Interaction Between GAD65 Antibodies and Dietary Fish Intake or Plasma Phospholipid n-3 Polyunsaturated Fatty Acids on Incident Adult-Onset Diabetes: The EPIC-InterAct Study

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OBJECTIVE

Islet autoimmunity is associated with diabetes incidence. We investigated whether there was an interaction between dietary fish intake or plasma phospholipid n-3 polyunsaturated fatty acid (PUFA) concentration with the 65-kDa isoform of GAD (GAD65) antibody positivity on the risk of developing adult-onset diabetes.

RESEARCH DESIGN AND METHODS

We used prospective data on 11,247 incident cases of adult-onset diabetes and 14,288 noncases from the EPIC-InterAct case-cohort study conducted in eight European countries. Baseline plasma samples were analyzed for GAD65 antibodies and phospholipid n-3 PUFAs. Adjusted hazard ratios (HRs) for incident diabetes in relation to GAD65 antibody status and tertiles of plasma phospholipid n-3 PUFA or fish intake were estimated using Prentice-weighted Cox regression. Additive (proportion attributable to interaction [AP]) and multiplicative interactions between GAD65 antibody positivity (≥65 units/mL) and low fish/n-3 PUFA were assessed.

RESULTS

The hazard of diabetes in antibody-positive individuals with low intake of total and fatty fish, respectively, was significantly elevated (HR 2.52 [95% CI 1.76–3.63] and 2.48 [1.79–3.45]) compared with people who were GAD65 antibody negative and had high fish intake, with evidence of additive (AP 0.44 [95% CI 0.16–0.72] and 0.48 [0.24–0.72]) and multiplicative ($P = 0.0465$ and 0.0103) interactions. Individuals with high GAD65 antibody levels (≥167.5 units/mL) and low total plasma phospholipid n-3 PUFAs had a more than fourfold higher hazard of diabetes (HR 4.26 [2.70–6.72]) and an AP of 0.46 (0.12–0.80) compared with antibody-negative individuals with high n-3 PUFAs.

CONCLUSIONS

High fish intake or relative plasma phospholipid n-3 PUFA concentrations may partially counteract the increased diabetes risk conferred by GAD65 antibody positivity.

Circulating islet autoantibodies are a marker of increased risk of type 1 diabetes (1,2), but they are also associated with adult-onset diabetes with a type 2–like phenotype (3). The most common autoantibody found in patients with autoimmune adult diabetes is directed against the 65-kDa isoform of GAD (GAD65) (4), whereas in children, multiple antibodies are usually present at diagnosis of type 1 diabetes (5). Several environmental risk factors have been suggested to modify progression from islet autoimmunity to clinical diabetes, but evidence about these modifying factors is limited (6).

Long-chain n-3 polyunsaturated fatty acids (PUFAs), mainly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) from marine food sources, have potential protective effects on diseases with an inflammatory component, such as autoimmune diabetes, owing to their anti-inflammatory, immunomodulatory, and gene expression regulatory properties (7). A Norwegian study showed that the risk of type 1 diabetes in children was diminished in those who had cod liver oil supplementation during the first year of life (8), suggesting a possible protective effect on the risk of autoimmune diabetes. In a separate study of genetically susceptible children in a U.S. cohort, total estimated dietary n-3 PUFAs as well as n-3 PUFA concentration measured in erythrocyte membranes, were inversely associated with the development of islet autoimmunity and multiple autoantibodies and type 1 diabetes, with similar hazard ratios (HRs) observed in separate analyses focused on marine n-3 PUFAs (9). Studies in adults are scarce, but we previously reported a lower risk of latent autoimmune diabetes in adults (LADA) in those who regularly consume fatty fish (10). However, the results from other studies have not confirmed these findings, and others have reported the absence of an association between serum or erythrocyte n-3 PUFAs and childhood islet autoimmunity (11) or type 1 diabetes (12). Our aim was to investigate the potential interaction between GAD65 antibody positivity and dietary fish intake or relative plasma phospholipid concentration of n-3 PUFAs in relation to incident adult-onset diabetes using prospective data from the European Prospective Investigation into Cancer and Nutrition (EPIC)-InterAct case-cohort study.

RESEARCH DESIGN AND METHODS

Study Design and Population

The design and methods of the EPIC-InterAct case-cohort study have previously been described (13). A total of 340,234 EPIC participants, who were free of known diabetes at baseline, in 8 of the 10 EPIC study countries (26 centers in Denmark, France, Germany, Italy, the Netherlands, Spain, Sweden, and the U.K.) were followed for 3.99 million person-years (median follow-up 10.9 years). During this time (1991–2007), 12,403 incident cases of diabetes were ascertained and verified. Ascertainment of incident diabetes involved multiple sources of evidence, including self-report and linkage to primary care registers, secondary care registers, medication use (drug registers), hospital admissions, and mortality data. A minimum of two data sources were required to confirm the diagnosis. In Denmark and Sweden, cases were identified through local and national diabetes and pharmaceutical registers, and hence, all ascertained cases were considered verified. Follow-up was censored at the date of diagnosis, 31 December 2007, or the date of death, whichever occurred first. All included cases were a diagnosis of type 2 diabetes, and diagnosis of other diabetes forms (e.g., type 1 diabetes, gestational diabetes mellitus) were not included as cases. Since GAD65 antibodies were measured at baseline (described below), the antibody status at diagnosis of the cases is unknown. Thus, some cases will most likely meet the commonly used criteria for LADA (antibody positivity, onset $\geq$35 years, and remaining insulin secretion often indicated by absence of insulin requirement for 6–12 months following diagnosis) (14). For this reason, the studied outcome is referred to as adult-onset diabetes.

A center-stratified subcohort of 16,835 (4.9% of the entire EPIC cohort) individuals was selected at random. We excluded 548 individuals with known prevalent diabetes and 133 with unknown diabetes status at baseline. From the case-cohort study, we also excluded 483 participants without plasma phospholipid fatty acid data, 352 with insufficient sample volume for GAD65 antibody measurement, 717 in the top 1% or bottom 1% of the ratio of energy intake to basal metabolic rate, and 692 with missing data on covariates used in the analysis. The sample for analysis, therefore, included 25,535 participants, of whom 11,247 were incident cases and 14,981 were in the subcohort that included 693 of the cases by design (Supplementary Fig. 1). The number of case and subcohort participants per country are presented in Supplementary Table 1. All study participants gave written informed consent, and the investigation was carried out in accordance with the Declaration of Helsinki as revised in 2008.

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Where authors are identified as personnel of the International Agency for Research on Cancer/World Health Organization, the authors alone are responsible for the views expressed in this article, and they do not necessarily represent the decisions, policy, or views of the International Agency for Research on Cancer/World Health Organization.

O.R. and N.J.W. contributed equally.

Plasma Sample Measurements

Baseline blood samples from all participants were stored at −196°C in liquid nitrogen at the EPIC coordinating center (International Agency for Research on Cancer) or in local biorepositories, with the exception of Umeå (−80°C) and Denmark (maximum −150°C). Plasma antibody levels were determined at the University of Washington School of Medicine by analyzing for GAD65 antibodies in a radioligand binding assay and expressed as relative units compared with the World Health Organization standard (15). GAD65 antibody levels ≥65 units/mL were defined as positive, as previously described (16), with a sensitivity of 85% and specificity of 99% (3). The assay used performs well compared with the gold standard sensitivity of 64% and specificity of 99% (17).

Fatty acids were analyzed in the plasma phospholipid fraction using a previously described method (18). In short, the plasma phospholipid fraction was obtained through solid-phase extraction followed by hydrolyzation and methylation. The resulting fatty acid methyl esters were analyzed by gas chromatography (HP-88, 30-m length; J&W) equipped with flame ionization detection (7890N GC; Agilent Technologies, Santa Clara, CA). The fatty acids were compared with commercial standards by their separate retention times and expressed as the percentage of total phospholipid fatty acids (mol%), which has been previously described in detail (19). The fatty acid profiling was performed at Medical Research Council Human Nutrition Research, Cambridge.

Dietary Assessment

Dietary data were collected at baseline using locally developed and validated quantitative questionnaires with individual average portion sizes (France, Spain, the Netherlands, Germany, and Italy) or semiquantitative food frequency questionnaires (Denmark, Naples, Sweden, and U.K.) (20,21). The EPIC Nutrient Database was used to standardize dietary data from each EPIC cohort (22). For this analysis, we used data on lean fish (fat content of <4 g/100 g) and fatty fish (fat content ≥4 g/100 g) intake, and total fish intake was calculated as the sum of those. Germany ($n = 3,462$) was excluded from the analyses of total fish and lean fish because this center had no data on lean fish intake. Intakes of shellfish and other types of fish (fish products/fish in crumbs and nonspecific or combined fish) were not included in the analyses in the current study.

Other Covariate Assessment

Standardized data on health and lifestyle factors were collected in baseline questionnaires (21). Height, weight, and waist circumference were measured by trained staff during standardized baseline health examinations, except in parts of Oxford (U.K.) and France, where anthropometric data were self-reported, and in Umeå, where waist circumference was not measured. BMI was calculated as weight divided by squared height (kg/m^2). Occupational and leisure time physical activity was assessed by questionnaire and categorized into the validated four-scale Cambridge physical activity index (23).

Statistical Analysis

All analyses were performed using SAS 9.4 statistical software (SAS Institute, Cary, NC). Baseline characteristics were summarized as means (SD) or medians (interquartile ranges) for continuous variables and frequencies for categorical variables. *P* values for comparisons between GAD65 antibody-negative and antibody-positive participants in the subcohort and among all incident cases, respectively, were calculated using χ^2 test (proportions), Student *t* test (means), or Kruskal-Wallis H test (medians). Spearman rank correlation coefficients were calculated for each possible combination of plasma phospholipid fatty acids (total n-3 PUFAs, EPA, docosapentaenoic acid [DPA], DHA) and dietary fish variables (total, fatty, lean) on the basis of the subcohort.

Prentice-weighted Cox regression models (24) were used to estimate HRs and 95% CIs of incident diabetes in relation to baseline GAD65 antibody status, plasma phospholipid n-3 PUFAs, fish intake, and various combinations of GAD65 antibody status and categories of n-3 PUFAs or fish intake. Categories of plasma phospholipid n-3 PUFAs and dietary fish intake were defined using tertiles of the distributions in the overall subcohort. GAD65 antibody status was analyzed both as a binary exposure (negative, positive) and as three levels (negative, 65 to <167.5 units/mL [low], and ≥167.5 units/mL [high]). The cutoff used to define high antibody levels corresponds to the median GAD65 antibody level among all GAD65 antibody–positive individuals in the study (3). Models were adjusted for age (as the underlying time scale), sex, education (none, primary, technical/professional, secondary, or higher), smoking status (never, former, or current), Cambridge physical activity index (inactive, moderately inactive, moderately active, or active), BMI (continuous), total energy intake (continuous), alcohol intake (none, >0 to <6, 6 to <12, 12 to <24, ≥24 g/day), and fruit and vegetable intake (g/day). The baseline hazard function was stratified by center (25). When GAD65 antibody status was the exposure, the model did not include dietary variables.

The interaction between GAD65 antibody status (positive vs. negative and high or low antibody level vs. negative) and plasma phospholipid n-3 PUFA or dietary fish intake was assessed as departure from additivity of effects (26). This form of interaction was deemed most suitable because we were interested in estimating the synergistic impact of being exposed both to GAD65 antibodies and to low relative n-3 PUFA plasma phospholipid concentration on diabetes incidence. Subgroups were created by modeling mutually exclusive indicator variables for the combinations of the risk factors and evaluated by the proportion attributable to interaction (AP). The AP estimate indicates the proportion of double-exposed cases that may be attributed to the interaction between the two exposures and that would be prevented by removal of one of the risk factors. Of note, this interpretation of AP rests on the assumption of causality (27). The equation for AP is as follows: $AP = (HR_{11} - HR_{10} - HR_{01} + 1)/HR_{11}$, where HR_{11} indicates doubly exposed (e.g., low fish intake, GAD65 antibody positivity) and HR_{01} or HR_{10} indicate either one exposure (e.g., high fish and GAD65 antibody positivity, low fish and GAD65 antibody negativity). The highest tertile of plasma phospholipid n-3 PUFAs or fish intake in combination with GAD65 antibody negativity was used as the reference category since this combination would confer the lowest risk when considering the two exposures jointly (26). The 95% CIs for the AP estimates are Wald confidence limits from delta approximation of the variances (28). The presence of interaction is scale dependent; therefore, we also report the *P*

value for the multiplicative interaction term of GAD65 antibody status (in two and three levels) and tertiles of fish intake or plasma phospholipid n-3 PUFAs. Interaction that is present on both the additive and the multiplicative scales may be considered the strongest form of interaction (29).

Sensitivity Analysis

Sensitivity analyses using the main interaction model for total fish and total n-3 PUFAs included exclusion of individuals with baseline HbA_{1c} ≥6.5% (≥48 mmol/mol), exclusion of diabetes cases diagnosed within 2 years after baseline, additional adjustment for family history of type 2 diabetes (data available for 49% of sample), and adjustment for waist circumference (missing for Umeå center $n = 1,647$). Mutual adjustments for fatty and lean fish were included in the analyses of specific types of fish. Potential confounding from additional dietary factors was assessed by adding intakes (g/day) of cereal products, red meat, processed meat, dairy products, coffee, tea, and soft drinks to the main interaction model, both one by one and all factors simultaneously. The dietary fish analysis was additionally adjusted for intakes of dietary fiber, fat, saturated fatty acids, monounsaturated fatty acids, cholesterol, and protein. The analysis of total n-3 PUFAs was additionally adjusted for plasma phospholipid saturated fatty acids. In post hoc analyses restricted to GAD65 antibody–positive individuals, adjusted HRs of diabetes in relation to plasma n-3 PUFAs and dietary fish were estimated.

RESULTS

Baseline Characteristics

The baseline prevalence of GAD65 antibody positivity in the subcohort was 2.0% ($n = 299$) and 3.6% ($n = 392$) among the incident diabetes cases. There were no major differences in baseline characteristics by GAD65 antibody status in the subcohort (Table 1). Among the cases, GAD65 antibody–positive individuals were more likely to have lower BMI compared with those who were GAD65 antibody negative. Mean follow-up time was 6.9 years for GAD65 antibody–negative cases and 6.5 years for GAD65-positive cases. Follow-up time did not differ significantly between the low-titer and high-titer GAD65 antibody groups (6.8 years and 6.3 years, respectively, $P = 0.19$) (data not shown).

Correlations Between Plasma Phospholipid n-3 PUFA Measurements and Fish Intake and Their Association With GAD65 Antibody Status

On the basis of the subcohort, total plasma phospholipid n-3 PUFAs had small positive correlations with intakes of total fish ($r = 0.26$), fatty fish ($r = 0.26$), and lean fish ($r = 0.15$) (Supplementary Table 2). Of the individual fatty acids, the positive correlation with total fish was highest for DHA ($r = 0.35$) and EPA ($r = 0.15$), but DPA was negatively correlated ($r = -0.17$). For that reason, and the fact that it is a metabolic intermediate

Table 1—Baseline characteristics by GAD65 antibody status among all eligible subcohort and incident diabetes cases: the EPIC-InterAct Study

	Subcohort			Incident diabetes cases		
	GAD65 antibody negative	GAD65 antibody positive	*P* value*	GAD65 antibody negative	GAD65 antibody positive	*P* value†
Individuals, *n*	14,682‡	299§		10,855	392	
Time of follow-up (years)	12.0 (2.3)	11.9 (2.4)	0.59	6.9 (3.3)	6.5 (3.5)	0.07
Age (years)	52.3 (9.1)	52.8 (9.2)	0.34	55.5 (7.6)	55.0 (8.4)	0.26
Female sex	62.5	64.9	0.39	50.2	58.2	0.002
BMI (kg/m^2)	26.0 (4.2)	25.8 (4.2)	0.43	29.8 (4.7)	28.4 (5.1)	<0.001
Family history of type 2 diabetes‖	13.5	19.0	0.10	36.3	31.0	0.12
High education	20.7	20.4	0.91	13.2	13.8	0.74
Current smoking	25.7	23.1	0.31	27.6	27.8	0.93
Physical activity, active according to index	20.1	19.4	0.77	16.8	18.6	0.35
Alcohol intake (g/day)	6.3 (0.9, 18.3)	6.0 (1.3, 17.8)	0.76	6.1 (0.6, 20.2)	5.4 (0.6, 15.3)	0.09
Energy intake (kcal/day)	2,136 (633)	2,152 (664)	0.68	2,177 (674)	2,068 (612)	<0.001
Fruit intake (g/day)	193.3 (103.5, 316.6)	207.1 (115.4, 328.4)	0.23	182.6 (96.3, 309.7)	183.3 (48.7, 300.5)	0.59
Vegetable intake (g/day)	156.3 (101.6, 239.4)	151.9 (97.7, 228.4)	0.78	150.0 (95.1, 235.9)	147.6 (97.5, 221.6)	0.43
Total dietary fish¶ (g/day)	20.0 (6.9, 39.2)	21.6 (7.2, 38.2)	0.45	22.8 (7.7, 42.3)	16.9 (5.3, 34.2)	<0.001
Fatty fish (g/day)	6.0 (0.6, 14.8)	7.3 (1.2, 15.7)	0.10	6.6 (0.6, 15.8)	6.6 (1.1, 13.6)	0.67
Lean fish¶ (g/day)	9.5 (0.0, 23.0)	9.7 (0.5, 21.2)	0.57	10.7 (0.0, 24.4)	7.0 (0, 20.1)	0.02
Plasma phospholipid n-3 PUFAs (mol%)	6.42 (5.35, 7.75)	6.49 (5.46, 7.77)	0.76	6.52 (5.42, 7.83)	6.34 (5.22, 7.67)	0.06
Plasma phospholipid EPA (mol%)	1.04 (0.72, 1.52)	1.05 (0.71, 1.50)	0.83	1.13 (0.79, 1.65)	1.06 (0.73, 1.55)	0.03
Plasma phospholipid DHA (mol%)	4.17 (3.39, 5.06)	4.15 (3.48, 5.08)	0.68	4.16 (3.42, 5.04)	4.04 (3.30, 5.03)	0.04

Data are mean (SD), %, or median (interquartile range) unless otherwise indicated. *P for the comparison between GAD65 antibody–positive and –negative subcohort participants. †P for the comparison between GAD65 antibody–positive and –negative cases. ‡Of which 670 (4.6%) developed diabetes during follow-up. §Of which 23 (7.7%) developed diabetes during follow-up. ‖Family history information available for 49.1% of the subcohort (47.2% for GAD65 antibody positive) and 48.8% (53.6% for GAD65 antibody positive) of incident diabetes cases. ¶Excluding participants in Germany ($n = 3,462$, including 1,523 incident cases of which 30 were GAD65 antibody positive) because of no available lean fish intake data.

between EPA and DHA, DPA was excluded from further analyses of individual fatty acids. Median levels of fish intake and plasma phospholipid n-3 PUFAs by intake of fatty and lean fish are shown in Supplementary Table 3.

Associations Among GAD65 Antibody Positivity, Plasma Phospholipid n-3 PUFAs/Fish Intake, and Incident Diabetes

GAD65 antibody positivity was associated with a higher incidence of diabetes (HR 1.81 [95% CI 1.49–2.20]), with a stronger association for high GAD65 antibody levels (≥167.5 units/mL) versus GAD65 antibody negativity (2.93 [2.27–3.79]) (Table 2). Neither fish intake nor plasma phospholipid n-3 PUFA was associated with incident diabetes (high vs. low category of total fish intake: 1.03 [0.93–1.15]; high vs. low category of total plasma phospholipid n-3 PUFAs: 1.00 [0.92–1.10]). The exception is plasma phospholipid EPA, for which a positive association with incident diabetes was observed in the comparison between highest and lowest tertile.

Interaction Between GAD65 Antibody Status and Plasma Phospholipid n-3 PUFAs/Fish Intake and Incident Diabetes

Relative to the combination of GAD65 antibody negativity and high fish intake, there was a positive association with incident diabetes for the combination of GAD65 antibody positivity and low intake of total fish (HR 2.52 [95% CI 1.76–3.63]) or fatty fish (2.48 [1.79–3.45]), with evidence of both additive and multiplicative interactions (Table 3). Similar associations were seen for GAD65 antibody positivity and plasma phospholipid n-3 PUFAs but without evidence of either additive or multiplicative interaction, except for EPA where there was evidence of additive interaction (AP 0.36 [95% CI 0.06–0.66]). For individuals with high levels of GAD65 antibodies in combination with low total plasma phospholipid n-3 PUFAs or DHA, the HRs were 4.26 (2.70–6.72) and 4.30 (2.86–6.47), respectively (Fig. 1), with evidence of additive interaction (AP 0.46 [0.12–0.80] and 0.43 [0.08–0.77], respectively) but not multiplicative interaction (Supplementary Table 4).

Table 2—Incident diabetes in relation to GAD65 antibody status, plasma phospholipid n-3 PUFAs, and dietary fish intake in tertiles on the basis of the distribution in the subcohort: the EPIC-InterAct Study

Exposure and category	Cases, *n*	HR (95% CI)
GAD65 antibody status*		
Negative	10,855	1.00 (reference)
Positive	392	1.81 (1.49–2.20)
Low GAD65 antibody	167	1.20 (0.91–1.57)
High GAD65 antibody	225	2.93 (2.27–3.79)
Total plasma phospholipid n-3 PUFAs (mol%)†		
Low (>0 to <5.69)	3,534	1.00 (reference)
Moderate (5.69 to <7.25)	3,786	0.99 (0.91–1.07)
High (≥7.25)	3,927	1.00 (0.92–1.10)
Plasma phospholipid EPA (mol%)†		
Low (>0 to <0.83)	3,155	1.00 (reference)
Moderate (0.83 to <1.34)	3,801	1.08 (0.99–1.17)
High (≥1.34)	4,291	1.14 (1.03–1.25)
Plasma phospholipid DHA (mol%)†		
Low (>0 to <3.65)	3,648	1.00 (reference)
Moderate (3.65 to <4.75)	3,991	0.97 (0.90–1.06)
High (≥4.75)	3,608	0.92 (0.84–1.00)
Total fish intake (g/week)†		
Low (<69.3)	2,896	1.00 (reference)
Moderate (69.3 to <222.9)	3,327	0.99 (0.89–1.09)
High (≥222.9)	3,501	1.03 (0.93–1.15)
Fatty fish intake (g/week)†		
Low (<12.2)	3,650	1.00 (reference)
Moderate (12.2 to <78.1)	3,650	0.88 (0.81–0.97)
High (≥78.1)	3,947	0.96 (0.87–1.05)
Lean fish intake (g/week)†		
Low (<13.2)	3,099	1.00 (reference)
Moderate (13.2 to <125.1)	3,152	0.95 (0.86–1.05)
High (≥125.1)	3,473	1.02 (0.92–1.14)

*HRs adjusted for age (underlying time scale), center, sex, education level, smoking status, physical activity, and BMI. †HRs additionally adjusted for total energy intake and intake of alcohol, fruits, and vegetables.

Sensitivity Analyses

The joint effects of GAD65 antibody positivity and low total plasma phospholipid n-3 PUFAs or total fish intake remained largely unchanged in most sensitivity analyses. As an exception, results were sensitive to adjustment for family history of type 2 diabetes. In analysis restricted to those with available information on family history, the HR associated with GAD65 antibody positivity combined with low total fish intake was 2.87 (95% CI 1.78–4.61; AP 0.32 [95% CI −0.13–0.78]) before adjustment and 3.54 (2.24–5.58; AP 0.44 [0.06–0.82]) after adjustment. Exclusion of individuals with elevated baseline HbA_{1c} (≥6.5% [≥48 mmol/mol]) resulted in a significant interaction between antibody positivity and total n-3 PUFAs (AP 0.35 [0.004–0.69]). Mutual adjustments for fatty and lean fish had no impact on the results in the analyses of specific types of fish. Analyses restricted to GAD65 antibody–positive individuals were hampered by small numbers but indicated a lower HR of diabetes among those with high compared with low fatty fish intake (Supplementary Table 5).

CONCLUSIONS

In this study, we identified evidence of an interaction between GAD65 antibody positivity and dietary fish intake or plasma phospholipid n-3 PUFAs on incident adult-onset diabetes. This means that the incidence is increased among antibody-positive individuals who have low dietary fish intake and that this increase may be higher than expected from the sum or product of the two individual exposures. Thus, our findings suggest that fish intake or the relative plasma phospholipid n-3 PUFA concentration may partly counteract the increased incidence of adult-onset diabetes in individuals who are GAD65 antibody positive. This fits with previous observations in childhood type 1 diabetes in which incidence was inversely associated with

Table 3—Incident diabetes for combinations of GAD65 antibody status and categories of plasma phospholipid n-3 PUFAs or dietary fish intake: the EPIC-InterAct Study

	GAD65 antibody negative		GAD65 antibody positive			
Exposure	Cases, *n*	HR* (95% CI)	Cases, *n*	HR* (95% CI)	AP† (95% CI)	*P* value‡
Total plasma phospholipid n-3 PUFAs						
High	3,798	1.00 (reference)	129	1.63 (1.19–2.24)		
Moderate	3,666	0.98 (0.91–1.06)	120	1.65 (1.20–2.26)		
Low	3,391	0.98 (0.90–1.08)	143	2.26 (1.54–3.30)	0.28 (−0.07 to 0.64)	0.34
Plasma phospholipid EPA						
High	4,159	1.00 (reference)	132	1.49 (1.08–2.06)		
Moderate	3,670	0.94 (0.87–1.02)	131	1.69 (1.20–2.40)		
Low	3,026	0.87 (0.79–0.95)	129	2.14 (1.55–2.95)	0.36 (0.06–0.66)	0.09
Plasma phospholipid DHA						
High	3,491	1.00 (reference)	117	1.61 (1.16–2.24)		
Moderate	3,872	1.06 (0.98–1.15)	119	1.88 (1.37–2.57)		
Low	3,492	1.08 (0.99–1.17)	156	2.28 (1.59–3.27)	0.26 (−0.09 to 0.61)	0.54
Total fish intake						
High	3,400	1.00 (reference)	101	1.47 (1.05–2.07)		
Moderate	3,198	0.95 (0.87–1.04)	129	1.57 (1.11–2.22)		
Low	2,764	0.95 (0.85–1.06)	132	2.52 (1.76–3.63)	0.44 (0.16–0.72)	0.0465
Fatty fish intake						
High	3,821	1.00 (reference)	126	1.26 (0.90–1.76)		
Moderate	3,509	0.91 (0.84–0.99)	141	2.09 (1.52–2.86)		
Low	3,525	1.03 (0.94–1.13)	125	2.48 (1.79–3.45)	0.48 (0.24–0.72)	0.0103
Lean fish intake						
High	3,371	1.00 (reference)	102	1.51 (1.07–2.13)		
Moderate	3,025	0.92 (0.84–1.00)	127	1.80 (1.35–2.42)		
Low	2,966	0.97 (0.87–1.08)	133	1.99 (1.32–2.99)	0.26 (−0.14 to 0.65)	0.41

*HRs adjusted for age (underlying time scale), center, sex, education level, smoking status, physical activity, BMI, total energy intake, and intake of alcohol, fruits, and vegetables. †AP is calculated for the combination of GAD65 antibody positivity and low plasma n-3 PUFAs/fish intake. ‡*P* for the multiplicative interaction term between GAD65 antibody status and categories of plasma n-3 PUFAs/fish intake.

fish oil supplementation in Norway (8) and fish consumption in the U.S. (9). High intake of fatty fish was associated with a lower risk of LADA in our previous study that was based on Swedish data (10). We are aware of only one previous study that specifically aimed to assess the impact of n-3 PUFAs on the progression from islet autoimmunity to clinical diabetes (12). In contrast to our findings, that study found no association of n-3 PUFAs with childhood type 1 diabetes. However, the study included only 45 participants and was restricted to children with high genetic risk.

Our results suggest that fish intake or n-3 PUFAs may delay the progression from islet autoimmunity to onset of diabetes, especially among those with more pronounced autoimmunity as indicated by high levels of GAD65 antibodies. The n-3 PUFAs may also have protective effects on the initiation of islet autoimmunity as suggested by previous studies (9,30), including a Finnish study that reported an inverse association between serum DHA at 6 months of age and insulin autoantibodies (30), while other studies found no support for an association with islet autoimmunity (11). In our study, GAD65 antibodies and n-3 PUFAs were measured in blood samples taken at the same time point. For that reason, n-3 PUFAs may not reflect exposure linked to the process of seroconversion, which may take place several years before the onset of diabetes (31). Hence, we could not study a potential effect of n-3 PUFAs on the initiation of islet autoimmunity.

Hypothetically, n-3 PUFAs may reduce diabetes risk by moderating the effects of an underlying autoimmune process. Autoimmune diabetes and several other autoimmune diseases have been described as having inflammatory components (7,32). EPA and DHA have been described to exert anti-inflammatory effects through a wide range of mechanisms, including disruption of lipid rafts important for signaling in T cells and other immune-related cells, and to inhibit activation of the transcription factor nuclear factor-κB (NF-κB), leading to decreased production of proinflammatory cytokines (7). A protective effect of n-3 PUFAs on pancreatic β-cell destruction has been shown in an animal model of type 1 diabetes, with effects attributed to decreased NF-κB expression and absent cytokine production (33). Cytokines and other inflammatory mediators may also have a role in the development of peripheral insulin resistance (32), which is part of the pathophysiology of diabetes, both with and without an autoimmune component (34). However, a meta-analysis of 26 randomized controlled trials did not find an overall effect of n-3 PUFAs on measures of insulin resistance (35). In the current study, we did not have information on either islet cell–specific T-cell reactivity, NF-κB, and cytokines or insulin resistance. However, we have previously reported the absence of an association between total dietary fish intake and plasma phospholipid n-3 PUFA levels and incident diabetes overall (19,36), which argues against an n-3 PUFA–mediated effect on insulin sensitivity as an explanation for our findings. In the current study, we observed a positive association between plasma phospholipid EPA and incidence of diabetes. However, a meta-analysis did not find such an association

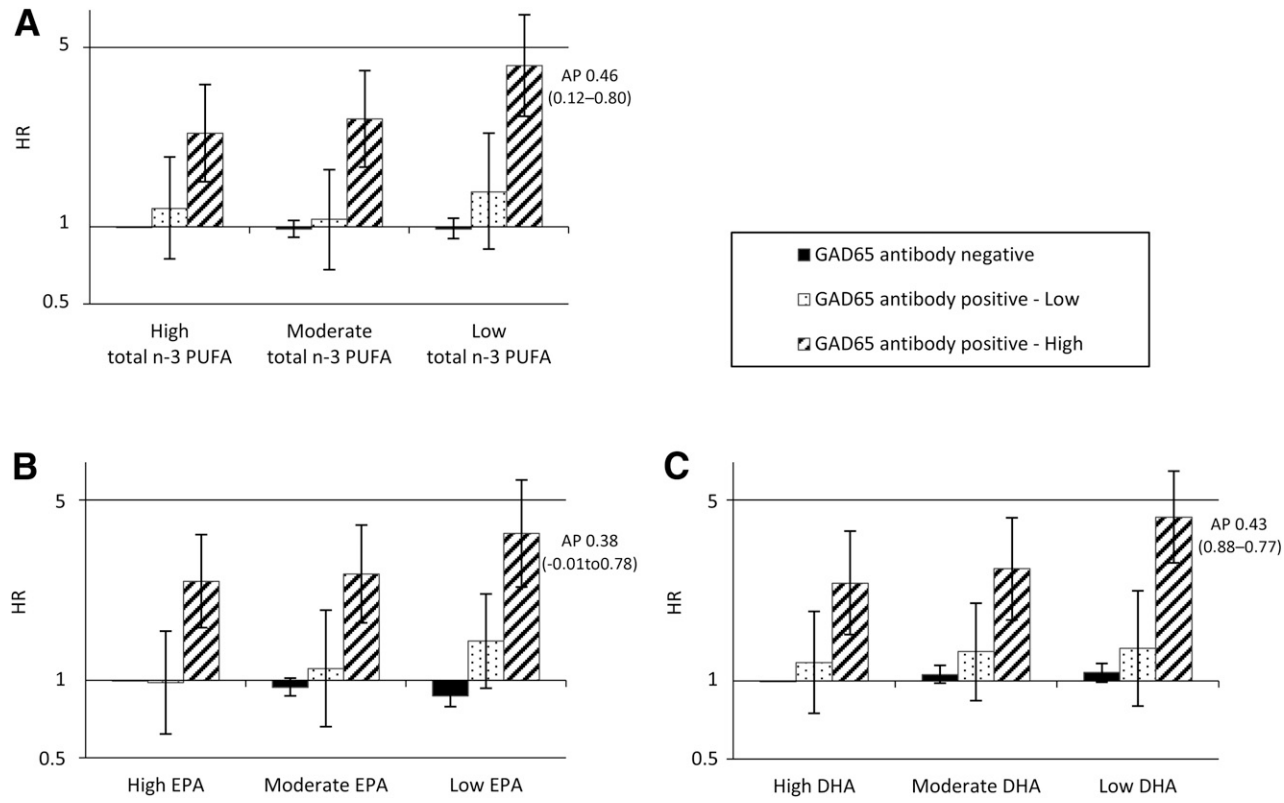

Figure 1—Adjusted HR (95% CI) of incident diabetes for combinations of GAD65 antibody status and tertiles of relative plasma phospholipid total n-3 PUFAs (*A*), EPA (*B*), and DHA (*C*). The reference group is the combination of GAD65 antibody negativity and the highest fatty acid tertile. The EPIC-InterAct Study analyses were adjusted for age (underlying time scale), center, sex, education level, smoking status, physical activity, BMI, total energy intake, and intake of alcohol, fruits, and vegetables.

(19), so this finding should be interpreted with caution.

Strengths of this study are the large sample size and the geographical and cultural diversity of the populations involved. These enabled us to analyze a larger number of GAD65 antibody–positive adults in relation to plasma phospholipid n-3 PUFA levels and dietary fish intake. The observed association is unlikely to be due to the presence of participants with undiagnosed diabetes at baseline because the results were unchanged in sensitivity analyses that excluded participants with high HbA_{1c} levels at baseline and individuals diagnosed with diabetes within the first 2 years of follow-up. Dietary self-report has measurement error, but this is unlikely to be differential with respect to autoantibodies and future diabetes. Thus, this would most likely lead to dilution of the association between dietary fish intake and diabetes, including in the interaction analysis. The use of plasma phospholipid fatty acid measurements in this study adds to the confidence in the results because they are an independent objective marker of fish intake. All determination of GAD65 antibody levels were undertaken in the same laboratory in Seattle by the same technician using the same method (16). Similarly, all plasma phospholipid fatty acid measurements were conducted in one laboratory in Cambridge, which minimizes systematic measurement variation.

There are also limitations to the study. Blood samples were taken at baseline, often several years before diabetes diagnosis. Other individuals, in both the case and the noncase groups, may have developed GAD65 antibodies during follow-up. Moreover, some GAD65-positive subcohort participants were probably diagnosed with type 1 diabetes or even LADA during follow-up, which we could not account for. However, given the probably very low incidence of newly developed autoantibody positivity or autoimmune diabetes during follow-up, this is unlikely to have any impact on the observed associations. GAD65 antibodies were the only type of antibody measured, and thus, it is possible that some individuals were positive for other antibodies. It may be that the level of GAD65 antibodies reflects different stages in the progression toward disease rather than level of autoimmunity, since studies on type 1 diabetes have reported decreasing antibody titers before onset of overt diabetes (37). Still, we noted that the mean follow-up time to diagnosis was similar in the high- versus low-titer GAD65 antibody group. There was a low to moderate correlation between self-reported fish intake and plasma phospholipid n-3 PUFAs. The relative n-3 PUFA concentrations in plasma phospholipids reflect dietary intake over the past few days (38) to weeks (39), whereas the food frequency questionnaires were aimed at assessing habitual food intake over the past year. Despite our attempts to take other important dietary, health, and lifestyle factors into consideration in our analyses, we cannot rule out unmeasured confounding or residual confounding from inadequately measured factors related to the dietary fish intake or plasma phospholipid n-3 PUFA status. We also cannot rule out the possibility of chance findings arising from multiple

testing. It is likely that a large proportion of the GAD65 antibody–positive participants with diabetes could be classified as having LADA (14). However, we were unable to differentiate cases of type 2 diabetes and LADA since GAD65 antibody status at the time of diagnosis was unknown. Notably, GAD65 antibody positivity was associated with HLA genotypes linked to autoimmune diabetes, including LADA (40), in the cohort (3).

In conclusion, our results suggest that dietary fish intake and plasma phospholipid n-3 PUFAs may modify the association between GAD65 antibody positivity and adult-onset diabetes and that the excess risk of diabetes conferred by GAD65 antibody positivity is amplified in individuals with low n-3 PUFA status. This suggests that intake of dietary fish, especially fatty fish, may prevent or prolong diabetes onset in GAD65 antibody–positive individuals. Whether our results can be explained by a causal effect of n-3 PUFAs remains to be established. Nevertheless, this study contributes to the limited body of knowledge about factors that influence the progression from islet autoimmunity to diabetes in adults and may aid in the identification of modifiable factors that could contribute to the prevention or delayed clinical manifestation of autoimmune diabetes.

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Author Contributions. J.E.L. performed the statistical analyses and drafted the manuscript. J.E.L., S.C., and O.R. conceptualized the research objectives and analysis design. N.G.F., S.J.S., and N.J.W. coordinated the InterAct project, with N.J.W. as chief investigator. C.S.H., O.R., and N.J.W. coordinated and initiated the GAD65 antibody measurements, with C.S.H. leading the laboratory undertaking these analyses. A.K. led the laboratory that undertook the phospholipid fatty acid measurements. All authors contributed to the interpretation of data, critical review and revision of the manuscript, and approval of the final version of the manuscript. N.J.W. is the guarantor of this work and, as such, had full access to all the data and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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