH D value >150 nmol/L.

studies have identified weak associations between polymorphisms in genes related to vitamin D metabolism and type 1 diabetes (22,23), but it is not likely that these polymorphisms in the mother or offspring would materially influence the association between maternal 25-OH D status and the risk of type 1 diabetes in the children. Despite some initial indications of a potential association between vitamin D receptor polymorphisms and the risk of type 1 diabetes, a systematic review concluded that there was no evidence to support such an association for any of the studied polymorphisms (24).

The exact mechanisms by which vitamin D acts in a potential protective manner against type 1 diabetes are not known, but many steps in the immune process may be altered under the influence of $1\alpha,25(OH)_2D$ in the direction of better tolerance, which is of general importance in preventing autoimmune processes (3,25,26). For instance, maturation of dendritic cells has been shown to be inhibited, and the cytokine production from T-helper cells shifted toward an anti-inflammatory pattern.

In conclusion, our results indicate an association between lower maternal serum concentrations of 25-OH D during pregnancy and increased risk of type 1 diabetes development in childhood. Given future replication in independent cohorts, this could provide support for the initiation of a randomized intervention trial to prevent type 1 diabetes in children by enhancing maternal 25-OH D status during pregnancy.

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I.M.S. wrote the manuscript. I.M.S. and L.C.S. contributed to the data analysis. G.J., A.E., and L.C.S. contributed to the conception and planning. P.A.J. contributed to organizing the original cohort study. P.A.T. was responsible for the 25-OH D assay. L.C.S. is the guarantor for this article. All authors commented on the manuscript.

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