Serum n–6 polyunsaturated fatty acids, Δ5- and Δ6-desaturase activities, and risk of incident type 2 diabetes in men: the Kuopio Ischaemic Heart Disease Risk Factor Study[1,2]

*Teymoor Yary,[3] Sari Voutilainen,[3] Tomi-Pekka Tuomainen,[3] Anu Ruusunen,[4] Tarja Nurmi,[3] and Jyrki K Virtanen[3]**

[3]Institute of Public Health and Clinical Nutrition, University of Eastern Finland, Kuopio, Finland; and [4]Department of Psychiatry, Kuopio University Hospital, Kuopio, Finland

ABSTRACT

Background: The role of n–6 (ω-6) polyunsaturated fatty acids (PUFAs) in type 2 diabetes (T2D) is inconclusive. In addition, little is known about how factors involved in PUFA metabolism, such as zinc, may affect the associations.

Objectives: We investigated the associations of serum n–6 PUFAs and activities of enzymes involved in PUFA metabolism, Δ5 desaturase (D5D) and Δ6 desaturase (D6D), with T2D risk to determine whether serum zinc concentrations could modify these associations.

Design: The study included 2189 men from the prospective Kuopio Ischaemic Heart Disease Risk Factor Study, aged 42–60 y and free of T2D at baseline in 1984–1989. T2D was assessed by self-administered questionnaires, by fasting and 2-h oral-glucose-tolerance test blood glucose measurement at re-examination rounds 4, 11, and 20 y after baseline, and by record linkage to the hospital discharge registry and the reimbursement register on diabetes medication expenses. Multivariate-adjusted Cox proportional hazards regression models were used to analyze associations.

Results: During the average follow-up of 19.3 y, 417 men developed T2D. Those with higher estimated D5D activity (extreme-quartile HR: 0.55; 95% CI: 0.41, 0.74; *P*-trend < 0.001) and higher concentrations of total n–6 PUFAs (HR: 0.54; 95% CI: 0.41, 0.73; *P*-trend < 0.001), linoleic acid (LA; HR: 0.52; 95% CI: 0.39, 0.70; *P*-trend < 0.001), and arachidonic acid (AA; HR: 0.62; 95% CI: 0.46, 0.85; *P*-trend = 0.007) had a lower risk and those with higher concentrations of γ-linolenic acid (GLA; HR: 1.28; 95% CI: 0.98, 1.68; $P = 0.021$) and dihomo-γ-linolenic acid (DGLA; HR: 1.38; 95% CI: 1.04, 1.84; *P*-trend = 0.005) and higher D6D activity had a higher (HR: 1.50; 95% CI: 1.14, 1.97; *P*-trend < 0.001) multivariate-adjusted risk of T2D. Zinc mainly modified the association with GLA on T2D risk, with a higher risk observed among those with serum zinc concentrations above the median (*P*-interaction = 0.04).

Conclusions: Higher serum total n–6 PUFA, LA, and AA concentrations and estimated D5D activity were associated with a lower risk of incident T2D, and higher GLA and DGLA concentrations and estimated D6D activity were associated with a higher risk. In addition, a higher serum zinc concentration modified the association of GLA on the risk of T2D. *Am J Clin Nutr* 2016;103:1337–43.

Keywords: polyunsaturated fatty acids, desaturase enzymes, serum zinc, type 2 diabetes, prospective study

INTRODUCTION

Type 2 diabetes (T2D)[5] is a major chronic disease and a leading cause of morbidity and mortality worldwide (1, 2). In addition to genetic factors, aging, obesity, smoking, and physical inactivity, dietary factors may also have a significant role in the prevention or development of T2D. Among dietary factors, n–6 PUFAs may have the potential to prevent T2D because of the beneficial impact of the major n–6 PUFA, linoleic acid (LA; 18:2n–6), on insulin resistance (3). LA, an essential fatty acid for humans, must be obtained from the diet; its primary sources are vegetable oils. In the body, LA is converted to other n–6 PUFAs, that is, γ-linolenic acid (GLA; 18:3n–6), dihomo-γ-linolenic acid (DGLA; 20:3n–6), and arachidonic acid (AA; 20:4n–6). These fatty acids can also be obtained from the diet in minor amounts, and AA is derived especially from animal products, but their concentrations in the body mainly reflect the endogenous formation from LA (4). In the conversion process, 2 enzymes, Δ5 desaturase (D5D) and Δ6 desaturase (D6D), have a central role by adding double bonds to the PUFAs. These enzymes have been quite consistently associated with insulin resistance and T2D: D5D activity with a lower risk and D6D activity with a higher risk (5). There is also fairly strong evidence for an inverse association between circulating LA and the risk of T2D, but the associations with the other n–6 PUFAs are less consistent (6–14). Although their concentrations in the circulation are much lower than that of LA, they may have a role

[1] Supported by a Jalmari and Rauha Ahokas Foundation grant and a Centre for International Mobility (CIMO) Fellowship (TM-14-9374) to TY and the University of Eastern Finland.

[2] Supplemental Tables 1 and 2 are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://ajcn.nutrition.org.

*To whom correspondence should be addressed. E-mail: jyrki.virtanen@uef.fi.

[5] Abbreviations used: AA, arachidonic acid; CRP, C-reactive protein; D5D, Δ5 desaturase; D6D, Δ6 desaturase; DGLA, dihomo-γ-linolenic acid; *FADS1*, fatty acid desaturase 1; *FADS2*, fatty acid desaturase 2; GLA, γ-linolenic acid; HOMA, homeostasis model assessment; KIHD, Kuopio Ischaemic Heart Disease Risk Factor; LA, linoleic acid; T2D, type 2 diabetes.

Received December 11, 2015. Accepted for publication February 22, 2016.
First published online March 23, 2016; doi: 10.3945/ajcn.115.128629.

in the development of T2D. For example, AA is a precursor of eicosanoids, some of which are proinflammatory (15).

Previously, in a subset of 895 middle-aged and older men from the Kuopio Ischaemic Heart Disease Risk Factor (KIHD) Study, serum LA concentration was inversely associated with impaired fasting glucose and risk of T2D after a 4-y follow-up (16). In the current study we extended these analyses and examined the associations of 4 serum n–6 PUFAs, the estimated D5D and D6D activities, and the risk of T2D during a 19-y follow-up in the whole study population of 2189 men. As a secondary analysis we also investigated whether serum zinc concentration may modify the associations between D5D and D6D activities or n–6 PUFAs and risk of T2D. Zinc is an essential cofactor for the desaturase enzymes (17), and we previously found in the KIHD study that higher serum zinc concentrations were associated with a higher risk of T2D (18).

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 2682 men who were 42, 48, 54, or 60 y old at baseline (82.9% of those eligible) were recruited in 2 cohorts. The first cohort consisted of 1166 men aged 54 y enrolled from 1984 to 1986, and the second cohort included 1516 men aged 42, 48, 54, or 60 y enrolled from 1986 to 1989. The baseline examinations were followed by a 4-y examination round (1991–1993) in which 1038 men from the second cohort (88% of those eligible) participated. At the 11-y examination round (1998–2001), all men from the second cohort were invited, and 854 men (95% of those eligible) participated. During the 20-y examination round, all eligible participants from the first and second cohorts were invited to the study site. A total of 1241 men (80% of those eligible) participated. The baseline characteristics of the entire study population have been described (19). The KIHD protocol was approved by the Research Ethics Committee of the University of Kuopio. All subjects gave written informed consent for participation. Participants with T2D (n = 162), impaired fasting glucose (n = 132), or unknown diabetes status (n = 38) at baseline, or who were missing data on serum n–6 PUFAs (n = 138) or serum zinc (n = 23), were excluded, which left 2189 men.

Measurements

Fasting venous blood samples were collected between 0800 and 1000 at the baseline examinations. Subjects were instructed to abstain from ingesting alcohol for 3 d and from smoking and eating for 12 h before 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) were published earlier. Physical activity was assessed on the basis of the 12-mo Leisure-time Physical Activity Questionnaire (21). The most common leisure-time physical activities, including the frequency, average duration, and intensity of each activity, were recorded. The energy expenditure of physical activity was expressed in kcal/d. Alcohol consumption (g/wk) was assessed with a structured quantity-frequency method by using the Nordic Alcohol Consumption Inventory for drinking behavior over the previous 12 mo (22). A study nurse measured the weight and height of the participants, and BMI was calculated as the ratio of weight in kilograms to the square of height in meters. Resting blood pressure was measured between 0800 and 1000 on the first examination day by one study nurse with a random-zero mercury sphygmomanometer. After a supine rest of 5 min, the measurement protocol included 3 measurements while in a supine position, 1 measurement while standing, and 2 measurements in a sitting position with 5-min intervals. The mean of all 6 systolic blood pressure values was used in the present analyses as the systolic blood pressure and the mean of all 6 diastolic measurements was used as the diastolic blood pressure (20). The consumption of foods and total energy intake at the study baseline were assessed with a guided 4-d food recording with the use of household measures (23). Education was assessed in years by using a self-administered questionnaire. Family history of diabetes was defined as positive if a first-degree relative of the subject had a history of diabetes. Smoking habits were also reported, including ever-smoking, current smoking (number of cigarettes, cigars, or pipes smoked per day), and duration of regular smoking. A trained study nurse checked and completed the questionnaires during an interview.

Plasma glucose was measured by 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). Insulin resistance and sensitivity and β cell function were estimated by the homeostasis model assessment (HOMA) computer algorithm (24). Serum C-reactive protein (CRP) was measured with an immunometric assay (Immulite High Sensitivity CRP Assay; Diagnostic Products Corporation).

Serum fatty acids were determined in a gas chromatography run without preseparation, as previously described (16). Serum fatty acids were extracted with chloroform-methanol. The 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. Each analyte had an individual reference standard, and the internal standard was eicosane. Fatty acids were chromatographed in an NB-351 capillary column (HNU-Nordion) with the use of a Hewlett-Packard 5890 Series II gas chromatograph (Agilent Technologies) with a flame ionization detector. Results were obtained in micromoles per liter. The CV was 9.6% for LA, 11.7% for GLA, 8.3% for DGLA, and 9.2% for AA. Desaturase enzyme activities were estimated as the ratio of product to precursor and were calculated as the ratio of AA to DGLA for D5D activity and as the ratio of GLA to LA for D6D activity.

Serum zinc concentrations were determined from frozen samples stored at –20°C for 1–5 y before analyses. A Perkin-Elmer 306 atomic absorption spectrophotometer was used for measurements. Seronorm (Nycomed) control serum samples were included in all daily batches (20, 25).

Diagnostic criteria for T2D

T2D was defined as self-reported, physician-diagnosed T2D and/or fasting plasma glucose ≥7.0 mmol/L or 2-h

oral-glucose-tolerance test plasma glucose ≥11.1 mmol/L at reexamination rounds 4, 11, and 20 y after 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 T2D for the entire study period until the end of the follow-up on 31 December 2010. Impaired fasting glucose at baseline was defined by using the WHO criterion of fasting plasma glucose of 6.1–6.9 mmol/L.

Statistical analysis

The univariate relations between serum n–6 PUFAs and D5D and D6D activities with baseline characteristics were assessed by means and linear regression (for continuous variables) or by chi-square tests (for bivariate relations). The average serum n–6 PUFA concentrations and desaturase activities by quartile of serum zinc concentration were estimated by using ANCOVA and linear regression. Cox proportional hazards regression models were used to estimate HRs of incidence of T2D in quartiles of n–6 PUFAs and desaturase activities. The confounders in the analyses were selected on the basis of established risk factors for T2D, previously published associations in the KIHD study, or on associations with exposures or outcomes in the present analysis. Model 1 included age (y) and examination year. Multivariable model 2 was further adjusted for potential confounders, including family history of T2D (yes or no), BMI (kg/m^2), smoking (never-smoker; previous smoker; current smoker, <20 cigarettes/d; or current smoker, ≥20 cigarettes/d), education (y), leisure-time physical activity (kcal/d), intakes of alcohol (g/wk) and energy (kcal/d), and serum long-chain n–3 PUFA concentrations (%). We did not adjust for HOMA indexes, blood glucose, serum insulin, or serum CRP, because those variables can be considered as mediators for the associations between serum PUFAs and risk of T2D. All quantitative variables were entered in the models as continuous variables. Cohort means were used to replace missing values in covariates (<0.5%). 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. The increase or reduction in absolute risk was calculated by multiplying the absolute risk in the reference group by the multivariable-adjusted HR increase or reduction in the comparison group. Statistical significance of the interactions on a multiplicative scale was assessed by likelihood ratio tests with the use of a cross-product term. All P values were 2-tailed ($\alpha = 0.05$). Data were analyzed by using SPSS 21.0 for Windows (IBM Corporation).

RESULTS

Table 1 shows the baseline characteristics of study participants according to quartile of serum total n–6 PUFAs. Men with higher serum total n–6 PUFAs were younger, had a higher education, smoked less, and had higher leisure-time physical activity and HOMA–insulin sensitivity score. They also had lower alcohol intakes and lower serum long-chain n–3 PUFA concentrations, BMI, CRP, fasting blood glucose, serum insulin, HOMA-IR, as well as HOMA–β cell function. Baseline characteristics in quartiles of serum LA and AA were generally similar to the quartiles of serum total n–6 PUFAs but the univariate associations of GLA and DGLA with glucose metabolism markers were generally opposite to those of LA and AA (**Supplemental Table 1**). Similar differences were observed with D5D and D6D activities: D5D activity was associated with generally more favorable and D6D activity with less favorable baseline characteristics (**Supplemental Table 2**).

TABLE 1
Baseline characteristics according to quartile of serum total n–6 PUFAs in 2189 men from the KIHD study[1]

	Serum total n–6 PUFAs				
	Q1 (13.28–28.65%)	Q2 (28.66–31.84%)	Q3 (31.85–34.80%)	Q4 (34.81–46.54%)	P-trend
Participants, n	547	547	548	547	
Age, y	53.6 ± 4.7	53.2 ± 5.0	52.9 ± 5.2	52.1 ± 5.7	<0.001
Education, y	8.4 ± 3.1	8.2 ± 3.2	8.8 ± 3.6	9.3 ± 3.7	<0.001
Current smokers, %	29.8	23.1	26.2	20.2	<0.001
Family history of diabetes, %	24.1	22.7	25.3	27.9	0.181
Alcohol intake, g/wk	88 ± 135	75 ± 118	70 ± 152	55 ± 87	<0.001
Leisure-time physical activity, kcal/d	131 ± 178	130 ± 159	139 ± 171	159 ± 189	0.020
BMI, kg/m^2	27.9 ± 3.7	27.0 ± 3.2	26.0 ± 2.9	25.4 ± 2.8	<0.001
Total energy intake, kcal/d	2335 ± 653	2388 ± 647	2390 ± 600	2417 ± 603	0.181
Fasting blood glucose, mmol/L	4.62 ± 0.38	4.53 ± 0.38	4.51 ± 0.41	4.43 ± 0.40	<0.001
Serum insulin, mU/L	13.60 ± 8.30	10.84 ± 5.14	9.95 ± 5.37	8.90 ± 3.48	<0.001
HOMA–IR	3.05 ± 1.63	2.46 ± 1.23	2.24 ± 1.15	1.99 ± 0.83	<0.001
HOMA–insulin sensitivity	71.23 ± 31.18	84.34 ± 33.80	93.10 ± 39.21	99.09 ± 38.54	<0.001
HOMA–β cell function	127.08 ± 44.38	115.90 ± 37.20	109.63 ± 34.1	106.78 ± 29.71	<0.001
CRP, mg/L	3.05 ± 5.98	2.19 ± 3.03	2.09 ± 3.38	1.80 ± 2.86	<0.001
Serum zinc, mg/L	0.94 ± 0.13	0.94 ± 0.11	0.93 ± 0.11	0.94 ± 0.11	0.445
Serum linoleic acid, %	21.09 ± 2.53	25.28 ± 1.34	28.01 ± 1.28	32.21 ± 2.60	<0.001
Serum γ-linolenic acid,%	0.31 ± 0.12	0.28 ± 0.10	0.28 ± 0.10	0.27 ± 0.11	<0.001
Serum dihomo-γ-linolenic acid, %	1.36 ± 0.39	1.36 ± 0.28	1.33 ± 0.26	1.30 ± 0.26	0.001
Serum arachidonic acid, %	4.30 ± 0.95	4.81 ± 0.94	4.98 ± 0.93	5.09 ± 0.99	0.001

[1]Values are means ± SDs unless otherwise indicated. P values for trend were assessed with linear regression (continuous variables) and by chi-square test (bivariate relations). CRP, C-reactive protein; HOMA, homeostasis model assessment; KIHD, Kuopio Ischaemic Heart Disease Risk Factor; Q, quartile.

TABLE 2
HRs (95% CIs) of incident T2D during the average follow-up of 19.3 y according to quartile of serum n–6 PUFAs and Δ5- and Δ6-desaturase activities in men[1]

	Serum n–6 PUFAs and Δ5- and Δ6-desaturase activities					
	Q1 (n = 547)	Q2 (n = 547)	Q3 (n = 548)	Q4 (n = 547)	P-trend	P-interaction
Total n–6 PUFAs, %	13.28–28.65	28.66–31.84	31.85–34.80	34.81–46.54		
Events, n	147	109	82	79		
Incidence rate/1000 PY	15.5	10.1	7.6	7.1		
Model 1	1	0.60 (0.46, 0.76)	0.45 (0.34, 0.59)	0.40 (0.30, 0.53)	<0.001	
Model 2	1	0.68 (0.53, 0.87)	0.58 (0.44, 0.77)	0.54 (0.41, 0.73)	<0.001	
Serum zinc <median	1	0.61 (0.41, 0.91)	0.56 (0.36, 0.85)	0.51 (0.32, 0.79)	0.002	
Serum zinc ≥median	1	0.74 (0.53, 1.02)	0.60 (0.41, 0.88)	0.58 (0.40, 0.85)	0.002	0.757
Linoleic acid, %	10.10–23.64	23.65–26.69	26.70–29.59	29.60–41.43		
Events, n	143	105	95	74		
Incidence rate, %	14.8	9.8	8.9	6.7		
Model 1	1	0.61 (0.47, 0.79)	0.55 (0.43, 0.72)	0.40 (0.30, 0.53)	<0.001	
Model 2	1	0.71 (0.55, 0.92)	0.68 (0.52, 0.89)	0.52 (0.39, 0.70)	<0.001	
Serum zinc <median	1	0.60 (0.39, 0.91)	0.70 (0.47, 1.05)	0.48 (0.30, 0.78)	0.005	
Serum zinc ≥median	1	0.81 (0.59, 1.13)	0.66 (0.46, 0.96)	0.56 (0.38, 0.83)	0.002	0.915
γ-Linolenic acid, %	0.04–0.21	0.22–0.27	0.28–0.35	0.36–1.02		
Events, n	99	81	106	131		
Incidence rate/1000 PY	9.4	7.6	10.1	12.5		
Model 1	1	0.83 (0.62, 1.11)	1.10 (0.84, 1.45)	1.37 (1.06, 1.78)	0.002	
Model 2	1	0.85 (0.63, 1.13)	1.03 (0.79, 1.36)	1.28 (0.98, 1.68)	0.021	
Serum zinc <median	1	0.73 (0.47, 1.14)	0.88 (0.59, 1.32)	0.96 (0.63, 1.44)	0.996	
Serum zinc ≥median	1	0.95 (0.63, 1.42)	1.23 (0.84, 1.80)	1.63 (1.13, 2.34)	0.002	0.041
Dihomo-γ-linolenic acid, %	0.57–1.15	1.16–1.33	1.34–1.50	1.51–7.78		
Events, n	84	98	102	133		
Incidence rate/1000 PY	8.0	9.4	9.5	12.8		
Model 1	1	1.20 (0.90, 1.61)	1.17 (0.88, 1.57)	1.65 (1.25, 2.17)	<0.001	
Model 2	1	1.12 (0.84, 1.50)	1.06 (0.79, 1.42)	1.38 (1.04, 1.84)	0.005	
Serum zinc <median	1	1.16 (0.75, 1.79)	0.86 (0.54, 1.37)	1.46 (0.95, 2.26)	0.148	
Serum zinc ≥median	1	1.09 (0.73, 1.64)	1.22 (0.83, 1.80)	1.36 (0.93, 1.98)	0.090	0.913
Arachidonic acid, %	1.36–4.10	4.11–4.72	4.73–5.40	5.41–9.24		
Events, n	124	104	104	85		
Incidence rate/1000 PY	12.4	9.8	9.7	7.8		
Model 1	1	0.77 (0.59, 1.00)	0.76 (0.58, 0.98)	0.60 (0.46, 0.80)	<0.001	
Model 2	1	0.76 (0.58, 0.99)	0.78 (0.60, 1.03)	0.62 (0.46, 0.85)	0.007	
Serum zinc <median	1	0.53 (0.35, 0.80)	0.52 (0.34, 0.80)	0.45 (0.28, 0.72)	0.001	
Serum zinc ≥median	1	1.04 (0.73, 1.47)	1.10 (0.76, 1.58)	0.82 (0.54, 1.24)	0.432	0.110
Δ6-Desaturase activity[2]	0.001–0.007	0.008–0.010	0.011–0.013	0.014–0.051		
Events, n	89	81	109	138		
Incidence rate/1000 PY	8.2	7.6	10.4	13.6		
Model 1	1	0.94 (0.69, 1.26)	1.29 (0.98, 1.71)	1.77 (1.35, 2.31)	<0.001	
Model 2	1	0.87 (0.64, 1.17)	1.12 (0.85, 1.49)	1.50 (1.14, 1.97)	<0.001	
Serum zinc <median	1	0.97 (0.63, 1.51)	0.95 (0.62, 1.46)	1.42 (0.94, 2.15)	0.084	
Serum zinc ≥median	1	0.77 (0.51, 1.17)	1.27 (0.88, 1.86)	1.55 (1.07, 2.23)	0.001	0.431
Δ5-Desaturase activity[2]	1.01–2.98	2.99–3.52	3.53–4.28	4.29–11.23		
Events, n	133	114	96	74		
Incidence rate/1000 PY	13.2	10.9	8.8	6.9		
Model 1	1	0.94 (0.69, 1.26)	0.65 (0.50, 0.85)	0.52 (0.39, 0.69)	<0.001	
Model 2	1	0.81 (0.63, 1.04)	0.65 (0.50, 0.84)	0.55 (0.41, 0.74)	<0.001	
Serum zinc <median	1	0.64 (0.43, 0.96)	0.46 (0.30, 0.72)	0.50 (0.33, 0.77)	0.002	
Serum zinc ≥median	1	0.93 (0.67, 1.29)	0.80 (0.57, 1.12)	0.58 (0.39, 0.88)	0.006	0.474

[1] n = 2189. Cox proportional hazards regression models were used to obtain HRs and 95% CIs. Model 1 was adjusted for age and examination year; model 2 was adjusted as for model 1 plus family history of T2D (yes or no), smoking (never-smoker; previous smoker; current smoker, <20 cigarettes/d; or current smoker, ≥20 cigarettes/d), education years, leisure-time physical activity (kcal/d), BMI (kg/m^2), serum long-chain n–3 PUFAs (%), and intakes of alcohol (g/wk) and energy (kcal/d). The analyses with Δ6-desaturase and Δ5-desaturase activities were not adjusted for serum long-chain n–3 PUFAs. PY, person-years; Q, quartile; T2D, type 2 diabetes.

[2] Calculation of Δ6-desaturase and Δ5-desaturase activities: Δ6 desaturase = γ-linolenic acid/linoleic acid and Δ5 desaturase = arachidonic acid/dihomo-γ-linolenic acid.

During the mean ± SD follow-up of 19.3 ± 6.5 y, 417 men (19.0%) developed T2D (42,247 person-years). After adjustment for age and examination year, those in the highest compared with the lowest quartile of serum total n–6 PUFAs had a 60% (95% CI: 47%, 70%; *P*-trend across quartiles < 0.001) lower HR of incident T2D (model 1 in **Table 2**; absolute risk in the reference group: 26.9%; absolute risk reduction in the highest quartile: 16.1%). The association was slightly attenuated after further adjustment for potential confounders but remained highly significant (Table 2, model 2). When fatty acids were evaluated individually, serum LA and AA had similar inverse associations with the risk of T2D (Table 2). After multivariable adjustments (Table 2, model 2), those in the highest compared with the lowest serum LA quartile had a 48% (95% CI: 30%, 61%; *P*-trend < 0.001) lower HR (absolute risk: 26.1%; absolute risk reduction: 12.5%) and those in the highest compared with the lowest AA quartile had a 38% (95% CI: 15%, 54%; *P*-trend = 0.007) lower HR (absolute risk: 22.7%; absolute risk reduction: 8.6%) of incident T2D. In contrast, higher serum GLA and DGLA concentrations were associated with a higher risk of T2D. Those in the highest GLA quartile had a 28% (95% CI: −2%, 68%; *P*-trend = 0.021; absolute risk in the lowest quartile: 18.1%; absolute risk increase: 5.1%) higher multivariate-adjusted HR and those in the highest DGLA quartile had a 38% (95% CI: 4%, 84%; *P*-trend = 0.005; absolute risk in the lowest quartile: 15.4%; absolute risk increase: 6.0%) higher HR of T2D when compared with the lowest quartile (Table 2, model 2).

After multivariable adjustment, those in the highest compared with the lowest quartile of D6D activity had a 50% (95% CI: 14%, 97%; *P*-trend < 0.001) higher HR of incident T2D (Table 2, model 2; absolute risk in the reference group: 16.3%; absolute risk increase in the highest quartile: 8.2%) and those in the highest compared with the lowest quartile of D5D activity had a 45% (95% CI: 26%, 59%; *P*-trend < 0.001; absolute risk in the lowest quartile: 24.3%, absolute risk reduction: 10.9%) lower HR of incident T2D.

In secondary analyses, a higher serum zinc concentration was associated with lower D5D activity and lower total n–6 PUFA and LA concentrations and with higher D6D activity and higher concentrations of GLA and DGLA at baseline (**Table 3**). When analyses of desaturase activities and n–6 PUFAs with T2D risk were stratified by median serum zinc concentration, serum GLA was associated with a higher risk of T2D only among the men with serum zinc above the median (extreme-quartile HR: 1.63; 95% CI: 1.13, 2.34; *P*-trend = 0.002, *P*-interaction = 0.041; absolute risk in the lowest quartile: 18.2%; absolute risk increase: 6.7%). In contrast, although the *P*-interaction did not reach significance (*P* = 0.11), an inverse association between AA and T2D risk was observed only among those with lower serum zinc concentrations (HR: 0.45; 95% CI: 0.28, 0.72; *P*-trend = 0.001; absolute risk in the lowest quartile: 23.5%; absolute risk reduction: 10.6%). The associations with the other n–6 PUFAs or desaturase activities were not appreciably affected by serum zinc concentrations (Table 2). In sensitivity analyses we excluded the T2D events that occurred during the

TABLE 3
Mean serum values for n–6 PUFA concentrations and Δ5- and Δ6-desaturase activities at baseline according to quartile of serum zinc in men[1]

	Serum zinc (mg/L)				
	Q1 (0.54–0.86) (*n* = 581)	Q2 (0.87–0.92) (*n* = 485)	Q3 (0.93–1.00) (*n* = 611)	Q4 (1.01–1.62) (*n* = 512)	*P*-trend
Total n–6 PUFAs, %					
Model 1	31.83 (31.45, 32.22)	32.16 (31.75, 32.58)	31.75 (31.38, 32.13)	31.17 (30.76, 31.58)	0.009
Model 2	31.82 (31.45, 32.19)	32.03 (31.63, 32.42)	31.84 (31.49, 32.20)	31.21 (30.82, 31.60)	0.024
Linoleic acid, %					
Model 1	26.78 (26.34, 27.08)	27.02 (26.62, 27.43)	26.71 (26.35, 27.06)	26.16 (25.76, 26.55)	0.024
Model 2	26.72 (26.37, 27.07)	26.91 (26.53, 27.29)	26.78 (26.44, 27.12)	26.16 (25.79, 26.53)	0.027
γ-Linolenic acid, %					
Model 1	0.28 (0.27, 0.29)	0.28 (0.27, 0.29)	0.28 (0.28, 0.29)	0.30 (0.29, 0.31)	0.001
Model 2	0.28 (0.27, 0.29)	0.28 (0.27, 0.29)	0.28 (0.27, 0.29)	0.30 (0.29, 0.31)	0.003
Dihomo-γ-linolenic acid, %					
Model 1	1.30 (1.28, 1.32)	1.33 (1.30, 1.36)	1.33 (1.31, 1.36)	1.40 (1.37, 1.42)	<0.001
Model 2	1.31 (1.29, 1.34)	1.34 (1.32, 1.37)	1.32 (1.30, 1.35)	1.38 (1.36, 1.41)	0.001
Arachidonic acid, %					
Model 1	4.84 (4.77, 4.93)	4.86 (4.77, 4.95)	4.76 (4.69, 4.84)	4.71 (4.62, 4.80)	0.047
Model 2	4.82 (4.75, 4.89)	4.83 (4.75, 4.91)	4.78 (4.71, 4.85)	4.75 (4.67, 4.83)	0.428
Δ6-Desaturase activity[2]					
Model 1	0.0108 (0.0104, 0.0113)	0.0108 (0.0104, 0.0113)	0.0111 (0.0107, 0.0114)	0.0121 (0.0116, 0.0126)	<0.001
Model 2	0.0108 (0.0104, 0.0112)	0.0109 (0.0105, 0.0113)	0.0110 (0.0106, 0.0114)	0.0121 (0.0116, 0.0125)	<0.001
Δ5-Desaturase activity[2]					
Model 1	3.87 (3.78, 3.96)	3.81 (3.71, 3.91)	3.71 (3.62, 3.80)	3.51 (3.42, 3.61)	<0.001
Model 2	3.81 (3.74, 3.89)	3.76 (3.68, 3.84)	3.75 (3.67, 3.82)	3.59 (3.51, 3.67)	<0.001

[1] *n* = 2189. Mean values and 95% CIs were obtained by using ANCOVA. Model 1 was adjusted for age, examination year, and energy intake; model 2 was adjusted as for model 1 plus family history of type 2 diabetes (yes or no), smoking (never-smoker; previous smoker; current smoker, <20 cigarettes/d; or current smoker, ≥20 cigarettes/d), education years, leisure-time physical activity (kcal/d), BMI (kg/m^2), serum long-chain n–3 PUFAs (%), and intake of alcohol (g/wk). The analyses with Δ6-desaturase and Δ5-desaturase activities were not adjusted for serum long-chain n–3 PUFAs. Q, quartile.

[2] Calculation of Δ6-desaturase and Δ5-desaturase activities: Δ6 desaturase = γ-linolenic acid/linoleic acid and Δ5 desaturase = arachidonic acid/dihomo-γ-linolenic acid.

first 2 y of follow-up (n = 3) but that had no effect on the associations (data not shown).

DISCUSSION

Our findings showed that concentrations of serum n–6 PUFAs, including LA and AA, were inversely associated with the risk of incident T2D and that higher serum concentrations of GLA and DGLA were associated with a higher risk in middle-aged and older Finnish men. In addition, D6D activity was associated with higher risk and D5D activity with lower risk of incident T2D. We also found that GLA was a risk factor for T2D only among those with higher serum zinc concentrations.

Cell membrane properties, including permeability, flexibility, fluidity, and the activity of membrane-bound enzymes, are affected by the bioavailability of PUFAs (26). The structural components of cell membranes play an important role in the binding of insulin to its receptors to exert its action (27). For example, a high concentration of LA in the cell membrane has been shown to increase the number of high-affinity sites with a concomitant decrease of low-affinity sites for insulin (28). LA has also been inversely associated with insulin resistance (29) and inflammation (30), both risk factors for T2D. Our results with regard to the inverse association with circulating LA are supported by several previous studies (6–14).

AA is a substrate for some proinflammatory metabolites (15); therefore, in theory, a high serum AA concentration could increase the risk of T2D. However, in our study and in another Finnish study (12), circulating AA was inversely associated with risk, although most other studies did not find this association (9, 10, 13, 14, 31). In addition to the proinflammatory metabolites, AA is also a precursor to several anti-inflammatory metabolites (15). Therefore, the associations of AA with disease risk are difficult to predict solely on the basis of its effects on proinflammatory factors. Animal models have also shown that AA may protect pancreatic β cells from chemical-induced cytotoxicity by enhancing the antioxidant status and suppressing production of cytokines, which could preserve insulin production capacity (32, 33).

We found a higher risk of T2D incidence with higher serum DGLA and GLA concentrations. This is somewhat unexpected, because GLA is the direct precursor of DGLA, which competes with EPA and AA for lipoxygenase and cyclooxygenase and produces potential anti-inflammatory eicosanoids (34). However, similar direct associations were also observed in other studies (7, 8, 11, 13, 35), although some studies did not find any association (10, 12, 31). There is also evidence that DGLA is associated with the development of insulin resistance (36) and inflammation (30), thereby supporting our findings.

The associations of D6D and D5D activities with the risk of incident T2D have been investigated in previous prospective studies, and the majority of these studies found that high D5D activity was associated with a lower risk and high D6D activity with a higher risk of T2D (5, 37), which supports our findings. Furthermore, the Mendelian randomization approach, which investigated the fatty acid desaturase 1 (*FADS1*) and fatty acid desaturase 2 (*FADS2*) genes that encode D5D and D6D, respectively, strongly supported the role of D6D and D5D in the development of T2D (6). The mechanism underlying the relation between D6D and D5D activities and T2D is largely unknown but may be related to the association of these enzymes with glucose homeostasis and inflammatory markers (38). For example, a role of D5D activity in the management of insulin sensitivity (12) and insulin resistance (36) has been suggested.

We previously observed in the KIHD study population that a high serum zinc concentration was associated with a higher risk of T2D (18). Zinc is an essential cofactor for desaturase enzymes (17), and animal studies have indicated that zinc has an important role in the metabolism of n–6 PUFAs (39). In the current study we found that those with a higher serum zinc concentration had higher D6D activity and higher concentrations of its end product, GLA, and GLA was associated with a higher risk of T2D only among those with higher serum zinc concentrations. In contrast, the activity of D5D was lower and there was no difference in the concentrations of its end product, AA, with higher serum zinc concentrations. However, AA was associated with a significantly lower risk of T2D only among those with lower serum zinc concentrations. These findings suggest that zinc may have a role in determining the impact of n–6 PUFAs on the risk of T2D and may also offer a potential explanation for the association of higher serum zinc concentration with increased risk of T2D in this study population (18). However, further studies in other populations are required to confirm our observations.

Strengths of our study include the use of objective biomarkers, serum fatty acids and zinc, as exposures; 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. A potential limitation is the use of a single exposure measurement, which may cause random error due to misclassification and therefore underestimates the true associations. In addition, this study included only middle-aged and older men, so our findings may not be generalizable to women or younger populations.

In conclusion, in this prospective population-based study, serum LA and AA concentrations and D5D activity were associated with a lower risk of incident T2D and GLA and DGLA concentrations and D6D activity with a higher risk among middle-aged and older men from eastern Finland. In addition, our results suggest that higher serum zinc concentrations may modify the associations of n–6 PUFAs on the risk of T2D. The results indicate that n–6 PUFAs may have a significant impact on the risk of T2D. However, the role of the mainly endogenously produced, minor n–6 PUFAs, GLA and DGLA, needs to be elucidated. Further research is also needed to elucidate how zinc and other factors involved in PUFA metabolism could modify the impact of PUFAs on the risk of T2D and other chronic diseases.

The authors' responsibilities were as follows—TY: analyzed the data and drafted the manuscript; JKV: analyzed the data and had primary responsibility for the final content; SV, T-PT, AR, TN, and JKV: acquired the data and critically revised the manuscript for important intellectual content; and all authors: designed the research and read and approved the final manuscript. None of the authors had a conflict of interest.

REFERENCES

1. Rhodes ET, Prosser L, Hoerger T, Lieu T, Ludwig D, Laffel L. Estimated morbidity and mortality in adolescents and young adults diagnosed with type 2 diabetes mellitus. Diabet Med 2012;29:453–63.
2. Herman WH, Zimmet P. Type 2 diabetes: an epidemic requiring global attention and urgent action. Diabetes Care 2012;35:943–4.

3. Risérus U, Willett WC, Hu FB. Dietary fats and prevention of type 2 diabetes. Prog Lipid Res 2009;48:44–51.
4. Arab L. Biomarkers of fat and fatty acid intake. J Nutr 2003;133:925S–32S.
5. Murff HJ, Edwards TL. Endogenous production of long-chain polyunsaturated fatty acids and metabolic disease risk. Curr Cardiovasc Risk Rep 2014;8:418.
6. Kröger J, Zietemann V, Enzenbach C, Weikert C, Jansen EH, Doring F, Joost HG, Boeing H, Schulze MB. Erythrocyte membrane phospholipid fatty acids, desaturase activity, and dietary fatty acids in relation to risk of type 2 diabetes in the European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam Study. Am J Clin Nutr 2011;93:127–42.
7. Alhazmi A, Stojanovski E, Garg ML, McEvoy M. Fasting whole blood fatty acid profile and risk of type 2 diabetes in adults: a nested case control study. PLoS One 2014;9:e97001.
8. Lankinen MA, Stančáková A, Uusitupa M, Ågren J, Pihlajamäki J, Kuusisto J, Schwab U, Laakso M. Plasma fatty acids as predictors of glycaemia and type 2 diabetes. Diabetologia 2015;58:2533–44.
9. Mahendran Y, Cederberg H, Vangipurapu J, Kangas AJ, Soininen P, Kuusisto J, Uusitupa M, Ala-Korpela M, Laakso M. Glycerol and fatty acids in serum predict the development of hyperglycemia and type 2 diabetes in Finnish men. Diabetes Care 2013;36:3732–8.
10. Mahendran Y, Agren J, Uusitupa M, Cederberg H, Vangipurapu J, Stancakova A, Schwab U, Kuusisto J, Laakso M. Association of erythrocyte membrane fatty acids with changes in glycemia and risk of type 2 diabetes. Am J Clin Nutr 2014;99:79–85.
11. Hodge AM, English DR, O'Dea K, Sinclair AJ, Makrides M, Gibson RA, Giles GG. Plasma phospholipid and dietary fatty acids as predictors of type 2 diabetes: interpreting the role of linoleic acid. Am J Clin Nutr 2007;86:189–97.
12. Takkunen MJ, Schwab US, de Mello VD, Eriksson JG, Lindström J, Tuomilehto J, Uusitupa MI; DPS Study Group. Longitudinal associations of serum fatty acid composition with type 2 diabetes risk and markers of insulin secretion and sensitivity in the Finnish Diabetes Prevention Study. Eur J Nutr 2015 May 1 (Epub ahead of print; DOI: 10.1007/s00394-015-0911-4).
13. Wang L, Folsom AR, Zheng ZJ, Pankow JS, Eckfeldt JH, ARIC Study Investigators. Plasma fatty acid composition and incidence of diabetes in middle-aged adults: the Atherosclerosis Risk in Communities (ARIC) Study. Am J Clin Nutr 2003;78:91–8.
14. Vessby B, Aro A, Skarfors E, Berglund L, Salminen I, Lithell H. The risk to develop NIDDM is related to the fatty acid composition of the serum cholesterol esters. Diabetes 1994;43:1353–7.
15. Harris WS, Shearer GC. Omega-6 fatty acids and cardiovascular disease: friend, not foe? Circulation 2014;130:1562–4.
16. Laaksonen DE, Lakka T, Lakka H, Nyyssönen K, Rissanen T, Niskanen L, Salonen JT. Serum fatty acid composition predicts development of impaired fasting glycaemia and diabetes in middle-aged men. Diabet Med 2002;19:456–64.
17. Russo C, Olivieri O, Girelli D, Guarini P, Pasqualini R, Azzini M, Corrocher R. Increased membrane ratios of metabolite to precursor fatty acid in essential hypertension. Hypertension 1997;29:1058–63.
18. Yary T, Virtanen JK, Ruusunen A, Tuomainen T, Voutilainen S. Serum zinc and risk of type 2 diabetes incidence in men: the Kuopio Ischaemic Heart Disease Risk Factor Study. J Trace Elem Med Biol 2016;33:120–4.
19. 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.
20. Salonen JT, Nyyssonen K, Korpela H, Tuomilehto J, Seppanen R, Salonen R. High stored iron levels are associated with excess risk of myocardial infarction in eastern Finnish men. Circulation 1992;86:803–11.
21. Lakka TA, Venalainen 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 in men. N Engl J Med 1994;330:1549–54.
22. Kauhanen J, Julkunen J, Salonen JT. Coping with inner feelings and stress: heavy alcohol use in the context of alexithymia. Behav Med 1992;18:121–6.
23. Voutilainen S, Rissanen TH, Virtanen J, Lakka TA, Salonen JT. Low dietary folate intake is associated with an excess incidence of acute coronary events: the Kuopio Ischemic Heart Disease Risk Factor Study. Circulation 2001;103:2674–80.
24. Wallace TM, Matthews D. The assessment of insulin resistance in man. Diabet Med 2002;19:527–34.
25. Salonen JT, Salonen R, Seppanen K, Kantola M, Suntioinen S, Korpela H. Interactions of serum copper, selenium, and low density lipoprotein cholesterol in atherogenesis. BMJ 1991;302:756–60.
26. Stillwell W, Wassall SR. Docosahexaenoic acid: membrane properties of a unique fatty acid. Chem Phys Lipids 2003;126:1–27.
27. Das UN. A defect in $\Delta 6$ and $\Delta 5$ desaturases may be a factor in the initiation and progression of insulin resistance, the metabolic syndrome and ischemic heart disease in South Asians. Lipids Health Dis 2010;9:130.
28. Bruneau C, Staedel-Flaig C, Crémel G, Leray C, Beck J, Hubert P. Influence of lipid environment on insulin binding in cultured hepatoma cells. Biochim Biophys Acta 1987;928:287–96.
29. Summers LK, Fielding B, Bradshaw H, Ilic V, Beysen C, Clark M, Moore N, Frayn K. Substituting dietary saturated fat with polyunsaturated fat changes abdominal fat distribution and improves insulin sensitivity. Diabetologia 2002;45:369–77.
30. Perreault M, Roke K, Badawi A, Nielsen DE, Abdelmagid SA, El-Sohemy A, Ma DW, Mutch DM. Plasma levels of 14:0, 16:0, 16:1n-7, and 20:3n-6 are positively associated, but 18:0 and 18:2n-6 are inversely associated with markers of inflammation in young healthy adults. Lipids 2014;49:255–63.
31. Patel PS, Sharp SJ, Jansen E, Luben RN, Khaw KT, Wareham NJ, Forouhi NG. Fatty acids measured in plasma and erythrocyte-membrane phospholipids and derived by food-frequency questionnaire and the risk of new-onset type 2 diabetes: a pilot study in the European Prospective Investigation into Cancer and Nutrition (EPIC)-Norfolk cohort. Am J Clin Nutr 2010;92:1214–22.
32. Suresh Y, Das U. Protective action of arachidonic acid against alloxan-induced cytotoxicity and diabetes mellitus. Prostaglandins Leukot Essent Fatty Acids 2001;64:37–52.
33. Krishna Mohan I, Das U. Prevention of chemically induced diabetes mellitus in experimental animals by polyunsaturated fatty acids. Nutrition 2001;17:126–51.
34. Kapoor R, Huang Y. Gamma linolenic acid: an antiinflammatory omega-6 fatty acid. Curr Pharm Biotechnol 2006;7:531–4.
35. Krachler B, Norberg M, Eriksson JW, Hallmans G, Johansson I, Vessby B, Weinehall L, Lindahl B. Fatty acid profile of the erythrocyte membrane preceding development of type 2 diabetes mellitus. Nutr Metab Cardiovasc Dis 2008;18:503–10.
36. Kurotani K, Sato M, Ejima Y, Nanri A, Yi S, Pham NM, Akter S, Poudel-Tandukar K, Kimura Y, Imaizumi K. High levels of stearic acid, palmitoleic acid, and dihomo-γ-linolenic acid and low levels of linoleic acid in serum cholesterol ester are associated with high insulin resistance. Nutr Res 2012;32:669–75.e3.
37. Kröger J, Schulze MB. Recent insights into the relation of Delta5 desaturase and Delta6 desaturase activity to the development of type 2 diabetes. Curr Opin Lipidol 2012;23:4–10.
38. Tosi F, Sartori F, Guarini P, Olivieri O, Martinelli N. Delta-5 and Delta-6 desaturases: crucial enzymes in polyunsaturated fatty acid-related pathways with pleiotropic influences in health and disease. Adv Exp Med Biol 2014;824:61–81.
39. Huang YS, Cunnane S, Horrobin D, Davignon J. Most biological effects of zinc deficiency corrected by γ-linolenic acid (18:3ω6) but not by linoleic acid (18:2ω6). Atherosclerosis 1982;41:193–207.