Diabetologia (2010) 53:1599–1607
DOI 10.1007/s00125-010-1734-8

ARTICLE

Maternal intake of vitamin D during pregnancy and risk of advanced beta cell autoimmunity and type 1 diabetes in offspring

L. Marjamäki · S. Niinistö · M. G. Kenward · L. Uusitalo · U. Uusitalo · M.-L. Ovaskainen · C. Kronberg-Kippilä · O. Simell · R. Veijola · J. Ilonen · M. Knip · S. M. Virtanen

Received: 24 November 2009 / Accepted: 3 March 2010 / Published online: 6 April 2010

Abstract
Aims/hypothesis We evaluated the intake of vitamin D by pregnant Finnish women and examined associations between maternal intake of vitamin D and the development of advanced beta cell autoimmunity and type 1 diabetes in their offspring.
Methods The research was carried out within the Diabetes Prediction and Prevention study (DIPP), which is a population-based birth cohort of infants at genetic risk of type 1 diabetes. Mothers of 3,723 infants born between 1997 and 2002 completed a validated 181-item food frequency questionnaire, which included questions on dietary supplements. The offspring were observed at 3 to 12 month intervals for the appearance of autoantibodies associated with type 1 diabetes and for the development of clinical type 1 diabetes.
Results Maternal mean daily intake of vitamin D was 5.1 μg from food and 1.3 μg from supplements. The maternal intake of vitamin D, either from food or from supplements, was not associated with the risk of advanced beta cell autoimmunity/type 1 diabetes in offspring (HR [95% CI] for intake of vitamin D from food 1.25 [0.80–1.95], for vitamin D intake from supplements 1.05 [0.95–1.16]), or with the risk of type 1 diabetes alone (HR [95% CI] for intake of vitamin D from food 0.84 [0.41–1.72], for vitamin D intake from supplements 1.09 [0.99–1.20]).

L. Marjamäki · L. Uusitalo · S. M. Virtanen
Tampere School of Public Health, University of Tampere,
Tampere, Finland

S. Niinistö · L. Uusitalo · U. Uusitalo · M.-L. Ovaskainen · C. Kronberg-Kippilä · S. M. Virtanen
Department of Lifestyles and Inclusion,
National Institute for Health and Welfare,
Helsinki, Finland

S. Niinistö (✉)
Department of Public Health, University of Helsinki,
Box 41, FI-00014 Helsinki, Finland
e-mail: sari.niinisto@helsinki.fi

M. G. Kenward
Department of Epidemiology and Population Health,
Medical Statistics Unit,
London School of Hygiene and Tropical Medicine,
London, UK

O. Simell
Department of Pediatrics, University of Turku,
Turku, Finland

R. Veijola
Department of Pediatrics, University of Oulu,
Oulu, Finland

J. Ilonen
Immunogenetics Laboratory, University of Turku,
Turku, Finland

J. Ilonen
Department of Clinical Microbiology,
University of Eastern Finland,
Kuopio, Finland

M. Knip · S. M. Virtanen
Department of Pediatrics, Tampere University Hospital,
Tampere, Finland

M. Knip
Hospital for Children and Adolescents and Folkhälsan Research Center, University of Helsinki,
Helsinki, Finland

S. M. Virtanen
Research Unit, Tampere University Hospital,
Tampere, Finland

Conclusions/interpretation Maternal intake of vitamin D either from food or supplements during pregnancy is not associated with advanced beta cell autoimmunity/type 1 diabetes or with type 1 diabetes alone in Finnish offspring carrying increased genetic susceptibility to type 1 diabetes.

Keywords Beta cell autoimmunity · Children · Cohort · Population-based studies · Pregnancy · Type 1 diabetes · Vitamin D

Abbreviations

DIPP	Diabetes Prediction and Prevention Study
FFQ	Food frequency questionnaire
GADA	Autoantibodies to 65 kDa isoform of GAD
IA-2A	Antibodies to tyrosine phosphatase-related islet antigen 2
IAA	insulin autoantibodies
ICA	Islet cell antibodies
IU	International unit

Introduction

Type 1 diabetes is an autoimmune disease resulting from selective, progressive destruction of insulin-secreting beta cells in the pancreatic islets [1, 2]. In studies with NOD mice, lack of vitamin D at an early age has been found to increase the later risk of autoimmune diabetes [3, 4]. In several NOD mice studies, pharmacological doses of bioactive vitamin D in the form of 1,25-dihydroxyvitamin D_3 or its analogues were seen to protect from autoimmune diabetes [4–7] and other autoimmune diseases [8]. Vitamin D has several effects on the immune system that could be of relevance in the pathogenesis of type 1 diabetes [8, 9]. The process leading to the destruction of the beta cells might be initiated even before birth [10], therefore the effect of early environmental determinants in utero may be of crucial importance. Because maternal vitamin D intake and status during pregnancy affect neonatal vitamin D status [11–13], the former is an important area to study when searching for factors potentially involved in the development of type 1 diabetes.

Maternal intake of vitamin D from food or use of vitamin D-containing supplements during pregnancy were weakly associated with decreased risk of early beta cell autoimmunity in two cohort studies [14, 15] and with clinical type 1 diabetes in one case–control study [16]. However, the results of these studies remain inconsistent. In a Swedish study, the use of vitamin D-containing supplements during pregnancy was associated with decreased autoimmunity against one autoantibody when children were 1 year old but not at later ages [14]. In a US cohort, maternal intake of vitamin D from food but not from supplements during pregnancy was associated with a decreased risk of islet autoimmunity appearance in the offspring [15]. In a Norwegian pilot case–control study, maternal cod liver oil supplementation (rich in vitamin D) during pregnancy was associated with a reduced risk of type 1 diabetes in the offspring [16]. However, this association was not confirmed in a larger series [17]. In neither of these Norwegian studies was vitamin D supplementation other than cod liver oil during pregnancy significantly associated with the risk of type 1 diabetes in the offspring.

The use of vitamin D-containing supplements in early childhood was associated with a reduced risk of type 1 diabetes in one case–control study [18] and in one cohort study [19]. In the Norwegian study, use of cod liver oil but not vitamin D supplementation as such during the first year of life was associated with lower risk of type 1 diabetes [17].

In Finland all pregnant women are recommended to have a vitamin D intake of 10 μg/day and also to use supplementation during the darkest period of the year from October to March. The purpose of the present study was to examine in a birth cohort of children carrying increased genetic risk of type 1 diabetes whether maternal vitamin D intake from food and/or supplements during pregnancy is associated with development of advanced beta cell autoimmunity or clinical type 1 diabetes in the offspring.

Methods

Participants The present investigation is part of the Finnish Type 1 Diabetes Prediction and Prevention (DIPP) Study, which is a large prospective, population-based birth cohort. Screening of the infants for HLA-conferred susceptibility to type 1 diabetes is offered to all parents after delivery in the Turku, Tampere and Oulu University Hospitals. Families with an infant carrying increased genetic risk are invited to take part in the DIPP study. Increased genetic risk is defined as: high-risk genotype: *HLA-DQB1*02/*0302* or moderate-risk genotype *HLA-DQB1*0302/x*, where *x*=other than **02*, **0301* or **0602*. Genetic screening is performed from a sample obtained from cord blood. Infants with congenital anomalies or severe systemic diseases are excluded, as are families in which both parents do not understand Finnish, Swedish or English. Ethical approval for this study was obtained from the local Ethical Committees. All the parents have given their written consent to the study.

The present series comprised 4,297 children with increased genetic risk of type 1 diabetes, who were born between 20 October 1997 and 31 December 2002 (79.4% of those invited). Mothers of 3,723 children (86.6%) provided dietary data.

Assessment of maternal nutrition Maternal food consumption was evaluated using a validated food frequency questionnaire (FFQ) [20] completed 1 to 3 months after delivery. The FFQ focused on maternal intake of food during the eighth month of pregnancy (the month preceding pregnancy leave in Finland) and the use of nutritional supplements throughout pregnancy. All returned FFQ forms were checked by a trained study nurse. If more than ten questions were not answered, the FFQ was excluded (n=52). The FFQ contained questions enquiring about the frequency (number of times per day, week or month) and the amount of foods consumed, in units of common serving sizes. In Finland butter spreads and margarines are commonly fortified with vitamin D. Fat-free and low-fat milks have been fortified with vitamin D up to the level naturally available in high-fat milk. These fortified foods are included in the calculation of total vitamin D intake from food sources. From the beginning of 2003, vitamin D fortification of dietary fats was increased and since then larger amounts of vitamin D have been added to milk and liquid milk products. These procedures did not influence the intake of vitamin D by the mothers of the present study, as their dietary data were collected until the end of 2002 only. The daily nutrient intakes were calculated using the Finnish Food Composition Database and in-house software of the National Institute for Health and Welfare. The content of the FFQ and data processing have been described in detail by Erkkola et al. [20].

With the FFQ, the mothers received written instructions on how to record the vitamin and mineral supplements as well as natural products and drugs used by brand names. The amounts of supplements used were recorded as tablets, drops, spoonfuls or millilitres. The intake of vitamin D from all kinds of supplements was assessed. Nutritional information on dietary supplements registered as drugs in Finland was obtained from the Finnish Pharmacopoeia. Information on dietary supplements other than drugs was obtained from the National Food Administration and from the manufacturers. Nutritional information on specific vitamin or mineral supplement without exact product specification (for example ‘vitamin D drops’) was estimated as the mean of nutrients from the same type of supplement products used by other mothers in the present study.

Socio-demographic and perinatal characteristics Socio-demographic factors, sex of child, maternal age, maternal education and familial diabetes were registered by a structured questionnaire completed by the parents after delivery. Data on neonatal and pregnancy characteristics (number of earlier deliveries, duration of gestation, route of delivery and maternal smoking during pregnancy) were received from the Medical Birth Registries at Oulu and Tampere University Hospitals.

Measurement of autoantibodies and definition of outcome The DIPP children were monitored for the appearance of signs of beta cell autoimmunity by analysing primarily islet cell antibodies (ICA). If a child tested positive for ICA, all his or her samples obtained from birth were analysed for insulin autoantibodies (IAA), autoantibodies to 65 kDa isoform of GAD (GADA) and antibodies to tyrosine phosphatase-related islet antigen 2 (IA-2A). Autoantibody samples were obtained at each study centre visit, which were scheduled to take place at the age of 3, 6, 12, 18 and 24 months, and subsequently at an interval of 12 months. If the child became positive for ICA, the visit interval was shortened to 3 months. The autoantibodies were measured in the Research Laboratory, Department of Pediatrics, University of Oulu. ICA, IAA, GADA and IA-2A were quantified as described previously [21].

We used two different endpoints: (1) advanced beta cell autoimmunity/type 1 diabetes; and (2) clinical type 1 diabetes alone. We defined advanced beta cell autoimmunity as being repeatedly positive for ICA and one or more of the three other autoantibodies analysed. The children were observed for a mean time of 4.3 years (range 0.2 to 8.9 years). Altogether 144 of the 4,297 (3.4%) children seroconverted to repeated ICA positivity and positivity to one of the three other autoantibodies during this period. Of the children, 74 (1.7%) had progressed to clinical type 1 diabetes (ascertained on the basis of WHO criteria [http://who.int/diabetesactiononline/diabetes/basics/en/print.html, accessed 2006]) at a median age of 4.1 years (range 1.0–7.9 years). Of the children progressing to type 1 diabetes, 53 had been repeatedly positive for ICA and at least one other autoantibody. However, nine of the remaining 21 children who progressed to type 1 diabetes had or had had one or more autoantibodies in one single sample only before or at the time of diagnosis. Moreover, four children who developed type 1 diabetes had been persistently seronegative, with a last blood sample drawn 3.3 to 5.5 years before diagnosis. Eight children were not subjected to any autoantibody analyses before the diagnosis of diabetes [22]. Therefore we decided to include clinical type 1 diabetes in the autoantibody endpoint termed advanced beta cell autoimmunity/type 1 diabetes. This resulted in 165 children (3.8% of all children) with either clinical type 1 diabetes or repeated positivity for ICA and at least one other autoantibody. The endpoint clinical type 1 diabetes alone includes only cases with confirmed clinical type 1 diabetes.

Statistical methods Because intake of vitamin D from food was normally distributed after logarithmical transformation, analysis of variance was used to study associations of the socio-demographic and perinatal variables with intake of vitamin D from diet. The associations between possible confounders and use of vitamin D-containing supplements

were assessed by two-dimensional contingency tables and tested by χ^2 test.

In aetiological analyses, effective sample size was 3,723, of whom 138 had developed the advanced beta cell autoimmunity/type 1 diabetes endpoint and 55 the clinical type 1 diabetes endpoint. Maternal vitamin D intake from food was logarithmically transformed and energy-adjusted by Willett's residual method [23]. The advanced beta cell autoimmunity/type 1 diabetes endpoint is interval-censored. To accommodate this structure, a piece-wise exponential survival model was used [24]. We defined the time variables for the piece-wise survival model as follows. For children who fulfilled the criteria for 'advanced beta cell autoimmunity', the time interval was defined to be between the last negative measurement and the first positive measurement (appearance of ICA plus at least one of the other three autoantibodies). For children who did not have repeated autoimmunity positivity before diagnosis of clinical type 1 diabetes, the time interval was defined to be between the last negative measurement and the date of diabetes diagnosis. For children who did not have blood samples before diagnosis of clinical type 1 diabetes, the 'event' time was defined to be the date of diabetes diagnosis. The models were fitted using maximum likelihood in SAS PROC NLMIXED, with standard errors of estimates derived from the observed information matrix. SAS version 9.1.3 (SAS Institute, Cary, NC, USA) was used in the analyses. The proportionality of the hazards was tested by adding interaction terms of the exposure variables with time interval to the models.

The association between maternal vitamin D intake from food and supplements with the development of clinical type 1 diabetes was analysed by Cox proportional hazards analysis because the exact date of type 1 diabetes diagnosis was known. The proportionality of the hazards was examined by log–log plot. These analyses were performed with SPSS version 15.0 (SPSS).

Possible confounding factors were controlled for by adding background variables as covariates to the statistical models. All background variables used in the analyses are associated with vitamin D intake or with the outcome according to the earlier literature or the present results. Two different models were used: one including only familial diabetes and genetic risk group; and one including also sex, gestational age, maternal age, maternal education, delivery hospital, route of delivery, number of earlier deliveries and smoking during pregnancy. Statistical significance was taken as 5%.

Results

Among the 3,723 women who provided the food consumption data, women with a high level of education, living in the Tampere region and not smoking during pregnancy, as well as women expecting their first child were slightly over-represented compared with mothers who did not return the FFQ.

Vitamin D intake from food Maternal and offspring characteristics, as well as vitamin D intake from food and use of vitamin D-containing supplements are summarised in Table 1. Maternal mean absolute daily intake of vitamin D from food was 5.1 μg (SD 2.6 μg). Higher intake of vitamin D from food correlated positively with age of the mother, as well as with the education of the mother and father, and with non-smoking during pregnancy. The most important food sources of vitamin D were fish and shellfish (28% of total intake), vitamin D-fortified margarines, butter–oil mixes and butter (26% of total intake), and milk and milk products (9% of total intake).

Vitamin D intake from supplements Mean daily intake of vitamin D from supplements was 1.3 μg (SD 2.6 μg) (20% of total intake). Only 30% of the women used vitamin D-containing supplements during pregnancy. In the group who used vitamin D-containing supplements, the following were over-represented compared with women who did not use supplementation: women with an academic education or with a husband with academic education; women expecting their first child; non-smokers during pregnancy; women with BMI at first antenatal visit below 25 kg/m^2; women living in the Tampere region; and women experiencing a complicated vaginal delivery. Women who had consumed a lot of fish and received the highest amounts of vitamin D from diet also used vitamin D-containing supplements more frequently.

Vitamin D intake and risk of advanced beta cell autoimmunity/type 1 diabetes and clinical type 1 diabetes alone Maternal intake of vitamin D, either from food or from supplements, or from combined sources was not associated with the risk of advanced beta cell autoimmunity/type 1 diabetes in children, when adjusted for genetic risk and familial type 1 diabetes (Table 2). Adjusting for several other socio-demographic and perinatal putative confounding factors did not change the results. Neither was any association observed between maternal vitamin D intake and clinical type 1 diabetes alone in children (Table 2). Adjusting for intake of *n*-3 fatty acids did not change the results (data not shown).

Discussion

In the present cohort study, maternal intake of vitamin D, either from food or supplements, during pregnancy was not

Table 1 Characteristics of the mothers and their offspring in relation to maternal daily intake of vitamin D from food and use of vitamin D-containing supplements

Characteristic	Offspring (*n*)	Mothers	Maternal vitamin D intake	
	With endpoint 1[a]	Distribution, *n* (% of total)	From food (μg/day), mean (SD)	From supplements (% of *n*)
n		4,297	3,723[b]	3,723[b]
Maternal age, *p* value			<0.0001	NS
<25 years	37	831 (19.3)	4.53 (2.25)	28.2
25–29 years	47	1,435 (33.4)	4.84 (2.46)	31.8
30–34 years	43	1,270 (29.6)	5.35 (2.83)	30.0
≥35 years	36	753 (17.5)	5.63 (2.81)	29.1
Missing data	2	8 (0.2)	–	–
Earlier deliveries, *p* value			NS	0.0001
0 deliveries	63	1,835 (42.7)	5.00 (2.75)	36.6
1 delivery	62	1,372 (31.9)	5.12 (2.57)	25.0
2 deliveries	19	628 (14.6)	5.09 (2.44)	24.1
≥3 deliveries	16	436 (10.1)	5.20 (2.46)	23.8
Missing data	5	26 (0.6)	–	–
Delivery hospital, *p* value			NS	<0.0001
Tampere	86	2,417 (56.2)	5.07 (2.72)	34.2
Oulu	79	1,880 (43.9)	5.05 (2.42)	24.2
Maternal education, *p* value			<0.0001	<0.0001
None	16	303 (7.1)	4.33 (2.23)	22.7
Vocational school/training	49	1,262 (29.4)	4.90 (2.59)	26.3
Upper secondary/vocational	54	1,698 (39.5)	5.06 (2.55)	30.7
Academic	37	867 (20.2)	5.53 (2.85)	37.7
Missing data	9	167 (3.9)	5.11 (2.02)	18.8
Smoking during pregnancy, *p* value			0.016	0.016
No	141	3,688 (85.8)	5.10 (2.63)	30.6
Yes	12	446 (10.4)	4.81 (2.60)	24.7
Missing data	12	163 (3.8)	4.74 (2.06)	33.6
Genetic risk group				
High risk (*DQB1*02/*0302*)	51	827 (19.2)	5.05 (2.71)	32.8
Moderate risk (*DQB1*0302/x* [c])	114	3,470 (80.8)	5.06 (2.58)	29.5
Familial diabetes, *p* value			NS	NS
Yes	25	295 (6.9)	5.42 (3.11)	29.9
No	140	4,002 (93.1)	5.04 (2.56)	30.1
Gestational age, *p* value			NS	NS
1st quartile: <39 weeks	54	1,079 (25.1)	5.02 (2.65)	30.3
2nd quartile: 39 weeks	32	1,026 (23.9)	5.02 (2.44)	29.1
3rd quartile: 40–40.7 weeks	38	1,043 (24.1)	5.04 (2.53)	28.0
4th quartile: ≥40.8 weeks	31	1,113 (25.9)	5.18 (2.85)	32.6
Missing data	10	45 (1.0)	–	–
Route of delivery, *p* value			NS	0.027
Uncomplicated vaginal	136	3,451 (80.3)	5.06 (2.53)	29.8
Complicated vaginal	5	248 (5.8)	4.82 (2.39)	38.0
Caesarean section	14	560 (13.0)	5.22 (3.30)	28.4
Missing data	10	38 (0.9)	–	–

[a] Advanced beta cell autoimmunity/type 1 diabetes

[b] Women with dietary data

[c] *x* not equal to **02*, **0301*, **0602*

Table 2 Hazard ratios (95% CI) for maternal vitamin D intake (μg) during pregnancy and risk of islet autoimmunity and type 1 diabetes in the offspring

Variables	Advanced beta cell autoimmunity/type 1 diabetes					Clinical type 1 diabetes alone				
	Positivity, *n* (*n* total)	HR (95% CI)[a]	*p* value	Adjusted HR[b] (95% CI)	*p* value	Positivity, *n* (*n* total)	HR (95% CI)[a]	*p* value	Adjusted HR[b] (95% CI)	*p* value
Energy-adjusted vitamin D intake										
From food	138 (3,395)	1.25 (0.82–1.90)	0.30	1.25 (0.80–1.95)	0.33	55 (3,723)	1.07 (0.56–2.08)	0.83	0.84 (0.41–1.72)	0.63
From food (per quartile)										
1st quartile	34 (934)	1		1		14 (934)	1		1	
2nd quartile	27 (931)	0.76 (0.46–1.27)	0.30	0.74 (0.44–1.25)	0.26	14 (931)	0.99 (0.47–2.08)	0.99	1.00 (0.48–2.11)	0.99
3rd quartile	36 (923)	1.03 (0.64–1.65)	0.90	1.12 (0.69–1.82)	0.64	13 (923)	0.94 (0.44–1.99)	0.86	0.84 (0.38–1.83)	0.66
4th quartile	41 (935)	1.15 (0.73–1.82)	0.54	1.17 (0.73–1.90)	0.51	14 (935)	0.99 (0.47–2.08)	0.98	0.77 (0.35–1.72)	0.52
Total vitamin D intake [c]	138 (3,395)	1.22 (0.90–1.64)	0.20	1.26 (0.92–1.73)	0.15	55 (3,723)	1.18 (0.74–1.87)	0.49	1.08 (0.65–1.79)	0.77
Per quartile total vitamin D[c]										
1st quartile	30 (924)	1		1		12 (924)	1		1	
2nd quartile	31 (925)	1.06 (0.64–1.76)	0.81	1.20 (0.72–2.00)	0.49	14 (925)	1.23 (0.57–2.66)	0.60	1.25 (0.57–2.73)	0.58
3rd quartile	38 (926)	1.24 (0.77–2.00)	0.38	1.33 (0.81–2.19)	0.27	9 (926)	0.74 (0.31–1.76)	0.50	0.64 (0.26–1.59)	0.34
4th quartile	39 (948)	1.27 (0.79–2.05)	0.32	1.38 (0.83–2.28)	0.21	20 (948)	1.63 (0.79–3.33)	0.18	1.50 (0.70–3.20)	0.29
Vitamin D from supplements	138 (3,395)	1.05 (0.95–1.15)	0.33	1.05 (0.95–1.16)	0.35	55 (3,723)	1.07 (0.98–1.17)	0.15	1.09 (0.99–1.20)	0.09

[a] Adjusted for genetic risk and familial type 1 diabetes

[b] Adjusted for genetic risk, familial type 1 diabetes, sex, gestational age, maternal age, maternal education, delivery hospital, route of delivery, number of earlier deliveries and smoking during pregnancy

[c] From food and supplements

associated with advanced beta cell autoimmunity/type 1 diabetes or clinical type 1 diabetes alone in Finnish children with HLA-conferred susceptibility to type 1 diabetes.

The main strengths of our study are a cohort setting and our definition of an endpoint that reflects advanced beta cell autoimmunity. We defined advanced beta cell autoimmunity as being repeatedly positive for ICA and for one or more of the three other autoantibodies analysed. This can be considered to be a relatively strong predictor of clinical type 1 diabetes [25]. It was also possible to study clinical type 1 diabetes alone separately, although the number of participants with this endpoint was relatively small. Another strength of our study is that vitamin D intake from food and supplements was taken into account and that putative confounding by other dietary factors could be evaluated. We used a FFQ that was developed and validated especially for the present study. The validation study was done in exactly the same way as the study proper. In the validation study of the FFQ, the correlation for vitamin D intake measured by FFQ and dietary records was 0.44 [20], suggesting that the FFQ is an acceptable method for estimating the amount of vitamin D from food.

A major limitation of this study is that we did not have data on 25-hydroxyvitamin D_3 concentrations in maternal serum. Another limitation is that we could not adjust the results for the child's intake of vitamin D because all information on children's intake of vitamin D was not available for the present cohort. In Finland the authorities recommend that children should be given vitamin D supplements at a daily dose of 10 μg from 2 weeks until 3 years of age and thereafter 7.5 μg/day.

In the present study, the maternal intake of vitamin D was substantially lower than the amount recommended, with only 15% of the pregnant women receiving the recommended 10 μg/day dose, while 44% had received below 5 μg/day. Only 30% of the pregnant women studied used supplements. Because the FFQ somewhat overestimates overall food intake and therefore also the amount of vitamin D compared with food records, the intake of vitamin D from food was in reality probably even more below the recommended amounts than can be concluded from the present results. Thus it is possible that the intake of vitamin D from food and supplements was generally so low, with a low SD, in our study, that only a few mothers received a potentially effective dose.

Unlike in a smaller US cohort study [15], we did not observe any inverse association between maternal intake of vitamin D from food and beta cell autoimmunity in the children. In the US study [15], the study population comprised 233 pregnant women and 16 children who were positive for one or more of IAA, GADA and IA-2A on at least one occasion (early pre-type 1 diabetes). Our survey, in comparison, involved 3,723 pregnant women and 138 children with repeated positivity for ICA and at least one other autoantibody (advanced pre-type 1 diabetes). Thus our population should be large enough to show a potential protective effect of maternal vitamin D intake from food. In both the US [15] and our study, the intake of vitamin D from food was evaluated with a FFQ focusing on the third trimester and completed 1 to 3 months after delivery. Also the average follow-up time was similar at around 4 years, as was the average intake of vitamin D from food. Thus in the US cohort, vitamin D intake from food was 6.3 μg (252.3 international units [IU]) in the unaffected group and 4.2 μg (167.6 IU) in the affected group, while in our study it was 5.1 μg among all mothers.

Similarly to the US cohort [15] and the Norwegian case–control studies [16, 17], no association was observed between maternal use of vitamin D supplements and clinical or preclinical type 1 diabetes in our series. In these studies, however, the use of supplements was assessed differently. In our study and the Norwegian reports, participants were asked about the use of supplements during the entire duration of pregnancy, while in the US survey, only the last trimester was focused on. We used vitamin D intake from supplements as a continuous variable, whereas in the US report, analyses were performed with dichotomic categories with a cut-off of 10 μg. The Norwegian studies did not include information on amounts taken, but only on frequency of use. Compared with our study, a larger proportion of mothers in the US study (81.3% in the affected group, 56.3% in the unaffected group) received a daily average of more than 10 μg from supplements. Although neither of the two outcomes in our study showed any significant association with maternal intake of vitamin D, there was an unexpected indication that maternal vitamin D intake from supplements during pregnancy might enhance the risk of type 1 diabetes in offspring (HR 1.09, 95% CI 0.99–1.20, $p=0.09$). Moreover, the results of the US study tended in the same direction, as when daily intake of vitamin D from supplements was ≥10 μg, the HR for autoimmunity was 3.09 ($p=0.11$) [15]. We think that these observations may be due to chance, but it is important to consider possible untoward effects of maternal vitamin D supplementation on the developing fetus.

In contrast to the reports discussed above, an extensive Swedish cohort study observed that the maternal intake of vitamin D at a dose of at least 5 μg/day from supplements during pregnancy protects the infant from autoimmunity at 1 year of age but not at later ages [14]. However, the Swedish study included some methodological differences compared with the other studies. For example, in that report diabetes-related autoimmunity was defined as being positive for a single autoantibody, GADA or IA-2A, or both [14], and the cut-off for antibody positivity was set at the

95th percentile of the cut-off for non-diabetic children [14], whereas the present and the US [15] study used a 99th percentile cut-off. In addition, the Swedish cohort was derived from the general population [14].

Vitamin D supplementation to offspring during infancy was associated with a reduced risk of type 1 diabetes in a European multinational case–control study [18], as well as in a Finnish prospective cohort study [19]. In the Finnish study, the supplementation dose and frequency were inversely associated with risk of clinical type 1 diabetes [19]. The cohort consisted of children born in 1966, when the recommended vitamin D supplementation was fivefold higher (50 μg [2,000 IU/]) than the current recommendation. Similarly, the doses in studies on NOD mice, in which a protective association was reported, were pharmacological; in addition vitamin D was administered in the form of the bioactive hormone, i.e. 1,25-dihydroxyvitamin D3 or its analogues [4–7].

In the Norwegian studies, maternal intake of cod liver oil during pregnancy [16], as well as the child's own exposure to cod liver oil during the first year of life [17] were associated with a decreased risk of type 1 diabetes. Because vitamin D supplements other than cod liver oil did not have any association, the protective effect of cod liver oil might have been due to other nutrients than vitamin D, e.g. *n*-3 fatty acids. The long-chain *n*-3 fatty acids eicosapentaenoic acid and docosahexaenoic acid have anti-inflammatory effects, which may be protective against type 1 diabetes [26, 27]. The results of a Norwegian intervention study in healthy young adults indicate that the bioavailability of vitamin D is similar, regardless of whether it is received from fish oil capsules or multivitamin supplements, because after 4 weeks daily oral intake of these supplements (both containing 10 μg cholecalciferol) increased serum 25-hydroxyvitamin D_3 concentrations to equal degrees [28]. In Finland it is not recommended to use vitamin-A-containing supplements such as cod liver oil during pregnancy and therefore cod liver oil is seldom taken. Our data included only a few users of cod liver oil and therefore the effect of cod liver oil could not be analysed separately.

In conclusion, the present findings from a relatively large mother–child cohort suggest that maternal intake of vitamin D at low dose either from food or supplements during pregnancy is not associated with the risk of preclinical or clinical type 1 diabetes among offspring with increased HLA *DQB1*-conferred susceptibility to type 1 diabetes. Until now, the evidence for the effects of maternal intake of vitamin D during pregnancy on subsequent development of type 1 diabetes in the child is rather limited. However, additional studies are definitively justified, because of the ongoing discussion about optimal levels of vitamin D intake. In particular, a cohort study determining the intake of vitamin D and serum concentrations of 25-hydroxyvitamin D_3 would be helpful. The epidemiological surveys performed so far have not taken into account the gene polymorphism for the vitamin D receptor or another gene involved in vitamin D metabolism, which might be linked to type 1 diabetes [29–32].

Acknowledgements This work was supported by the Doctoral Programs in Public Health, the Academy of Finland (grants 63672, 79685, 79686, 80846, 201988, 210632), the Finnish Diabetes Research Foundation, the Finnish Pediatric Research Foundation, the Juho Vainio Foundation, the Yrjö Jahnsson Foundation, the European Foundation for the Study of Diabetes, Medical Research Funds, Turku, Oulu and Tampere University Hospitals, JDRF (grants 197032, 4-1998-274, 4-1999-731, 4-2001-435), Novo Nordisk Foundation and EU Biomed 2 Program (BMH4-CT98-3314). We express our gratitude to the children and parents who participated. We would also like to thank the DIPP research nurses, doctors, nutritionists and laboratory staff for excellent collaboration over the years. Many thanks also to J. Nevalainen, H. Sinkko and H. Tapanainen for their skilful technical assistance. An Abstract of the present study has published at the 9th International Congress of Immunology of Diabetes Society and American Diabetes Association Research Symposium, 14–18 November 2007.

Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

References

1. Atkinson MA, Maclaren NK (1994) The pathogenesis of insulin-dependent diabetes mellitus. N Engl J Med 24:1428–1436
2. Atkinson MA, Eisenbarth GS (2001) Type 1 diabetes: new perspective on disease pathogenesis. Lancet 358:221–229
3. Mathieu C, van Etten E, Decallone B et al (2004) Vitamin D and 1, 25-dihydroxyvitamin D_3 as modulator in the immune system. J Steroid Biochem Mol Biol 89–90:449–452
4. Zella J, McCary LC, DeLuca HF (2003) Oral administration of 1,25-dihydroxyvitamin D_3 completely protects NOD mice from insulin-dependent diabetes mellitus. Arch Biochem Biophys 417:77–80
5. Mathieu C, Waer M, Laureys J, Rutgeerts O, Bouillon R (1994) Prevention of autoimmune diabetes in NOD mice by 1,25 dihydroxyvitamin D3. Diabetologia 37:552–558
6. Mathieu C, Waer M, Casteels K, Laureys J, Boullion R (1995) Prevention of type 1 diabetes by non-hypercalcemic doses of a new structural analogue of 1,25(OH)2D3, KH1060. Endocrinology 136:866–872
7. Casteels K, Waer M, Bouillon R et al (1998) 1,25 Dihydroxyvitamin D3 restores sensitivity to cyclophosphamide-induced apoptosis in non-obese diabetic (NOD) mice and protects against diabetes. Clin Exp Immunol 112:181–187
8. DeLuca HF, Cantorna MT (2001) Vitamin D: its role and uses in immunology. FASEB J 15:2579–2585
9. Holick MF (2004) Vitamin D: importance in the prevention of cancers, type 1 diabetes, heart disease, and osteoporosis. Am J Clin Nutr 79:362–371
10. Lindberg B, Ivarsson SA, Landin-Olsson M, Sundkvist G, Svanberg L, Lernmark A (1999) Islet autoantibodies in cord blood from children who developed type 1 (insulin-dependent) diabetes mellitus before 15 years of age. Diabetologia 42:181–187
11. Maghbooli Z, Hossein-Nezhad A, Shafaei AR, Karimi F, Madani FS, Larijani B (2007) Vitamin D status in mothers and their newborns in Iran. BMC Pregnancy Childbirth 7:1

12. Zeghoud F, Vervel C, Guillozo H, Walrant-Debray O, Boutignon H, Garabedian M (1997) Subclinical vitamin D deficiency in neonates: definition and response to vitamin D supplements. Am J Clin Nutr 65:771–778
13. Okonofua F, Houlder S, Bell J, Dantona P (1986) Vitamin D nutrition in pregnant Nigerian women at term and their newborn infants. J Clin Pathol 39:650–653
14. Brekke HK, Ludvigsson J (2007) Vitamin D supplementation and diabetes-related autoimmunity in the ABIS study. Pediatr Diabetes 8:11–14
15. Fronczak C, Baron A, Chase HP et al (2003) In utero dietary exposures and risk of islet autoimmunity in children. Diabetes Care 26:3237–3242
16. Stene LC, Ulriksen J, Magnus P, Joner G (2000) Use of cod liver oil during pregnancy associated with lower risk of type 1 diabetes in the offspring. Diabetologia 43:1093–1098
17. Stene LC, Joner G, Norwegian Childhood Diabetes Study Group (2003) Use of cod liver oil during the first year of life is associated with lower risk of childhood-onset type 1 diabetes: a large, population-based, case–control study. Am J Clin Nutr 78:1128–1134
18. The EURODIAB Substudy 2 Study Group (1999) Vitamin D supplement in early childhood and risk for type 1 (insulin-dependent) diabetes mellitus. Diabetologia 42:51–54
19. Hyppönen E, Läärä E, Reunanen A, Järvelin M-R, Virtanen SM (2001) Intake of vitamin D and risk of type 1 diabetes: a birth-cohort study. Lancet 358:1500–1503
20. Erkkola M, Karppinen M, Javanainen J, Räsänen L, Knip M, Virtanen SM (2001) Reproducibility and validity of a food frequency questionnaire during pregnancy. Am J Epidemiol 154:466–476
21. Kukko M, Kimpimäki T, Korhonen S et al (2005) Dynamics of diabetes-associated autoantibodies in young children with human leukocyte antigen-conferred risk for type 1 diabetes recruited from the general population. J Clin Endocrinol Metab 90:2712–2717
22. Uusitalo L, Nevalainen J, Niinistö S et al (2008) Serum α- and γ-tocopherol concentrations and risk of advanced beta cell autoimmunity in children with HLA-conferred susceptibility to type 1 diabetes mellitus. Diabetologia 51:773–780
23. Willett W (1998) Energy-adjusted or residual method. In: Willett W (ed) Nutritional epidemiology, 2nd edn. Oxford University Press, New York, pp 288–291
24. Virtanen SM, Kenward MG, Erkkola M (2006) Age at introduction of new foods and advanced beta-cell autoimmunity in young children with HLA-conferred susceptibility to type 1 diabetes. Diabetologia 49:1512–1521
25. Kulmala P, Savola K, Petersen JS (1998) Prediction of insulin-dependent diabetes mellitus in siblings of children with diabetes. A population-based study. J Clin Invest 101:327–336
26. Norris JM, Yin X, Lamb MM et al (2007) Omega-3 polyunsaturated fatty acid intake and islet autoimmunity in children at increased risk for type 1 diabetes. JAMA 298: 1420–1428
27. Hughes DA, Pinder AC (2000) n-3 polyunsaturated fatty acids inhibit the antigen-presenting function of human monocytes. Am J Clin Nutr 71(Suppl):357S–360S
28. Holvik K, Madar AA, Meyer HE et al (2007) A randomised comparison of increase in serum 25-hydroxyvitamin D concentration after 4 weeks of daily oral intake of 10 μg cholecalciferol from multivitamin tablets or fish oil capsules in healthy young adults. Br J Nutr 98:620–625
29. McDermott MF, Ramachandran A, Ogunkolade BW et al (1997) Allelic variation in the vitamin D receptor influences susceptibility to IDDM in Indian Asians. Diabetologia 40:971–975
30. Shimada A, Kanzawa Y, Motohashi Y et al (2008) Evidence for association between vitamin D receptor BsmI polymorphism and type 1 diabetes in Japanese. J Autoimmun 30:4207–4211
31. Mimbacas A, Trujillo J, Gascue C, Javiel G, Cardoso H (2007) Prevalence of vitamin D receptor gene polymorphism in a Uruguayan population and its relation to type 1 diabetes mellitus. Genet Mol Res 5:534–542
32. Bailey R, Cooper JD, Zeitels L et al (2007) Association of the vitamin D metabolism gene CYP27B1 with type 1 diabetes. Diabetes 56:2616–2621