Serum Omega-3 Polyunsaturated Fatty Acids and Risk of Incident Type 2 Diabetes in Men: The Kuopio Ischaemic Heart Disease Risk Factor Study

Short title: Serum omega-3 PUFA and risk of type 2 diabetes

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ABSTRACT

OBJECTIVE—The relationship between fish or omega-3 polyunsaturated fatty acids (PUFA) and type 2 diabetes is inconclusive. Even contaminants in fish, such as mercury, may modify the effects. We investigated the associations between serum omega-3 PUFAs eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), docosahexaenoic acid (DHA) and alpha-linolenic acid (ALA), hair mercury and risk of incident type 2 diabetes in middle-aged and older Finnish men.

RESEARCH DESIGN AND METHODS—A total of 2212 men from the prospective, population-based Kuopio Ischaemic Heart Disease Risk Factor study, aged 42-60 years and free of type 2 diabetes at baseline in 1984-1989, were investigated. Serum PUFA and hair mercury were used as biomarkers for exposure. Dietary intakes were assessed with 4-day food recording. Type 2 diabetes was assessed by self-administered questionnaires, fasting and 2-h oral glucose tolerance test blood glucose measurement at re-examination rounds 4, 11 and 20 years after the baseline, and by record linkage to hospital discharge registry and reimbursement register on diabetes medication expenses. Cox proportional hazards models were used to analyze associations.

RESULTS—During the average follow-up of 19.3 years, 422 men developed type 2 diabetes. Men in the highest vs. the lowest serum EPA+DPA+DHA quartile had 33% lower multivariate-adjusted risk for type 2 diabetes (95% CI 13-49%, *P*-trend 0.01). No statistically significant associations were observed with serum or dietary ALA, dietary fish or EPA+DHA, or hair mercury.

CONCLUSIONS—Serum long-chain omega-3 PUFA concentration, an objective biomarker for fish intake, was associated with long-term lower risk of type 2 diabetes.

Diet and other lifestyle factors have a major role in the development of type 2 diabetes (1). Among dietary factors, the long-chain omega-3 polyunsaturated fatty acids (PUFA) eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) from fish and other seafood have gained special interest, because of their beneficial association with the risk of cardiovascular diseases and several risk factors for diabetes, including inflammation, adiposity, hypertension and dyslipidemia (2, 3). In animal models, the long-chain omega-3 PUFA have also been shown to decrease insulin resistance (4), but the results from randomized controlled trials in humans have generally found little benefits on glucose-insulin homeostasis (5, 6). In prospective studies, the association between fish or EPA+DHA intake and risk of incident type 2 diabetes has been mixed (7-10); in studies in the USA the association has even been positive (7, 8). The random error inherited in dietary assessment methods can attenuate associations in dietary studies and thus could explain the null findings. Only a few studies have used an objective biomarker, circulating long-chain omega-3 PUFA concentration, as the exposure (7). However, none of these studies found an association with incident type 2 diabetes, either (7). Interestingly, these studies did not find an increased risk with higher concentrations in the studies from the USA, suggesting that the higher risk could at least partly be related to the dietary assessment method (7).

Previously in a subsample of 895 men from the Kuopio Ischaemic Heart Disease Risk Factor (KIHD) Study, serum linoleic acid, an n-6 PUFA, was associated with lower risk of the combined endpoint of type 2 diabetes and impaired fasting glycemia and lower risk of adverse changes in insulin and glucose concentrations during 4-year follow-up, but no associations were found with other PUFAs (11). To better elucidate the role of the long-chain omega-3 PUFA on the risk of type 2 diabetes in this study population, we investigated the association of the long-chain omega-3 PUFA with the risk of incident type 2 diabetes during the mean follow-up of over 19 years in 2212 middle-aged and older men from KIHD. We also investigated the association with the intermediate-chain length omega-3 PUFA alpha-linolenic acid (ALA, 18:3n-3), an essential fatty acid that is derived from plant sources in the diet and which can also be elongated to longer-chain omega-3 PUFAs in humans. ALA has been found to improve insulin sensitivity in animal models (12), and in some (13, 14), but not all (15) short-term randomized trials ALA or flaxseed oil, a rich source of ALA, has moderately improved fasting plasma glucose and markers of insulin resistance in humans. In a meta-analysis of prospective studies, dietary or circulating ALA was found to have a non-significant trend towards moderately lower risk of type 2 diabetes (7).

The previous null findings with fish and long-chain omega-3 PUFA could also be related to the environmental contaminants in fish, such as methylmercury, which has been associated with insulin resistance and with higher risk of type 2 diabetes (16, 17). Previously in KIHD, methylmercury exposure was associated with higher risk of cardiovascular diseases and it also attenuated the benefits of the serum long-chain omega-3 PUFAs on the risk (18). Therefore, we also investigated the impact of methylmercury exposure, assessed by hair mercury concentration, on the risk of type 2 diabetes.

RESEARCH DESIGN AND METHODS

Study population

The KIHD study was designed to investigate risk factors for CVD, atherosclerosis, and related outcomes in a population-based, randomly selected sample of men from eastern Finland (19). The baseline examinations were carried out in 1984-1989. A total of 2682 men who were 42, 48, 54 or 60 years old at baseline (82.9% of those eligible) were recruited in two cohorts. The first cohort consisted of 1166 men who were 54 years old, enrolled in 1984-1986, and the second cohort included 1516 men who were 42, 48, 54 or 60 years old, enrolled in 1986-1989. The baseline examinations were followed by the 4-year examination round (1991-1993) in which 1038 men from the second cohort (88% of the eligible) participated. At the 11-year examination round (1998-2001), all men from the second cohort were invited and 854 men (95% of the eligible) participated. During the 20-year examination round, all eligible participants from the first and second cohorts were invited to the study site. A total of 1241 men (80% of the eligible) participated. The baseline characteristics of the entire study population have been described (19). The KIHD study protocol was approved by the Research Ethics Committee of the University of Kuopio. All subjects gave written informed consent for participation.

Subjects with type 2 diabetes (n=167), impaired fasting glucose (n=127) or unknown diabetes status (n = 38) at baseline, or with missing data on serum fatty acids (138) were excluded, leaving 2212 men. Dietary intakes were available for 2194 participants. For the analyses with hair mercury, complete data was available for 1977 men.

Measurements

Fasting venous blood samples and hair samples were collected between 8AM and 10AM at the baseline examinations. Subjects were instructed to abstain from ingesting alcohol for three days and from smoking and eating for 12 hours prior to giving the sample. Detailed descriptions of the determination of serum lipids and lipoproteins (20), assessment of medical history and medications (20), family history of diseases (20), smoking (20), alcohol consumption (20), blood pressure (20), and physical activity (21) have been published. Plasma glucose was measured using a glucose dehydrogenase method after precipitation of proteins by trichloroacetic acid. The serum samples for insulin determination were stored frozen at –80°C. Serum insulin was determined with a Novo Biolabs radioimmunoassay kit (Novo Nordisk, Bagsvaerd, Denmark). Homeostasis model of assessment (HOMA) was calculated using the HOMA2 calculator (www.dtu.ox.ac.uk). Education was assessed in years by using self-administered questionnaire. Annual income was obtained from a self-administered questionnaire. The family history of diabetes was defined as positive, if a first degree relative of the subject had diabetes history. Mercury in hair was determined by flow injection analysis-cold vapor atomic absorption spectrometry and amalgamation (22).

Measurement of serum fatty acids

Serum esterified and nonesterified fatty acids were determined in one gas chromatographic run without preseparation (11). Serum fatty acids were extracted with chloroform-methanol. Chloroform phase was evaporated and treated with sodium methoxide, which methylated esterified fatty acids. Quantification was carried out with reference standards purchased from Nu-Check Prep Inc. (MN, USA). Each analyte had individual reference standard and recovery of analytes was confirmed with an internal standard eicosan (arachidic acid $C_{20}H_{40}O_2$). Fatty acids were chromatographed in an NB-351 capillary column (HNU-Nordion, Helsinki, Finland) by a Hewlett-Packard 5890 Series II gas chromatograph (Hewlett-Packard Company, Avondale, Pa, USA, since 1999 Agilent Technologies Inc., USA) with a flame ionization detector. Results were obtained in μmol/L. The coefficient of variation (CV) for repeated measurements of major esterified fatty acids was ~5%. Because the relative degree of saturation of fatty acids varies among esterified fatty acid types, the esterified fatty acid concentrations were adjusted for serum LDL and HDL cholesterol and triglyceride concentrations. The CV for major nonesterified fatty acids was ~15%. No adjustment was conducted for nonesterified fatty acids.

Assessment of dietary intakes

The consumption of foods at the study baseline was assessed with an instructed 4-day food recording by household measures (23). The instructions were given and the completed food records were checked by a nutritionist. The intakes of nutrients were estimated using the NUTRICA® version 2.5 software (Social Insurance Institution, Helsinki, Finland). The databank of the software is mainly based on Finnish values of nutrient composition of foods. The nutrients were adjusted for energy intake using the residual method (24). We did not have information on docosapentaenoic acid (DPA, 22:5n-3) intake.

Diagnostic criteria for type 2 diabetes

Type 2 diabetes was defined as a self-reported physician-set diagnosis of type 2 diabetes and/or fasting plasma glucose ≥7.0 mmol/L or 2-hour oral glucose tolerance test (OGTT) plasma glucose ≥11.1 mmol/L at re-examination rounds 4, 11 and 20 years after the baseline, and by record linkage to the national hospital discharge registry, and to the Social Insurance Institution of Finland register for reimbursement of medicine expenses used for type 2 diabetes for the entire study period until the end of the follow-up in Dec 31, 2010. Impaired fasting glucose at baseline was defined using WHO criteria, fasting plasma glucose 6.1-6.9 mmol/L. OGTT was not done at the study baseline.

Statistical analysis

The univariate relationships between serum EPA+DPA+DHA and ALA and baseline characteristics were assessed by means and linear regression (for continuous variables) or χ^2-tests (for categorical variables). Cox proportional hazards regression models were used to estimate hazard ratios (HR) in quartiles of fatty acids. The validity of the proportional hazards assumption was evaluated by using Schoenfeld residuals. The multivariable model (Model 2) was adjusted for potential confounders, including age (years), examination year, body mass index (kg/m^2), family history of type 2 diabetes (yes/no), smoking (never smoker, previous smoker, current smoker <20 cigarettes/day and current smoker ≥20 cigarettes/day), education years, leisure-time physical activity (kcal/week), intake of alcohol (g/day), and serum linoleic acid (%). Cohort mean was used to replace missing values in covariates (<0.5%). Statistical significance of the interactions on a multiplicative scale was assessed by likelihood ratio tests using a cross-product term. Tests of linear trend were conducted by assigning the median values for each category of exposure variable and treating those as a single continuous variable. All *P*-values were 2-tailed (α=0.05). Data were analyzed using SPSS 19.0 for Windows (SPSS Inc., Chicago, IL).

RESULTS—At baseline, men with higher serum long-chain omega-3 PUFA concentration had a higher BMI, income, education, leisure-time physical activity, serum LDL cholesterol, and hair mercury concentration and higher intakes of fruits, berries and vegetables, EPA+DHA and alcohol, and lower serum triglyceride, insulin and linoleic acid concentrations, lower HOMA-IR, and lower energy, fiber and saturated fatty acid intakes (Table 1). Men with higher serum ALA concentration were younger, had a lower BMI, waist-to-hip ratio, hair mercury, and lower intakes of EPA+DHA, saturated fatty acids and alcohol, and higher physical activity, income, education, serum triglyceride and linoleic acid concentrations, and higher fiber, fruit, berry and vegetable intake and ALA intake. They were also less likely to be smokers and have coronary heart disease at baseline.

Serum long-chain omega-3 PUFAs, except for DPA, had a fairly strong correlation with EPA+DHA intake (Table 2). The correlations between the long-chain omega-3 PUFAs and serum or dietary ALA were weak and generally inverse. Serum and dietary ALA showed a moderate intercorrelation (Table 2).

During the average follow-up of 19.3 years (SD 6.5 years), 422 men (19.2%) developed type 2 diabetes. In the multivariate-adjusted models (Model 2 in Table 3), men in the highest quartile of serum long-chain omega-3 PUFA had 33% lower risk (95% CI 13-49%) for incident type 2 diabetes, compared to the men in the lowest quartile. Among individual fatty acids, DHA and DPA had similar inverse associations with the risk, whereas the association with EPA was weaker and nonsignificant (Table 3). Serum ALA was associated with lower risk of type 2 diabetes after adjusting for age and examination year (Model 1 in Table 3), but further adjustments attenuated the association (Model 2).

In the secondary analyses, dietary intakes of fish, EPA+DHA or ALA were not associated with the risk of type 2 diabetes (Table 4), although after multivariate adjustments the direction of the association with fish and EPA+DHA intakes was similar to the findings with serum long-chain omega-3 PUFA.

Hair mercury was not associated with the risk of type 2 diabetes (Table 4). Further adjusting the Model 2 for serum long-chain omega-3 PUFA concentration further attenuated the association (HR in the highest quartile 1.00, 95% CI 0.72-1.38; *P* for trend 0.57). Hair mercury did not modify the associations of the serum or dietary long-chain omega-3 PUFAs or fish intake with the risk of type 2 diabetes, either (*P* for interactions >0.40).

In the sensitivity analyses we excluded from the analyses the type 2 diabetes events that occurred during the first two years of follow-up. Only three men had a diagnosis for type 2 diabetes during that time period and excluding them had no effect on the associations (data not shown).

DISCUSSION—In this prospective, population-based cohort study among middle-aged and older men, serum long-chain omega-3 PUFA concentration, an objective biomarker of fish and omega-3 fatty acid intake during the previous weeks (25), was associated with a lower risk of incident type 2 diabetes. In contrast, dietary fish or EPA+DHA intakes, assessed with 4-day food recording, were not associated with the risk. After adjustments, serum or dietary ALA, or hair mercury were not associated with the risk of type 2 diabetes, either.

In the recent meta-analyses of prospective studies, intakes of fish or EPA+DHA were not found to be associated with lower risk of incident type 2 diabetes (7-10). However, there was significant heterogeneity across the study results based on the region of the study population. No association was found in the studies from Europe, whereas in the studies from Asia/Australia fish or EPA+DHA intakes were associated with lower risk of type 2 diabetes and in the studies from the USA with higher risk (7-10). These geographical differences in the risk may reflect, for example, genetic differences, gene-diet interactions or differences in the type of fish consumed (fatty fish vs. lean fish) and in fish preparation methods (raw/steamed/boiled vs. deep-fried). However, the number of studies in other regions than USA is limited, which reduces the generalizability of the findings in non-US populations. Interestingly, in the US studies where an objective biomarker was used, circulating long-chain omega-3 PUFA concentrations were not found to be associated with higher risk, suggesting that the increased risk observed with dietary intakes may be related to the dietary assessment method (7). We also found the association with type 2 diabetes to differ whether we used dietary intakes or serum concentrations as the exposure. In our study, however, we observed a significant inverse association with serum long-chain omega-3 PUFA concentrations, whereas there were no statistically significant associations with dietary EPA+DHA or fish intakes, although the direction of the associations was similar compared to the serum concentrations. This most likely reflects the inability of the 4-day food recording to accurately assess intakes of foods that are usually consumed at most 1-2 times per week, such as fish. This would cause exposure misclassification and bias associations towards the null, thus making it more difficult to find true associations between dietary intakes and risk of type 2 diabetes. This kind of bias does not affect serum measurements, however.

The long-chain omega-3 PUFA could have beneficial effects on glucose homeostasis and type 2 diabetes due to the impact on adiposity, hypertension and dyslipidemia that are risk factors for diabetes (2, 3). They can also potentially inhibit inflammation and suppress gene expression related to lipid metabolism (26-29). However, experimental studies with fish or fish oil supplements have generally found no benefits on glucose metabolism (5), although there is some evidence for increased insulin sensitivity (5) and improved insulin secretion and glucose disposal (30). Higher serum long-chain omega-3 PUFA concentration could also reflect higher intake of fish in place of red meat and lower intake of saturated fatty acids. Especially processed red meat consumption has been associated with modestly higher risk of type 2 diabetes (31), and saturated fatty acids have been associated with impaired insulin sensitivity (32). However, in our study there were no differences in meat consumption in the long-chain omega-3 PUFA quartiles (Table 1), and although higher serum long-chain omega-3 PUFA concentration was associated with lower saturated fatty acid intake (Table 1), adjustment for saturated fatty acid intake did not change the associations (data not shown).

In the recent meta-analysis of prospective studies, dietary and circulating ALA showed a non-significant trend towards lower risk of type 2 diabetes, with low heterogeneity between studies (7). Although there was a non-significantly lower risk of type 2 diabetes with higher serum ALA concentrations also in our study, we did not find associations with dietary ALA. However, circulating ALA concentration may not be a good biomarker for typical dietary intakes, because a large proportion of ALA is oxidized or, in limited amounts, converted to longer-chain omega-3 PUFAs (33). Therefore, considering the associations with both dietary and circulating ALA, our results do not suggest a significant role for ALA in the prevention of type 2 diabetes in this study population.

Mercury exposure has been shown to cause pancreatic islet β-cell dysfunction in experimental models (34), which could lead to development of diabetes. Because fish is a major source of mercury in humans, the previous null findings or the observed higher risk of type 2 diabetes with higher fish consumption in some studies (7, 8) could potentially relate to mercury exposure. However, none of the studies have controlled for mercury exposure. Only little data exists about the relationship between mercury exposure and diabetes risk in humans. Patients with diabetes or metabolic syndrome have been found to have higher hair mercury levels than healthy controls (35-37), and blood mercury was found to associate with insulin resistance (16). Recently, higher toenail mercury levels were associated with higher risk of type 2 diabetes in a prospective study of American young adults, aged 20-32 at baseline (17). Our findings do not support these results, despite much higher average mercury levels. In that study the median toenail mercury in the highest quintile was 0.607 μg/g, whereas in our study the median hair concentration in the highest quartile was 4.2 μg/g, corresponding to approximately 1.6 μg/g in toenails. Mercury levels in both toenails and hair are considered as indicators for long-term mercury exposure (38). More research is clearly needed for elucidating the role of mercury exposure in type 2 diabetes.

A major strength of the study is the use of objective biomarkers, serum fatty acids and hair mercury, as exposure. Other strengths include the population-based recruitment, prospectively collected data, extensive examinations for potential confounders, long follow-up with a large number of events, and no loss to follow-up. Potential limitations include the single exposure measurement, which may cause random error due to misclassification and therefore underestimate the true associations. Because serum long-chain omega-3 PUFA concentration was associated with generally healthier lifestyle and biochemical characteristics (Table 1), the impact of residual confounding cannot be completely excluded. However, serum long-chain omega-3 PUFA concentration is not uniformly associated with a healthier lifestyle in this study population; for example, men with higher serum long-chain omega-3 PUFA had a higher body mass index, higher alcohol intake and lower fiber intake (Table 1). Our study population included only middle-aged and older men, so the findings may not be generalizable to other age groups or to women. Besides mercury, we did not have information on other environmental contaminants in fish, such as persistent organic pollutants (POPs), which have been associated with insulin resistance and higher risk of type 2 diabetes (39, 40). Of special interest would be to investigate the potential joint effects of simultaneous mercury and POP exposures, of which very little is currently known (16).

In summary, our results from this prospective, population-based cohort study with a long follow-up suggest that the serum long-chain omega-3 PUFA concentration, an objective biomarker for fish consumption, is associated with lower risk of incident type 2 diabetes in middle-aged and older men from Eastern Finland. In contrast, ALA, the plant-based intermediate-chain length omega-3 PUFA, or mercury, were not associated with the risk. Further research from diverse study populations and with objective biomarkers of exposure is needed to elucidate the role of the omega-3 PUFAs on the risk of type 2 diabetes.

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Table 1—Baseline characteristics (1984-1989) according to the serum omega-3 polyunsaturated fatty acids: The Kuopio Ischaemic Heart Disease Risk Factor Study (n = 2,212)

	EPA+DPA+DHA quartile (%)			ALA quartile (%)		
	Lowest (<3.62)	Highest (>5.33)	*P* for trend	Lowest (<0.57)	Highest (>0.87)	*P* for trend
Number of subjects	553	553		553	553	
Age (years)	52.9 ± 5.4	53.1 ± 5.1	0.24	54.0 ± 3.9	52.2 ± 6.0	<0.001
Body mass index (kg/m^2)	26.4 ± 3.4	26.9 ± 3.4	0.01	27.1 ± 3.7	26.2 ± 3.0	<0.001
Waist-to-hip ratio	0.95 ± 0.07	0.95 ± 0.06	0.47	0.96 ± 0.06	0.94 ± 0.06	<0.001
Leisure-time physical activity (kcal/day)	133 ± 170	155 ± 195	0.05	116 ± 139	139 ± 152	0.06
Income (euro)	12490 ± 7390	14580 ± 9950	<0.001	11600 ± 8940	14860 ± 8660	<0.001
Education (years)	8.6 ± 3.2	9.1 ± 3.7	0.01	7.9 ± 3.0	9.3 ± 3.9	<0.001
Serum linoleic acid (%)	27.3 ± 5.3	25.9 ± 4.2	<0.001	25.6 ± 5.1	27.6 ± 4.1	<0.001
Hair mercury (µg/g)	1.29 ± 1.46	2.76 ± 2.36	<0.001	2.33 ± 2.17	1.73 ± 1.96	<0.001
Serum LDL cholesterol (mmol/L)	3.83 ± 0.97	4.17 ± 1.02	<0.001	4.01 ± 1.03	3.97 ± 0.97	0.34
Serum triglycerides (mmol/L)	1.55 ± 0.95	1.06 ± 0.52	<0.001	1.04 ± 0.53	1.55 ± 0.94	<0.001

Blood glucose (mmol/L)	4.52 ± 0.40	4.55 ± 0.40	0.07	4.52 ± 0.41	4.51 ± 0.37	0.55
Serum insulin (mU/L)	11.94 ± 8.29	10.46 ± 4.83	0.001	11.05 ± 1.03	10.83 ± 5.80	0.48
HOMA2-IR	1.49 ± 0.83	1.35 ± 0.61	0.01	1.40 ± 0.75	1.39 ± 0.64	0.55
Dietary intakes						
Energy (kcal/day)	2410 ± 676	2296 ± 605	<0.001	2372 ± 685	2370 ± 563	0.65
Meat and meat products (g/day)	157 ± 79	153 ± 78	0.27	158 ± 83	156 ± 77	0.60
Fruits, berries and vegetables (g/day)	243 ± 151	269 ± 165	0.01	218 ± 142	275 ± 154	<0.001
Saturated fatty acids (% of energy)	18.2 (4.7)	17.3 (4.0)	<0.001	18.8 (4.5)	17.4 (4.3)	<0.001
Fiber (g/day)	26.5 (10.1)	24.3 (8.5)	<0.001	23.6 (8.4)	26.2 (8.7)	<0.001
EPA+DHA (g/day)	0.11 ± 0.20	0.59 ± 0.60	<0.001	0.35 ± 0.43	0.28 ± 0.38	0.01
ALA (g/day)	1.52 ± 0.64	1.48 ± 0.70	0.21	1.21 ± 0.56	1.76 ± 0.66	<0.001
Alcohol intake (g/week)	55.7 ± 93.1	80.5 ± 102.2	0.001	88.0 ± 128.2	56.9 ± 100.8	<0.001
Current smoker (%)	34	29	0.09	35	28	0.003
Coronary heart disease (%)	25	22	0.53	28	19	<0.001
Family history of type 2 diabetes (%)	26	28	0.36	25	29	0.07

Values are means (SD) or percentages.

EPA = eicosapentaenoic acid; DPA = docosapentaenoic acid; DHA = docosahexaenoic acid; ALA=alpha-linolenic acid; HOMA=Homeostasis model assessment.

Table 2—Mean values of serum and dietary omega-3 polyunsaturated fatty acids and Spearman correlation coefficients

	Serum EPA+DPA+DHA (%)	Serum EPA (%)	Serum DPA (%)	Serum DHA (%)	Dietary EPA+DHA (g/day)	Serum ALA (%)	Dietary ALA (g/day)
Mean (SD)	4.68 (1.59)	1.67 (0.90)	0.55 (0.10)	2.45 (0.73)	0.32 (0.42)	0.74 (0.24)	1.49 (0.66)
Correlations							
Serum EPA+DPA+DHA	1	0.92*	0.62*	0.91*	0.52*	-0.14*	-0.04*
Serum EPA		1	0.56*	0.69*	0.47*	-0.18*	-0.11*
Serum DPA			1	0.70*	0.22*	0.01	0.02
Serum DHA				1	0.49*	-0.09*	0.03
Dietary EPA+DHA					1	-0.08*	0.05*
Serum ALA						1	0.35*
Dietary ALA							1

*P value<0.05

EPA = eicosapentaenoic acid; DPA = docosapentaenoic acid; DHA = docosahexaenoic acid; ALA=alpha-linolenic acid.

Table 3—Risk of incident type 2 diabetes in quartiles of serum omega-3 polyunsaturated fatty acids

	Serum fatty acid quartile				*P* for trend
	1 (n=553)	2 (n=553)	3 (n=553)	4 (n=553)	
EPA+DPA+DHA (%)	<3.62	3.62-4.34	4.35-5.33	>5.33	
Incidence rate/1000 PY	11.7	8.7	9.9	9.4	
Model 1*	1	0.71 (0.54-0.93)	0.84 (0.64-1.09)	0.78 (0.60-1.01)	0.20
Model 2	1	0.71 (0.54-0.93)	0.78 (0.60-1.02)	0.67 (0.51-0.87)	0.01
EPA (%)	<1.10	1.10-1.47	1.48-1.97	>1.97	
Incidence rate/1000 PY	9.6	10.5	9.9	9.6	
Model 1	1	1.10 (0.84-1.44)	1.05 (0.80-1.39)	1.05 (0.79-1.38)	0.91
Model 2	1	1.07 (0.81-1.40)	0.96 (0.73-1.27)	0.85 (0.64-1.13)	0.15
DPA (%)	<0.48	0.48-0.54	0.55-0.61	>0.61	
Incidence rate/1000 PY	12.9	9.0	9.0	9.0	
Model 1	1	0.67 (0.51-0.87)	0.65 (0.50-0.85)	0.65 (0.50-0.84)	0.002
Model 2	1	0.77 (0.59-1.01)	0.73 (0.56-0.96)	0.72 (0.55-0.94)	0.02
DHA (%)	<1.95	1.95-2.34	2.35-2.84	>2.84	

Incidence rate/1000 PY	11.6	9.8	9.0	9.3	
Model 1	1	0.84 (0.65-1.10)	0.76 (0.58-1.00)	0.77 (0.59-1.01)	0.05
Model 2	1	0.81 (0.62-1.06)	0.71 (0.54-0.94)	0.66 (0.51-0.87)	0.003
ALA (%)	<0.57	0.57-0.70	0.71-0.87	>0.87	
Incidence rate/1000 PY	11.6	9.6	9.5	9.0	
Model 1	1	0.80 (0.62-1.05)	0.77 (0.59-1.01)	0.70 (0.53-0.93)	0.02
Model 2	1	0.87 (0.67-1.14)	0.88 (0.67-1.17)	0.82 (0.62-1.10)	0.22

*Values are hazard ratio (95% confidence interval).

EPA=eicosapentaenoic acid; DPA=docosapentaenoic acid; DHA=docosahexaenoic acid; PY=person-years; ALA=alpha-linolenic acid.

Model 1 is adjusted for age and examination year.

Model 2 is adjusted for Model 1 and body mass index (kg/m2), family history of type 2 diabetes (yes/no), smoking (never smoker, previous smoker, current smoker <20 cigarettes/day and current smoker ≥20 cigarettes/day), education years, leisure-time physical activity (kcal/week), intake of alcohol (g/day), and serum linoleic acid (%).

Table 4—Risk of incident type 2 diabetes in quartiles of fish intake, energy-adjusted omega-3 polyunsaturated fatty acid intakes and hair mercury

	Exposure Quartile				*P* for trend
	1 (n=548)	2 (n=549)	3 (n=549)	4 (n=548)	
Fish (g/day)	<5	5-35	36-75	>75	
Incidence rate/1000 PY	9.7	9.9	10.1	10.1	
Model 1*	1	1.03 (0.78-1.34)	1.03 (0.79-1.35)	1.07 (0.82-1.41)	0.62
Model 2	1	0.98 (0.75-1.29)	0.97 (0.74-1.27)	0.89 (0.68-1.18)	0.40
EPA+DHA (g/day)	<0.05	0.05-0.19	0.20-0.43	>0.43	
Incidence rate/1000 PY	10.2	9.0	10.6	10.2	
Model 1	1	0.90 (0.68-1.19)	1.06 (0.81-1.38)	1.05 (0.80-1.37)	0.49
Model 2	1	0.80 (0.61-1.06)	0.91 (0.70-1.19)	0.85 (0.65-1.12)	0.54
ALA (g/day)	<1.02	1.02-1.41	1.42-1.83	>1.83	
Incidence rate/1000 PY	10.4	9.7	10.5	9.4	
Model 1	1	0.86 (0.65-1.13)	0.94 (0.72-1.23)	0.82 (0.62-1.08)	0.23
Model 2	1	0.91 (0.69-1.20)	1.09 (0.82-1.45)	1.06 (0.79-1.43)	0.47

Hair mercury (µg/g)[†]	<0.7	0.7-1.3	1.4-2.7	>2.7	
Incidence rate/1000 PY	9.0	10.8	9.6	9.4	
Model 1	1	1.23 (0.93-1.63)	1.11 (0.82-1.49)	1.11 (0.82-1.50)	0.82
Model 2	1	1.19 (0.90-1.59)	0.95 (0.70-1.28)	0.91 (0.67-1.24)	0.25

*Values are hazard ratio (95% confidence interval).

[†]The number of participants in quartiles of hair mercury is 494, 492, 496 and 495.

EPA=eicosapentaenoic acid; DHA=docosahexaenoic acid; PY=person-years; ALA=alpha-linolenic acid.

Model 1 is adjusted for age and examination year.

Model 2 is adjusted for Model 1 and body mass index (kg/m2), family history of type 2 diabetes (yes/no), smoking (never smoker, previous smoker, current smoker <20 cigarettes/day and current smoker ≥20 cigarettes/day), education years, leisure-time physical activity (kcal/week), intake of alcohol (g/day), and serum linoleic acid (%).