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RESEARCH ARTICLE

Vitamin D-binding protein and 25-hydroxyvitamin D during pregnancy in mothers whose children later developed type 1 diabetes

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Abstract

Background Levels of 25-hydroxyvitamin D (25-OH D) during late pregnancy have been linked to type 1 diabetes risk in the offspring. Vitamin D-binding protein increases in concentration during pregnancy. We aimed to test whether concentrations of vitamin D-binding protein and 25-OH D throughout pregnancy differed between women whose offspring later developed type 1 diabetes (cases) and controls.

Methods A nested case–control study was conducted within a cohort of pregnant women from all over Norway in 1992–1994. Offspring registered in The Norwegian Childhood Diabetes Registry, diagnosed with type 1 diabetes before age 15, defined the case women, giving 113 cases in the study. Two hundred twenty controls were randomly selected within the same cohort. One to four serum samples from each participant drawn at different time points during pregnancy were analysed for vitamin D-binding protein and 25-OH D by radioimmunoassay.

Results Vitamin D-binding protein and 25-OH D significantly increased by gestational week ($p < 0.001$) and tended to be lower in cases than in controls, −0.27 μmol/L (95% CI −0.57, 0.03) and −5.01 nmol/L (95% CI −8.03, −0.73), respectively. While first and second trimester concentrations of vitamin D-binding protein and 25-OH D alone were not significantly different, lower third trimester concentrations tended to be associated with higher risk of type 1 diabetes in the offspring, albeit at borderline significance after mutual adjustment.

Conclusions In this first study of maternal vitamin D-binding protein measured throughout pregnancy and risk of type 1 diabetes in offspring, lower concentration, particularly in the third trimester, tended to be associated with type 1 diabetes. Copyright © 2016 John Wiley & Sons, Ltd.

Keywords 25-hydroxyvitamin D; biomarkers; epidemiology; pregnancy; type 1 diabetes; vitamin D-binding protein

Introduction

Vitamin D, well-known for its action in bone mineral metabolism, is now established as a contributor in different other cells and tissues [1,2]. Vitamin D receptors (VDR) are found in pancreatic β-cells as well as immune cells like macrophages and lymphocytes [3]. In addition, the enzyme 1α-hydroxylase (CYP27B1), which converts 25-hydroxyvitamin D (25-OH D) to the biological

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active 1,25-$(OH)_2$ D, is present in both immune cells and in the pancreatic islet cells [4], suggesting that the need for pre-conversion to 1,25-$(OH)_2$ D in the kidneys are circumvented in these cells [2]. Vitamin D metabolites are fat-soluble and are transported in the blood bound to vitamin D-binding protein (DBP, also known as group-specific component, Gc-protein). Vitamin D-binding protein is a multifunctional protein and may also affect the immune system [5]. Vitamin D-binding protein acts for instance as an activator of macrophages and has been associated to surfaces of T- and B-cells and pancreatic acinar cells [5,6]. Vitamin D or insufficiency of the vitamin has in some studies been suggested to play a role in type 1 diabetes development [7,8]. A recent study found lower concentrations of DBP in patients with type 1 diabetes when compared with their first-degree relatives and healthy controls [9]. Vitamin D-binding proteins are known to increase throughout pregnancy [10] and may influence on total 25-OH D level. We have previously found an association between lower maternal 25-OH D concentrations in late pregnancy and higher risk of autoimmune type 1 diabetes in the offspring [11], whereas Miettinen *et al* found no association between childhood type 1 diabetes an maternal 25-OH D in early pregnancy [12].

Development of the immune system starts in early foetal life, but in the second and third trimester, the spleen and lymph nodes become more active as lymphoid organs by forming and educating both T- and B-lymphocytes [13]. The positive and negative selection of lymphocytes during late foetal life is the start of a good defence against infections and tolerance to own tissue. In most cases of type 1 diabetes, autoantibodies are present in blood months to years before diagnosis indicating a process starting long before the clinical presentation of the disease [14,15]. The foetal period of life when the immune system is going through an extensive education, with positive and negative selection of T- and B- lymphocytes might be a vulnerable period for factors stimulating immune tolerance. Prenatal factors have previously been described to influence later risk of type 1 diabetes [16].

In this study, we investigated for the first time both 25-OH D and DBP throughout pregnancy by repeated measurements to examine if there were any differences in concentrations between women whose offspring later developed type 1 diabetes compared with controls.

Materials and methods

Subjects and samples

In a cohort of 35 940 pregnant women who gave birth in Norway during 1992–1994, serum samples were collected at health care centres at regular maternity check-ups [17]. The serum samples taken throughout pregnancy were stored in a biobank at −20 °C after collection. Subsequently, 29 072 women consented to participate in further research. The women were equally distributed from the entire country. Their offspring were identified in the Medical Birth Registry of Norway, and children who developed type 1 diabetes before 15 years of age were identified in the Norwegian Childhood Diabetes Registry. The completeness of the registry is estimated to 91% [18].

The offspring of 119 women were identified to have type 1 diabetes. One or more serum samples from 113 of these women were available for analysis (cases). From 318 controls, randomly selected among non-cases in the cohort, samples were available from 220 women (Figure S1). One control was subsequently excluded because of chronic kidney disease, leaving 220 controls. The samples were drawn mainly from first maternity check-up (week 8–12), around week 22 and week 38, and in this study categorized as samples from first, second or third trimester of pregnancy (Table 1). In both cases and controls, >80% of the women were represented with two or three blood samples from throughout pregnancy. Three per cent had four samples, and in 16%, only one sample was available.

The study was approved by the Regional Committee for Medical and Health Research Ethics and by the Norwegian Data Protection Authority.

Laboratory analyses

Serum 25-OH D was measured using a competitive radioimmunoassay (DiaSorin, Stillwater, MN, USA). The reference range for the normal adult population in Norway is 37–131 nmol/L. The intra-assay coefficient of variation (CV) was 6%, and the total CV was 13% at low values (38 nmol/L), 16% at middle values (75 nmol/L) and 14% at high values (148 nmol/L). Vitamin D-binding protein were analysed by an in house competitive radioimmunoassay. The reference range was 3.0–5.3 μmol/L. The intra-assay CV was 5%, the total CV at low concentrations (3.7 μmol/L) was 17%, and at high concentrations (6.4 μmol/L) it was 16%. Assays were run at the Hormone Laboratory at Oslo University Hospital, which participates in the Vitamin D External Quality Assessment Scheme and Labquality.

Statistical analyses

Statistical analyses were carried out using SPSS version 18 (SPSS, Chicago, IL, USA) and STATA version 12.0 (Stata, College Station, TX, USA). We modelled repeated measures of DBP, and 25-OH D in separate linear mixed

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Table 1. Characteristics of the study sample

	Case subject [a]	Control subject[a]	*p*-value
n of subjects	113	220	
Age of child at diagnosis of type 1 diabetes (years) [mean (SD)]	9.0 (3.5)[b]		
Sex of child, female [*n* (%)]	65 (57.5)	98 (44.3)	0.03
Mother's age at delivery (years) [mean (SD)]	28.2 (5.6)	28.3 (5.3)	0.89
Caesarean section [*n* (%)]	15 (13.3)	31 (14.0)	0.85
Maternal diabetes [*n* (%)][c]	5 (4.4)	1 (0.5)	0.05
Previous pregnancies [*n* (%)][d]			0.79[e]
0	47 (41.6)	93 (42.1)	
1	45 (39.8)	92 (41.6)	
> = 2	20 (17.7)	35 (15.8)	
Season of delivery [*n* (%)]			0.25[e]
January–March	29 (25.7)	51 (23.1)	
April–June	34 (30.1)	55 (24.9)	
July–September	27 (23.9)	61 (27.6)	
October–December	23 (20.4)	54 (24.4)	
Gestational week of blood sample [median (IQR)]			
First trimester (0–12 weeks)	9 (7–10)	9 (8–10.8)	0.63
Second trimester (13–27 weeks)	22 (21–24)	22 (20–23)	0.14
Third trimester (28 weeks +)	37 (36–38)	38 (37–38)	0.54
Season of blood sample [*n* (%)]	*n* = 260	*n* = 521	0.20[f]
January–March	67 (25.8)	148 (28.4)	
April–June	63 (24.2)	129 (24.8)	
July–September	63 (24.2)	136 (26.1)	
October–December	67 (25.8)	108 (20.7)	
Region of residence [*n* (%)]			
Northern Norway	9 (8.0)	19 (8.6)	0.43[e]
Central Norway	29 (25.7)	67 (30.3)	
Western Norway	31 (27.4)	56 (25.3)	
Eastern Norway	44 (38.9)	79 (35.7)	

[a]Cases: women who delivered a child who developed type 1 diabetes before 15 years of age. Controls: random sample of women in the cohort whose child had not developed type 1 diabetes by 15 years of age.
[b]Information on age at diagnosis was missing in 17 case subjects.
[c]Some five women in the case group and one woman in the control group were reported to have diabetes during pregnancy, unknown type.
[d]Information on previous pregnancies was missing in one case and one control subject.
[e]Global test for association.
[f]Global test for association all samples: $p = 0.20$ (within first trimester $p = 0.62$, second trimester $p = 0.11$, third trimester $p = 0.98$).
IQR, interquartile range; SD, standard deviation.

models with random intercept and random slope for week of gestation at blood sampling specific for each woman. This was performed using the xtmixed command in STATA, with unstructured covariance structure and maximum likelihood estimation. Case–control status was entered as an independent variable, together with week of gestation and other potential confounding variables such as season of blood sampling, maternal diabetes and sex of the child. For DBP, we also included a square term for week of gestation to model the non-linear relation. Possible interaction between case/control status and week of gestation was tested. The analyses for associations between DBP and type 1 diabetes, and between 25-OH D and type 1 diabetes development were conducted using logistic regression models with DBP and 25-OH D divided into quartiles based on the values from the control group. Samples were grouped into trimesters. Potential confounders (see previous text) were included in the models. Test for linear trend was obtained using 25-OH D and DBP as continuous variables in the logistic regression models, in which the possible interaction between 25-OH D and DBP also were tested. A 95% CI for the odds ratio (OR) excluding 1.00 or a p value < 0.05 was considered statistically significant.

Results

Characteristics of the study population are shown in Table 1. Women in the case group did not differ materially from the control subjects, except for more women among cases and more men among controls than expected, as previously reported [11]. Median week of blood sampling in cases and controls was 9th, 22nd and 37th/38th in first, second and third trimester, respectively.

The mean concentrations of DBP and 25-OH D increased significantly with increasing week of gestation in cases and controls, $p < 0.001$, and the level of both tended to be lower in cases than in controls (Figure 1A and B).

The linear mixed model showed that the mean DBP tended to be lower in cases than in controls

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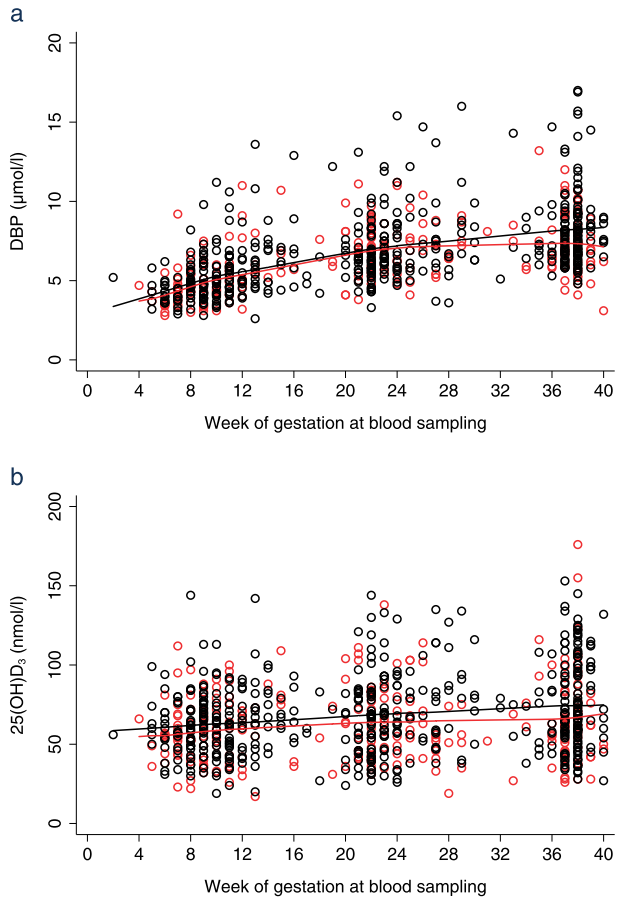

Figure 1. Levels of (A) vitamin D-binding protein and (B) 25-hydroxyvitamin D during pregnancy in women whose child later developed type 1 diabetes (red) and controls (black). Lines are smoothed using locally weighted scatterplot smoothing (LOWESS).

(−0.27 µmol/L, 95% CI: −0.57, 0.03), although this difference was borderline non-significant after adjustment for season of sampling, maternal diabetes and sex of the child (Table S1). Adding 25-OH D into the model further attenuated the estimated difference, −0.20 µmol/L, 95% CI: −0.50, 0.11, (Table S1).

When stratifying data by trimester, lower DBP in the third trimester was significantly associated with higher risk of type 1 diabetes (Table 2). The linear trend remained statistically significant, even after adjusting for 25-OH D, although the difference between the upper and lower quartile was no longer significant in the fully adjusted model (Table 2). First and second trimester DBP alone were not significantly associated with offspring type 1 diabetes.

The overall 25-OH D was significantly lower for cases compared with controls, (mean difference adjusted for sex of the child, season of blood sample and maternal diabetes: −5.01 nmol/L, (95% CI: −9.28, −0.73), $p = 0.022$. Adding DBP to the model resulted in a slightly smaller, borderline non-significant difference between cases and controls, −3.82 nmol/L, 95% CI: −8.03, 0.40, $p = 0.076$ (Table S2).

Low third trimester 25-OH D was associated with higher risk of childhood onset type 1 diabetes, but this became borderline non-significant in the fully adjusted model including also DBP (Table 3). Second and first trimester maternal 25-OH D were not significantly associated with risk of type 1 diabetes in the children (Table 3).

Additional adjustments for region of residence in Norway did not influence on the results for DPB or 25-OH D analyses (data not shown). Vitamin D-binding protein and 25-OH D were significantly positively linearly correlated in all three trimesters with Pearson's correlation coefficients of 0.19–0.25 (Figure S2).

There was a tendency that the increase in both 25-OH D and DBP per week of gestation was slightly lower for cases than for controls, but the interaction between case/control status and week of gestation was not significant for neither DBP ($p = 0.15$) nor 25-OH D ($p = 0.7$) in the mixed models reported in Table S1 and S2.

Finally, we did some exploratory analyses; first of the potential interaction between maternal third trimester 25-OH D and DBP in logistic regression model with type 1 diabetes as the dependent variable. The interaction term was not significant, OR 1.003, $p = 0.31$ (data not shown). Second, we explored the patterns of 25-OH D and DBP during pregnancy to see if there were any differences between cases and controls. We used two different methods (Supporting Information Materials and Methods) to assess the potential effect of consistently low, medium or high levels and changes up or down during pregnancy. No clear patterns emerged. The free fraction of 25-OH D was estimated using a published formula and an assumed constant albumin concentration, but there were no significant differences between cases and controls (methods with references and results are reported in Supporting Information Materials and Methods and Figure S3).

Discussion

In this study, we found that the average DBP throughout pregnancy tended to be lower in women whose offspring later developed type 1 diabetes compared with controls, albeit at borderline statistical significance. A similar pattern has previously been found for 25-OH D concentrations in late pregnancy [11], and this present study found a systematically lower 25-OH D throughout pregnancy for mothers whose children later developed type 1 diabetes compared with controls. The latter difference was borderline non-significant after adjustments for DBP.

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Table 2. Maternal DBP by trimester of pregnancy in cases whose child developed type 1 diabetes and in control subjects

	Case subject	Control subject	Unadjusted OR (95% CI)	Adjusted OR* (95% CI)
Third trimester samples				
No.	71	146		
DBP (μmol/L) [means (SD)]	7.38 (1.90)	8.18 (2.29)		
DBP (quartiles) [*n* (%)]				
1. < 6.7 μmol/L	26 (36.6)	34 (23.3)	2.29 (1.02–5.15)	2.04 (0.87–4.80)
2. > = 6.7 and <7.6 μmol/L	19 (26.8)	37 (25.3)	1.54 (0.67–3.56)	1.36 (0.56–3.28)
3. > = 7.6 and <9.0 μmol/L	13 (18.3)	36 (24.7)	1.08 (0.44–2.64)	0.90 (0.35–2.33)
4. > = 9.0 μmol/L	13 (18.3)	39 (26.7)	1.0 (reference)	1.0 (reference)
Test for trend continuous			$p = 0.014$	$p = 0.039$
Second trimester samples				
No. women/no. samples	76	146		
DBP (μmol/L) [means (SD)]	6.99 (1.76)	6.96 (2.06)		
DBP (quartiles) [*n* (%)]				
1. < =5.6 μmol/L	21 (26.3)	36 (24.7)	0.98 (0.17–2.03)	1.16 (0.51–2.63)
2. >5.6 and < =6.5 μmol/L	9 (11.3)	36 (24.7)	0.41 (0.25–1.26)	0.28 (0.10–0.77)
3. >6.5 and < =7.78 μmol/L	24 (30.0)	38 (26.0)	1.03 (0.50–2.16)	1.02 (0.47–2.19)
4. >7.78 μmol/L	22 (27.5)	36 (24.7)	1.0 (reference)	1.0 (reference)
Test for trend continuous			$p = 0.90$	$p = 0.97$
First trimester samples				
No. women/no. samples	86	149		
DBP (μmol/L) [means (SD)]	4.81 (1.37)	5.05 (1.43)		
DBP (quartiles) [*n* (%)]				
1. < =4.1 μmol/L	24 (27.9)	35 (23.5)	1.49 (0.69–3.24)	1.37 (0.59–3.17)
2. >4.1 and < =4.8 μmol/L	25 (29.1)	39 (26.2)	1.40 (0.65–2.99)	1.41 (0.63–3.13)
3. >4.8 and < =5.7 μmol/L	20 (23.3)	38 (25.5)	1.15 (0.52–2.52)	1.00 (0.43–2.28)
4. >5.7 μmol/L	17 (19.8)	37 (24.8)	1.0 (reference)	1.0 (reference)
Test for trend continuous			$p = 0.20$	$p = 0.27$

CI, confidence interval; DBP, vitamin D-binding protein; OR, odds ratio; SD, standard deviation.
*Adjusted for season of blood sample (January–March, April–June, July–September and October–December), sex of the child, maternal diabetes and 25-hydroxyvitamin D.

Based on the previous evaluation of maternal serum levels of 25-OH D in late pregnancy [11], this present study were extended to include samples from three time points during pregnancy. We then had the possibility to evaluate any changes during pregnancy and the possible total influence of 25-OH D on the offspring. In addition, this study included analyses of DBP. We wanted to evaluate any influence of levels of vitamin D-binding protein on 25-OH D levels as total 25-OH D includes both the bound (to DBP and albumin) and the unbound fraction. We found significant correlation between DBP and 25-OH D.

Previous studies have reported preclinical lower intake or measured levels of 25-OH D to be associated with higher risk of type 1 diabetes in children and young adults [19,20], whereas another study found no such associations [21]. Previous descriptions of DBP in relation to type 1 diabetes development are sparse. In one study, lower levels of DBP was found in patients with type 1 diabetes compared with first-degree relatives or healthy controls [9], but it is unclear whether the differences were a cause or a consequence of the disease. Another smaller study did not find any difference in plasma DBP between type 1 diabetes patients and their controls during puberty [22]. Vitamin D-binding protein is a polymorphic serum protein [23]. The binding affinity for 25-OH D, and levels of both 25-OH D and 1,25-OH D_2 have been described to vary with genotype [24]. A previous study reported that polymorphisms in genes coding for DBP were associated with IA-2 auto antibodies in type 1 diabetes patients when compared with first-degree relatives and controls [25], whereas another study could not find associations between DBP alleles and type 1 diabetes [26]. We did not have access to DNA for genotyping in our study, and could thus not make any distinction between different forms of DBP.

Levels of DBP are known to increase throughout pregnancy, which was also found in this study. Vitamin D-binding protein is produced in the liver and cleared by the kidneys [27]. Impaired production or increased excretion in liver or kidney disease might influence circulating levels. Previous studies describe decreased levels of DBP in people with chronic liver disease and nephrotic syndrome [27,28]. We therefore excluded women with liver and kidney disease from the analysis. 25-OH D is stored in the body fat. Previous studies have found obesity to be associated with lower serum concentrations of 25-OH D [29]. We had no information on BMI or percent of fat mass in this study. Further, 25-OH D levels have been found to differ between different races or ethnic groups

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Table 3. Maternal serum 25-OH D by trimester of pregnancy in cases whose child developed type 1 diabetes and in control subjects

	Case subject	Control subject	Unadjusted OR (95% CI)	Adjusted OR* (95% CI)
Third trimester samples				
No.	72	160		
25-OH D (nmol/L) [means (SD)]	65.9 (28.5)	75.0 (27.9)		
25-OH D (quartiles) [*n* (%)]				
1. < 55 nmol/L	24 (33.3)	38 (23.8)	2.53 (1.07–5.98)	2.59 (0.96–6.98)
2. > = 55 and <69 nmol/L	21 (29.2)	39 (24.4)	2.15 (0.90–5.16)	1.92 (0.71–5.21)
3. > = 69 and <96 nmol/L	17 (23.6)	43 (26.9)	1.58 (0.65–3.86)	1.56 (0.61–3.99)
4. > = 96 nmol/L	10 (13.9)	40 (25.0)	1.0 (reference)	1.0 (reference)
Test for trend continuous			$p = 0.024$	$p = 0.065$
Second trimester samples				
No. women/no. samples	80	155		
25-OH D (nmol/L) [means (SD)]	65.6 (24.2)	68.4 (24.4)		
25-OH D (quartiles) [*n* (%)]				
1. < =51 nmol/L	21 (27.6)	39 (25.2)	1.28 (0.59–2.77)	1.32 (0.55–3.16)
2. >51 and < =67 nmol/L	9 (11.8)	39 (25.2)	0.97 (0.44–2.17)	0.95 (0.39–2.30)
3. >67 and < =83 nmol/L	24 (31.6)	43 (27.7)	0.88 (0.40–1.96)	0.97 (0.41–2.33)
4. >83 nmol/L	22 (28.9)	34 (21.9)	1.0 (reference)	1.0 (reference)
Test for trend continuous			$p = 0.39$	$p = 0.73$
First trimester samples				
No. women/no. samples	91	158		
25-OH D (nmol/L) [means (SD)]	57.9 (18.8)	62.0 (19.1)		
25-OH D (quartiles) [*n* (%)]				
1. < =48.8 nmol/L	26 (28.6)	39 (24.7)	1.73 (0.80–3.76)	1.42 (0.59–3.42)
2. >48.8 and < =61.5 nmol/L	30 (33.0)	40 (25.3)	1.95 (0.91–4.17)	1.53 (0.64–3.58)
3. >61.5 and < =74.3 nmol/L	20 (22.0)	40 (25.3)	1.30 (0.58–2.90)	0.97 (0.41–2.34)
4. >74.3 nmol/L	15 (16.5)	39 (24.7)	1.0 (reference)	1.0 (reference)
Test for trend continuous			$p = 0.10$	$p = 0.30$

25-OH D, 25-hydrosyvitamin D; CI, confidence interval; OR, odds ratio; SD, standard deviation.
*Adjusted for sex of the child, maternal diabetes and season of blood sample (January–March, April–June, July–September, October–December) and levels of vitamin D-binding protein.

[30]. The differences might be due to different skin colours, and it could partly be due to different genetic variants of DBP (further discussed later). But as for BMI, we had no possibility to adjust for ethnicity as this information was lacking in the study population.

The samples utilized in this study were stored in a freezer at −20 °C for a long time, and due to previous analyses, there had been some freeze thaw cycles before the current study. Previous studies indicate 25-OH D to tolerate long time storage at −20 °C without deterioration [31,32] as well as being unaffected by number of freeze-thaw cycles [33]. Vitamin D-binding proteins on the other hand are somewhat less robust to higher storage temperature [34,35] and are more vulnerable to freeze thaw cycles. The samples from both cases and controls were handled in the same way. A deterioration of the samples would affect the samples equally. A real difference between cases and controls would most likely be attenuated by the degradation.

The mean serum level of total 25-OH D was positively correlated with mean DBP level, consistent with previous studies on healthy young adults [36] and on pregnant women [37]. Another study found no correlation between 25-OH D and DBP in any of the study groups [9]. The mechanism behind the observed correlation between 25-OH D and DBP is not clear, and it is not obvious whether the two can be viewed as confounders for each other in the relationship with type 1 diabetes in the offspring. We presented results before and after adjustment, even though it may be questioned whether adjustment provides the most correct estimate. The answer depends on whether DBP act as a confounder, a mediator, or neither, in the potential causal pathway. Results from different assays of 25-OH D measurements might be influenced by DBP concentration, but the DiaSorin radioimmunoassay used in our study was apparently not influenced by level of DBP, in contrast to some other commercial 25-OH D assays [38].

The measurements in this study have focused on total levels of 25-OH D throughout pregnancy. Based on the free hormone hypothesis and recent results in other fields [36], we could have expected the free fraction of 25-OH D to be more strongly associated with risk of type 1 diabetes in the offspring. By using a known formula for estimating free levels of 25-OH D [36,39] in our samples, we found no association with the free levels of the pre-hormone and type1 diabetes development in the child (Supporting Information material).

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Our results on 25-OH D in pregnancy in this study add to two previous publications in this field [11,12] and suggest a possible explanation for the apparent discrepancy in results. It seems that 25-OH D in late pregnancy is a stronger predictor of type 1 diabetes in the offspring than early pregnancy levels. By strict dividing samples into trimesters of pregnancy, first and second trimester samples alone showed no association between maternal 25-OH D levels and childhood type 1 diabetes. This was in accordance with Miettinen *et al* [12] who found no association between maternal 25-OH D levels in first trimester of pregnancy and risk of type 1 diabetes in the offspring.

Possible mechanisms by which vitamin D might act in a potential protective manner against type 1 diabetes have previously been reviewed [8,40]. Whether the tendency to an increased risk of type 1 diabetes in offspring of mothers with lower levels of DBP and 25-OH D suggested in this study are most related to the levels of DBP, total level of 25-OH D, or both is not clear. In the kidneys, 25-OH D bound to DBP is actively brought to the site of $1,25(OH)_2D$ synthesis by megalin-mediated endocytosis [41]. This process might take place in other cells as well [42]. Vitamin D-binding protein has been suggested to be involved in inflammation. A positive correlation between total level of DBP and macrophage activation has been found [43]. This modification of the binding protein might be induced by signals from activated B- and T-cells [44]. One common DBP haplotype has been linked to reduced macrophage activity and thereby protection against other inflammatory disease like in COPD. The protein encoded by the same haplotype has a lower affinity for vitamin D compared with other haplotypes [23,45]. The foetal period of life when the immune system is going through an extensive education, with positive and negative selection of T- and B- lymphocytes might be a vulnerable period for factors stimulating immune tolerance. Note, however, that this study is a descriptive study, unable to describe causality. Furthermore, any future study in this field would benefit from larger sample size.

In conclusion, by repeated measurements of vitamin D-binding protein and 25-hydroxyvitamin D throughout pregnancy, we found a tendency of inverse associations with type 1 diabetes development in the offspring. Differences between case and control women tended to be greater towards late pregnancy.

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Ingvild Menes Sørensen designed and conducted the research, analysed the data and performed the statistical analysis and finally wrote the article. Geir Joner designed the research. Pål A. Jenum organized the original cohort. Anne Eskild designed the research. Cathrine Brunborg analysed the data and performed the statistical analysis. Peter A. Torjesen provided essential reagents. Lars Christian Stene designed and conducted the research, analysed the data and performed the statistical analysis and had the primary responsibility for final content. All authors commented on manuscript.

Conflicts of interest

The authors have no conflicts of interest.

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