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Running head: Serum fatty acids and risk of pre-type 1 diabetes

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Abstract

Background/ Objectives: N-3 (omega-3) fatty acids have been reported to decrease the risk for development of β cell autoimmunity and clinical type 1 diabetes. We set out to examine whether different serum fatty acids are associated with the development of advanced β cell autoimmunity in children carrying HLA-DQB1-conferred susceptibility to type 1 diabetes.

Subjects/Methods: Within a cohort, serum total fatty acid composition of 108 children with advanced β cell autoimmunity and of 216 matched persistently autoantibody-negative controls was analyzed using gas chromatography. Non-fasting serum samples were obtained annually at the ages of 1 to 6 years. Conditional logistic regression was applied to analyze the associations between advanced β cell autoimmunity and serum fatty acids.

Results: The serum fatty acid profile of myristic acid (OR =1.48, 95% CI 1.09-2.00, *p*=0.011), pentadecanoic acid (OR=1.65 95% CI: 1.19-2.28, *p*=0.003), palmitoleic acid isomers 16:1n-7 (omega-7) (OR=1.41, 95% CI 1.03- 1.92, *p*=0.030) and 16:1n-9 (omega-9) (OR=1.45, 95% CI 1.05-2.01, *p*=0.026), and conjugated linoleic acid (OR=1.67, 95% CI 1.16-2.41, *p*=0.006) closest to the time of the appearance of multiple autoantibodies were positively associated with the risk of advanced β cell autoimmunity after adjustment for potential confounding factors. Serum linoleic acid showed inverse, marginal association with the endpoint.

Conclusions: Serum biomarkers of milk and ruminant meat fat consumption are directly and linoleic acid inversely associated with advanced β cell autoimmunity in children with HLA-conferred susceptibility to type 1 diabetes.

Keywords: Serum fatty acids, Diabetes mellitus, Type 1-associated autoantibodies, Child, Cohort studies

Introduction

Experimental studies in cell cultures, animals and humans indicate that fatty acids may modulate the functions of the immune system (Hughes *et al.*, 1996, Wallace *et al.*, 2001, Kleemann *et al.*, 1998, Kew *et al.*, 2004, Fritsche, 2006). Consequently fatty acids might affect the development of type 1 diabetes, which results from an autoimmune inflammatory destruction of the insulin-producing β cells. So far, only a few studies have addressed the possible role of fatty acids in the development of type 1 diabetes. Studies in rats suggest that long-chain fatty acids are able to prevent alloxan-induced diabetes (Mohan & Das, 2001, Suresh & Das, 2001). Consumption during the first year of life of cod liver oil, which is rich in n-3 (omega-3) fatty acids, was associated with reduced risk of type 1 diabetes in a case-control study (Stene *et al.*, 2003). In a cohort of children with increased genetic risk for type 1 diabetes, the total intake of n-3 fatty acids and their content in erythrocyte membranes was inversely associated with β cell autoimmunity characterizing pre-type 1 diabetes (Norris *et al.*, 2007). However, the putative effects of other fatty acids on type 1 diabetes development have not been evaluated in man. Certain fatty acids function as biomarkers of milk and ruminant meat fat intake (Wolk *et al.*, 2001) and essential fatty acids in serum reflect vegetable oil consumption (Zock *et al.*, 1997). Cow's milk intake during infancy and childhood has been linked to development of type 1 diabetes (e.g., Virtanen *et al.*, 1991, Verge *et al.*, 1994, Virtanen *et al.*, 2000). We aimed at examining in a birth cohort of children carrying increased HLA-DQB1-conferred risk for type 1 diabetes, which fatty acids in serum show association with the development of advanced β cell autoimmunity, i.e. positivity for islet cell autoantibodies (ICA) and at least one other autoantibody out of the three additional antibodies commonly analyzed for predictive purposes.

Subjects and Methods

Study design

Within a large prospective, population-based birth cohort of Finnish children at increased genetic risk for type 1 diabetes in the Type 1 Diabetes Prediction and Prevention Study (DIPP), serum fatty acids were analyzed in 108 children developing advanced β cell autoimmunity or type 1 diabetes (cases) and 216 persistently autoantibody-negative control children (controls). The high-risk genotype was defined as HLA-DQB1*02/*0302 and moderate-risk genotypes as HLA-DQB1*0302/x; with x = other than *02, *0301, or *0602). The children were born between October 1, 1996 and July 6, 2004 in Oulu and Tampere University Hospitals (n=5787, 76% of the children invited). Ethical approval has been obtained from the local Ethics Committees. A written informed consent was obtained from the parent(s). A detailed description of the genetic screening methods and enrolment criteria in the DIPP Study have been published (Kupila *et al.*, 2001). DIPP Study has been registered in www.clinicaltrials.gov (NCT00223613).

The DIPP children were monitored for the appearance of signs of β cell autoimmunity by analyzing primarily ICA. If a child tested positive for ICA, all his or her samples obtained since birth were analyzed for autoantibodies to insulin (IAA), antibodies to the 65 kD of glutamic acid decarboxylase (GAD), and the tyrosine phosphatase-related IA-2 molecule (IA-2A). Autoantibody samples were obtained at each study center visit 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 turned positive for ICA, the visit interval was shortened to 3 months. The autoantibodies were analyzed in the Research Laboratory, Department of Pediatrics, University of Oulu as described (Kukko *et al.*, 2005).

We defined advanced β cell autoimmunity as being repeatedly positive for ICA and for one or more of the three other antibodies analyzed. By September 30, 2004, among the 5787 children enrolled in DIPP in Oulu and Tampere, 119 (2.1%) tested repeatedly positive for ICA and at least one other autoantibody. By November, 2004, 45 (0.8%) children had progressed to clinical type 1 diabetes at a median age of 3.1 years (range 1.0–6.4 years). Clinical type 1 diabetes was included in the autoantibody endpoint as described in Uusitalo *et al.* (2008). This resulted in 128 children (2.2% of all children) with either clinical type 1 diabetes or repeated positivity for ICA and at least one other autoantibody. The term "advanced β cell autoimmunity" (multiple seroconversion) will be used to describe jointly these two groups of children.

For each case, two controls matched for gender, genetic risk group, site and time of birth (range ± 3 months) were selected as described in (Uusitalo *et al.*, 2008). Sociodemographic factors and familial diabetes were registered by a structured questionnaire completed by the parents after the delivery. We obtained data on neonatal and pregnancy characteristics from the Medical Birth Registries at Oulu and Tampere University Hospitals (Table 1). The children were measured for length/height and weight during the study centre visits. Body mass index (BMI) was calculated as ratio of weight to height^2.

Non-fasting serum samples for fatty acid analyses were taken annually by venipuncture, protected from light and stored at -70˚C (Uusitalo *et al.*, 2008). The samples from each case-control set were taken for analysis until the time when the case child progressed to advanced β cell autoimmunity. Of the 128 case children, 20 were excluded because no serum samples were available. Consequently, serum fatty acids of 108 cases and 216 controls were analyzed. On the average, two serum samples were available from each child. All samples from each

case-control set were measured in the same batch by a bioanalyst who was blinded for the case-control status of the children.

We determined serum fatty acids as follows. Fat was extracted from 50 µl of serum with dichloromethane - methanol (2:1, volume:volume) (Folch *et al.,* 1957). Total fatty acids were methylated with acidic (5 weight-% H_2SO_4) methanol (Stoffel *et al.,* 1959). The percentage composition of methylated total fatty acids from 14:0 to 22:6 n-3 was determined in a HP 6890 gas chromatograph (Hewlett Packard, Palo Alto, CA) equipped with Chemstation (version A.06.03) having a DB225 column (30-m long I.D. 0.32 mm, phase layer 0.25 µm, Agilent J&W GC), using split injection and hydrogen as carrier gas. A temperature program from 160ºC to 230ºC was used. The percentage composition of fatty acid methyl esters was normalized to 100%. Between-series variability of control samples was 2–7% for fatty acid peaks over 1% and 6–18% for smaller peaks. We determined altogether 17 individual fatty acids and, in addition, calculated palmitoleic to palmitic acid ratio which reflects stearoyl-CoA desaturase activity. The fatty acids were expressed as percentages of total fatty acids in serum (Table 2). Serum cholesterol concentrations were analyzed manually in the Laboratory of Analytical Biochemistry by an Olli-C photometer (Thermo Fisher Scientific Vantaa, Finland) using an enzymatic (CHOD-PAP) method.

Statistical analysis

The estimation of risk is based on a nested case-control design set up within the cohort. In a nested case-control design each case is associated with k randomly selected matched controls (in the present study k=2). The standard procedure of estimation in nested case-control designs is the conditional likelihood logistic regression analysis (Borgan & Samuelsen, 2003).

This approach yields consistent and asymptotically normal estimates of the regression parameters (Borgan *et al.*, 1995). We used SAS version 9.1.3 (SAS Institute, Cary, NC, USA) PROC LOGISTIC with the strata-option to fit the models. Statistical significance was taken as 5%. The differences between cases and controls in the background characteristics were tested with the Wald test obtained from the conditional likelihood analysis of logistic regression.

The number of measurements per child is small and does not, at this early stage of the follow-up, permit the use of multiple lagged covariates models. Because this exploratory nature of the analysis approach, it is important to investigate the changes in point estimates (odds ratios) across different choices of time lags. The endpoint analyses were performed in three settings. First, the serum fatty acid composition at 1 year of age (the first measurement) was used as the explanatory variable. Secondly, the nearest fatty acid profile at least 0.5 year before the appearance of advanced β cell autoimmunity was applied. Thirdly, the most recent available single measurement value for fatty acid composition at or before the time when the case turned out to have advanced β cell autoimmunity was used. To remove potential time trends in the serum fatty acid composition during early childhood, we computed the z-scores of the measurements within each time point prior to the analysis and used these in the model. The associations were studied both on continuous and categorical scales (the latter defined by falling into intervals given by the first and the third sample quartile within each age group). Because no suggestion for nonlinearity of the associations was observed, only the results for the continuous fatty acid variables are presented.

Results

The serum fatty acids consisted mainly of oleic, linoleic and palmitic acids in both case and control children. E.g., at the age of 1 year in the serum of the controls linoleic acid represented 27.2%, arachidonic acid 5.2%, other n-6 polyunsaturated fatty acids 1.7%, n-3 polyunsaturated fatty acids 3.8%, oleic acid 27.2%, other monounsaturated fatty acids plus conjugated linoleic acid 3.9%, palmitic acid 21.6%, and other saturated fatty acids 9.4% of the total fatty acids. Figure 1 shows the distribution of myristic acid, pentadecanoic acid, and linoleic acid at 1 year of age and in the most recent measurement in case and control children.

The odds ratios from different time lags reveal that the effect tends to increase in magnitude the closer the measurement is to the time of seroconversion. At or closest before the appearance of multiple autoantibodies, the proportions of myristic acid and pentadecanoic acid were significantly associated with increased risk for advanced β cell autoimmunity (Table 2). These associations were strengthened when the models were adjusted for the listed confounding factors. In an adjusted model, the proportion of palmitoleic acid isomers 16:1n-7 and 16:1n-9, and that of the conjugated linoleic acid isomers were also significantly associated with increased risk of advanced β cell autoimmunity as was the ratio of palmitoleic acid to palmitic acid. A nearly significant inverse association was observed between the risk of advanced β cell autoimmunity and the proportions of linoleic acid (p=0.051) in serum in the adjusted model. In the cholesterol-adjusted model, the inverse association between the risk of advanced β cell autoimmunity and the proportion of linoleic acid became significant (linoleic acid OR=0.68, 95% CI 0.48, 0.96, p=0.028). Interestingly, the estimated effect of linoleic acid, even though marginal, seems to be invariant to the choice of the time lag used in the analysis. Adjusting for cholesterol did not change the other associations (data not shown).

The BMI of the children at 1 year of age, at least 0.5 year before or at the time of the appearance of advanced β cell autoimmunity was not related to the endpoint (OR 1.13, 95% CI 0.96-1.35, OR 1.11, 95% CI 0.89-1.39 and OR 1.09, 95% 0.92-1.30, respectively). The serum fatty acid pattern was associated with BMI. At the age of 1 year, serum stearic (r=-0.125), eicosapentaenoic (r=-0.125), docosahexaenoic (r=-0.155), and arachidonic acid (r=-0.127) were inversely correlated to BMI at the same age ($p<0.05$). At the time of the appearance of advanced β cell autoimmunity, positive correlations were observed between myristic (r=0.169), palmitic (r=0.225) and oleic acid (r=0.148) and BMI at the same time and inverse correlations between serum stearic (r=-0.127), eicosapentaenoic (r=-0.199), docosahexaenoic (r=-0.247), docosapentaenoic (r=-0.117), linoleic acid (r=-0.113) and arachidonic acid (r=-0.217). When adjusted for BMI, the associations observed between serum fatty acids and advanced β cell autoimmunity remained mostly unchanged: at the time of the appearance of advanced β cell autoimmunity the associations between myristic, palmitoleic acid (both n-7 and n-9) and ratio of palmitoleic acid (n-7) to palmitic acid became of borderline significance (Table 2).

Discussion

We observed among 315 children carrying HLA-conferred susceptibility to type 1 diabetes that the profiles of individual saturated serum fatty acids, myristic acid and pentadecanoic acid, and monounsaturated palmitoleic acid isomers 16:1n-7 and 16:1n-9 as well as the two polyunsaturated fatty acid isomers jointly called conjugated linoleic acid, were positively associated with the risk of advanced β cell autoimmunity at or before the time of seroconversion. These serum fatty acids are excellent biomarkers of the dietary intake of milk or meat fat of the ruminants (Wolk *et al.*, 2001). On the other hand, linoleic acid, which

reflects vegetable oil intake (Zock *et al.*, 1997), was inversely associated with the risk of advanced β cell autoimmunity.

One of the strengths of present study is the population-based nested case-control design, where upon the results are not influenced by selection bias. Further, the serum samples of the cases and controls were collected, handled, stored and analyzed in the same standardized way.

A limitation of the present study was that only one non-fasting serum sample at each age point was available as well as that the total fatty acid composition was determined instead of analyzing fatty acids in isolated lipid fractions. The daily intra-individual variation is more marked in the fatty acid profile of triglycerides and free fatty acids than in the fatty acid profile of cholesterol esters and phospholipids (Moilanen, 1987). The lipid fractions in serum differ in their fatty acid composition (Nikkari, 1983, Moilanen, 1987,) and changes in relative proportions of lipid fractions also affect the percentages of fatty acids in serum. For this reason we applied a separate statistical model, which was adjusted for serum cholesterol concentration. We observed an inverse association between the risk of β cell autoimmunity and linoleic acid composition in serum. This result is in line with the finding that serum biomarkers of milk and meat fat were associated with an increased risk of advanced β cell autoimmunity, since linoleic acid profile is low if the profile of saturated fatty acids is high. The observation could also reflect that the impact of linoleic acid on advanced β cell autoimmunity may be protective in accordance with some experimental animal studies (Mohan & Das, 2001, Suresh & Das, 2001).

Statistical analyses were done in three settings: first, using fatty acid measurements from 1 year of age (the first age point available); secondly, at least 0.5 years before the appearance of

multiple autoantibodies; and thirdly, using the most recent measurement in relation to the appearance of advanced β cell autoimmunity. Our findings suggest that the associations observed between fatty acids and advanced β cell autoimmunity become clearer the closer the time of seroconversion you are. We cannot exclude that the seroconversion to positivity for multiple autoantibodies could affect the serum fatty acid pattern, although we are not aware of any putative mechanism for such an association. It is also possible that the type of fat in the diet might act rather as a promoter than as an initiator of β cell destruction.

Unlike Norris and coworkers (2007) in their case-cohort analysis with 35 children repeatedly positive for at least one autoantibody, we did not observe any association between autoimmunity and the n-3 fatty acid status among the children studied. Most likely the discrepancy in results is caused by different methods used for the assessment of fatty acid status. The fatty acid composition of erythrocyte membranes describes the intake of n-3 fatty acids over a period of 4 to 6 weeks (Baur *et al.*, 2000), while our method reflects the intake during the preceding 1 to 2 weeks (Moilanen, 1987, Katan *et al.*, 1997, Arab, 2003). An important source of n-3 fatty acids in the diet is fatty fish which is consumed rather rarely by young children (Ylönen *et al.*, 1996). Therefore a long-term biomarker of n-3 fatty acid intake is probably more suitable for the estimation of their intake than a short-term biomarker. The mean age of the cases was higher in the previous than in the present study (5.3 vs. 2.5 years, respectively). Because the intake of n-3 fatty acids increases with age in children, this difference in age may partly explain our failure to confirm this suggested protective association. On the other hand, milk products and meat are frequently consumed by children (Ylönen *et al.*, 1996), and thus the determination of serum total fatty acids should reflect their consumption rather well.

All the fatty acids which were positively associated with advanced β cell autoimmunity in the present study reflect dietary intake of milk and/or meat fat of ruminants (Wolk *et al.*, 2001, Crowe *et al.*, 2006). Especially pentadecanoic and conjugated linoleic acid are not available from other dietary sources and the amounts of them available from ruminant meat compared to milk are very small. A major part of palmitoleic acid 16:1n-7 is endogenously formed from the desaturation of palmitic acid (Crowe *et al.*, 2006), and thus the content of palmitoleic acid in serum may reflect intake of palmitic acid from cow's milk and meat.

In previous studies early introduction of cow's milk in infancy has been linked to the development of preclinical and clinical type 1 diabetes (Virtanen *et al*., 1991, Åkerblom *et al.*, 2005). Findings from the pilot of a randomized double-blind trial suggest that the appearance of type 1 diabetes-associated autoantibodies can possibly be reduced by 40–60% by weaning high-risk infants to a highly hydrolyzed formula (Åkerblom *et al.*, 2005). Also the amount of cow's milk used later in childhood could be related to the risk of type 1 diabetes (Verge *et al.*, 1994, Virtanen *et al.*, 2000, Wahlberg *et al.*, 2006). It has been suggested that either milk proteins or insulin in milk might trigger the immunization leading to type 1 diabetes (Vaarala *et al.* 1999, Scherezenmeir & Jagla 2000) However, until now there is no unequivocal evidence for a mechanism or for the fraction of milk which possibly facilitates progression to type 1 diabetes.

Our findings in the area of pre-type 1 diabetes resemble associations observed between fatty acids and insulin resistance, obesity and type 2 diabetes. Long-chain unsaturated fatty acids have been associated inversely and saturated fatty acid positively with insulin resistance and obesity (Innis, 2007, Aguilera *et al.*, 2008, Sabin *et al.*, 2007). Plasma palmitoleic (n-7) content and stearoyl-CoA desaturase activity (ratio of palmitoleic to palmitic acid) were

positively associated with abdominal obesity in children (Okada *et al.*, 2005) and in the present series with advanced β cell autoimmunity. Whether our finding is explained through increased weight gain in childhood and its association with the development of type 1 diabetes (Hyppönen *et al.*, 2000), remains to be evaluated. Adjustment for BMI had a minor effect on the associations between serum fatty acids and the autoimmunity endpoint. If increased weight gain would be involved in the causal pathway between serum fatty acids and autoimmunity, this adjustment would not be justified. The fact that the associations observed between certain fatty acids and advanced β cell autoimmunity remain after adjustment for BMI, speaks in favour of independent associations between these fatty acids and the endpoint.

In conclusion, our results suggest that serum biomarkers of milk and meat fat are positively and serum linoleic acid inversely associated with increased risk of advanced β cell autoimmunity in children with HLA-conferred susceptibility to type 1 diabetes. Further research is needed to confirm or contest these associations.

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FIGURE 1 Composition of myristic acid, pentadecanoic acid and linoleic acid in serum in case (circles, mean values) and control (triangles, mean values) children at the age of 1 year (a) and closest to the time of the appearance of advanced β cell autoimmunity (b). The central bar in the box plots represents the median concentration, the box represents the interquartile range (IQR), and the whiskers represent the smallest and largest non-outlier concentrations. The asterisks represent outliers, i.e. observations that lie more than 1.5xIQR lower than the first quartile or 1.5xIQR higher than the third quartile. For statistical testing between cases and controls see columns for unadjusted odds ratios in Table 2.

TABLE 1 Characteristics of children with advanced β cell autoimmunity and seronegative controls

	Cases N=108	Controls[1] N=207	p value[2]
First degree relative affected by type 1 diabetes, n (%)			0.010
Yes	16 (14.8)	11 (5.3)	
No	88 (81.5)	186 (89.9)	
Missing information	4 (3.7)	10 (4.8)	
Maternal age at delivery, years			0.126
<25	21 (19.4)	34 (16.4)	
25-30	34 (31.5)	73 (35.3)	
30-35	24 (22.2)	66 (31.2)	
>35	29 (26.9)	34 (16.4)	
Mean (SD)gestational age, weeks	39.3 (2.1)	39.7 (1.9)	0.063
Number of earlier deliveries			0.224
0	39 (36.1)	91 (44.0)	
1	42 (38.9)	58 (28.0)	
2	15 (13.9)	36 (17.4)	
≥3	12 (11.1)	22 (10.6)	
Maternal vocational education, n (%)			0.004
None	11 (10.2)	6 (2.9)	
Vocational school or course	28 (25.9)	53 (25.6)	
Upper secondary vocational education	37 (34.3)	105 (50.7)	
Academic	29 (26.9)	35 (16.1)	
Missing information	3 (2.8)	8 (3.9)	
Paternal vocational education, n (%)			0.958
None	5 (4.6)	8 (3.9)	
Vocational school or course	40 (37.0)	78 (37.7)	
Upper secondary vocational education	37 (34.3)	66 (31.9)	
Academic	22 (20.4)	42 (20.3)	
Missing information	4 (3.7)	13 (6.3)	
Maternal smoking during pregnancy			0.809
Yes	8 (7.4)	14 (6.8)	
No	96 (88.9)	185 (89.4)	
Missing information	4 (3.7)	8 (3.8)	
Mean (SD) body mass index of the child at the age of 1 year, kg/m^2	17.3 (1.5)	17.1 (1.5)	0.131

[1]Eight children served as controls for two cases but were included only once in the total number of controls. One of the controls became a case at a later date and was counted only as a case.

[2] The difference between cases and controls was tested with the Wald test obtained from the conditional likelihood analysis of logistic regression.

TABLE 2 Associations of serum fatty acid composition (expressed as % of total fatty acids in serum) with the risk of advanced β cell autoimmunity in conditional and multiple conditional logistic regression models in children with HLA-DQB1 conferred susceptibility to type 1 diabetes. In the analyses the risk has been explained for each individual of the case-control set by the fatty acid composition. Odds ratio (OR) describes change in risk, when fatty acid composition is changed by an amount corresponding to its standard deviation.

	Fatty acid composition at 1 yr of age (97 case and 199 control children)[1]		Fatty acid composition ≥ 0.5 yr before the appearance of advanced β cell autoimmunity[2] (83 case and 168 control children)		Fatty acid composition at the time of or closest to the appearance of advanced β cell autoimmunity[3] (108 case and 216 control children)		
	OR (95% CI)	Adjusted OR (95% CI)[4]	OR (95% CI)	Adjusted OR (95% CI)[4]	OR (95% CI)	Adjusted OR (95% CI)[4]	Adjusted OR (95% CI)[5]
Myristic acid 14:0	1.17 (0.91, 1.49)	1.29 (0.92, 1.80)	1.25 (0.95,1.64)	1.37 (0.98, 1.91)	**1.31 (1.04, 1.64)**[6]	**1.48 (1.09, 2.00)**[6]	1.37 (0.96,1.95)
Pentadecanoic acid 15:0	1.04 (0.81, 1.34)	1.31 (0.94, 1.83)	1.03 (0.78,1.35)	1.33 (0.95, 1.87)	**1.35 (1.05, 1.72)**[6]	**1.65 (1.19, 2.28)**[7]	**1.60 (1.10,2.32)**[6]
Palmitic acid 16:0	0.96 (0.75, 1.22)	1.15 (0.82, 1.61)	1.07 (0.82,1.39)	1.32 (0.93,1.87)	0.99 (0.79, 1.24)	1.17 (0.86, 1.58)	1.05 (0.75,1.48)
Stearic acid 18:0	0.89 (0.70, 1.14)	0.87 (0.64, 1.18)	1.24 (0.93,1.64)	1.20 (0.85,1.70)	0.99 (0.77, 1.26)	1.05 (0.78, 1.42)	1.00 (0.72,1.40)
Palmitoleic acid 16:1(n-7)	1.01 (0.79, 1.29)	1.15 (0.85, 1.55)	0.98 (0.76,1.25)	1.08 (0.81,1.43)	1.21 (0.95, 1.55)	**1.41 (1.03, 1.92)**[6]	1.40 (0.99,1.97)
Palmitoleic acid 16:1(n-9)	1.06 (0.83, 1.37)	1.15 (0.84, 1.56)	1.08 (0.83,1.40)	1.15 (0.85,1.54)	1.18 (0.92, 1.50)	**1.45 (1.05, 2.01)**[6]	1.41 (0.97,2.05)
Oleic acid 18:1(n-9)	1.29 (1.00, 1.66)	1.24 (0.89, 1.72)	1.12 (0.88,1.43)	1.17 (0.86,1.59)	1.07 (0.84, 1.36)	1.13 (0.84, 1.53)	1.08 (0.78,1.51)
Cis vaccenic acid 18:1(n-7)	0.99 (0.77, 1.27)	1.02 (0.71, 1.47)	1.06 (0.81,1.38)	0.95 (0.67,1.36)	1.09 (0.86, 1.37)	1.11 (0.81, 1.53)	1.15 (0.82,1.62)
Ratio 16:1 (n-7) to 16:0[8]	1.03 (0.80, 1.32)	1.14 (0.85, 1.55)	0.97 (0.75,1.26)	1.05 (0.78,1.41)	1.24 (0.97, 1.59)	**1.40 (1.03, 1.91)**[6]	1.40 (1.00,1.97)
Alphalinolenic acid 18:3n-3	1.04 (0.83, 1.31)	1.07 (0.80, 1.43)	0.97 (0.75,1.24)	0.86 (0.62, 1.20)	0.93 (0.73, 1.18)	0.93 (0.69, 1.25)	0.88 (0.62,1.27)
Eicosapentaenoic acid 20:5n-3	0.96 (0.75, 1.22)	1.00 (0.72, 1.40)	0.91 (0.71, 1.17)	0.97 (0.73, 1.30)	0.90 (0.70, 1.15)	0.88 (0.63, 1.22)	0.91 (0.62,1.33)
Docosahexaenoic acid 22:6n-3	0.89 (0.70, 1.13)	0.90 (0.67, 1.21)	1.00 (0.76, 1.31)	1.00 (0.71, 1.41)	0.92 (0.73, 1.16)	0.90 (0.68, 1.20)	0.97 (0.70,1.34)
Docosapentaenoic acid 22:5n-3	0.88 (0.69, 1.14)	0.90 (0.65, 1.23)	0.88 (0.68, 1.14)	0.87 (0.63, 1.21)	1.04 (0.82, 1.31)	1.00 (0.75, 1.33)	1.08 (0.78,1.48)
Linoleic acid 18:2n-6	0.86 (0.67, 1.11)	0.77 (0.54, 1.08)	0.78 (0.59, 1.02)	**0.67 (0.48, 0.94)**[6]	0.84 (0.66, 1.08)	0.73 (0.53, 1.00)	0.77 (0.54,1.09)
Arachidonic acid 20:4n-6	0.93 (0.73, 1.18)	0.90 (0.67, 1.21)	1.06 (0.83, 1.35)	1.06 (0.79, 1.42)	1.11 (0.88, 1.40)	0.97 (0.73, 1.29)	1.10 (0.81,1.49)
Gammalinolenic acid 18:3n-6	1.20 (0.94, 1.53)	1.21 (0.89, 1.64)	1.11 (0.85, 1.43)	1.15 (0.86, 1.55)	1.23 (0.96, 1.57)	1.07 (0.80, 1.44)	1.04 (0.76,1.42)
Dihomogammalinolenic acid 20:3n-6	1.08 (0.85, 1.38)	1.15 (0.85, 1.56)	1.14 (0.89, 1.46)	1.21 (0.90, 1.63)	1.15 (0.91, 1.44)	1.09 (0.82, 1.45)	1.15 (0.85,1.57)
Ratio of total n-6:total n-3[9]	1.05 (0.82, 1.35)	0.96 (0.71, 1.31)	1.01 (0.75, 1.35)	0.93 (0.65, 1.32)	1.01 (0.78, 1.30)	0.92 (0.66, 1.29)	0.90 (0.62,1.31)
Conjugated linoleic acid[10]	1.06 (0.81, 1.40)	**1.55 (1.03, 2.32)**[6]	1.00 (0.75, 1.32)	1.46 (0.99, 2.16)	1.29 (0.99, 1.67)	**1.67 (1.16, 2.41)**[7]	**1.52 (1.03,2.25)**[6]

[1] Median (range) time lag between the fatty acid measurement and advanced β cell autoimmunity 1 (0-5.4) years

[2]Median (range) time lag between the fatty acid measurement and advanced β cell autoimmunity 1 (0.5-3.3) years

[3] Median (range) time lag between the fatty acid measurement and advanced β cell autoimmunity 0 (0-3.3) years

[4]Multiple logistic regression model is adjusted for vocational education of the mother and the father, maternal age, duration of gestation, type 1 diabetes in a first-degree relative, number of earlier deliveries, and maternal smoking during pregnancy

[5]Multiple logistic regression model is adjusted for body mass index at the time of or closest to the appearance of advanced β cell autoimmunity, vocational education of the mother and the father, maternal age, duration of gestation, type 1 diabetes in a first-degree relative, number of earlier deliveries, and maternal smoking during pregnancy

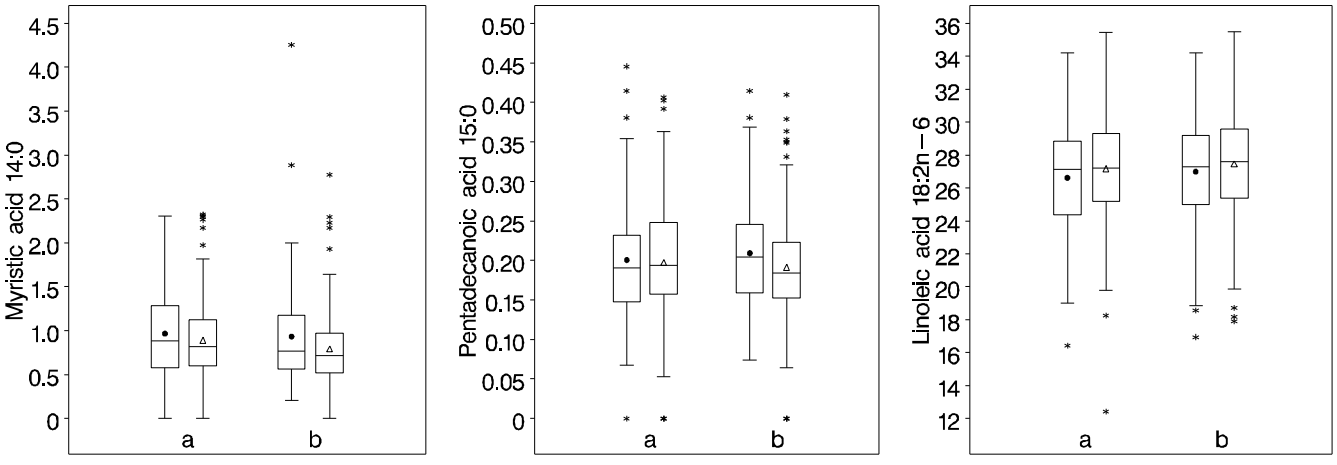
[6] $p<0.05$. The statistically significant ($p<0.05$) findings have been bolded

[7] $p<0.01$. The statistically significant ($p<0.05$) findings have been bolded

[8]Palmitoleic to palmitic acid ratio reflects stearoyl-CoA desaturase activity

[9]Total n-6 fatty acids is calculated as sum of linoleic acid, arachidonic acid, gammalinolenic acid, and dihomogammalinolenic acid and total n-3 fatty acids is calculated as sum of alpha-linolenic acid, eicosapentaenoic acid, docosahexaenoic acid and docosapentaenoic acid

[10] Sum of isomers 10-trans, 12-cis-18:2 and 9-cis, 11-trans-18:2


Myristic acid 14:0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
a
b
Pentadecanoic acid 15:0
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0
a
b
Linoleic acid 18:2n−6
36
34
32
30
28
26
24
22
20
18
16
14
12
a
b