

Serum fatty acids and risk of developing islet autoimmunity: A nested case-control study within the TRIGR birth cohort

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Abstract

Background: Circulating fatty acids have been linked to development of type 1 diabetes.

Objectives: To study the prospective associations of serum fatty acids with the risk of islet autoimmunity in high-risk children.

Methods: A nested case-control selection was carried out within the TRIGR cohort, which included infants with HLA (DQB1 or DQA1)-conferred disease susceptibility and a first-degree relative with type 1 diabetes, born between 2002 and 2007 in 15 countries and followed-up until 2017. The present study included 244 case children positive for at least two islet autoantibodies (ICA, IAA, GADA, and IA-2A) and two control children were matched for country and age. Proportions of 26 serum fatty acids at cord blood and at 6, 12, and 18 months of age were assessed using gas-chromatography.

Results: The average proportions of the following fatty acids were associated with an increased risk of islet autoimmunity, adjusted for sex, HLA risk, and maternal type

Abbreviations: CLA, conjugated linoleic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; GADA, GAD autoantibodies; HDA, heptadecanoic acid; IA-2A, islet antigen 2 antibodies; IAA, insulin autoantibodies; ICA, Islet cell antibodies; PDA, pentadecanoic acid; TRIGR, Trial to Reduce IDDM in the Genetically at Risk.

Leena Hakola and Iris Erlund shared first authorship; Sari Niinistö and Suvi M. Virtanen shared last authorship.

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1 diabetes: pentadecanoic acid (15:0) (OR 3.41: 95% CI 1.70, 6.85), heptadecanoic acid (*iso* 17:0) (2.64: 1.62, 4.28) and (*anteiso* 17:0) (2.27: 1.39, 3.70), stearic acid (18:0) (23.8: 2.32, 244.6), and conjugated linoleic acid (18:2n-7) (2.60: 1.47, 4.59). Breastfeeding and not having maternal type 1 diabetes were positively associated with levels of the above-mentioned fatty acids. N-3 fatty acids were not consistently associated with islet autoimmunity.

Conclusions: We found direct associations of pentadecanoic acid, heptadecanoic acid, stearic acid, and conjugated linoleic acid with the risk of islet autoimmunity. Further studies are needed to understand the complex role of fatty acids in the development of type 1 diabetes.

KEYWORDS

autoimmune disease, children, diabetes mellitus, type 1, fatty acids, HLA, islet autoimmunity

1 | INTRODUCTION

Type 1 diabetes is an autoimmune disease with a genetic background, but likely affected by several environmental risk factors and protective factors.¹ Few studies have investigated the role of fatty acid metabolism or dietary fatty acids in the development of type 1 diabetes. Fatty acids play an important role in several physiological pathways, such as inflammation,^{2,3} energy balance and endocrine responses and they might modify the epigenome and gut microbiome.⁴⁻⁷

Serum fatty acid biomarkers (i.e., fatty acid proportions in serum) are well established biomarkers of fatty acid status. They reflect both endogenous fatty acid biosynthesis and, to a varying degree, intake from dietary sources.⁸⁻¹⁰ Saturated fatty acid biomarkers in serum mainly reflect endogenous biosynthesis.¹⁰ Fatty acids that are considered as biomarkers of dietary exposure include the long-chain unsaturated n-3 fatty acids eicosapentaenoic acid (EPA, 20:5n-3), docosapentaenoic acid (DPA, 22:5n-3), and docosahexaenoic acid (DHA, 22:6n-3) They are obtained from fish and other marine foods and to a minor degree, produced endogenously from dietary alpha-linolenic acid. The odd chain fatty acids pentadecanoic acid (PDA, 15:0) and heptadecanoic acid (HDA, 17:0), as well as conjugated linoleic acid (CLA) have been linked to dairy,¹¹ human milk,¹² or dietary fiber intake.¹³

In type 1 diabetes research, main focus has been on n-3 fatty acids, due to their anti-inflammatory properties.^{7,14} In fact, higher intake or levels of these fatty acids in infancy have been associated with decreased risk of developing islet autoimmunity or type 1 diabetes.^{12,15,16} In two prospective studies, erythrocyte DPA (22:5n-3)¹⁶ and serum DHA (22:6n-3)¹² were associated with decreased risk of islet autoimmunity in susceptible children, the latter only among the non-breastfed children.¹² Few studies have investigated the other fatty acids.^{12,17} One study observed that fatty acids linked to dairy were associated with islet autoimmunity,¹⁷ which is in line with several prospective studies linking cow's milk consumption with development of type 1 diabetes.¹⁸⁻²²

Our aim was to study the prospective associations between serum fatty acids and the risk of developing islet autoimmunity in a large multicenter study of children with high genetic risk of type 1 diabetes. We hypothesized that fatty acid biomarkers are associated with the risk of islet autoimmunity, and that n-3 fatty acids in particular show a protective association.

2 | METHODS

2.1 | Study design and population

This study is based on samples collected in the Trial to Reduce IDDM in the Genetically at Risk (TRIGR) study (ClinicalTrials.gov registration no. NCT00179777). TRIGR is a double-blind randomized clinical trial of 2159 infants recruited between 2002 and 2007 in 15 countries.²³ The inclusion criteria were having at least one first-degree relative with type 1 diabetes, a risk HLA genotype, and a signed consent.²³ Exclusion criteria have been described before.²³ All participants were observed until the youngest child turned 10-year-old in 2017. Written informed consent was collected from all families, signed by the legal guardian of the child. The study was approved by the ethics committees of all participating centers. There was no difference in the incidence of islet autoimmunity²⁴ or type 1 diabetes²³ between children randomized to receive extensively hydrolyzed casein infant formula and those randomized to receive regular cow's milk based one.

In the present Divia study, an ancillary study of the TRIGR, 244 case children with islet autoimmunity and two matched control children (n = 488) for each case were selected (Figure S1). Case children (n = 244) were defined as those with positivity for two or more of the following autoantibodies; ICA, IAA, IA-2A, GADA, or positivity to at least one autoantibody and type 1 diabetes in June 2016. Two control children for each case child were randomly selected among those who met the matching criteria: age (± 1 year) and country, and who were not positive to two or more diabetes-related autoantibodies.

TABLE 1 Background characteristics of children with positivity for at least two autoantibodies and matched control children, the TRIGR Divia Study

	Case children n = 244	Control children n = 488	P for difference ^a
Sex			0.006
Boys, No. (%)	148 (60.7)	244 (50.0)	
Girls, No. (%)	96 (39.3)	244 (50.0)	
HLA risk ^b			<0.001
High risk, No. (%)	90 (36.9)	117 (24.0)	
Moderate risk, No. (%)	96 (39.3)	208 (42.6)	
Mild risk, No. (%)	58 (23.8)	163 (33.4)	
Family member with type 1 diabetes ^c			0.008
Mother, No. (%)	99 (40.6)	249 (51.0)	
Father or full sibling, No. (%)	145 (59.4)	239 (49.0)	
Treatment group in TRIGR study			0.129
Casein hydrolysate, No. (%)	135 (55.3)	241 (49.4)	
Control formula, No. (%)	109 (44.7)	247 (50.6)	

^aP values are from Chi-square tests.

^bHigh risk: HLA-DQB1*0302/DQB1*02; Moderate risk: HLA-DQB1*0302/x (x not DQB1*02, DQB1*0301, or DQB1*0602); Mild risk: HLA-DQA1*05-DQB1*02/y (y not DQA1*0201-DQB1*02, DQB1*0301, DQB1*0602, or DQB1*0603) and HLA-DQA1*03-DQB1*02/y (y not DQA1*0201-DQB1*02, DQB1*0301, DQB1*0602, or DQB1*0603).

^cThe variable “Family member with type 1 diabetes” was formed based on maternal type 1 diabetes (yes/no). Children with a mother with type 1 diabetes may have another 1st degree relative with type 1 diabetes, but those in group “Father or full sibling with type 1 diabetes,” do not have a mother with type 1 diabetes.

2.2 | Assessments

The cord blood was collected according to hospital practices and was a mixture of arterial and venal cord blood. Blood samples were collected at 3–12 months' intervals at study visits.²³

HLA genotyping for the selected *DQB1* and *DQA1* alleles (Table 1) was performed using sequence-specific oligonucleotide hybridization and genotypes categorized as high, moderate and mild risk of type 1 diabetes.²³

ICAs were detected using indirect immunofluorescence. IAA, IA-2A, and GADA were quantified with the use of specific radio-binding assays in the Scientific Laboratory, Children's Hospital, University of Helsinki, Helsinki, Finland.²³

Serum fatty acids at ages 0, 6, 12, and 18 months were determined by gas-chromatography using an Agilent 6890 gas chromatograph (Hewlett Packard, Palo Alto, California) with a split injector and hydrogen as the carrier gas at Finnish Institute for Health and Welfare, Finland. We employed a capillary column Omegawax 320 (length: 30 m, I.D.: 0.32 mm, phase layer: 0.25 μ m; Supelco, Bellefonte, Pennsylvania). Coefficient of variation (CV%) for the control samples are presented in Table S1. The laboratory was blinded regarding the case-control status of the samples. The samples were analyzed according to a pre-determined list, with the matched cases and controls sorted together to minimize variation in results. Also, results are reported as proportions (%) of total fatty acids, rather than absolute concentrations, which is the most commonly used approach in fatty acid related studies.

Altogether 26 individual fatty acids were detected. Furthermore, we calculated the sum of n-3-marine fatty acid (EPA 20:5n-3, DPA 22:5n-3, and DHA 22:6n-3), n-3 fatty acid (n-3 marine and algalinolenic acid 18:3n-3), and n-6 fatty acid proportions (linoleic acid 18:2n-6, dihomo-gammalinolenic acid 20:3n-6, arachidonic acid 20:4n-6, docosatetraenic acid 22:4n-6), as well as n6:n3 ratio. The categorization of the fatty acids is presented in Table 2. The CLA isomer detected was trans-10 cis-12. For secondary analyses, we calculated fatty acid ratios as listed in Table S2.

The clinical and background variables were derived from hospital records, questionnaires, and assessments during follow-up. The following variables were considered as potential confounders: maternal type 1 diabetes (yes, no), child's HLA-conferred risk, sex, duration of any breastfeeding (<3 months; 3–5.99 months; \geq 6 months), weight for age and sex z-scores, maternal education (years), season of birth (summer, fall, winter, spring), gestational age (<38, \geq 38 weeks), and mode of delivery (Caesarean section, vaginal delivery).

2.3 | Statistical analyses

We used the centered log-ratio (CLR) transformed fatty acid status for statistical comparisons, except for the ratio variables. This was done because serum fatty acids are presented as proportions. Differences in background variables between case and control children were compared using the Chi-square test or the Fisher's Exact Test (based on cell size). Differences in serum fatty acid proportion by

TABLE 2 Risk of islet autoimmunity by the average fatty acid proportions in ages 0 to 18 months before the first seroconversion, the TRIGR Divia Study

	Centered log-ratio transformed fatty acid proportion mean (SD)		Adjusted for sex, HLA risk and maternal type 1 diabetes			Corrected P value
	Case children, n 244	Control children, n 488	OR	95% CI	P value	
Saturated fatty acids						
Myristic acid 14:0	0.001 (0.281)	-0.017 (0.251)	1.51	0.82-2.79	0.19	
Pentadecanoic acid 15:0	0.018 (0.256)	-0.048 (0.259)	3.41	1.70-6.85	0.001	0.003
Palmitic acid 16:0	-0.001 (0.079)	0.005 (0.057)	0.47	0.03-6.49	0.57	
Heptadecanoic acid <i>iso</i> 17:0	0.055 (0.351)	-0.064 (0.394)	2.64	1.62-4.28	<0.001	<0.001
Heptadecanoic acid <i>anteiso</i> 17:0	0.036 (0.366)	-0.061 (0.368)	2.27	1.39-3.70	0.001	0.003
Stearic acid 18:0	0.019 (0.079)	0.003 (0.085)	23.8	2.32-244.6	0.008	0.20
Eicosanoid acid 20:0	0.023 (0.145)	0.013 (0.148)	2.07	0.63-6.82	0.23	
Docosanoic acid 22:0	0.000 (0.156)	0.012 (0.177)	0.66	0.24-1.80	0.42	
Tetracosanoic acid 24:0	0.002 (0.159)	0.015 (0.177)	0.64	0.24-1.67	0.36	
Monounsaturated fatty acids						
Palmitoleic acid 16:1n-7	0.017 (0.227)	0.017 (0.256)	1.23	0.60-2.53	0.57	
Palmitoleic acid 16:1n-9	0.030 (0.194)	0.017 (0.179)	1.37	0.52-3.61	0.53	
Cis vaccenic acid 18:1n-7	0.019 (0.150)	0.013 (0.158)	1.72	0.54-5.47	0.36	
Oleic acid 18:1n-9	-0.009 (0.098)	-0.007 (0.098)	1.30	0.19-8.70	0.79	
11-eicosenoic acid 20:1n-9	-0.011 (0.173)	-0.015 (0.166)	1.35	0.46-3.93	0.59	
Polyunsaturated fatty acids						
<i>n</i> -6						
Linoleic acid 18:2n-6	-0.002 (0.090)	-0.001 (0.095)	0.46	0.06-3.27	0.44	
Dihomogammalinolenic acid 20:3n-6	-0.024 (0.164)	-0.025 (0.183)	0.95	0.33-2.72	0.92	
Arachidonic acid 20:4n-6	0.028 (0.251)	0.020 (0.237)	1.08	0.51-2.30	0.85	
Docosatetraenoic acid 22:4n-6	0.045 (0.264)	0.025 (0.255)	1.31	0.64-2.68	0.47	
Docosatetraenoic acid 22:4n-6	0.037 (0.265)	0.026 (0.261)	1.10	0.50-2.41	0.81	
<i>n</i> -3						
Alphalinolenic acid 18:3n-3	0.019 (0.277)	-0.004 (0.291)	1.20	0.61-2.34	0.60	
Eicosapentaenoic acid 20:5n-3	-0.036 (0.351)	-0.046 (0.340)	1.00	0.55-1.80	0.99	
Eicosapentaenoic acid 20:5n-3	0.003 (0.440)	-0.043 (0.468)	1.43	0.90-2.29	0.14	
Docosapentaenoic acid 22:5n-3	0.005 (0.269)	-0.030 (0.305)	1.40	0.73-2.67	0.31	
Docosahexaenoic acid 22:6n-3	0.063 (0.404)	0.035 (0.408)	1.09	0.69-1.72	0.72	
<i>n</i> -3 marine ^a	0.030 (0.340)	-0.003 (0.356)	1.23	0.72-2.10	0.46	
Transfatty acids						
Conjugated linoleic acid 18:2n-7	0.038 (0.354)	-0.039 (0.351)	2.60	1.47-4.59	0.001	0.003
Other						
Nervonic acid 24:1n-9	0.005 (0.201)	0.011 (0.204)	0.80	0.33-1.94	0.62	
Dimethylacetal form of 16:0	0.010 (0.185)	0.007 (0.191)	1.01	0.42-2.42	0.98	
Dimethylacetal form of 18:0	0.004 (0.255)	-0.031 (0.268)	1.49	0.78-2.83	0.23	
Ratio						
n6:n3-ratio	12.014 (3.605)	12.460 (3.848)	0.963	0.92-1.01	0.15	

Notes: The P values and OR's (95% CI's) are from conditional logistic regression adjusted for sex, HLA risk, and maternal type 1 diabetes. OR's are per one unit increase in log-transformed fatty acid proportions. The n6:n3-ratio variables are based on the original %-values (not transformed). Corrected P values are based on multiple testing correction with false discovery rate method, a step-up procedure using 0.05 level as the criterion, 150 tests.

^aIncludes eicosapentaenoic acid, docosapentaenoic acid and docosahexaenoic acid.

maternal type 1 diabetes and breastfeeding status were compared using the Student's *t*-test for paired samples.

The prospective associations between serum fatty acids and the risk of islet autoimmunity were studied with conditional logistic

regression. To account for possible confounding, we evaluated the associations between background variables and fatty acid proportions.

The main analyses compared islet autoimmunity by the mean fatty acid proportions of the transformed fatty acid proportions before the

seroconversion sample, adjusted for sex, HLA, and maternal type 1 diabetes. To study potential nonlinearity of the associations, we added quadratic terms in the models of those fatty acids that showed statistically significant linear association in the main analyses. However, none of the quadratic terms were statistically significant suggesting that linear models are suitable. Since no difference in the type 1 diabetes development was detected between the treatment and control arm in the original TRIGR cohort, no adjustment for treatment arm was used. As the effects of fatty acids may be different depending on age of the child, we studied also the associations of fatty acids with the risk of islet autoimmunity separately at each age point.

As secondary analyses, we studied whether maternal type 1 diabetes, treatment arm, HLA genotype, child's sex, and breastfeeding at 6 months modified the associations between average fatty acids and risk of islet autoimmunity by adding corresponding interaction terms into the conditional logistic regression model. When the P value for interaction was <0.05 we stratified the analyses according to the modifying variable.

The analyses were performed using SAS v9.4 and RStudio 3.5.3. Multiple testing was addressed by using a P value adjustment method that controls the false discovery rate (FDR; a step-up procedure using 0.05 level as the criterion, 150 tests (Figure 1; Table 2). P values <0.05 were considered statistically significant.

3 | RESULTS

The median (interquartile range) age for the first seroconversion was 2.0 (1.0, 4.0) years among the 244 case children. The autoantibody combinations among the case children at time of case selection were ICA, IAA, and GADA ($n = 55$), ICA and GADA ($n = 55$), ICA and IAA ($n = 43$), ICA, IAA, GADA, and IA-2A ($n = 36$), IAA and GADA ($n = 22$), ICA, GADA, and IA-2A ($n = 12$), ICA, IAA, IA-2A ($n = 6$), GADA and IA-2A ($n = 4$), ICA and IA-2A ($n = 4$), IAA, GADA and IA-2A ($n = 2$), and IAA and IA-2A ($n = 2$). Three children were positive to only one autoantibody (GADA, $n = 2$; IAA $n = 1$) and clinical type 1 diabetes. Altogether, 144 (59%) of the case children developed type 1 diabetes during TRIGR follow-up. Case children were more often male than female, had more often high-risk HLA-genotypes, and had less often a mother with type 1 diabetes compared with control children (Table 1). Among the participants, 24.6% were from Northern Europe (Finland, Sweden), 27.8% from Canada, 16.0% from United States, 3.7% from Australia, 15.6% from Central Europe I (Czech Republic, Estonia, Hungary, and Poland), 9.4% from Central Europe II (Germany, Luxembourg, Netherlands, and Switzerland), and 2.9% from Southern Europe (Italy and Spain).

3.1 | Serum fatty acid proportions across age-points and the risk of islet autoimmunity

The mean PDA (15:0), HDA (*iso* 17:0), HDA (*anteiso* 17:0), stearic acid (18:0), and CLA (18:2n-7) proportions across age-points preceding the

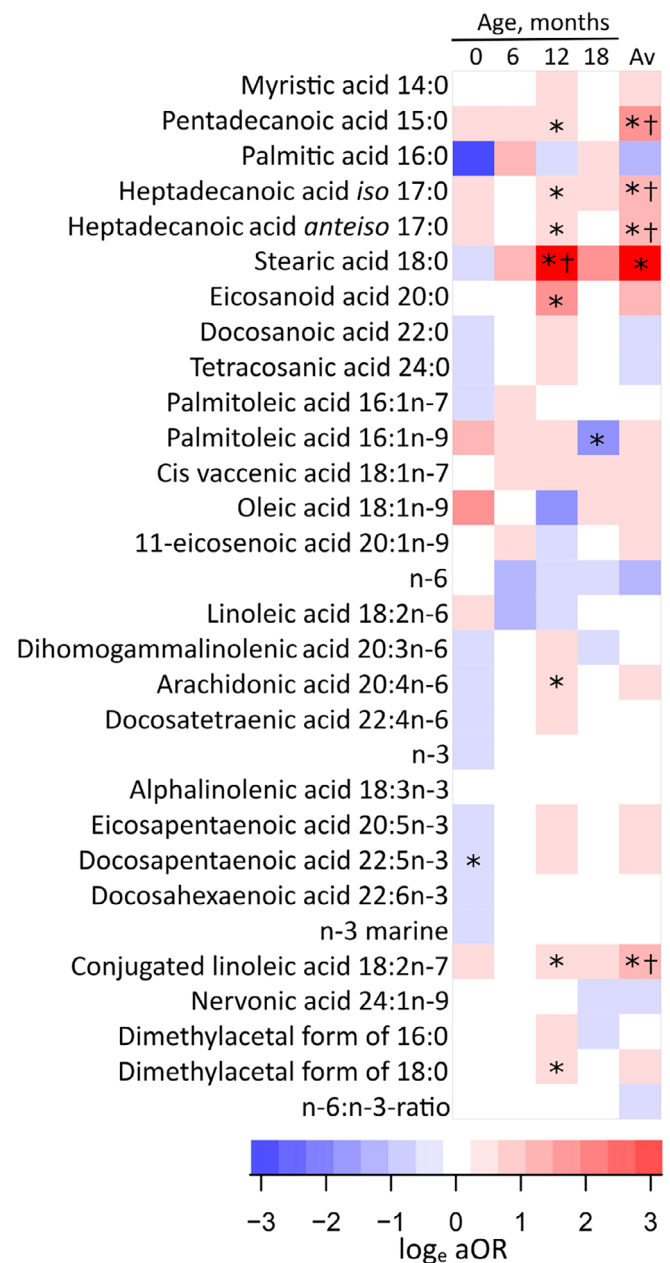


FIGURE 1 Associations of fatty acid proportions at ages 0, 6, 12, and 18 months, and average proportions across age points (Av) with the risk of islet autoimmunity in 244 case children and 488 control children. The colors indicate odds ratios adjusted for HLA genotype, maternal type 1 diabetes, and sex after logarithmic transformation. Red color indicates increased risk and blue color indicates decreased risk of islet autoimmunity and white corresponds to the original odds ratios that were close to 1. * indicates statistical significance with P value <0.05 before multiple testing correction. † indicates P value <0.05 after multiple testing correction (False discovery rate method, a step-up procedure using 0.05 level as the criterion, 150 tests). Fatty acids were available for number of cases/controls as follows: 210/428 at 0 months, 225/462 at 6 months, 233/454 at 12 months, and 193/399 at 18 months. n-3 marine includes eicosapentaenoic acid, docosapentaenoic acid, and docosahexaenoic acid

first seroconversion were associated with an increased risk of islet autoimmunity in a model adjusted for sex, HLA risk, and maternal type 1 diabetes (Table 2; Figure 1). Further adjustment for weight z-score did not change the results, nor did additional adjustment for season of birth, maternal education, gestational age, nor mode of delivery. Additional adjustment for breastfeeding duration strengthened the associations between the risk of islet autoimmunity and PDA (15:0) (OR 5.13; 95% CI 2.33, 11.3), HDA (*iso* 17:0) (4.09; 2.29, 7.30) and (*anteiso* 17:0) (3.31; 1.86, 5.91), stearic acid (18:0) (36.1; 3.21, 404.3), and CLA (18:2n-7) (4.06; 2.07, 7.93). Further, after additional adjustment for breastfeeding the inverse association between n6:n3 fatty acid ratio and islet autoimmunity was statistically significant (OR 0.94; 95% CI 0.89, 1.00, $P = 0.043$). When studying average proportions across age points, none of the n-3 fatty acids was associated with islet autoimmunity (Table 2). Among the fatty acid ratios, only stearic acid/palmitic acid (C18:0/C16:0) was positively associated with islet autoimmunity (Table S4). PDA (15:0), HDA (*iso* 17:0) and HDA (*anteiso* 17:0) and CLA (18:2n-7) showed a strong positive correlation with each other, and weaker positive correlation with stearic acid (18:0) (Table S3).

3.2 | Associations between fatty acids and risk of islet autoimmunity by age

The adjusted ORs for islet autoimmunity by serum fatty acids at individual age points are visualized in Figure 1. The DPA (22:5n-3) at cord blood was inversely associated with islet autoimmunity (OR 0.59, 95% CI 0.36, 0.96, $P = 0.03$), adjusted for sex, HLA, and maternal type 1 diabetes. Other associations, that were unlike those based on the averaged analyses, were found for 18-month palmitoleic acid (16:1n-9) (OR 0.31, 95% CI 0.12, 0.79, $P = 0.014$), 12-month eicosanoid acid (20:0) (OR 3.14, 95% CI 1.16, 8.48, $P = 0.024$), 12-month arachidonic acid (20:4n-6) (OR 1.83, 95% CI 1.09, 3.06, $P = 0.023$), and 12-month dimethylacetal form of 18:0, (OR 1.87, 95% CI 1.16, 3.00, $P = 0.010$). After multiple testing correction the average PDA (15:0), HDA (*iso* 17:0), HDA (*anteiso* 17:0), and CLA (18:2n-7) proportions and the 12-month stearic acid (18:0) proportion remained statistically significantly associated with islet autoimmunity (Figure 1).

3.3 | Associations of age, maternal type 1 diabetes, and breastfeeding with serum fatty acid proportions

Fatty acid proportions varied by age (Table S4). Maternal type 1 diabetes and breastfeeding were associated with several fatty acids in children (Table S5). Differences in fatty acid proportions between children with and without a mother with type 1 diabetes were seen already at birth (Table S5). Only minor differences were seen in serum fatty acid proportions between treatment and control arms. At 6 months of age, children in the casein hydrolysate versus children in control arm had higher proportion of palmitoleic acid (16:1n-9) (0.33

vs 0.32, $P = 0.004$), HDA (*anteiso* 17:0) (0.09 vs. 0.08, $P = 0.01$), arachidonic acid (20:4n-6) (5.0 vs 4.7, $P = 0.005$), and nervonic acid (24:1n-9) (0.52 vs 0.49, $P = 0.009$), and lower proportion of eicosanoid acid (20:0) (0.21 vs 0.21, $P = 0.02$).

3.4 | Maternal type 1 diabetes and intervention arm modified the associations between serum fatty acids and risk of islet autoimmunity

We observed an interaction between maternal type 1 diabetes and the average of the following fatty acids: myristic acid (14:0), eicosanoid acid (20:0), algalinolenic acid (18:3n-3), and DPA (22:5n-3) on the risk of islet autoimmunity (Table S6). A stronger association between myristic acid (14:0), algalinolenic acid (18:3n-3), and DPA (22:5n-3) was observed in children of mothers with type 1 diabetes compared with children of unaffected mothers. In addition, we observed an interaction between average HDA (*anteiso* 17:0) proportion across ages (0–18 months) and treatment arm on the risk of islet autoimmunity. HDA (*anteiso* 17:0) was associated with islet autoimmunity only among children randomized to be weaned to the extensively hydrolyzed casein infant formula (adjusted OR 3.56, 95% CI 1.84, 6.89) but not in the control group (OR 1.25, 95% CI 0.62, 2.54), P for interaction = 0.03 We did not observe interactions ($P < 0.05$) between average fatty acids and HLA genotype, child sex, and breastfeeding at 6 months on the risk of islet autoimmunity.

4 | DISCUSSION

In this prospective study of children carrying high genetic risk of type 1 diabetes, we observed that higher serum PDA (15:0), HDA (*iso* 17:0) and *anteiso* 17:0), stearic acid (18:0), and CLA (18:2n-7) proportions were associated with increased risk of islet autoimmunity. In addition, we observed a weak protective association for cord blood DPA on the risk of islet autoimmunity but not for other n-3 fatty acids. Finally, maternal type 1 diabetes and breastfeeding status were strongly associated with children's serum fatty acid proportions.

The strengths of the study include repeated assessment of various individual serum fatty acids and islet autoantibodies in a large group of children from several countries. Another strength is the ability to adjust for several potential confounding factors and to study interactions. The current study included children with very high susceptibility to type 1 diabetes. In addition to the increased HLA-conferred genetic risk, the children had a first-degree relative/s with type 1 diabetes. This is a strength in terms of potential interest to intervene in the highest risk children to prevent type 1 diabetes. However, generalizability of the results to other populations is unknown. A limitation is that as an observational study, causality of observed associations is unclear. Furthermore, while fatty acids are not independent from one another, but correlated because of shared metabolic pathways and dietary sources, the associations of each individual fatty acid cannot always be distinguished from one another. In other words,

several fatty acids likely reflect the same phenomenon. Additional limitations include the fact that genetics related to fatty acid metabolism could not be considered.

We found that higher proportions of PDA (15:0), HDA (*iso* 17:0 and *anteiso* 17:0), stearic acid, and CLA (18:2n-7) were associated with increased risk of islet autoimmunity. Some saturated fatty acids have been suggested to be pro-inflammatory,²⁵ and by modulating the inflammatory milieu, they could facilitate developing islet autoimmunity. PDA, HDA, and stearic acid are saturated fatty acids, and could therefore affect the risk of type 1 diabetes. On the other hand, observed associations may reflect something else in the environment, such as breastfeeding or cow's milk consumption and not causality. Cow's milk consumption has been consistently linked to risk of islet autoimmunity or type 1 diabetes in several prospective studies,¹⁸⁻²² and milk proteins have been suspected but not confirmed to facilitate the association.²³ The clinical meaning of the observed associations is unclear as the changes in fatty acid proportions per presented odds ratios are quite large for some fatty acids.

Previous findings related to fatty acid proportions and islet autoimmunity are limited and partly inconsistent with the present findings. In the DIPP study, fatty acids related to breastfeeding, such as PDA (15:0) at 6 months, was associated with decreased risk of islet autoimmunity.¹² However, the PDA and CLA proportions later in childhood were associated with increased risk of islet autoimmunity in DIPP study.¹⁷ In addition, serum triglycerides containing odd-chain fatty acids were higher in children with compared with those without islet autoimmunity in the BABYDIAB study.²⁶

Stearic acid (18:0) was associated with increased risk of islet autoimmunity in the present study. Furthermore, the stearic acid/palmitic acid (18:0/16:0) ratio was directly associated with islet autoimmunity. The conversion of palmitic acid to stearic acid is regulated by the elongation of very long chain fatty acids protein 6 (ELOVL6) which has previously been linked to diabetes, obesity and energy metabolism.^{27,28} Animal studies, which are important from a mechanistic point of view, have shown that the regulation of fatty acid metabolism (e.g., elongase and desaturase expression) may play an important role in managing lipid composition in response to changes in dietary and hormonal status.²⁷ Clearly, further studies are needed to corroborate our finding and to investigate whether ELOVL6 might play a role in type 1 diabetes development.

Among the n-3 fatty acids, only DPA in cord blood was weakly protectively associated with islet autoimmunity. The results do not support fully the hypotheses that n-3 fatty acids in general would protect from islet autoimmunity. However, DPA showed protective association with islet autoimmunity also in the US DAISY study¹⁶ suggesting a potential importance of this specific fatty acid. N-3 fatty acids may also be especially meaningful in the early infancy. This is supported by the present finding, and the DIPP study, in which 3-month DHA showed protective association with islet autoimmunity among the nonbreastfed children.¹² However, association of the cord blood DPA with islet autoimmunity did not hold multiple testing correction. Further, our stratified analyses suggested that DPA across

age points was associated with increased risk of islet autoimmunity among children with maternal type 1 diabetes.

As we observed no strong association between serum n-3 fatty acids and islet autoimmunity, it can be speculated that in higher risk children, n-3 polyunsaturated fatty acids play a smaller role than in other children. This is in line with a previous trial reporting that DHA supplementation induced a weaker lowering effect than anticipated on inflammatory cytokine production in children with high genetic and familial type 1 diabetes risk.¹⁴

The inconsistent findings based on previous^{12,16,17} and the present study could be explained by several differences between the studies: participating countries, birth year of children, type of fatty acid biomarkers, age points, islet autoimmunity definitions, and the participant's risk profiles for type 1 diabetes. It should be noted that the present study included children with higher type 1 diabetes risk than the previous ones. As for the type of biomarker, we used fatty acid composition in serum, which is the most commonly employed biomarker. It should be noted that the result might be somewhat different if more long-term biomarkers, measured from erythrocytes, were used.

We observed that fatty acid status was different in children with mothers affected by type 1 diabetes compared with children with an affected father and/or sibling. Differences were seen in several fatty acid biomarkers, including the fatty acids that were associated with islet autoimmunity. This is in line with previous studies reporting differing fatty acid profiles in cord blood of children with or without maternal type 1 diabetes.^{29,30} It is known that mothers with type 1 diabetes have on average higher body mass index, shorter duration of pregnancy, higher rates of caesarean section, differing fatty acid status, and offspring with higher cord blood insulin, higher birth weight, shorter duration of breastfeeding, and different breast milk fatty acid profile,^{29,31-33} all of which might affect the serum fatty acid proportion in the offspring. Therefore, it is plausible that maternal type 1 diabetes, or other maternal characteristics linked to it, may affect the children's fatty acid profile, especially at the early ages.

Fatty acids that were associated with increased risk of islet autoimmunity were higher in children who were breastfed compared with the nonbreastfed children. Adjustment with breastfeeding duration strengthened the observed associations, which suggests that the fatty acid association is not explained by breastfeeding. We aimed to study the independent association of fatty acids by adjusting for maternal type 1 diabetes and breastfeeding, however, there might be residual confounding. Importantly, adjustment for weight z-score did not change the results.

In conclusion, we observed that higher serum PDA (15:0), HDA (*iso* 17:0 and *anteiso* 17:0), stearic acid (18:0), and CLA (18:2n-7) proportions were associated with increased risk of developing islet autoimmunity. Furthermore, the results do not indicate strong protective associations for the n-3 fatty acids. Overall, our understanding about the potential role of fatty acids in type 1 diabetes development is still limited and more research is needed to identify modifiable factors affecting risk, especially in children at very high risk.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

Leena Hakola, Sari Niinistö, Iris Erlund, Mikael Knip, and Suvi M. Virtanen were responsible for conception and design of the study. Sari Niinistö, Iris Erlund, Anita M. Nucci, Jeffrey P. Krischer, Mikael Knip, and Suvi M. Virtanen were responsible for the acquisition of data. David Cuthbertson analyzed the data. Iris Erlund supervised laboratory analysis of fatty acids from serum samples. Reija Autio and Leena Hakola produced the Figure 1. Leena Hakola drafted the article with contributions from Sari Niinistö, Iris Erlund, David Cuthbertson, and Suvi M. Virtanen. Leena Hakola, Iris Erlund, David Cuthbertson, Maija E Miettinen, Reija Autio, Anita M. Nucci, Taina Härkönen, Jarno Honkanen, Outi Vaarala, Heikki Hyöty, Mikael Knip, Jeffrey P. Krischer, Sari Niinistö, and Suvi M. Virtanen contributed to the interpretation of the data, critically reviewed and approved the version to be published. Mikael Knip and Suvi M. Virtanen are the guarantors of this work.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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