

Fatty acid status in infancy is associated with the risk of type 1 diabetes-associated autoimmunity

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Abstract

Aims/hypothesis We investigated the association of early serum fatty acid composition with the risk of type 1 diabetes-associated autoimmunity. Our hypothesis was that fatty acid status during infancy is related to type 1 diabetes-associated autoimmunity and that long-chain *n*-3 fatty acids, in particular, are associated with decreased risk.

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Methods We performed a nested case–control analysis within the Finnish Type 1 Diabetes Prediction and Prevention Study birth cohort, carrying HLA-conferred susceptibility to type 1 diabetes ($n = 7782$). Serum total fatty acid composition was analysed by gas chromatography in 240 infants with islet autoimmunity and 480 control infants at the age of 3 and 6 months. Islet autoimmunity was defined as repeated positivity for islet cell autoantibodies in combination with at least one of three selected autoantibodies. In addition, a subset of 43 infants with primary insulin autoimmunity (i.e. those with insulin autoantibodies as the first autoantibody with no concomitant other autoantibodies) and a control group ($n = 86$) were analysed. A third endpoint was primary GAD autoimmunity defined as GAD autoantibody appearing as the first antibody without other concomitant autoantibodies (22 infants with GAD autoimmunity; 42 infants in control group). Conditional logistic regression was applied, considering multiple comparisons by false discovery rate <0.05 .

Results Serum fatty acid composition differed between breastfed and non-breastfed infants, reflecting differences in the fatty acid composition of the milk. Fatty acids were associated with islet autoimmunity (higher serum pentadecanoic, palmitic, palmitoleic and docosahexaenoic acids decreased risk; higher arachidonic:docosahexaenoic and *n*-6:*n*-3 acid ratios increased risk). Furthermore, fatty acids were associated with primary insulin autoimmunity, these associations being stronger (higher palmitoleic acid, *cis*-vaccenic, arachidonic, docosapentaenoic and docosahexaenoic acids decreased risk; higher α -linoleic acid and arachidonic:docosahexaenoic and *n*-6:*n*-3 acid ratios increased risk). Moreover, the quantity of breast milk consumed per day was inversely associated with primary insulin autoimmunity, while the quantity of cow's milk consumed per day was directly associated.

Conclusions/interpretation Fatty acid status may play a role in the development of type 1 diabetes-associated

autoimmunity. Fish-derived fatty acids may be protective, particularly during infancy. Furthermore, fatty acids consumed during breastfeeding may provide protection against type 1 diabetes-associated autoimmunity. Further studies are warranted to clarify the independent role of fatty acids in the development of type 1 diabetes.

Keywords Autoimmunity · Breast milk · Fatty acid status · Infant · *n*-3 fatty acids · Type 1 diabetes

Abbreviations

AA	Arachidonic acid
ALA	α -Linolenic acid
CLA	Conjugated linoleic acid
DIPP	Type 1 diabetes prediction and prevention study
DHA	Docosahexaenoic acid
DPA	Docosapentaenoic acid
EPA	Eicosapentaenoic acid
FDR	False discovery rate
GADA	Autoantibodies to 65 kDa isoform of GAD
GLA	γ -Linolenic acid
IAA	Insulin autoantibodies
IA-2A	Antibodies to tyrosine phosphatase-related islet antigen 2
ICA	Islet cell autoantibodies
LA	Linoleic acid
MUFA	Monounsaturated fatty acid
PUFA	Polyunsaturated fatty acid
SFA	Saturated fatty acid

Introduction

Type 1 diabetes is an autoimmune disease characterised by the destruction of pancreatic beta cells. Inflammatory reactions and immunological dysfunction precede the disease [1, 2], often beginning in infancy or early childhood [3]. Fatty acids may play a role in the development of type 1 diabetes-associated autoimmunity, as they have been shown to modulate the immune system and inflammatory reactions [4, 5]. Furthermore, early fatty acid status may be important because it can affect programming and development of an infant's immune system, as well as the maturation of the gut, with long-term consequences [6, 7]. Other lipids could also play a role. Interestingly, early lipidomic profile was associated with progression to type 1 diabetes [8]. Long-chain *n*-3 polyunsaturated fatty acid (PUFA) intake and/or status were protectively related to type 1 diabetes-associated autoimmunity or clinical disease in several [9–11], but not all [12, 13], epidemiological

studies. Maternal intake and status of fish-derived fatty acids during pregnancy were not associated with the risk of developing type 1 diabetes in the offspring [9, 14, 15], with the exception of one case–control study reporting a protective association [16]. Saturated fatty acids (SFAs) and monounsaturated fatty acids (MUFAs) have also been associated with the risk of type 1 diabetes-associated autoimmunity. In the Finnish type 1 diabetes prediction and prevention (DIPP) study, high serum levels of cow's milk-derived fatty acids were associated with a higher risk of islet autoimmunity [12]. This is in line with the observed association between milk fat consumption and increased risk of islet autoimmunity [17].

During early infancy, breast milk and infant formulas are the main dietary sources of fatty acids and therefore the type of milk feeding is likely the most important determinant of fatty acid status. Breastfeeding, measured as breastfeeding duration, was not found to be associated with the risk of islet autoimmunity or type 1 diabetes in cohort studies [17–20]. Looking at the other side of the coin, cow's milk consumption (measured as the quantity consumed) during infancy and childhood was related to increased risk of islet autoimmunity and/or type 1 diabetes in several prospective cohort series [17, 21–24]. The cause is unknown and several components in milk may be involved [25–27].

Our hypothesis was that fatty acid status during infancy is related to type 1 diabetes-associated autoimmunity and that, in particular, high levels of long-chain *n*-3 PUFAs are associated with decreased risk.

Methods

Study design The study was carried out in a nested case–control design within the Finnish DIPP Nutrition Study and included children at increased genetic risk for type 1 diabetes ($n = 7782$). Increased genetic risk was determined by the presence in cord blood of 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*). Children were recruited during the years 1997 and 2004. Informed consent was obtained from the families. DIPP children are monitored for islet autoimmunity and their blood samples are collected by venepuncture at the ages of 3, 6, 9, 12, 18 and 24 months and subsequently at 12 month intervals up to the age of 15 years. Ethical approval for this study was obtained from the local ethics committees.

For the nested case–control design, all cases of islet autoimmunity and of type 1 diabetes without preceding islet autoimmunity were identified at the end of the follow-up period

(Fig. 1). The participants were monitored for the appearance of markers for islet autoimmunity and clinical type 1 diabetes. Islet cell autoantibodies (ICA) were used as the primary screening tool for beta cell autoimmunity. When a child seroconverted to positivity for ICA for the first time, all that child's preceding and subsequent samples 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) and at this point the follow-up interval was shortened to 3 months.

Autoantibody assays ICA were quantified by a standard indirect immunofluorescence method on sections of frozen human pancreas from a blood group O donor [28]. IAA were quantified by microassay [29, 30] and GADA and IA-2A were analysed using specific radiobinding assays [31–33]. Details of the autoantibody methods are described by Kimpimäki et al [34].

Definition of outcomes Islet autoimmunity was defined as repeated positivity for ICA in combination with at least one of the biochemical autoantibodies analysed (IAA, GADA or IA-2A), or the presence of clinical type 1 diabetes. The median age of islet autoimmunity endpoint was 3.0 years (range 0.5–9.2). The diagnosis of diabetes was based on the WHO criteria. As a second endpoint within our islet autoimmunity case children, we used primary insulin autoimmunity, defined as IAA appearing as the first antibody without any other concomitant autoantibodies. The median age of primary insulin autoimmunity was 1.0 years (range 0.2–4.2). As a third endpoint, we used primary GAD autoimmunity in children with islet autoimmunity. Primary GAD autoimmunity was defined as GADA appearing as the first antibody without other

concomitant autoantibodies. The median age of primary GAD autoimmunity was 2.0 years (range 0.2–7.1). Distributions of seroconversion age for islet autoimmunity, primary insulin autoimmunity and GAD autoimmunity are presented in Table 1.

Selection of controls Matching criteria for the control children were the same sex, HLA-conferred DQB1 risk group (moderate or high risk), delivery hospital, the date of birth within 3 months in relation to the case children and not being from the same family as the case children. From all potential control children, two seronegative children were randomly selected at the age point at which the respective case child turned out to have islet autoimmunity or type 1 diabetes. For the random selection, one child could serve as a control for more than one case and could subsequently become a case. Thirty-one children served as control for two or more case children and ten of the control children later became a case. Finally, the total fatty acid composition was analysed in 240 children with islet autoimmunity or type 1 diabetes and 480 matched seronegative control children from the 3 and 6 month serum samples (Table 2).

Measurement of serum fatty acid status Serum fatty acid proportions were determined by using a gas chromatographic method (Hewlett Packard, Palo Alto, CA, USA) as described earlier [12]. Each case–control set was analysed in the same batch. Laboratory staff were blinded for case–control status.

Assessment of the child's diet Dietary information was obtained at the age of 3 and 6 months by a 3 day food record (including two weekdays and one weekend day [17]) and a dietary questionnaire in which families recorded the infant's age at introduction of new foods and breastfeeding. Infant

Fig. 1 Flow chart of the study. Islet autoimmunity was defined as repeated positivity for ICA in combination with at least one of the biochemical markers analysed (IAA, GADA or IA-2A) or presence of clinical type 1 diabetes. Primary insulin autoimmunity was defined as IAA appearing as the first autoantibody without any other concomitant autoantibodies. Primary GAD autoimmunity was defined as being first positive only for GADA out of the four autoantibodies measured (ICA, IAA, GADA, IA-2A). T1D, type 1 diabetes

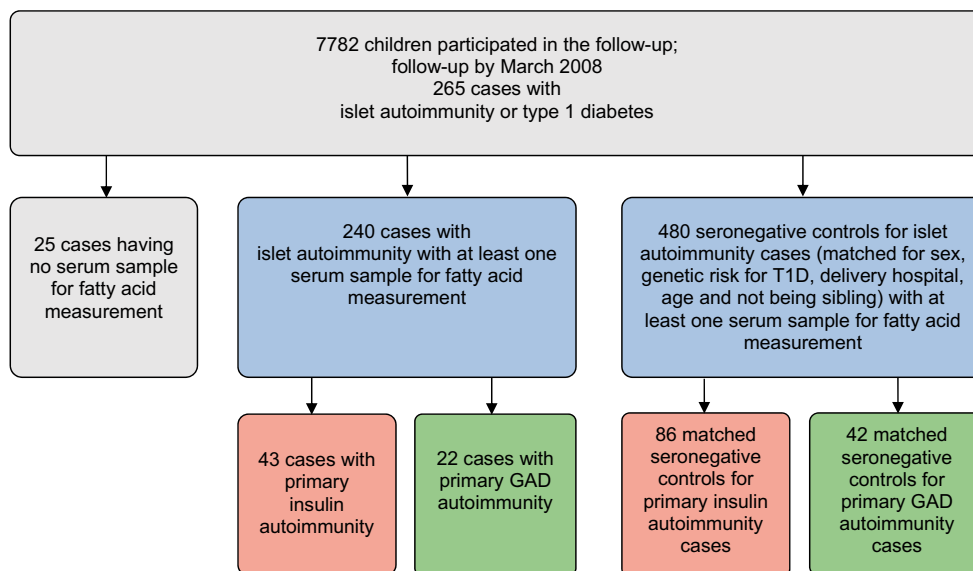


Table 1 Age of seroconversion for islet autoimmunity and for primary insulin and GAD autoimmunity

Age in months	Islet autoimmunity ^a (<i>N</i> = 240)	Primary insulin autoimmunity ^b (<i>N</i> = 43)	Primary GAD autoimmunity ^c (<i>N</i> = 22)
0–2.9	0 (0)	1 (2.3)	1 (4.6)
3–5.9	1 (0.4)	1 (2.3)	0 (0)
6–8.9	1 (0.4)	3 (7.0)	1 (4.6)
9–11.9	8 (3.3)	7 (16.3)	1 (4.6)
12–17.9	40 (16.7)	16 (37.2)	4 (18.2)
18–23.9	30 (12.5)	6 (14.0)	4 (18.2)
24–35.9	45 (18.8)	5 (11.6)	6 (27.3)
36–47.9	45 (18.8)	3 (7.0)	0 (0)
≥48	70 (29.2)	1 (2.3)	5 (22.7)

Data are shown as *n* (%)

^a Islet autoimmunity was defined as repeated positivity for ICA in combination with at least one of the biochemical autoantibodies analysed (IAA, GADA or IA-2A), or presence of clinical type 1 diabetes

^b Primary insulin autoimmunity was defined as IAA appearing as the first autoantibody without any other concomitant autoantibodies. The primary insulin autoimmunity cases were selected from among the islet autoimmunity cases

^c Primary GAD autoimmunity was defined as GADA appearing as the first autoantibody without any other concomitant autoantibodies. The GAD autoimmunity cases were selected from among the islet autoimmunity cases

feeding pattern, including feeding in the delivery hospital, was also established by interview during the clinical visits (3, 6, 9, 12 months). Families were given oral and written instructions by trained research nurses in how to fill in the food records, day care personnel were provided with written instructions and the records were checked by trained research nurses at the clinical visit [17].

Our definition of exclusive breastfeeding was that breast milk was the only drink or food to which the infant was exposed, except for water and dietary supplementation (not containing energy). The composition of breast milk was taken from the Finnish national food composition database (FINELI) which was updated based on Finnish breast milk samples collected during the years 2011–2012 from 40 women whose children were 1–6 months of age. Fatty acid composition of infant formula was based on data reported by the manufacturers.

Calculation of the quantity of breast milk consumed The quantity of breast milk consumed was calculated using estimated energy requirements and growth [35]. The length and weight of the children were measured every 3 months during the first year of life at the study centre visits. Energy need was estimated based on energy requirements for weight and age, plus the estimated amount of incremental energy needed to support tissue accretion (energy deposition) [35]. Estimated energy requirement equalled total energy requirement plus energy deposition. Estimated energy from breast milk equalled estimated energy requirement minus energy intake from food records. Based on this, the quantity of breast milk consumed per day was calculated.

Statistical analyses Background characteristics of children comprising cases and their controls were compared using conditional logistic regression analysis. This accounts for the matching of cases and controls and is the standard method of estimation in nested case–control study designs. To analyse the serum fatty acid status in breastfed and non-breastfed infants, they were categorised according to their breastfeeding status at the age of 3 months: (1) exclusively breastfed; (2) breastfed, but not exclusively breastfed and (3) non-breastfed. At the age of 6 months we used only two categories for breastfeeding: (1) breastfed and (2) non-breastfed. This was because there were only six children who were exclusively breastfed at that age. The differences in serum status between breastfeeding categories were tested using mixed linear model with matched sets used as random effects.

To assess the associations between serum fatty acid proportions and islet autoimmunity, primary insulin autoimmunity or primary GAD autoimmunity, conditional logistic regression was applied. To account for potential confounders, we included cow's milk consumption, familial diabetes and maternal vocational education in the multiple conditional likelihood logistic regression models. We used cow's milk consumption as an adjusting factor because it is a more accurate variable (measured by food records) than breastmilk consumption (estimate based on growth). The quantity of cow's milk and breast milk consumed cannot be included as adjusting factors in the same model because of their strong correlation. Children with missing confounder data were excluded from the adjusted analysis. Multiple testing was addressed by using a *p* value adjustment method that controls the false discovery rate (FDR) [36]. We considered FDR <0.05 to be significant. Interactions between fatty acids and breastfeeding with the

Table 2 Characteristics of the study children with islet autoimmunity and seronegative controls and risk of islet autoimmunity associated with the background variables

Characteristic	Children with islet autoimmunity ^a (<i>N</i> = 240)	Control children ^b (<i>N</i> = 434)	OR (95% CI)	<i>p</i> value ^c
First degree relative affected by type 1 diabetes				
No	209 (88.6)	396 (95.0)	1	0.001
Yes	27 (11.4)	21 (5.0)	2.85 (1.52, 5.35)	
Missing information	4	17		
Maternal vocational education				0.002
None	25 (10.6)	18 (4.3)	1	
Vocational school or course	63 (26.8)	116 (27.6)	0.36 (0.18, 0.73)	
Upper secondary vocational education	86 (36.6)	195 (46.3)	0.29 (0.15, 0.58)	
Academic	61 (26.0)	92 (21.9)	0.48 (0.24, 0.95)	
Missing information	5	13		
Mode of delivery				0.168
Vaginal	212 (89.8)	370 (85.6)	1	
Caesarean section	24 (10.2)	62 (14.4)	0.70 (0.42, 1.16)	
Missing information	4	2		
Maternal age, years	29.7 ± 5.9	29.1 ± 4.9	1.02 (0.99, 1.05)	0.142
Gestational age, pregnancy weeks	39.4 ± 2.0	39.7 ± 1.9	0.94 (0.86, 1.01)	0.106
No maternal smoking during pregnancy (reference group: yes) ^d	210 (90.1)	392 (92.7)	1.42 (0.80, 2.54)	0.229
Duration of exclusive breastfeeding				0.347
<1 month	103 (44.4)	180 (41.7)	1	
1–2.99 months	61 (26.3)	100 (23.1)	0.95 (0.65, 1.39)	
≥3 months	68 (29.3)	152 (35.2)	0.76 (0.52, 1.11)	
Missing information	8	2		
Duration of any breastfeeding				0.148
<3 months	52 (22.4)	83 (19.3)	1	
3–5.99 months	54 (23.3)	82 (19.0)	1.11 (0.69, 1.81)	
≥6 months	126 (54.3)	266 (61.7)	0.77 (0.51, 1.15)	
Missing information	8	3		
Quantity of breast milk consumed (including breastfed and not breastfed), g				0.288
At 3 months of age	654 ± 428	695 ± 411	0.90 (0.74, 1.09)	
At 6 months of age	337 ± 372	375 ± 384	0.86 (0.71, 1.04)	0.121
Quantity of cow's milk consumed, g				0.194
At 3 months of age	271 ± 367	232 ± 342	1.12 (0.94, 1.33)	
At 6 months of age	398 ± 344	340 ± 331	1.25 (1.05, 1.50)	0.013

Data are shown as means ± SD, *n* (%) or *n*

^a Islet autoimmunity is defined as repeated positivity for islet autoantibodies and also positivity for at least one biochemical autoantibody out of the three that were measured (IAA, GADA or IA-2A), or presence of clinical type 1 diabetes

^b Twenty-nine children served as control for two cases, one child for three cases and one child for four cases, but were included only once in the total number of control children. Ten of the control children became a case at a later date and were counted as cases. Of these ten children, two served as control for two cases

^c Difference between cases and controls was tested with the Wald test obtained from the conditional likelihood analysis of logistic regression

^d Data not available for 7 cases and 11 controls

endpoints were studied by adding an interaction term to the conditional logistic regression model. We reported results from the interaction models if there was an indication of an interaction (FDR <0.05). Spearman's correlations between serum fatty acids and milk feeding were also calculated. Analyses were performed using SAS version 9.2 (SAS Institute, Cary, NC, USA).

Results

Background variables and the risk of islet autoimmunity

Familial type 1 diabetes was associated with an increased risk

of islet autoimmunity, while maternal vocational education was associated with a decreased risk (Table 2).

Serum fatty acid status in relation to breastfeeding status

Serum fatty acid status differed between breastfed and non-breastfed infants at the ages of 3 months and 6 months, reflecting differences in fatty acid composition of breast milk and infant formulas (Table 3). Breastfed infants had higher serum levels of myristic, stearic, conjugated linoleic (CLA), palmitoleic, *cis*-vaccenic, dihomo- γ -linolenic (GLA), arachidonic (AA), eicosapentaenoic (EPA), docosapentaenoic (DPA) and docosahexaenoic (DHA) acids, compared with non-breastfed infants, at 3 and 6 months of age. Breastfed infants had lower

Table 3 Proportions of fatty acids in serum stratified by breastfeeding status at the ages of 3 and 6 months, and fatty acid composition of breast milk and cow's milk-based formulas consumed in Finland during the years 1997–2005

Fatty acid	Mean serum fatty acids at 3 months of age (% of total fatty acids)				Mean serum fatty acids at 6 months of age (% of total fatty acids)			Fatty acid composition (g/100 g milk)	
	Exclusively breastfed (<i>n</i> = 201)	Breastfed, but not exclusively (<i>n</i> = 280)	Not breastfed (<i>n</i> = 122)	<i>p</i> value ^a	Breastfed (<i>n</i> = 376)	Not breastfed (<i>n</i> = 260)	<i>p</i> value ^a	Breast milk	Cow's milk- based formula
SFA								1.490	1.410
Myristic acid 14:0	1.61	1.49	1.14	<0.0001	1.22	0.96	<0.0001	0.221	0.131
Pentadecanoic acid 15:0	0.23	0.21	0.21	0.003	0.20	0.20	0.065	0.017	0.016
Palmitic acid 16:0	23.78	23.47	23.44	0.819	23.36	23.41	0.131	0.769	0.882
Stearic acid 18:0	10.58	10.05	8.78	<0.0001	9.48	8.28	<0.0001	0.239	0.210
Trans								0.040	0.020
Conjugated LA 18:2 <i>n</i> -7/(9- <i>cis</i> , 11- <i>trans</i> -18:2)	0.27	0.24	0.23	<0.0001	0.23	0.20	<0.0001	0.008	0.006
MUFA								1.210	1.370
Palmitoleic acid 16:1 <i>n</i> -9	0.49	0.44	0.32	<0.0001	0.38	0.31	<0.0001	0.010	0.005
Palmitoleic acid 16:1 <i>n</i> -7	1.79	1.63	1.10	<0.0001	1.63	1.31	<0.0001	0.072	0.014
Oleic acid 18:1 <i>n</i> -9	30.24	29.93	32.38	<0.0001	28.79	31.76	<0.0001	1.105	1.310
<i>cis</i> -vaccenic acid 18:1 <i>n</i> -7	2.34	2.16	1.61	<0.0001	2.14	1.71	<0.0001	0.058	0.022
PUFA								0.480	0.600
<i>n</i> -6								0.367	0.502
LA 18:2 <i>n</i> -6	19.73	21.47	25.20	<0.0001	23.75	26.07	<0.0001	0.337	0.500
GLA 18:3 <i>n</i> -6	0.13	0.13	0.17	0.012	0.12	0.16	<0.0001	0.003	0.002
Dihomo-GLA 20:3 <i>n</i> -6	1.23	1.20	0.98	<0.0001	1.01	0.92	<0.0001	0.008	0.000
AA 20:4 <i>n</i> -6	4.37	4.31	2.55	<0.0001	4.32	2.65	<0.0001	0.009	0.001
<i>n</i> -3								0.098	0.090
ALA 18:3 <i>n</i> -3	0.78	0.80	0.81	0.301	0.83	0.89	0.033	0.069	0.090
EPA 20:5 <i>n</i> -3	0.42	0.39	0.15	<0.0001	0.38	0.19	<0.0001	0.004	0.000
DPA 22:5 <i>n</i> -3	0.36	0.37	0.23	<0.0001	0.40	0.26	<0.0001	0.006	0.000
DHA 22:6 <i>n</i> -3	1.65	1.69	0.70	<0.0001	1.77	0.73	<0.0001	0.011	0.000
Ratios									
AA:DHA	29.71	31.19	35.32	<0.0001	33.19	33.92	<0.0001	0.81	–
<i>n</i> -6: <i>n</i> -3	9.35	9.88	19.38	<0.0001	10.61	17.30	<0.0001	3.74	5.58

^a The differences in serum fatty acid status between breastfeeding categories were tested using mixed linear model, in which case-control status was considered

serum levels of oleic, linoleic (LA) and GLA compared with non-breastfed infants. In addition, at the age of 3 months, the breastfed infants had a higher serum level of pentadecanoic acid compared with non-breastfed infants. At 6 months of age, breastfed infants had a lower serum level of α -linolenic acid (ALA) (Table 3). The ratios of *n*-6:*n*-3 fatty acids and AA:DHA in serum at 3 and 6 months were lower in breastfed infants than in non-breastfed infants (Table 3). These results are in line with lower contents of oleic acid, LA and ALA and higher content of myristic, pentadecanoic, stearic, palmitoleic, *cis*-vaccenic and dihomo-GLA and CLA, AA, EPA, DPA and DHA in breast milk than in infant formula (Table 3).

Fatty acids that were present at higher proportions in serum of breastfed infants and present at comparatively high proportions in breast milk correlated directly with the amount of breast milk consumed, and inversely with the amount of cow's milk consumed, by control children at the age of 3 and/or 6 months (electronic supplementary material [ESM] Table 1). Respectively, fatty acids present at higher proportions in serum of non-breastfed infants, consumed in comparatively higher amounts in cow's milk, correlated directly with the amounts of cow's milk consumed and inversely with the amount of breast milk consumed (ESM Table 1).

Serum fatty acid status, type of milk feeding and the risk of islet autoimmunity Higher serum levels of palmitic and palmitoleic acid (16:1*n*-9) at the age of 3 months, as well as pentadecanoic acid at the age of 6 months, were associated with a decreased risk of islet autoimmunity (Table 4). On the other hand, serum AA:DHA ratio at the age of 3 months and serum *n*-6:*n*-3 ratio at the age of 6 months was associated with an increased risk of islet autoimmunity. Breastfeeding status modified the associations between serum DHA and the risk of islet autoimmunity at the age of 3 months ($p = 0.003$, FDR = 0.024). The proportion of DHA in the serum was associated with a decreased risk of islet autoimmunity only in non-breastfed children (Table 4).

Serum fatty acid status, milk feeding and the risk of primary insulin autoimmunity The proportions of palmitoleic acid, *cis*-vaccenic acid, AA, DPA and DHA were inversely associated with primary insulin autoimmunity at the age of 3 and/or 6 months (Table 5). ALA and high ratios of AA:DHA and *n*-6:*n*-3 were associated with increased risk. Higher breast milk consumption was associated with a decreased risk of primary insulin autoimmunity, whereas cow's milk consumption was associated with an increased risk.

Serum fatty acid status, milk feeding and the risk of primary GAD autoimmunity After multiple testing, fatty acid status in infancy was not associated with GAD autoimmunity (ESM Table 2).

Table 4 Associations between proportion of fatty acids in serum at 3 and 6 months of age and the risk of islet autoimmunity

Fatty acid	At 3 months of age (213 cases/436 controls)					At 6 months of age (222 cases/466 controls)						
	All				Breastfed at 3 months of age		Not breastfed at 3 months of age		All			
	OR	95% CI	<i>p</i> value	FDR	OR	95% CI	OR	95% CI	OR	95% CI	<i>p</i> value	FDR
SFA												
Myristic acid 14:0	0.98	0.82, 1.18	0.862	0.905					0.89	0.75, 1.06	0.200	0.367
Pentadecanoic acid 15:0	0.92	0.76, 1.10	0.364	0.472					0.74	0.61, 0.90	0.002	0.031 ^a
Palmitic acid 16:0	0.69	0.55, 0.87	0.002	0.031 ^a					0.75	0.59, 0.96	0.021	0.103
Stearic acid 18:0	0.86	0.71, 1.04	0.122	0.268					0.90	0.75, 1.09	0.293	0.444
Trans												
Conjugated LA 18:2 <i>n</i> -7/(9- <i>cis</i> , 11- <i>trans</i> -18:2)	0.83	0.68, 1.02	0.070	0.214					0.78	0.63, 0.95	0.014	0.094
MUFA												
Palmitoleic acid 16:1 <i>n</i> -9	0.75	0.62, 0.91	0.004	0.034 ^a					0.85	0.70, 1.03	0.089	0.219
Palmitoleic acid 16:1 <i>n</i> -7	0.88	0.74, 1.06	0.178	0.356					0.84	0.70, 1.01	0.062	0.208
Oleic acid 18:1 <i>n</i> -9	1.05	0.85, 1.29	0.661	0.765					1.06	0.88, 1.27	0.544	0.647
<i>cis</i> -vaccenic acid 18:1 <i>n</i> -7	0.84	0.69, 1.02	0.079	0.216					0.84	0.69, 1.02	0.073	0.214
PUFA												
<i>n</i>-6												
LA 18:2 <i>n</i> -6	1.28	1.05, 1.56	0.015	0.094					1.24	1.02, 1.51	0.030	0.118
GLA 18:3 <i>n</i> -6	1.08	0.86, 1.35	0.520	0.636					0.97	0.80, 1.18	0.758	0.855
Dihomo-GLA 20:3 <i>n</i> -6	1.10	0.90, 1.35	0.364	0.472					1.02	0.84, 1.24	0.845	0.905
AA 20:4 <i>n</i> -6	0.98	0.79, 1.23	0.878	0.905					0.90	0.73, 1.11	0.326	0.472
<i>n</i>-3												
ALA 18:3 <i>n</i> -3	1.17	0.98, 1.39	0.084	0.216					1.06	0.89, 1.27	0.497	0.625
EPA 20:5 <i>n</i> -3	0.91	0.75, 1.11	0.344	0.472					0.90	0.75, 1.08	0.254	0.444
DPA 22:5 <i>n</i> -3	0.87	0.66, 1.13	0.280	0.444					0.89	0.70, 1.14	0.349	0.472
DHA 22:6 <i>n</i> -3 ^b	0.84	0.67, 1.07	0.152	0.317	0.79	0.59, 1.04	0.16	0.05, 0.48 ^a	0.89	0.73, 1.09	0.275	0.444
Ratios												
AA:DHA	1.39	1.13, 1.70	0.002	0.031 ^a					1.22	1.03, 1.47	0.024	0.107
<i>n</i> -6: <i>n</i> -3	1.20	0.99, 1.45	0.057	0.208					1.32	1.10, 1.59	0.004	0.034 ^a
Quantity of cow's milk consumed, g/day	1.12	0.94, 1.33	0.194	0.367					1.24	1.04, 1.48	0.018	0.097
Quantity of breast milk consumed, g/day	0.90	0.75, 1.09	0.288	0.444					0.86	0.71, 1.04	0.121	0.268

Where there is an indication of interaction between fatty acid and breastfeeding with the development of islet autoimmunity (FDR <0.05), then OR for breastfed and non-breastfed groups are presented

Islet autoimmunity was defined as repeated positivity for islet autoantibodies and also positivity for at least one biochemical autoantibody out of the three that were measured (IAA, GADA or IA-2A), or presence of clinical type 1 diabetes

^a FDR $p < 0.05$

^b Indication of interaction with breastfeeding at 3 months of age (p value = 0.003, FDR = 0.024)

Adjustments for background factors and milk feeding

Adjusting conditional logistic regression models for familial diabetes and maternal education did not change the results (data not shown). Adjusting for cow's milk consumption did not change the association between fatty acids and islet autoimmunity substantially (ESM Fig. 1). Although this adjustment diluted the associations between SFAs and MUFAs and primary insulin autoimmunity, the associations between fish-derived fatty acids and primary insulin autoimmunity remained essentially unchanged (ESM Fig. 1). After correction for multiple testing, the results were non-significant ($FDR < 0.05$).

Discussion

Main findings Fatty acid status in infancy was related to type 1 diabetes-associated autoimmunity. Fish-derived fatty acids in particular appeared to be protective. In addition, fatty acids found in breast milk were protectively associated with islet autoimmunity and primary insulin autoimmunity. Generally, associations were stronger for primary insulin autoimmunity, the earliest endpoint investigated, compared with later.

Strengths Our study used a nested case–control design within a well-defined prospective birth cohort and had a comparatively large number of islet autoimmunity endpoints. Furthermore, established and well-functioning biomarkers were used; an earlier validation study from DIPP, comparing food consumption data with serum fatty acid composition, showed that fatty acid proportions in non-fasting, whole serum are suitable biomarkers in young children [37]. Importantly, the current study was performed at a time when DHA was not yet added to infant formulas in Finland and therefore its intake was low in formula-fed infants. This created a relatively large contrast in exposure, which is an advantage. Moreover, we investigated dietary factors in infancy and it is important to note that estimation of an infant's diet is comparatively easy and accurate because it is less variable than that of an older child.

Limitations Fatty acids found in the peripheral circulation of infants originate from infant and maternal diets and adipose tissue storage, as well as metabolic processes, some of which are affected by genetic factors [38, 39]. It can be considered a limitation that it was not possible to account for genetic factors involved in fatty acid metabolism. Furthermore, the study included a relatively low number of cases with primary insulin autoimmunity ($n = 43$) and primary GAD autoimmunity ($n = 22$) compared with the islet autoimmunity endpoint ($n = 240$). Thus, the statistical power may have been insufficient to show statistically significant associations between

fatty acid proportions and the primary GAD endpoint, particularly, after multiple testing ($FDR < 0.05$).

n-3 PUFAs and risk of type 1 diabetes-associated autoimmunity

Fish-derived fatty acids DHA and DPA were associated with decreased risk of primary insulin autoimmunity. For islet autoimmunity, a protective association was observed in non-breastfed infants at 3 months of age. Overall, these results suggest that long-chain *n*-3 PUFAs are protective during infancy. We reported earlier that long-chain *n*-3 PUFA proportions were not associated with islet autoimmunity in older children (1–6 years of age) in the DIPP cohort [12]. Altogether, the results indicate that long-chain *n*-3 PUFA status during the early months, a time when the immune system is maturing and being programmed, is critical.

Interestingly, a very strong association was found between DHA and decreased risk of islet autoimmunity in non-breastfed infants. These infants had the lowest levels of long-chain *n*-3 PUFAs because very few consumed fish and DHA was not present in formula at that time. This indicates an independent role for long-chain *n*-3 fatty acids in the development of type 1 diabetes-associated autoimmunity. It should be noted that this result is not confounded by breastfeeding.

Only 19% of the infants in the DIPP cohort had eaten fish by the age of 6 months and therefore the source of EPA, DPA and DHA was maternal diet (during pregnancy or lactation) or endogenous synthesis from their precursor ALA. ALA per se was associated with increased risk of primary insulin autoimmunity, which is in line with our earlier observation that maternal consumption of vegetable oils during lactation was associated with increased risk of islet autoimmunity in this cohort [40]. ALA in the maternal diet comes mainly from vegetable oils; in 3-month-old infants it is obtained from breast milk and formula. The ratios of *n*-6:*n*-3 and AA:DHA were associated with increased risk of islet autoimmunity and primary insulin autoimmunity. This may reflect the beneficial effect of long-chain *n*-3 fatty acids.

Our results concerning long-chain *n*-3 PUFAs are in line with the findings of a Norwegian case–control study suggesting that the use of cod liver oil (rich in EPA and DHA) during the first year of life protects against type 1 diabetes [9]. Furthermore, the prospective Diabetes Autoimmunity Study in the Young (DAISY), investigating older children (9 months to 8 years of age), found that high dietary intake and erythrocyte membrane content of *n*-3 PUFAs were associated with a decreased risk of islet autoimmunity (defined as repeated positivity for at least one autoantibody) [10, 11], but not with progression to type 1 diabetes [13]. Results from DAISY as well as the DIPP cohort support the view that early stages of autoimmunity are affected by long-chain *n*-3 PUFAs.

Fatty acids related to breast milk and risk of type 1 diabetes-associated autoimmunity Fatty acids present in

Table 5 Associations between proportions of fatty acids in serum at 3 and 6 months of age and the risk of primary insulin autoimmunity

Fatty acid	At 3 months of age (39 cases/77 controls)				At 6 months of age (41 cases/86 controls)			
	OR	95% CI	<i>p</i> value	FDR	OR	95% CI	<i>p</i> value	FDR
SFA								
Myristic acid 14:0	1.20	0.77, 1.88	0.417	0.540	0.99	0.67, 1.45	0.940	0.940
Pentadecanoic acid 15:0	1.30	0.83, 2.05	0.255	0.351	0.73	0.47, 1.12	0.146	0.230
Palmitic acid 16:0	0.98	0.58, 1.65	0.927	0.940	1.12	0.65, 1.94	0.677	0.787
Stearic acid 18:0	0.50	0.28, 0.90	0.021	0.060	0.61	0.38, 0.97	0.038	0.093
Trans								
Conjugated linoleic acid 18:2 <i>n</i> -7/ (9- <i>cis</i> , 11- <i>trans</i> -18:2)	0.74	0.45, 1.20	0.219	0.311	0.65	0.39, 1.10	0.113	0.191
MUFA								
Palmitoleic acid 16:1 <i>n</i> -9	0.54	0.35, 0.85	0.007	0.030 ^a	0.52	0.32, 0.85	0.009	0.032 ^a
Palmitoleic acid 16:1 <i>n</i> -7	0.81	0.53, 1.22	0.314	0.419	0.67	0.43, 1.05	0.078	0.149
Oleic acid 18:1 <i>n</i> -9	1.71	1.02, 2.87	0.042	0.097	1.78	1.07, 2.96	0.026	0.072
<i>cis</i> -vaccenic acid 18:1 <i>n</i> -7	0.65	0.41, 1.02	0.062	0.125	0.53	0.33, 0.85	0.008	0.032 ^a
PUFA								
<i>n</i> -6								
LA 18:2 <i>n</i> -6	1.20	0.75, 1.94	0.449	0.565	1.39	0.89, 2.17	0.146	0.230
GLA 18:3 <i>n</i> -6	0.90	0.53, 1.51	0.679	0.787	1.06	0.62, 1.84	0.824	0.906
Dihomo-GLA acid 20:3 <i>n</i> -6	0.62	0.38, 1.01	0.057	0.119	0.54	0.31, 0.94	0.029	0.075
AA 20:4 <i>n</i> -6	0.58	0.33, 1.02	0.057	0.119	0.33	0.16, 0.67	0.003	0.030 ^a
<i>n</i> -3								
ALA 18:3 <i>n</i> -3	1.95	1.23, 3.08	0.004	0.030 ^a	1.04	0.65, 1.68	0.865	0.906
EPA 20:5 <i>n</i> -3	0.90	0.55, 1.45	0.660	0.787	0.62	0.36, 1.07	0.088	0.159
DPA 22:5 <i>n</i> -3	0.35	0.17, 0.74	0.006	0.030 ^a	0.23	0.09, 0.56	0.001	0.030 ^a
DHA 22:6 <i>n</i> -3	0.59	0.33, 1.09	0.090	0.159	0.33	0.16, 0.68	0.003	0.030 ^a
Ratios								
AA:DHA	1.39	0.85, 2.26	0.185	0.281	1.89	1.20, 2.99	0.007	0.030 ^a
<i>n</i> -6: <i>n</i> -3	1.06	0.70, 1.60	0.797	0.899	2.45	1.40, 4.30	0.002	0.030 ^a
Quantity of cow's milk consumed, g/day	1.70	1.10, 2.64	0.018	0.057	1.88	1.15, 3.08	0.012	0.039 ^a
Quantity of breast milk consumed, g/day	0.46	0.27, 0.78	0.005	0.030 ^a	0.45	0.25, 0.80	0.007	0.030 ^a

Primary insulin autoimmunity was defined as being first positive only for IAA out of the four autoantibodies measured (IAA, ICA, GADA or IA-2A). Primary insulin autoimmunity cases were selected from the islet autoimmunity cases

^a FDR $p < 0.05$

higher quantities in breast milk, as well as serum of breastfed infants (pentadecanoic, palmitic, palmitoleic and *cis*-vaccenic acids and AA, DPA and DHA), were associated with decreased risk of islet autoimmunity or primary insulin autoimmunity, or both. Furthermore, the quantity of breast milk consumed was associated with decreased risk of primary insulin autoimmunity. The findings support the view that breastfeeding or some components of breast milk, including fatty acids, are protective, particularly against early autoimmunity. As far as we know, our study is the first one investigating the amount of breast milk consumed in this context, and having primary insulin and GAD autoimmunity as outcomes. Earlier findings on the associations between duration

of breastfeeding and risk of developing type 1 diabetes are inconsistent. Most prospective cohort studies do not show any association between breastfeeding duration and the risk of islet autoimmunity or type 1 diabetes [18–20] apart from a Swedish study that reported a protective association with islet autoimmunity [23] and a Norwegian study that reported a protective association for progression from islet autoimmunity to type 1 diabetes [41].

In our study, the quantity of cow's milk consumed was associated with an increased risk of primary insulin autoimmunity. Our findings are in line with previous prospective cohort studies on milk consumption during infancy and childhood and the development of islet autoimmunity and/or type 1 diabetes [17, 21–24].

Cow's milk as adjusting factor To investigate whether fatty acids are independently associated with type 1 diabetes autoimmunity, and not merely a reflection of the type of milk consumed, we adjusted the statistical models for the amount of cow's milk consumed. This did not substantially change results, suggesting that fatty acid status is indeed independently associated with islet autoimmunity. For primary insulin autoimmunity, as expected, most of the associations were diluted, because of the strong association between milk type and primary insulin autoimmunity (applied to SFA, MUFA, ratios of AA:DHA and $n-6:n-3$).

Differences between endpoints We observed stronger associations for primary insulin autoimmunity, the earliest endpoint investigated, compared with the later endpoints (islet autoimmunity and primary GAD). First, the explanation for why different associations are seen for different endpoints may be that their development differs by genotype and therefore the pathogenic processes may be different [42]. Second, measurements of fatty acid status and primary insulin autoimmunity are carried out within a short period and therefore there may be less confounding. It is possible that a protective association is more difficult to demonstrate at a later age, because the diet becomes more variable.

Conclusions Our results show that fatty acid status in infants strongly reflects the type of milk feeding. Higher proportions of fish-derived fatty acids as well as fatty acids related to breastfeeding may provide protection from type 1 diabetes-associated autoimmunity. Furthermore, breast milk consumption may reduce, and cow's milk consumption may increase, the risk of primary insulin autoimmunity. This study clarifies the complex associations between fatty acid status, milk type and type 1 diabetes development, but further studies on the subject are warranted.

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Data availability The datasets generated during and/or analysed during the current study are not publicly available due to the protection of the identity of the study participants and their clinical data, but data is available from the corresponding author on reasonable request.

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Contribution statement All authors were responsible for the study concept and design, acquisition of data and analysis or interpretation of the data. SN, IE, OV and SMV researched data. IE supervised serum fatty acid analyses. H-MT performed statistical analysis. SA supervised dietary data collection, processing and analysis. JT, RV and MK provided the clinical data used in this study and supervised the clinical work. RV and MK were responsible for the autoantibody analyses and JI for the genetic analyses. SN wrote the first version of the manuscript. IE contributed significantly to the writing and revision of the manuscript. SMV reviewed the manuscript. All authors edited the manuscript. All authors have approved the final version to be published. SMV is the guarantor of this work and, as such had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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