



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

Jyrki K. Virtanen,¹ Jaakko Mursu,¹
Sari Voutilainen,¹ Matti Uusitupa,^{1,2}
and Tomi-Pekka Tuomainen¹

OBJECTIVE

The relationship between fish or omega-3 polyunsaturated fatty acids (PUFAs) 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), α -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 2,212 men from the prospective, population-based Kuopio Ischemic 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 and 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 versus 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.

Diabetes Care 2014;37:189–196 | DOI: 10.2337/dc13-1504

¹University of Eastern Finland, Institute of Public Health and Clinical Nutrition, Kuopio, Finland

²Research Unit, Kuopio University Hospital, Kuopio, Finland

Corresponding author: Jyrki K. Virtanen, jyrki.virtanen@uef.fi.

Received 26 June 2013 and accepted 30 August 2013.

© 2014 by the American Diabetes Association. See <http://creativecommons.org/licenses/by-nc-nd/3.0/> for details.

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 (PUFAs) 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 PUFAs 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 U.S., 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 U.S., 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 Ischemic Heart Disease Risk Factor (KIHD) study, serum linoleic acid, an omega-6 PUFA, was associated with lower risk of the combined end point 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 2,212 middle-aged and older men from KIHD. We also investigated the association with the intermediate-chain length omega-3 PUFA α -linolenic acid (ALA; 18:3n-3), an essential fatty acid that is derived from plant sources in the diet and that 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 nonsignificant trend toward 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 cardiovascular disease, atherosclerosis, and related outcomes in a population-based, randomly selected sample of men from Eastern Finland (19). The baseline examinations were carried out from 1984 to 1989. A total of 2,682 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 1,166 men who were 54 years old, enrolled from 1984 to 1986, and the second cohort included 1,516 men who were 42, 48, 54, or 60 years old, enrolled from 1986 to 1989.

The baseline examinations were followed by the 4-year examination round (1991–1993) in which 1,038 men from the second cohort (88% of those 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 1,241 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 ($n = 127$), unknown diabetes status ($n = 38$) at baseline, or missing data on serum fatty acids ($n = 138$) were excluded, leaving 2,212 men. Dietary intakes were available for 2,194 participants. For the analyses with hair mercury, complete data were available for 1,977 men.

Measurements

Fasting venous blood samples and hair samples were collected between 8:00 A.M. and 10:00 A.M. at the baseline examinations. Subjects were instructed to abstain from ingesting alcohol for 3 days and from smoking and eating for 12 h 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 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 prepreparation (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 ν -Check Prep Inc. (MN). 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, since 1999 Agilent Technologies Inc.) with a flame ionization detector. Results were obtained in micromoles per liter. The coefficient of variation for repeated measurements of major esterified fatty acids was $\sim 5\%$. 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 coefficient of variation for major nonesterified fatty acids was $\sim 15\%$. 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-h oral glucose tolerance test 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 on Dec 31, 2010. Impaired fasting glucose at baseline was defined using World Health Organization criteria, fasting plasma glucose 6.1–6.9 mmol/L. Oral glucose tolerance test 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 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, BMI (kilograms per square meter), 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 (kilocalories/week), intake of alcohol (grams/day), and serum linoleic acid (percentage). 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 two-tailed ($\alpha = 0.05$). 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; higher intakes of fruits, berries, and vegetables, EPA + DHA, and alcohol; and lower serum triglyceride, insulin and linoleic acid concentrations, HOMA of insulin resistance, energy, and fiber and saturated fatty acid intakes (Table 1). Men with higher serum ALA concentration were younger; had a lower BMI, waist-to-hip ratio, and hair mercury; had lower intakes of EPA + DHA, saturated fatty acids, and alcohol; had higher physical activity, income, education, and serum triglyceride and linoleic acid concentrations; and had higher fiber, fruit, berry, vegetable, and ALA intake. They were also less likely to be smokers and to 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 with 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

Table 1—Baseline characteristics (1984–1989) according to the serum omega-3 PUFAs: The Kuopio Ischemic Heart Disease Risk Factor study

	EPA + DPA + DHA quartile (%)			ALA quartile (%)		
	Lowest (<3.62)	Highest (>5.33)	<i>P</i> for trend	Lowest (<0.57)	Highest (>0.87)	<i>P</i> 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
BMI (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)	12,490 ± 7,390	14,580 ± 9,950	<0.001	11,600 ± 8,940	14,860 ± 8,660	<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 of insulin resistance	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)	2,410 ± 676	2,296 ± 605	<0.001	2,372 ± 685	2,370 ± 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

N = 2,212. Values are means (SD) or percentages.

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 (hazard ratio 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

Table 2—Mean values of serum and dietary omega-3 PUFAs 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* < 0.05.

Table 3—Risk of incident type 2 diabetes in quartiles of serum omega-3 PUFAs

	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/1,000 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/1,000 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/1,000 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/1,000 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/1,000 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% CI), unless otherwise indicated. Model 1 is adjusted for age and examination year. Model 2 is adjusted for model 1 and BMI (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 (%). PY, person-years.

2 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).

CONCLUSIONS

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,

Table 4—Risk of incident type 2 diabetes in quartiles of fish intake, energy-adjusted omega-3 PUFA 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/1,000 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/1,000 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/1,000 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/1,000 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% CI), unless otherwise indicated. Model 1 is adjusted for age and examination year. Model 2 is adjusted for model 1 and BMI (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 (%). PY, person-years. †The number of participants in quartiles of hair mercury is 494, 492, 496, and 495.

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 U.S. with higher risk (7–10). These geographic differences in the risk may reflect, for example, genetic differences, gene–diet interactions, or differences in the type of fish consumed (fatty fish versus lean fish) and in fish preparation methods (raw/steamed/boiled versus deep-fried). However, the number of studies in other regions than U.S. is limited, which reduces the generalizability of the findings in non-U.S. populations. Interestingly, in the U.S. 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 with 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 toward 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 nonsignificant trend toward lower risk of type 2 diabetes, with low heterogeneity between studies (7). Although there was a nonsignificantly 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 exist 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 years 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 $\mu\text{g/g}$, whereas in our study the median hair concentration in the highest quartile was 4.2 $\mu\text{g/g}$, corresponding to approximately 1.6 $\mu\text{g/g}$ 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 BMI, 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, 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 persistent organic pollutant 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.

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

Author Contributions. J.K.V. came up with the concept and design, researched the data, wrote the manuscript, and reviewed and edited the manuscript. J.M., S.V., M.U., and T.-P.T. contributed to the discussion and reviewed and edited the manuscript. All authors have read and approved the final version of the manuscript. J.K.V. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

References

- Schulze MB, Hu FB. Primary prevention of diabetes: what can be done and how much can be prevented? *Annu Rev Public Health* 2005;26:445–467
- Poudyal H, Panchal SK, Diwan V, Brown L. Omega-3 fatty acids and metabolic syndrome: effects and emerging mechanisms of action. *Prog Lipid Res* 2011;50:372–387
- Mozaffarian D, Wu JH. Omega-3 fatty acids and cardiovascular disease: effects on risk factors, molecular pathways, and clinical events. *J Am Coll Cardiol* 2011;58:2047–2067
- Storlien LH, Kraegen EW, Chisholm DJ, Ford GL, Bruce DG, Pascoe WS. Fish oil prevents insulin resistance induced by high-fat feeding in rats. *Science* 1987;237:885–888
- Akinkuolie AO, Ngwa JS, Meigs JB, Djoussé L. Omega-3 polyunsaturated fatty acid and insulin sensitivity: a meta-analysis of randomized controlled trials. *Clin Nutr* 2011;30:702–707
- Hartweg J, Perera R, Montori V, Dinneen S, Neil HA, Farmer A. Omega-3 polyunsaturated fatty acids (PUFA) for type 2 diabetes mellitus. *Cochrane Database Syst Rev* 2008;1:CD003205
- Wu JH, Micha R, Imamura F, et al. Omega-3 fatty acids and incident type 2 diabetes: a systematic review and meta-analysis. *Br J Nutr* 2012;107(Suppl. 2):S214–S227
- Wallin A, Di Giuseppe D, Orsini N, Patel PS, Forouhi NG, Wolk A. Fish consumption, dietary long-chain n-3 fatty acids, and risk of type 2 diabetes: systematic review and meta-analysis of prospective studies. *Diabetes Care* 2012;35:918–929
- Zheng JS, Huang T, Yang J, Fu YQ, Li D. Marine N-3 polyunsaturated fatty acids are inversely associated with risk of type 2 diabetes in Asians: a systematic review and meta-analysis. *PLoS ONE* 2012;7:e44525
- Xun P, He K. Fish consumption and incidence of diabetes: meta-analysis of data from 438,000 individuals in 12 independent prospective cohorts with an average 11-year follow-up. *Diabetes Care* 2012;35:930–938
- Laaksonen DE, Lakka TA, Lakka HM, et al. Serum fatty acid composition predicts development of impaired fasting glycaemia and diabetes in middle-aged men. *Diabet Med* 2002;19:456–464
- Mustad VA, Demichele S, Huang YS, et al. Differential effects of n-3 polyunsaturated fatty acids on metabolic control and vascular reactivity in the type 2 diabetic ob/ob mouse. *Metabolism* 2006;55:1365–1374
- Wendland E, Farmer A, Glasziou P, Neil A. Effect of alpha linolenic acid on cardiovascular risk markers: a systematic review. *Heart* 2006;92:166–169
- Bloedon LT, Balikai S, Chittams J, et al. Flaxseed and cardiovascular risk factors: results from a double blind, randomized, controlled clinical trial. *J Am Coll Nutr* 2008;27:65–74
- Egert S, Fobker M, Andersen G, Somoza V, Erbersdobler HF, Wahrburg U. Effects of dietary alpha-linolenic acid, eicosapentaenoic acid or docosahexaenoic acid on parameters of glucose metabolism in healthy volunteers. *Ann Nutr Metab* 2008;53:182–187
- Chang JW, Chen HL, Su HJ, Liao PC, Guo HR, Lee CC. Simultaneous exposure of non-diabetics to high levels of dioxins and mercury increases their risk of insulin resistance. *J Hazard Mater* 2011;185:749–755
- He K, Xun P, Liu K, Morris S, Reis J, Guallar E. Mercury exposure in young adulthood and incidence of diabetes later in life: the CARDIA Trace Element Study. *Diabetes Care* 2013;36:1584–1589
- Virtanen JK, Voutilainen S, Rissanen TH, et al. Mercury, fish oils, and risk of acute coronary events and cardiovascular disease, coronary heart disease, and all-cause mortality in men in Eastern Finland. *Arterioscler Thromb Vasc Biol* 2005;25:228–233
- Salonen JT. Is there a continuing need for longitudinal epidemiologic research? The Kuopio Ischaemic Heart Disease Risk Factor study. *Ann Clin Res* 1988;20:46–50
- Salonen JT, Nyyssönen K, Korpela H, Tuomilehto J, Seppänen R, Salonen R. High stored iron levels are associated with excess risk of myocardial infarction in Eastern Finnish men. *Circulation* 1992;86:803–811
- Lakka TA, Venäläinen JM, Rauramaa R, Salonen R, Tuomilehto J, Salonen JT. Relation of leisure-time physical activity and cardiorespiratory fitness to the risk of acute myocardial infarction. *N Engl J Med* 1994;330:1549–1554
- Salonen JT, Seppänen K, Nyyssönen K, et al. Intake of mercury from fish, lipid peroxidation, and the risk of myocardial infarction and coronary, cardiovascular, and any death in Eastern Finnish men. *Circulation* 1995;91:645–655
- Voutilainen S, Rissanen TH, Virtanen J, Lakka TA, Salonen JT; Kuopio Ischaemic Heart Disease Risk Factor Study. Low dietary folate intake is associated with an excess incidence of acute coronary events: The Kuopio Ischaemic Heart Disease Risk Factor study. *Circulation* 2001;103:2674–2680
- Willett WC. *Nutritional Epidemiology*. New York, Oxford University Press, 2013
- Nikkari T, Luukkainen P, Pietinen P, Puska P. Fatty acid composition of serum lipid fractions in relation to gender and quality of dietary fat. *Ann Med* 1995;27:491–498
- González-Pérez A, Horrillo R, Ferré N, et al. Obesity-induced insulin resistance and hepatic steatosis are alleviated by omega-3

- fatty acids: a role for resolvins and protectins. *FASEB J* 2009;23:1946–1957
27. Oh DY, Talukdar S, Bae EJ, et al. GPR120 is an omega-3 fatty acid receptor mediating potent anti-inflammatory and insulin-sensitizing effects. *Cell* 2010;142:687–698
28. Reinders I, Virtanen JK, Brouwer IA, Tuomainen TP. Association of serum n-3 polyunsaturated fatty acids with C-reactive protein in men. *Eur J Clin Nutr* 2012;66:736–741
29. Deckelbaum RJ, Worgall TS, Seo T. n-3 fatty acids and gene expression. *Am J Clin Nutr* 2006;83(Suppl.):1520S–1525S
30. Lankinen M, Schwab U, Kolehmainen M, et al. Whole grain products, fish and bilberries alter glucose and lipid metabolism in a randomized, controlled trial: the Sysdimet study. *PLoS ONE* 2011;6:e22646
31. Pan A, Sun Q, Bernstein AM, et al. Red meat consumption and risk of type 2 diabetes: 3 cohorts of US adults and an updated meta-analysis. *Am J Clin Nutr* 2011;94:1088–1096
32. Risérus U, Willett WC, Hu FB. Dietary fats and prevention of type 2 diabetes. *Prog Lipid Res* 2009;48:44–51
33. Burdge GC. Metabolism of alpha-linolenic acid in humans. *Prostaglandins Leukot Essent Fatty Acids* 2006;75:161–168
34. Chen YW, Yang CY, Huang CF, Hung DZ, Leung YM, Liu SH. Heavy metals, islet function and diabetes development. *Islets* 2009;1:169–176
35. Serdar MA, Bakir F, Haşimi A, et al. Trace and toxic element patterns in nonsmoker patients with noninsulin-dependent diabetes mellitus, impaired glucose tolerance, and fasting glucose. *Int J Diabetes Dev Ctries* 2009;29:35–40
36. Flores CR, Puga MP, Wrobel K, Garay Sevilla ME, Wrobel K. Trace elements status in diabetes mellitus type 2: possible role of the interaction between molybdenum and copper in the progress of typical complications. *Diabetes Res Clin Pract* 2011;91:333–341
37. Park SB, Choi SW, Nam AY. Hair tissue mineral analysis and metabolic syndrome. *Biol Trace Elem Res* 2009;130:218–228
38. Roman HA, Walsh TL, Coull BA, et al. Evaluation of the cardiovascular effects of methylmercury exposures: current evidence supports development of a dose-response function for regulatory benefits analysis. *Environ Health Perspect* 2011;119:607–614
39. Carpenter DO. Environmental contaminants as risk factors for developing diabetes. *Rev Environ Health* 2008;23:59–74
40. Airaksinen R, Rantakokko P, Eriksson JG, Blomstedt P, Kajantie E, Kiviranta H. Association between type 2 diabetes and exposure to persistent organic pollutants. *Diabetes Care* 2011;34:1972–1979