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Erythrocyte membrane omega-3 fatty acid levels and omega-3 fatty acid intake are not associated with conversion to type 1 diabetes in children with islet autoimmunity: The Diabetes Autoimmunity Study in the Young (DAISY)

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Abstract

Aim—We investigated whether omega-3 fatty acid intake and erythrocyte membrane omega-3 fatty acid levels are associated with conversion to type 1 diabetes in children with islet autoimmunity (IA).

Methods—The Diabetes Autoimmunity Study in the Young is following children at increased genetic risk for type 1 diabetes for the development of persistent IA, as defined as being positive for glutamic acid decarboxylase 65, i, or insulin autoantibodies on two consecutive visits, and then for the development of type 1 diabetes, as diagnosed by a physician. One hundred and sixty-seven children with persistent IA were followed for a mean of 4.8 yr, and 45 of these developed type 1 diabetes at a mean age of 8.7 yr. Erythrocyte membrane fatty acids (as a percent of total lipid) and dietary fatty acid intake (estimated via food frequency questionnaire) were analyzed as time-varying covariates in proportional hazards survival analysis, with follow-up time starting at detection of the first autoantibody.

Results—Neither dietary intake of omega-3 fatty acids nor omega-6 fatty acids were associated with conversion to type 1 diabetes, adjusting for human leukocyte antigen (HLA)-DR, family history of type 1 diabetes, age at first IA positivity, maternal age, maternal education, and maternal ethnicity. Adjusting for HLA-DR, family history of type 1 diabetes and age at first IA positivity, omega-3 and omega-6 fatty acid levels of erythrocyte membranes were not associated with conversion to type 1 diabetes.

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Conflict of interest
The authors declare no conflict of interest.

Conclusions—In this observational study, omega-3 fatty acid intake and status are not associated with conversion to type 1 diabetes in children with IA.

Keywords

dietary intake; IA; omega-3 fatty acids; type 1 diabetes mellitus

Type 1 diabetes is an autoimmune disease characterized by the destruction of the insulin producing beta cells in the pancreatic islets. Islet autoimmunity (IA) precedes and is strongly predictive of type 1 diabetes development (1); and it is likely that both genetic and environmental factors play a role in the development of IA and subsequent progression to type 1 diabetes.

In the Diabetes Autoimmunity Study of the Young (DAISY), a longitudinal observational study following otherwise healthy children who are at increased risk of developing type 1 diabetes, we reported that higher omega-3 fatty acid intake was associated with a lower risk of IA [hazard ratios (HR): 0.45], and likewise, that higher percentage of omega-3 fatty acid in the erythrocyte membrane was associated with a lower risk of IA (HR: 0.63) (2). Given this association as well as the findings that children with type 1 diabetes were less likely than controls to have received fish oil in infancy (3) and had decreased percentage of docosahexaenoic acid (DHA) in erythrocyte membranes (4) as compared to controls, an obvious next question is whether omega-3 fatty acid intake or percentage of omega-3 fatty acid in erythrocyte membranes are associated with lower risk of type 1 diabetes in children who already have IA. Researchers, along with physicians and parents of children who test autoantibody positive, are likely to consider many possible ways to delay or prevent the onset of type 1 diabetes once autoimmunity has developed. This analysis would provide important information for efforts examining feasibility of interventional approaches in terms of timing and target population.

Methods

DAISY population

DAISY is a prospective study of two groups of young children at increased risk for developing type 1 diabetes mellitus. One group consists of first degree relatives of patients with type 1 diabetes mellitus, identified and recruited between birth and 8 yr of age; and the second group consists of babies born at St. Joseph's Hospital in Denver, CO, USA, screened by umbilical cord blood samples for diabetes-susceptibility alleles in the human leukocyte antigen (HLA) region. Cord blood or the first available blood sample (depending upon enrollment group) is sent to Roche Molecular Systems Inc., Alameda, CA, USA, for polymerase chain reaction-based HLA class II typing. The details of the newborn screening (5) and follow-up (2, 6) have been published elsewhere.

Recruitment took place from January 1994 to November 2006. DAISY has 2629 current and former participants. Written informed consent was obtained from the parents of study participants. The Colorado Multiple Institutional Review Board approved all study protocols.

Measurement of diabetes associated autoantibodies

Serum autoantibodies were tested at 9, 15, and 24 months, and annually thereafter. The three autoantibodies of interest were glutamic acid decarboxylase 65 (GAD65), insulinoma associated antigen-2, and insulin autoantibody. GAD and insulinoma autoantibody were measured with a combined radiobinding assay; insulin autoantibody was measured using microinsulin autoantibody. These methods are described elsewhere (2, 6).

If a subject tests positive for an autoantibody, they were put on an accelerated testing schedule that varied from every 3 to 6 months. Type 1 diabetes mellitus, which is diagnosed by a physician, was defined as having a random blood glucose >200 mg/dL and/or a HbA_{1c} (A1C) >6.2% with clinical symptoms of diabetes.

Measurement of membrane fatty acids

The protocol of collecting erythrocytes from blood samples collected at each DAISY visit began in 2000. Erythrocytes from the blood sample were separated within 30 min of blood draw, flash frozen in liquid nitrogen and stored at −70°C. Samples of erythrocytes were extracted for lipids following the method developed by Bligh and Dyer (7), and stored at −20°C in sealed cryotubes following flushing with nitrogen gas. The fatty acids present in the lipid isolates were subsequently methylated using the base-catalyzed procedures by Maxwell and Marmer (8) in preparation for analysis by gas chromatography (Hewlett-Packard 6890; Agilent, Santa Clara, CA, USA) with mass spectral detection (Hewlett-Packard 5973). The samples, separated across a CP-WAX column (25 m × 0.25 mm i.d., 0.2 μm film; Varian, Palo Alto, CA, USA), were identified by comparing the retention times and m/z of selected ions from analytes in the samples to those of authentic standards (NuCheckPrep; Elysian, MN, USA; Supelco; St. Louis, MO, USA). Quantitation was determined against five-point standard curves and fatty acid percentage is reported as a gram fatty acid/100 g red blood cell (RBC) lipid.

We measured the following fatty acids in the membranes: 18:2n-6 [linoleic acid (LA)], 20:4n-6 [arachidonic acid (ARA)], 18:3n-6 [gamma-linolenic acid (GLA)], 18:3n-3 [alpha-linolenic acid (ALA)], 20:5n-3 [eicosapentaenoic acid (EPA)], 22:6n-3 [docosahexaenoic acid (DHA)], and 22:5n-3 [docosapentaenoic acid (DPA)]. ALA, DHA, EPA, and DPA were combined to estimate total n-3 fatty acid intake; and LA, ARA, and GLA were combined to estimate total n-6 fatty acid intake. Measures of erythrocyte membrane fatty acids were expressed in percent of total lipids (gram of fatty acid/100 g RBC lipid).

Collection and analysis of supplement and dietary intake

During annual interviews, parents are asked to report the dietary supplements, including those containing omega-3 fatty acids that their children have taken in the last year. In addition, we prospectively measured early childhood diet using a 111-item semi-quantitative food frequency questionnaire (FFQ) that has been altered and validated for use in preschool children (9). Starting at the age of 2 yr, or at enrollment if after the age of 2 yr, the FFQ was administered annually and asked the mothers to recall the diets of their children in the previous year. Starting between the ages of 10 and 12 yr, children were asked to recall their own diets and complete the youth/adolescent questionnaire (YAQ), an FFQ geared toward adolescents (10, 11) that is based on the FFQ that we ask the parents to complete for the younger children. We conducted an instrument-comparison study in our DAISY population, in which we determined that data from these two instruments may be combined when an instrument indicator variable [i.e., type of FFQ (FFQ vs. YAQ)], was included in the model (12). A quantitative dietary assessment of polyunsaturated fatty acid (PUFA) intake was not available for the first year of life in DAISY; therefore we do not have intake data during infancy. This was an observational study; no dietary advice was given to the families.

In both the FFQ and the YAQ, to calculate intakes of n-3 and n-6 fatty acids and other nutrients, a commonly used unit or portion size for each food (e.g., one egg or 3–4 oz of fish, etc.) was specified on the FFQ and the parents were asked how often on average during the previous year their child had consumed that amount. Nine responses were possible, ranging from ‘never’ to ‘≥6 times/d’. Specifically, the questionnaire asked about the frequency of intake of canned tuna, dark-meat fish (mackerel, salmon, sardines, bluefish,

and swordfish), other fish (not specified), and shrimp, lobster, and scallops. The questionnaire also inquired about the kind of fat usually used for frying, sautéing, and baking (vegetable oil, solid vegetable oil shortening, butter, margarine, lard, or none). The intake of nutrients was computed for each child by multiplying the frequency of consumption of each unit of food by the nutrient content of the specified portions. Composition values for fatty acids and other nutrients were obtained from the Harvard University Food Composition Database, as derived from US Department of Agriculture sources (13) and supplemented by manufacturer information (14).

The total n-3 fatty acid intake variable was calculated by summing the intakes of the following fatty acids available in the FFQ data: ALA, EPA, DHA, and DPA. The calculation of EPA and DHA intake from this FFQ and database is described in detail elsewhere (15). The total n-6 fatty acid intake variable was calculated as the sum of LA, ARA, and GLA intake.

We compared intake of PUFAs as assessed by our FFQ to erythrocyte membrane composition of the same fatty acids in 404 DAISY children over time, for a total of 917 visits (16). Longitudinal analysis showed that estimates of energy-adjusted intakes of marine PUFAs, and total n-3 fatty acids were significantly correlated with the sums of EPA and DHA ($\rho = 0.23$, $p < 0.0001$) and all n-3 fatty acids ($\rho = 0.42$; $p < 0.0001$) (as a percent of total lipid) in the erythrocyte membrane, respectively.

Statistical methods

HR and 95% confidence intervals (CI) for the development of type 1 diabetes in relation to erythrocyte membrane fatty acid content or intake of n-3 and n-6 fatty acids were calculated by Cox proportional hazards regression. Both the erythrocyte fatty acid content variables and n-3 and n-6 fatty acid variables were analyzed as time-varying covariates, such that the values of erythrocyte fatty acid could vary with the clinical visits and reflect their change over time. Follow-up time began at detection of the first autoantibody. We calculated adjusted HR based on a standard deviation difference in the fatty acid level (percent of total lipids), or on a standard deviation in calculated intake from the FFQ. For the erythrocyte membrane fatty acid analysis, we adjusted for HLA-DR genotype (HLA DR3/4, *DQB1*0302* vs. other genotypes), whether or not the child had a first degree relative with type 1 diabetes, and age at first positive autoantibody. For the intake analysis, we additionally adjusted for total energy intake, type of FFQ (FFQ vs. YAQ), maternal age, maternal income, and maternal ethnicity.

When testing the hypothesis regarding intake of omega-3 fatty acids and type 1 diabetes risk, we attempted to assess both taking an omega-3 fatty acid supplement and intake via dietary sources in the same model. However, of the 155 children with both dietary intake and supplement data, only 18 of the 126 children who did not convert to type 1 diabetes and none of the 29 children who converted to type 1 diabetes were taking an omega-3 fatty acid supplement at their last visit, which made the HR inestimable. Therefore, we were only able to examine omega-3 fatty acid intake from dietary sources in this analysis.

Exposure data (dietary intake or erythrocyte membrane fatty acid percentage) were typically not collected at the visit at which the child was diagnosed with type 1 diabetes. In this situation, we took the exposure data collected at the DAISY follow-up visit just prior to diagnosis and carried it forward to the diagnosis visit to conduct the time-varying analysis, but only if that previous visit had taken place within 12 months prior to diagnosis.

Results

During DAISY follow-up, 167 children developed IA at a mean age of 5.4 yr. Age of development of IA is not different between individuals with a first degree relative with type 1 diabetes (5.8 yr) and those from the general population identified by newborn screen (5.1 yr; $p = 0.17$). These 167 autoantibody positive children were followed for a mean of 4.8 yr, with an 8% (14 children) lost-to-follow-up. Forty-five of these children developed type 1 diabetes at a mean age of 8.7 yr (Table 1). Children who developed type 1 diabetes were more likely to have a high-risk HLA genotype than children who did not develop type 1 diabetes. There were no differences in family history of diabetes, sex, maternal ethnicity, maternal age, and maternal education between children who did and did not develop type 1 diabetes. We obtained erythrocyte membrane fatty acid measures on 154 children and we obtained dietary intake data on 157 children in this cohort; with 144 children having both measures. Because we intended to analyze the hypotheses regarding fatty acid intake and fatty acid status separately, we did not require that children have both a dietary intake and a membrane fatty acid measurement to be included in the analysis cohort. There were no significant differences in the characteristics of those that had dietary fatty acid intake data and those that had erythrocyte membrane fatty acid data (data not shown). In order to describe the cohort's fatty acid intake and erythrocyte membrane fatty acid composition, which were collected at multiple time points throughout the autoimmune period, Table 2 presents mean levels at 3-, 6-, and 9-yr-old children in follow-up.

Intake of omega-3 fatty acids was not associated with risk of conversion to type 1 diabetes in children with IA, either unadjusted, or when adjusted for HLA-DR3/4 status, family history of type 1 diabetes, age at first autoantibody detection, ethnicity, income, maternal age, total energy intake, and type of FFQ (FFQ vs. YAQ) (Table 3). Similarly, intake of omega-6 fatty acids was not associated with risk of conversion to type 1 diabetes in children with IA (Table 3).

When examining fatty acid status, we found no association between erythrocyte membrane omega-3 fatty acids (as a percent of total lipids) and risk of conversion to type 1 diabetes in children with IA, either unadjusted or when adjusted for HLA-DR3/4 status, family history of type 1 diabetes and age at first autoantibody detection (Table 4). We saw a similar lack of association between erythrocyte membrane omega-6 fatty acid levels (as a percent of total lipids) and risk of conversion to type 1 diabetes in children with IA (Table 4).

Discussion

While our previous work in the DAISY cohort showed that increased intake and higher erythrocyte membrane omega-3 fatty acid levels were associated with a decreased risk of developing IA (2), our current study suggests that neither intake nor membrane levels of omega-3 or omega-6 fatty acids are associated with risk of developing of type 1 diabetes in children with IA.

The reasons why omega-3 fatty acids may play a role in prevention of IA, but do not play a similar role in development of type 1 diabetes once an individual has developed IA are not entirely clear. The exact mechanism by which omega-3 fatty acids might prevent development of IA is unknown. Long chain omega-3 fatty acids incorporated into cell membranes act as substrates for a class of anti-inflammatory eicosanoids, resolvins, and protectins (17). These molecules exert their anti-inflammatory effects by suppressing proinflammatory cytokines, acting as competing substrates for ARA (which produces proinflammatory eicosanoids), and reducing oxidative stress. One possibility is that there may be stage-dependent differences in the characteristics of islet inflammation. For

example, if classical inflammatory responses, such as interleukin-1β are critical early on, then DHA may be effective at an early time point. As the disease progresses, other aspects of inflammation or T cell activation may come into play, such as interferon-γ and cytotoxic T lymphocyte responses, where DHA or EPA may not be as effective.

Another possibility is that small differences in fatty acid status are enough to affect risk at the beginning of the disease process (i.e., conversion to IA) but are not large enough to influence the conversion to diabetes when the disease process is farther along and inflammation is greater. As DAISY is an observational study, the variation in omega-3 fatty acid status in these children is relatively small because it is based on typical dietary intake. This may explain why we observed a protective association early in the disease process but not later. This study does not exclude the possibility that higher doses of omega-3 fatty acids, such as those that would be used in an intervention study, would be effective in this phase of the disease. Alternatively, it is possible that once a child has developed persistent IA, he/she has progressed too far along in the process of developing type 1 diabetes for any intervention to be effective, as has been suggested in the nicotinamide and insulin interventions that have been attempted to date (18–21).

In contrast to our earlier findings that both erythrocyte membrane levels of omega-3 fatty acids and fatty acid intake are associated with a decreased hazard of development IA, we did not find a decreased hazard of conversion to type 1 diabetes for either intake or erythrocyte membrane levels. As our cohort of autoantibody positive children and number of type 1 diabetes cases are relatively small, it is important to confirm this finding in other populations. The nutritional intervention to prevent type 1 diabetes is a TrialNet feasibility study of an intervention examining whether nutritional supplements with DHA, given during the last trimester of pregnancy and the first few years of life, can prevent development of islet autoantibodies (22). This will provide valuable data regarding the feasibility of an intervention of this type in the earlier phase of the disease.

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Table 1

Descriptive characteristics of children with IA by whether or not they progressed to type 1 diabetes during DAISY follow-up

	Progressed to type 1 diabetes (n = 45)	Has not progressed to type 1 diabetes (n = 122)	Unadjusted HR (95% CI)	p
Variable	n (%)	n (%)		
High-risk genotype HLA-DR3/4, DQ8	22 (48.9)	35 (28.7)	2.05 (1.14–3.68)	**0.02**
Family history of type 1 diabetes	30 (66.7)	67 (54.9)	1.20 (0.64–2.23)	0.57
Female	22 (48.9)	60 (49.2)	1.26 (0.70–2.27)	0.44
Non-Hispanic White ethnicity	42 (93.3)	94 (77.1)	1.57 (0.48–5.11)	0.46
Maternal education >12 yr (n = 162)	39 (88.6)	96 (81.4)	1.68 (0.66–4.26)	0.28
Maternal age (mean, SD) (n = 164)	32.41 (5.23)	30.03 (5.59)	1.05 (0.99–1.11)	0.09
Mean age at first autoantibody positive visit (yr)	3.92 (2.97)	5.91 (3.44)	0.92 (0.83–1.02)	0.11
Mean length of follow-up (yr)	4.80 (2.87)	4.51 (3.28)	N/A	N/A
Mean age at diabetes diagnosis or last visit (yr)	8.71 (3.90)	10.42 (4.26)	N/A	N/A

CI, confidence interval; DAISY, Diabetes Autoimmunity Study of the Young; HLA, human leukocyte antigen; HR, hazard ratios; IA, islet autoimmunity; N/A, not available.

Table 2

Description of omega-3 and omega-6 dietary intake (top) and erythrocyte fatty acid levels (bottom) at three ages in children with IA*

	Group of children†, mean (SD)		
IA analysis cohort	**3 yr olds**	**6 yr olds**	**9 yr olds**
Dietary intake variables	n = 45	n = 45	n = 41
Energy intake (kcal/d)	2189.93 (775.94)	1942.08 (675.54)	2053.86 (799.70)
Total omega-3 fatty acids (g/d)‡	1.11 (0.54)	1.07 (0.51)	1.28 (0.63)
ALA	0.99 (0.44)	0.93 (0.47)	1.15 (0.56)
EPA	0.027 (0.035)	0.034 (0.051)	0.038 (0.056)
DHA	0.077 (0.072)	0.089 (0.084)	0.082 (0.083)
DPA	0.016 (0.012)	0.018 (0.017)	0.014 (0.011)
Total omega-6 fatty acids (g/d)§	11.03 (5.54)	10.65 (4.83)	11.56 (4.92)
LA	10.89 (5.48)	10.50 (4.78)	11.43 (4.85)
GLA	0.013 (0.007)	0.013 (0.008)	0.010 (0.007)
ARA	0.13 (0.07)	0.14 (0.08)	0.12 (0.07)
Erythrocyte fatty acid levels	n = 35	n = 61	n = 54
Total omega-3 fatty acids‡	4.13 (1.26)	4.73 (1.94)	4.06 (1.18)
ALA	0.25 (0.17)	0.23 (0.07)	0.22 (0.10)
EPA	0.20 (0.08)	0.26 (0.22)	0.24 (0.10)
DHA	2.14 (0.50)	2.53 (1.25)	2.32 (0.84)
DPA	1.54 (1.03)	1.70 (0.75)	1.27 (0.48)
Total omega-6 fatty acids‡	24.83 (4.24)	26.11 (3.10)	26.35 (2.05)
LA	13.37 (2.78)	14.30 (2.20)	14.55 (2.01)
GLA	0.11 (0.05)	0.12 (0.05)	0.14 (0.06)
ARA	11.35 (2.15)	11.70 (1.85)	11.66 (1.67)

ALA, alpha-linolenic acid; ARA, arachidonic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; GLA, gamma-linolenic acid; IA, islet autoimmunity; LA, linoleic acid.

*These data are from cross-sectional analysis of the cohort. The same child may be in one or all of these age group samples depending on whether fatty acid data were available at these ages.

†Age groups are determined as follows: 3-yr-olds are at least 3 yr of age but less than 4 yr of age; 6-yr-olds are at least 6 yr of age, but less than 7 yr of age; 9-yr-olds are at least 9 yr of age, but less than 10 yr of age.

‡Total omega-3 acids consisted of ALA (18:3n3), EPA (20:5n3), DHA (22:6n3), and DPA (22:5n3).

§Total omega-6 fatty acids consisted of LA (18:2n6), ARA (20:4n6), and GLA (18:3n6).

Table 3

Association between dietary omega-3 or omega-6 fatty acid intake and risk of developing type 1 diabetes* in children with IA

	Unadjusted HR		Adjusted HR	
	95% CI†	p	95% CI†,‡	p
Total omega-3 fatty acids	1.00 (0.53–1.89)	1.00	1.31 (0.65–2.62)	0.46
ALA	1.15 (0.61–2.16)	0.66	1.38 (0.70–2.69)	0.35
EPA	0.63 (0.31–1.28)	0.20	0.79 (0.39–1.62)	0.52
DHA	0.72 (0.41–1.29)	0.27	0.92 (0.50–1.69)	0.79
DPA	0.60 (0.33–1.10)	0.10	0.69 (0.56–1.33)	0.27
Total omega-6 fatty acids	1.20 (0.64–2.26)	0.57	1.50 (0.80–2.82)	0.21
LA	1.21 (0.65–2.26)	0.56	1.50 (0.80–2.80)	0.20
GLA	1.15 (0.69–1.93)	0.59	1.36 (0.79–2.33)	0.27
ARA	0.75 (0.41–1.37)	0.35	1.00 (0.54–1.84)	0.99

ALA, alpha-linolenic acid; ARA, arachidonic acid; CI, confidence interval; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; FFQ, food frequency questionnaire; GLA, gamma-linolenic acid; IA, islet autoimmunity; LA, linoleic acid.

*Based on 157 individuals with FFQ data. Of these, 30 had progressed to type 1 diabetes, and 127 had not.

†HR and 95% CI represent a one SD change in fatty acid variable. Fatty acid measured as intake in g/d. Values for fatty acid intake are adjusted for total energy intake and type of FFQ instrument (FFQ vs. YAQ).

‡Adjusted for total caloric intake, type of FFQ, age at first autoantibody positive visit, HLA DR3/4 status, family history of type 1 diabetes, maternal age, maternal education, and maternal ethnicity.

Table 4

Association between erythrocyte membrane omega-3 or omega-6 fatty acid content and risk of developing type 1 diabetes* in children with IA

	Unadjusted HR		Adjusted HR	
	95% CI†	p	95% CI†,‡	p
Total omega-3 fatty acids	0.99 (0.70–1.41)	0.96	1.06 (0.75–1.50)	0.73
ALA	1.02 (0.79–1.31)	0.89	0.99 (0.73–1.34)	0.96
EPA	1.10 (0.83–1.47)	0.52	1.14 (0.85–1.52)	0.39
DHA	1.13 (0.84–1.53)	0.43	1.21 (0.90–1.65)	0.21
DPA	0.81 (0.49–1.33)	0.41	0.86 (0.53–1.39)	0.54
Total omega-6 fatty acids	1.28 (0.89–1.84)	0.18	1.25 (0.88–1.77)	0.22
LA	1.25 (0.90–1.73)	0.18	1.19 (0.87–1.63)	0.29
GLA	0.97 (0.58–1.62)	0.91	0.96 (0.62–1.50)	0.87
ARA	1.13 (0.81–1.56)	0.48	1.16 (0.84–1.60)	0.38

ALA, alpha-linolenic acid; ARA, arachidonic acid; CI, confidence interval; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; GLA, gamma-linolenic acid; IA, islet autoimmunity; LA, linoleic acid.

*Based on 154 individuals with erythrocyte fatty acid measures. Of these, 39 had progressed to type 1 diabetes, and 115 had not.

†HR and 95% CI represent a one SD change in fatty acid variable. Fatty acid measured as a percent of total membrane lipids.

‡Adjusted for age at first autoantibody positive visit, HLA DR3/4 status, and family history of type 1 diabetes.