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Maternal dietary fatty acid intake during pregnancy and the risk of preclinical and clinical type 1 diabetes in the offspring

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Abstract

The aim of the present study was to examine the associations between the maternal intake of fatty acids during pregnancy and the risk of preclinical and clinical type 1 diabetes in the offspring. The study included 4887 children with human leucocyte antigen (HLA)-conferred type 1 diabetes susceptibility born during the years 1997–2004 from the Finnish Type 1 Diabetes Prediction and Prevention Study. Maternal diet was assessed with a validated FFQ. The offspring were observed at 3- to 12-month intervals for the appearance of type 1 diabetes-associated autoantibodies and development of clinical type 1 diabetes (average follow-up period: 4·6 years (range 0·5–11·5 years)). Altogether, 240 children developed preclinical type 1 diabetes and 112 children developed clinical type 1 diabetes. Piecewise linear log-hazard survival model and Cox proportional-hazards regression were used for statistical analyses. The maternal intake of palmitic acid (hazard ratio (HR) 0·82, 95 % CI 0·67, 0·99) and high consumption of cheese during pregnancy (highest quarter v. intermediate half HR 0·52, 95 % CI 0·31, 0·87) were associated with a decreased risk of clinical type 1 diabetes. The consumption of sour milk products (HR 1·14, 95 % CI 1·02, 1·28), intake of protein from sour milk (HR 1·15, 95 % CI 1·02, 1·29) and intake of fat from fresh milk (HR 1·43, 95 % CI 1·04, 1·96) were associated with an increased risk of preclinical type 1 diabetes, and the intake of low-fat margarines (HR 0·67, 95 % CI 0·49, 0·92) was associated with a decreased risk. No conclusive associations between maternal fatty acid intake or food consumption during pregnancy and the development of type 1 diabetes in the offspring were detected.

Key words: Pregnancy: Fatty acids: Type 1 diabetes: Foods: Children: Cohort studies

Finland has the world's highest, and still increasing, incidence of type 1 diabetes, with the increase being particularly conspicuous among children aged below 5 years[1]. Type 1 diabetes develops as the result of an immune-mediated inflammatory reaction that destroys β-cells in the pancreatic islets[2]. Available data suggest that type 1 diabetes is caused by a complex interplay between intestinal microbiota, gut permeability and mucosal immunity[2]. Prenatal modulation of the gut is dependent on which factors the fetus encounters via the placenta[3]. Accordingly, maternal nutrition during

pregnancy may modulate the development of the gut immune system in the offspring. Maternal fatty acid status during pregnancy influences the fatty acid status of the fetus and newborn infant[4]. The fatty acids may influence immune programming in many ways, e.g. by altering the lipid composition of fetal cell membranes, cellular metabolism, eicosanoid synthesis and gene expression regulation[4].

Epidemiological studies have suggested that the consumption of *n*-3 fatty acid-rich cod-liver oil during pregnancy or infancy might help in the prevention of the development of type 1 diabetes[5,6]. In contrast, in a US cohort, maternal *n*-3 and *n*-6 fatty acid intake has been shown to be not associated with the appearance of islet autoimmunity in the offspring[7], and in a Norwegian cohort study, maternal serum concentrations of *n*-3 fatty acids during pregnancy have been reported to be not associated with the risk of clinical type 1 diabetes in the offspring[8]. By contrast, in a US cohort, the intake of *n*-3 fatty acids and their content in erythrocyte membranes in children have been shown to be inversely associated with β-cell autoimmunity[9]. Animal studies have also indicated a protective effect of *n*-3 fatty acids[10,11].

There are only a few studies that have considered the effect of various fatty acids on the development of type 1 diabetes. In our previous nested case–control analysis in children, serum fatty acid biomarkers of milk consumption have been shown to be positively associated, and linoleic acid inversely associated, with the risk of advanced β-cell autoimmunity at or close to the time of seroconversion[12]. In our recent study in children, we have found that the intake of fat from all milk products is associated with a higher risk of preclinical type 1 diabetes[13]. Lipid-induced impairment of β-cell function (lipotoxicity) has been well established both *in vitro* and *in vivo* in animal models in the pathogenesis of type 2 diabetes[14]. This raises the question of whether lipotoxicity is involved in the pathogenesis of type 1 diabetes as well. By contrast, MUFA have been shown to protect β-cells in some *in vitro* studies[15].

In the present study, we evaluated the intake of fatty acids and foods important in relation to fatty acid intake among pregnant Finnish women and assessed associations with the development of preclinical and clinical type 1 diabetes in their 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 study of Finnish children at an increased genetic risk of type 1 diabetes. Increased human leucocyte antigen (HLA)-conferred disease susceptibility is defined by a high-risk genotype (*HLA-DQB1 *02/*0302*) or a moderate-risk genotype (*HLA-DQB1*0302/x*, where *x* = other than **02*, **0301* or **0602*). Details of the genetic screening methods and enrolment criteria have been published previously[16]. The present study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all

procedures involving human subjects were approved by the Ethical Committees of the University Hospitals of Oulu and Tampere, Finland. Written informed consent was obtained from all the subjects.

The study population comprised 5605 children with HLA-conferred susceptibility to type 1 diabetes. They were born in Tampere or Oulu University Hospital between October 1997 and beginning of September 2004 (72 % of those invited). FFQ were completed by the mothers of 4887 children during pregnancy (87 %).

Assessment of maternal nutrition

The participants completed a validated 181-item semi-quantitative FFQ concerning their habitual diet during 1 month. The mothers were asked about their food consumption during the eighth month of pregnancy (the month preceding maternity leave in Finland). If more than ten of the 181 food frequency questions were not answered, the FFQ was excluded (*n* 53). The FFQ contained questions about the frequency (number of times per d, week or month) and amount of foods consumed, in units of common serving sizes. In addition, the FFQ contained questions related to what type of fat was used in cooking and baking and in salad dressings as well as questions related to the extent of home baking. The individual type and quantity of fat were taken into account when calculating food and nutrient intakes. FFQ content, validity and data processing have been described earlier[17]. Information on the intake of fatty acids from all kinds of supplements throughout pregnancy was requested. Sociodemographic and other factors, including maternal education and diabetes in close relatives, were recorded using a structured questionnaire completed by the parents after delivery.

Autoantibody assays and definition of outcome

The DIPP children were monitored for the appearance of signs of β-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, autoantibodies to 65 kDa isoform of glutamic acid decarboxylase and antibodies to tyrosine phosphatase-related islet antigen 2. Autoantibody samples were obtained during each of the study centre visits, 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 interval between visits was shortened to 3 months. The autoantibodies were analysed in the Research Laboratory, Department of Pediatrics, University of Oulu. ICA, insulin autoantibodies, autoantibodies to 65 kDa isoform of glutamic acid decarboxylase and autoantibodies to tyrosine phosphatase-related islet antigen 2 levels were quantified as described previously[18].

We defined preclinical type 1 diabetes as being repeatedly positive for ICA and one or more of the three other autoantibodies analysed. Altogether, 283 of the 5605 (5·0 %) children seroconverted to repeated ICA positivity and positivity to one of the three other autoantibodies during this period at

a mean age of 3·6 (range 0·5–10·0) years. Of the children, 145 (2·6%) had progressed to clinical type 1 diabetes at a mean age of 4·6 (range 0·5–11·5) years. Among the children who progressed to clinical type 1 diabetes, 103 had been repeatedly positive for ICA and at least one other autoantibody. However, fifteen of the remaining forty-two children who progressed to type 1 diabetes had or had had one or more autoantibodies before or at the time of diagnosis. Moreover, ten children who developed type 1 diabetes had been persistently seronegative, with their last blood sample being drawn at a mean age of 4·0 (range 0·3–7·7) years before diagnosis. A total of seventeen children were not subjected to any autoantibody analysis before the diagnosis of diabetes. Thus, preclinical type 1 diabetes endpoint was defined as the first occurrence of either (1) repeated positivity for ICA in combination with positivity for one or more of the autoantibodies analysed or (2) clinical type 1 diabetes. Due to missing maternal FFQ data, statistical analyses were carried out with 240 preclinical and 112 clinical type 1 diabetes endpoints.

Statistical analyses

The endpoint of preclinical type 1 diabetes is interval censored and possibly dependent on siblings. To accommodate this structure, a piecewise linear log-hazard survival model was used to analyse the associations of fatty acid intake and food consumption with the risk of preclinical type 1 diabetes, assuming linear log-hazards in the intervals 0–1·99, 2–3·99 and >4 years. The results were not sensitive to the particular choice of intervals used. Observation intervals beyond positivity did not contribute to the analyses. The models were fitted using maximum likelihood in SAS PROC NLMIXED, with standard errors of the estimates derived from the observed information matrix. Random effects for family were introduced to accommodate familial dependence, and these were assumed to follow a normal distribution. The proportionality of the hazards was tested by adding linear interaction terms of the exposure variables with time to the models. To illustrate such time-varying effects, the endpoint analyses were carried out in two settings. First, all the data were used in the analyses. Second, data that included endpoint information only up to 3 years of age were used. There was no interval censoring in the diagnosis of clinical type 1 diabetes. Therefore, the associations of fatty acid intake and food consumption with the risk of clinical disease were analysed by Cox proportional-hazards regression.

Fatty acid variables were adjusted for energy intake by the residual method[19] after logarithmic transformation. Food variables were adjusted for energy intake by adding energy intake as a covariate to the survival model. Fatty acid and food variables were used as both continuous and categorical (the latter defined by falling into intervals given by the first and the third sample quartile) explanatory variables in the analyses. Some food variables were dichotomised because of a high proportion of non-users. If there is no indication of a non-linear association between dietary variables and endpoint, only results for continuous covariates are presented. Otherwise, results for categorical variables are presented. In addition to energy-adjusted fatty acid variables, analyses were carried out for the relative proportions of fatty acids (the percentages of total fatty acid intake). We also separately analysed the intakes of fat and protein from milk products.

The possible confounding by background characteristics (hospital of birth, familial diabetes and maternal vocational education) was controlled by adding background variables as covariates to the survival model; SAS version 9.2 (SAS Institute) was used in the analyses. Statistical significance was taken as less than 5%.

Results

The characteristics of the study children are given in Table 1. The maternal average daily intake of fatty acids from foods and mean consumption of foods during pregnancy are given in Tables 2 and 3, respectively. The number of users of supplements containing fatty acids was small (n 81, 1·7% of all

Table 1. Characteristics of the study participants

(Number of participants and percentages)

Characteristics	Distribution		With endpoint preclinical type 1 diabetes		With endpoint type 1 diabetes	
	n	Percentage of total	n	Percentage of total	n	Percentage of total
Total n	4887	100	240	4·9	112	2·3
Genetic risk group						
High risk (*DQB1*02/*0302*)	961	19·7	77	8·0	39	4·1
Moderate risk (*DQB1*0302/x**)	3926	80·3	163	4·2	73	1·9
Familial diabetes						
Yes	285	5·8	32	11·2	19	6·7
No	4421	90·5	202	4·6	91	2·1
Missing data	181	3·7	6	3·3	2	1·1
Maternal education						
None	297	6·1	27	9·1	11	3·7
Vocational school/training	1292	26·4	58	4·5	22	1·7
Upper secondary/vocational	2067	42·3	88	4·3	46	2·2
Academic	1103	22·6	60	5·4	29	2·6
Missing data	128	2·6	7	5·5	4	3·1

*x not equal to *02, *0301 and *0602.

Table 2. Maternal mean daily intake of fatty acids from food sources among pregnant Finnish women (n 4887) during the years 1997–2004

Fatty acids	Fresh and sour milk products		Cheese		Butter and butter-oil spreads		Low-fat margarines		High-fat margarines		Oils		Meat and meat products		Fish and fish products		Other food groups*		From all food groups	
	Mean	Percentage of total intake	Mean	Percentage of total intake	Mean	Percentage of total intake	Mean	Percentage of total intake	Mean	Percentage of total intake	Mean	Percentage of total intake	Mean	Percentage of total intake	Mean	Percentage of total intake	Mean	Percentage of total intake	Mean	Percentage of total intake
Total fatty acids (g)	14.1	13.4	14.9	14.2	10.2	9.8	1.0	0.9	5.7	5.5	11.5	10.9	20.8	19.8	1.6	1.6	24.0	22.9	104.7	100
SFA (g)	9.4	20.3	9.8	21.2	5.8	12.5	0.3	0.6	1.6	3.6	1.1	2.3	7.8	16.8	0.3	0.7	10.2	22.0	46.2	100
Myristic acid (mg)	1514	29.9	1548	30.6	924	18.3	20	0.4	143	2.8	12	0.2	345	6.8	54	1.1	497	9.8	5056	100
Pentadecanoic acid (mg)	212	32.3	215	32.7	143	21.7	1	0.0	1	0.1	0	0.0	44	6.7	7	1.0	34	5.2	658	100
Palmitic acid (mg)	4023	18.7	4056	18.8	2372	11.0	145	0.7	671	3.1	760	3.5	4532	21.7	219	1.0	4698	21.8	21564	100
Stearic acid (g)	2	14.4	2	15.8	1	8.7	0	0.3	0	3.1	0	1.9	3	23.5	0	0.4	4	31.6	11	100
MUFA (g)	3.3	8.8	3.7	9.7	3.0	7.9	0.4	1.1	2.5	6.7	6.1	16.2	9.3	24.7	0.7	1.7	8.4	22.2	37.7	100
Palmitoleic acid (n-7) (mg)	187	14.6	183	14.3	115	9.0	2	0.1	3	0.2	11	0.9	532	41.7	105	8.2	137	10.8	1277	100
Palmitoleic acid (n-9) (mg)	24	7.8	28	9.1	13	4.2	0	0.1	6	2.0	6	1.9	187	60.9	4	1.2	39	12.6	306	100
Oleic acid (mg)	262	1.2	152	0.7	119	0.6	226	1.0	1031	4.8	5700	26.3	7745	35.8	318	1.5	5809	26.8	21646	100
Cis-vaccenic acid (mg)	47	3.8	24	2.0	5	0.4	13	1.1	65	5.2	230	18.5	471	37.9	37	3.0	334	26.9	1244	100
PUFA (g)	0.4	2.6	0.4	2.8	0.8	5.2	0.3	1.8	1.2	8.0	3.8	25.8	2.5	17.3	0.6	3.9	4.3	29.1	14.6	100
n-3 (g)	0.1	1.9	0.1	2.2	0.2	6.1	0.0	1.5	0.4	11.2	0.9	28.6	0.4	11.4	0.4	12.1	0.7	22.5	3.2	100
α-Linolenic acid (mg)	61	2.2	72	2.6	197	7.2	50	1.8	364	13.3	927	33.9	245	9.0	30	1.1	706	25.8	2734	100
EPA (mg)	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	21	21.0	81	79.0	0	0.1	102	100
DHA (mg)	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	40	13.9	216	75.4	30	10.6	286	100
n-6 (g)	0.2	2.2	0.3	2.4	0.5	4.3	0.2	2.0	0.8	7.3	2.8	25.7	2.1	19.2	0.2	1.6	3.5	31.5	11.1	100
Linoleic acid (mg)	229	2.1	236	2.2	462	4.3	211	2.0	806	7.5	2843	26.3	1963	18.2	163	1.5	3453	32.0	10792	100
Arachidonic acid (mg)	3	2.9	1	0.7	0	0.0	0	0.0	0	0.0	0	0.0	76	67.2	8	7.3	25	21.8	113	100
Conjugated linoleic acid (mg)	70	36.0	72	36.7	35	17.9	0	0.2	1	0.4	5	2.5	6	3.3	0	0.2	5	2.7	195	100

* Eggs, snacks, sweets, chocolates, seeds and nuts, industrial and animal fats, bread and cereals, and other less important food groups in relation to fatty acid intake.

women). Thus, intakes of fatty acids only from food sources are presented and used in the aetiological analysis. The maternal mean daily intake of total fatty acids was 32·8% of energy intake (E%), SFA 14·4 E%, MUFA 11·7 E%, PUFA 4·7 E%, n-3 PUFA 1·0 E% and n-6 PUFA 3·5 E%. Fresh and sour milk, cheese, butter and butter-oil spreads were the most important sources of SFA, the most important of which were myristic, pentadecanoic and palmitic acids (Table 2). Red meat and meat products were the main sources of palmitoleic acid (both n-9 and n-7), stearic acid, oleic acid, cis-vaccenic acid and arachidonic acid. Linoleic acid and α-linolenic acid were derived mainly from oils and EPA and DHA from fish.

The maternal intake of saturated palmitic acid (HR 0·82, 95 % CI 0·67, 0·99; P=0·039) was weakly associated with a decreased risk of clinical type 1 diabetes (Table 4). For the endpoint clinical type 1 diabetes, interactions between fatty acids and time were significant for the following fatty acids: palmitoleic acid isomers 16 : 1n-7 (P=0·019) and 16 : 1n-9 (P=0·014); EPA (P=0·037); dihomo-γ-linolenic acid (P=0·013). To illustrate these apparently time-varying effects, analyses were carried out separately for children aged below 3 years. When the follow-up time was less than 3 years, mono-unsaturated palmitoleic acid (16 : 1n-7) (HR 0·54, 95 % CI 0·36, 0·86; P=0·014) was associated with a decreased risk of clinical type 1 diabetes, but not during the overall follow-up period.

High consumption of cheese was associated with a decreased risk of clinical type 1 diabetes (highest quarter v. intermediate half HR 0·52 (95 % CI 0·31, 0·87)) (Table 3). The consumption of sour milk was associated with an increased risk of preclinical type 1 diabetes (HR 1·14, 95 % CI 1·02, 1·28), and the consumption of low-fat margarines was associated with a decreased risk (HR 0·67, 95 % CI 0·49, 0·92). No significant interactions with time were detected for any of the foods, and results are thus presented for only the whole period. The adjustment for the putative confounding variables (genetic risk, hospital of birth, familial diabetes and maternal vocational education) modified the results slightly (Tables 3, 4 and 5).

To determine which fraction of milk products accounts for the observed associations with the development of preclinical or clinical type 1 diabetes, we separately examined the intakes of fat and protein from milk products. High intakes of fat from fresh milk (HR 1·43, 95 % CI 1·04, 1·96) and protein from sour milk (HR 1·15, 95 % CI 1·02, 1·29) were associated with an increased risk of preclinical type 1 diabetes (Table 5).

Discussion

In the present prospective cohort study with genetically susceptible offspring, it was found that maternal diet during pregnancy was weakly associated with the development of preclinical and clinical type 1 diabetes. The maternal intake of palmitic acid and consumption of cheese during pregnancy were inversely associated with the development of clinical type 1 diabetes. The consumption of sour milk products, intake of protein from sour milk and intake of fat from fresh milk products were associated with an increased risk of pre-clinical type 1 diabetes, and the intake of low-fat margarines was associated with a decreased risk.

Table 3. Associations of maternal food consumption during pregnancy with the risk of preclinical and clinical type 1 diabetes in the offspring*

(Mean values and standard deviations; hazard ratios (HR) and 95 % confidence intervals)

Food group (g/d per times per week)	Mean	SD	HR†	95 % CI	P‡	Adjusted HR§	95 % CI	P‡	HR†	95 % CI	P‡	Adjusted HR§	95 % CI	P‡
					Preclinical type 1 diabetes (n 4887, n 240)						Type 1 diabetes (n 4887, n 112)			
Cows' milk products (continuous) (g)	856	422	1·05	0·91, 1·21	0·521	1·02	0·88, 1·20	0·754	1·01	0·81, 1·25	0·943	1·01	0·81, 1·13	0·921
Fresh milk (g)	526	369												
Lowest quarter: <213			0·94	0·69, 1·28		0·94	0·68, 1·30		0·87	0·56, 1·36		0·88	0·56, 1·38	
Intermediate half			1		0·421	1		0·196	1		0·071	1		0·037
Highest quarter: >760			0·81	0·58, 1·12		0·73	0·51, 1·04		0·56	0·33, 0·94		0·51	0·30, 0·88	
Cheese (g)	65	46												
Lowest quarter: <31			0·82	0·60, 1·13		0·78	0·56, 1·09		0·65	0·40, 1·04		0·61	0·38, 1·00	
Intermediate half			1		0·100	1		0·127	1		0·015	1		0·023
Highest quarter: >86			0·71	0·51, 0·99		0·74	0·52, 1·04		0·52	0·31, 0·87		0·55	0·33, 0·94	
Sour milk (continuous) (g)	231	199	1·14	1·02, 1·28	0·030	1·16	1·03, 1·30	0·024	1·14	0·96, 1·35	0·155	1·15	0·97, 1·37	0·397
Butter and butter-oil spreads (continuous) (g)	15	15	0·92	0·80, 1·07	0·293	0·96	0·83, 1·11	0·540	0·90	0·73, 1·12	0·330	0·92	0·74, 1·14	0·421
Low-fat margarines (g)	2·5	6												
Users v. non-users			0·67	0·49, 0·92	0·010	0·66	0·48, 0·91	0·009	0·70	0·44, 1·10	0·104	0·66	0·41, 1·05	0·066
High-fat margarines (g)	7	13												
Users v. non-users			1·12	1·00, 1·26	0·378	1·12	0·85, 1·46	0·422	1·16	0·80, 1·69	0·431	1·18	0·81, 1·73	0·391
Oil (continuous) (g)	11	7	0·96	0·84, 1·11	0·677	0·96	0·82, 1·11	0·557	0·96	0·78, 1·17	0·661	1·03	0·83, 1·28	0·804
Red meat and meat products (continuous) (g)	127	61	1·10	0·94, 1·28	0·231	1·09	0·93, 1·27	0·308	1·15	0·92, 1·44	0·213	1·16	0·92, 1·45	0·208
Poultry (continuous) (g)	37	32	0·97	0·85, 1·12	0·718	1·02	0·89, 1·17	0·805	0·98	0·79, 1·20	0·831	1·03	0·83, 1·27	0·810
Fatty fish‖ (continuous) (times per week)	1·3	1·3	0·89	0·76, 1·03	0·108	0·91	0·78, 1·06	0·193	0·92	0·74, 1·14	0·414	0·91	0·73, 1·14	0·411
Lean fish¶ (continuous) (times per week)	0·7	0·7	1·00	0·88, 1·14	0·966	0·97	0·85, 1·12	0·687	1·05	0·88-1·26	0·604	0·99	0·81, 1·20	0·883

* For continuous food variables, HR describes the change in risk, when the food variable is changed by an amount corresponding to its standard deviation. For categorical food variables, reference category is the intermediate half, and for dichotomised variables, reference category is the non-users.
† Model was adjusted for maternal energy intake.
‡ Likelihood ratio test was used to test whether the model with and without the food variables differed.
§ Model was adjusted for maternal energy intake, genetic risk, hospital of birth, familial diabetes and maternal vocational education.
‖ Salmon, rainbow trout, Baltic herring, mackerel, lavaret, trout, European cisco, pike-perch, flounder, bream, rosefish, herring, anchovy and tuna.
¶ Fish fingers, frozen fish, perch, northern pike, burbot, shrimps and shell.

Table 4. Associations of maternal fatty acid intake during pregnancy with the risk of preclinical and clinical type 1 diabetes in the offspring*

(Hazard ratios (HR) and 95 % confidence intervals)

	Preclinical type 1 diabetes						Type 1 diabetes					
	All (n 4887, n 240)			All (n 4887, n 240)			All (n 4887, n 112)			All (n 4887, n 112)		
Fatty acids (g/mg)	HR	95 % CI	P†	Adjusted HR‡	95 % CI	P†	HR	95 % CI	P†	Adjusted HR‡	95 % CI	P†
Total fatty acids (g)	0·92	0·81, 1·05	0·247	0·92	0·80, 1·05	0·224	0·86	0·71, 1·05	0·134	0·86	0·71, 1·05	0·143
SFA (g)	0·90	0·79, 1·03	0·127	0·91	0·79, 1·04	0·163	0·83	0·68, 1·01	0·056	0·84	0·69, 1·03	0·087
Myristic acid (14 : 0) (mg)	0·90	0·79, 1·03	0·133	0·92	0·81, 1·06	0·246	0·82	0·67, 1·00	0·041	0·86	0·71, 1·05	0·132
Pentadecanoic acid (15 : 0) (mg)	0·91	0·80, 1·04	0·172	0·93	0·81, 1·06	0·172	0·84	0·69, 1·03	0·083	0·87	0·71, 1·06	0·153
Palmitic acid (16 : 0) (mg)	0·89	0·79, 1·02	0·099	0·90	0·79, 1·03	0·134	0·82	0·67, 0·99	0·039	0·84	0·69, 1·02	0·074
Stearic acid (18 : 0) (mg)	0·91	0·80, 1·03	0·150	0·91	0·79, 1·04	0·162	0·84	0·69, 1·03	0·085	0·85	0·70, 1·04	0·120
MUFA (g)	0·96	0·84, 1·09	0·551	0·96	0·82, 1·08	0·404	0·93	0·76, 1·12	0·427	0·91	0·75, 1·11	0·348
Palmitoleic acid (16 : 1n-7) (mg)	0·91	0·79, 1·03	0·145	0·93	0·81, 1·07	0·307	0·90	0·74, 1·09	0·265	0·95	0·78, 1·15	0·571
Palmitoleic acid (16 : 1n-9) (mg)	0·96	0·84, 1·10	0·562	0·98	0·86, 1·12	0·753	1·02	0·84, 1·24	0·833	1·05	0·87, 1·26	0·649
Oleic acid (18 : 1n-9) (mg)	0·93	0·82, 1·06	0·296	1·02	0·89, 1·16	0·802	0·92	0·76, 1·11	0·386	0·99	0·82, 1·20	0·926
Cis-vaccenic acid (18 : 1n-7) (mg)	0·95	0·84, 1·08	0·456	10·03	0·84, 1·08	0·693	0·97	0·80, 1·16	0·707	1·04	0·87, 1·26	0·660
PUFA (g)	1·01	0·89, 1·14	0·887	0·96	0·83, 1·09	0·502	1·00	0·83, 1·20	0·975	0·98	0·80, 1·19	0·798
n-3 (g)	0·97	0·86, 1·10	0·685	0·97	0·86, 1·10	0·685	1·02	0·85, 1·23	0·802	0·99	0·82, 1·20	0·917
α-Linolenic acid (18 : 3n-3) (mg)	0·99	0·87, 1·12	0·856	0·97	0·85, 1·10	0·607	1·02	0·85, 1·22	0·848	0·98	0·81, 1·19	0·844
EPA (20 : 5n-3) (mg)	0·94	0·82, 1·08	0·401	0·96	0·83, 1·11	0·560	1·03	0·86, 1·24	0·730	1·04	0·85, 1·26	0·719
DHA (22 : 6n-3) (mg)	0·96	0·84, 1·10	0·531	0·97	0·84, 1·12	0·708	1·04	0·87, 1·25	0·669	1·04	0·86, 1·27	0·687
n-6 (g)	1·02	0·90, 1·16	0·707	1·01	0·88, 1·15	0·910	1·00	0·83, 1·21	0·996	0·98	0·80, 1·19	0·219
Linoleic acid (18 : 2n-6) (mg)	1·01	0·89, 1·15	0·796	1·01	0·88, 1·15	0·942	1·00	0·83, 1·20	0·995	1·00	0·83, 1·20	0·995
Arachidonic acid (20 : 4n-6) (mg)	1·03	0·91, 1·17	0·608	1·04	0·92, 1·19	0·537	1·08	0·90, 1·30	0·397	1·08	0·90, 1·30	0·397
γ-Linolenic acid (18 : 3n-6) (mg)	0·92	0·78, 1·09	0·310	0·93	0·81, 1·07	0·293	0·84	0·65, 1·10	0·187	0·84	0·65, 1·10	0·187
Dihomo-γ-linolenic acid (20 : 3n-6) (mg)	0·96	0·84, 1·10	0·556	0·94	0·82, 1·08	0·384	1·01	0·84, 1·22	0·923	1·02	0·84, 1·22	0·874
Ratio of n-6:n-3	1·05	0·94, 1·18	0·407	1·06	0·94, 1·20	0·332	0·96	0·79, 1·17	0·698	0·98	0·81, 1·19	0·832
Conjugated linoleic acid (18 : 2n-6) (mg)	0·93	0·81, 1·06	0·757	0·94	0·82, 1·08	0·377	0·88	0·72, 1·07	0·186	0·89	0·73, 1·08	0·220

* Fatty acid variables used in statistical analyses were energy adjusted using Willett's residual method. HR describes change in risk, when fatty acid variable is changed by an amount corresponding to its standard deviation. These results are not adjusted for any potential confounders.

† Likelihood ratio test was used to test whether the model with and without the fatty acid variables differed.

‡ Model was adjusted for genetic risk, hospital of birth, familial diabetes and maternal vocational education.

Table 5. Associations of maternal intake of fat and protein from dairy products during pregnancy with the risk of preclinical and clinical type 1 diabetes in the offspring*

(Hazard ratios (HR) and 95 % confidence intervals)

Fat and protein from dairy products (g)	Preclinical type 1 diabetes (n 4887, n 240)						Type 1 diabetes (n 4887, n 112)					
	HR†	95 % CI	P‡	Adjusted HR§	95 % CI	P‡	HR†	95 % CI	P‡	Adjusted HR§	95 % CI	P‡
Fat from												
All cows' milk products (continuous)	0·89	0·75, 1·06	0·197	0·91	0·76, 1·08	0·275	0·87	0·68, 1·12	0·265	0·90	0·70, 1·16	0·408
Fresh milk												
Lowest quarter	1·35	0·98, 1·87		1·31	0·95, 1·82		1·62	1·04, 2·52		1·64	1·04, 2·59	
Intermediate half	1		0·046	1		0·081	1		0·114	1		0·112
Highest quarter	1·43	1·04, 1·96		1·39	1·01, 1·92		1·16	0·72, 1·86		1·17	0·72, 1·90	
Cheese (continuous)	0·86	0·74, 1·01	0·053	0·88	0·75, 1·03	0·101	0·89	0·71, 1·10	0·266	0·92	0·74, 1·15	0·459
Sour milk (continuous)	1·08	0·95, 1·22	0·280	1·07	0·94, 1·22	0·310	1·06	0·88, 1·28	0·549	1·06	0·88, 1·27	0·578
Protein from												
All cows' milk products (continuous)	0·94	0·80, 1·11	0·468	0·94	0·79, 1·11	0·480	0·92	0·73, 1·17	0·497	0·96	0·75, 1·22	0·713
Fresh milk												
Lowest quarter	0·94	0·68, 1·29		0·94	0·68, 1·29		0·88	0·56, 1·37		0·88	0·56, 1·39	
Intermediate half	1		0·340	1		0·155	1		0·077	1		0·045
Highest quarter	0·78	0·55, 1·09		0·71	0·50, 1·01		0·56	0·33, 0·95		0·52	0·30, 0·89	
Cheese												
Lowest quarter	0·92	0·67, 1·27		0·86	0·62, 1·19		0·80	0·51, 1·27		0·71	0·44, 1·15	
Intermediate half	1		0·177	1		0·241	1		0·070	1		0·088
Highest quarter	0·72	0·51, 1·02		0·75	0·53, 1·07		0·56	0·34, 0·94		0·60	0·36, 1·01	
Sour milk (continuous)	1·15	1·02, 1·29	0·030	1·16	1·03, 1·31	0·023	1·14	0·96, 1·36	0·147	1·16	0·98, 1·38	0·112

* For continuous fat and protein variables, HR describes change in risk, when the food variable is changed by an amount corresponding to its standard deviation. For categorical fat and protein variables, reference category is the intermediate half.
† Model was adjusted for energy intake.
‡ Likelihood ratio test was used to test whether the model with and without the food variables differed.
§ Model was adjusted for maternal energy intake, genetic risk, hospital of birth, familial diabetes and maternal vocational education.

Maternal fatty acid intake and diabetes risk

The strength of the present study is the population-based prospective cohort setting as well as the fact that the end-points used comprised both preclinical type 1 diabetes and, separately, clinical type 1 diabetes. The endpoint 'preclinical type 1 diabetes' is strongly associated with clinical disease[20]. The FFQ used in the present study was validated against two 5 d food records, and it estimated the consumption of food groups relatively well[17]. The consumption of milk products had very good validity (Pearson's correlation coefficients for different milk products varied between 0·58 and 0·86)[17].

The major limitation of the study is the high number of fatty acids and food groups analysed. Altogether, we tested over seventy associations. None of the associations was highly significant and may be false positive and occur due to chance. Therefore, the results must be interpreted with caution. Another limitation is that the children's diet during infancy was not taken into account, because the food records of all the children were not available. In the validation study, for some important food sources of fat, the correlations measured by FFQ and food records were low, as Pearson's correlation coefficients for the consumption of oils and low-fat spreads were 0·22 and 0·25, respectively[17].

The present observation is the first epidemiological one to indicate that the maternal intake of palmitic acid, which reflects the consumption of certain milk products, and consumption of cheese are inversely associated with clinical type 1 diabetes in the offspring. The results indicate that maternal exposure to certain milk products during pregnancy might induce tolerance to cows' milk in genetically susceptible offspring. A previous study has shown that the maternal intake of protein from cheese during pregnancy and maternal intake of protein from fresh milk products and cheese during lactation are marginally inversely associated with the humoral immune responses to cows' milk proteins in offspring with a high risk of type 1 diabetes[21]. An enhanced humoral immune response to various cows' milk proteins has been observed in infancy in children who later were diagnosed with type 1 diabetes[22].

In contrast to the inverse associations between the maternal intake of milk-related fatty acids and the risk of type 1 diabetes in the offspring in the present study, our previous case–control analysis in 315 children has shown that the fatty acid biomarkers of milk consumption are directly associated with the risk of islet autoimmunity at or before the time of sero-conversion[12]. Our newest findings in children were that the consumption of fresh milk products and cows' milk-based infant formulas and the intake of fat from all milk products and intake of protein from fresh milk products are weakly associated with a higher risk of advanced preclinical type 1 diabetes[13]. These findings support the hypothesis that the direct exposure of children to cows' milk leads to immunisation or the results could be partly explained by possible lipotoxicity. Several other case–control and cohort studies as well as randomised trial findings also support the hypothesis that cows' milk is associated with the development of type 1 diabetes, although there are conflicting results also[23,24]. Savilahti & Saarinen[25] observed that very early exposure to cows' milk in the maternity hospital may decrease the risk of type 1 diabetes in the offspring by the age of 8 years.

In the present study, the maternal intake of palmitoleic acid ($16:1n$-7) during pregnancy was inversely associated with the risk of clinical type 1 diabetes when the children were observed up to the age of 3 years. An inverse association of palmitoleic acid intake may reflect the maternal consumption of meat or dairy products. The observation could also reflect that the effect of palmitoleic acid may be protective in accordance with some *in vitro* studies[15].

Type 1 diabetes has been shown to be associated with increased inflammation, and n-3 fatty acids have been implicated as anti-inflammatory agents, while n-6 acids fatty acids have been shown to be pro-inflammatory agents[26]. In line with other cohort findings, the maternal intake of n-3 or n-6 fatty acids during pregnancy was not associated with the risk of preclinical or clinical type 1 diabetes in the present study[7,8].

In accordance with our previous analyses using a smaller dataset, the maternal intake of low-fat margarines during pregnancy was found to be associated with a decreased risk of preclinical type 1 diabetes[27]. In contrast to our earlier results[27], butter was not associated with the risk of prediabetes and sour milk was associated with an increased risk of prediabetes in the present study. However, in the present study, the maternal intake of protein from sour milk was also associated with an increased risk of prediabetes.

The present findings that the maternal intake of fat from fresh milk products is weakly associated with an increased risk of advanced preclinical type 1 diabetes may indicate lipotoxicity to be responsible for the pathogenesis of type 1 diabetes. However, palmitic acid intake exhibited a protective association with the clinical type 1 diabetes endpoint.

In other analyses carried out in the cohort of the present study, interesting findings emerged for fatty acids and other immune-mediated endpoints, and these suggest that maternal diet and fatty acid intake during pregnancy may influence the development of the immune system in the offspring. High maternal consumption of butter and a high ratio of n-6:n-3 fatty acids during pregnancy have been reported to be associated with an increased risk of allergic rhinitis in the offspring[28]. The maternal intakes of α-linoleic acid (n-3), total n-3 PUFA, SFA and palmitic acid during pregnancy have been reported to be associated with a decreased risk of asthma and that of arachidonic acid (n-6) with an increased risk[29]. These and present findings support the importance of fatty acids when studying relationships between nutrition and immune-mediated diseases.

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