Serum n–6 polyunsaturated fatty acids and risk of death: the Kuopio Ischaemic Heart Disease Risk Factor Study

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ABSTRACT

Background: The cardioprotective properties of linoleic acid (LA), a major n–6 (ω-6) polyunsaturated fatty acid (PUFA), have been recognized, but less is known about its associations with other causes of death. Relatively little is also known about how the minor n–6 PUFAs—γ-linolenic acid (GLA), dihomo-γ-linolenic acid (DGLA), and arachidonic acid (AA)—relate to mortality risk.

Objective: We investigated the associations of serum n–6 PUFAs, an objective biomarker of exposure, with risk of death in middle-aged and older men and whether disease history modifies the associations.

Design: We included 2480 men from the prospective Kuopio Ischaemic Heart Disease Risk Factor Study, aged 42–60 y at baseline in 1984–1989. The stratified analyses by baseline disease status included 1019 men with a history of cardiovascular disease (CVD), cancer, or diabetes and 1461 men without a history of disease.

Results: During the mean follow-up of 22.4 y, 1143 deaths due to disease occurred. Of these, 575 were CVD deaths, 317 were cancer deaths, and 251 were other-cause deaths. A higher serum LA concentration was associated with a lower risk of death from any cause (multivariable-adjusted HR for the highest compared with the lowest quintile: 0.57; 95% CI: 0.46, 0.71; P-trend < 0.001) and with deaths due to CVD (extreme-quintile HR: 0.54; 95% CI: 0.40, 0.74; P-trend < 0.001) and non-CVD or noncancer causes (HR: 0.48; 95% CI: 0.30, 0.76; P-trend $= 0.001$). Serum AA had similar, although weaker, inverse associations. Serum GLA and DGLA were not associated with risk of death, and none of the fatty acids were associated with cancer mortality. The results were generally similar among those with or without a history of major chronic disease (P-interaction > 0.13).

Conclusions: Our findings showed an inverse association of a higher biomarker of LA intake with total and CVD mortality and little concern for risk, thus supporting the current dietary recommendations to increase LA intake for CVD prevention. The finding of an inverse association of serum AA with the risk of death needs replication in other populations. *Am J Clin Nutr* 2018;107:427–435.

Keywords: mortality, polyunsaturated fatty acids, population study, prospective study, cardiovascular disease, cancer

INTRODUCTION

Dietary guidelines recommend partial replacement of saturated fat with n–3 and n–6 PUFAs to reduce the risk of cardiovascular disease (CVD). This is largely based on the established beneficial effect of this replacement on serum LDL-cholesterol concentrations (1). Effects on CVD have also been investigated in dietary fat modification trials, in which saturated fat sources, mainly butter, were replaced by predominantly n–6 PUFA [especially linoleic acid (LA)]–containing vegetable oils and margarines. However, many of these trials did not find a significant effect on CVD incidence, and meta-analyses from these trials have come to divergent conclusions (2–6). The majority of the previous studies were secondary-prevention trials, so the effect of dietary fat modification for primary prevention also remains unclear. The mixed evidence has led to ongoing debate with regard to the scientific validity of existing dietary advice to replace saturated fat with n–6 PUFA-rich vegetable oils and highlights the need for additional research to address this question of major clinical and public health importance.

Apart from CVD, concerns have also been raised with regard to the influence of n–6 PUFAs on the risk of several other diseases and overall mortality (7). A primary hypothesized mechanism that may lead to harm suggests that because LA and its endogenous metabolites (**Supplemental Figure 1**), particularly arachidonic acid (AA; 20:4n–6), are substrates for several proinflammatory mediators, increased LA intake may promote chronic low-level inflammation associated with several chronic diseases,

The authors reported no funding received for this study.

Supplemental Figures 1–4 and Supplemental Tables 1–9 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/ajcn/.

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Abbreviations used: AA, arachidonic acid; AR, absolute risk; ARR, absolute risk reduction; CHD, coronary artery disease; DGLA, dihomo-γ-linolenic acid; GLA, γ-linolenic acid; ICD, International Classification of Diseases; KIHD, Kuopio Ischaemic Heart Disease Risk Factor Study; LA, linoleic acid.

Received August 29, 2017. Accepted for publication December 4, 2017.

First published online March 16, 2018; doi: https://doi.org/10.1093/ajcn/nqx063.

such as cancer, diabetes, and neurodegeneration (8–10). In addition, because the metabolism of n–3 and n–6 PUFAs shares the same metabolic pathways, n–6 PUFAs could compete for the same enzymes as the n–3 PUFAs, thereby theoretically negating the anti-inflammatory and other beneficial effects of n–3 PUFAs. Yet, most of the observational evidence to date has focused on the association of n–6 PUFAs with cardiovascular outcomes, with relatively few studies assessing the relation of n–6 PUFAs with non-CVD endpoints and total mortality.

Because of the limited and mixed evidence, our purpose was to assess the prospective associations of serum n–6 PUFAs with the risk of death from any disease and of cause-specific mortality in middle-aged and older men from eastern Finland. By using circulating n–6 PUFAs as an exposure, we were able to assess LA and AA, in addition to 2 other, mainly endogenously produced, n–6 PUFAs: γ-linolenic acid (GLA; 18:3n–6) and dihomo-γ-linolenic acid (DGLA; 20:3n–6). In addition, to allow inferences on the potential of n–6 PUFAs for primary and secondary prevention, we also separately investigated the associations of n–6 PUFAs among those free of major chronic disease at baseline (CVD, cancer, or type 2 diabetes) and among those with a history of disease.

METHODS

Study population

The Kuopio Ischaemic Heart Disease Risk Factor Study (KIHD) was designed to investigate risk factors for CVD, atherosclerosis, and related outcomes in a prospective, population-based sample of men from eastern Finland (11). Therefore, the non-CVD outcomes used in the current study can be regarded as secondary endpoints and those results considered as exploratory. The baseline examinations were carried out in 1984–1989. A total of 2682 men (83% of those eligible) who were 42, 48, 54 or 60 y old at baseline were recruited in 2 cohorts (**Supplemental Figure 2**). The first cohort consisted of 1166 men aged 54 y who enrolled in 1984–1986, and the second cohort included 1516 men aged 42, 48, 54, or 60 y who enrolled in 1986–1989. The baseline examinations were followed by the 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 of the men from the second cohort were invited and 854 men (95% of those eligible) participated. The baseline characteristics of the entire study population have been described (12). The KIHD protocol was approved by the Research Ethics Committee of the University of Kuopio. All of the subjects gave written informed consent for participation. Subjects with missing baseline data on serum fatty acids ($n = 161$) were excluded, which left 2480 men for the main analyses. The stratified analyses by baseline disease status included 1019 men with a history of CVD ($n = 926$), cancer ($n = 48$), or type 2 diabetes ($n = 148$) and 1431 men without history of disease.

Measurements

Fasting venous blood samples were collected between 0800 and 0010 at the baseline examinations. Subjects were instructed to abstain from ingesting alcohol for 3 d and from smoking and eating for 12 h before providing the sample. Detailed descriptions of the determination of serum lipids and lipoproteins (13), assessment of medical history and medications at baseline (13), family history of diseases (13), smoking (13), alcohol intake (13), blood pressure (13), and physical activity (14) have been published. Serum C-reactive protein was measured with an immunometric assay (Immulite High Sensitivity CRP Assay; Diagnostics Products Corporation, USA). BMI was computed as the ratio of weight in kilograms to the square of height in meters. Education was assessed in years by using a self-administered questionnaire. Hypertension was defined as blood pressure >140/90 mm Hg or treatment for hypertension. Diabetes was defined as self-reported diabetes or fasting blood glucose of ≥6.7 mmol/L. Dietary intakes were assessed with 4-d food records (15).

Serum fatty acids

Serum esterified and nonesterified fatty acids were measured in 1991 from samples that had been stored at −80°C in 1 gas chromatographic run, as described previously (16). Serum fatty acids were extracted with chloroform-methanol. The chloroform phase was evaporated and treated with sodium methoxide, which methylated the esterified fatty acids. Quantification was carried out with reference standards purchased from NU-Check Prep, Inc. Each analyte had an individual reference standard, and the internal standard used was eicosane. Fatty acids were chromatographed in an NB-351 capillary column (HNU-Nordion) by a Hewlett-Packard 5890 Series II gas chromatograph (Hewlett-Packard Company; since 1999, Agilent Technologies, Inc.) with a flame ionization detector. Results are presented as a proportion of total serum fatty acids. The interassay CVs for repeated measurements were 8.7% for LA, 11.6% for GLA, 8.3% for DGLA, and 9.9% for AA.

Ascertainment of follow-up events

Deaths were ascertained by computer linkage to the national cause of death register with the use of the Finnish personal identification code (social security number). There were no losses to follow-up. All of the deaths were coded according to the International Classification of Diseases (ICD), 10th revision, codes. All disease deaths that occurred from the study entry to 31 December 2014 were included. ICD codes I00–I99 and C00–D48 were used to define CVD and cancer deaths, respectively. Deaths due to accidents or suicides (ICD codes S00–T98) were not included.

Statistical analysis

The univariate relations of serum n–6 PUFAs with baseline characteristics were assessed by means and linear regression (for continuous variables) or chi-square tests (for categorical variables). Cox proportional hazards regression models adjusted for relevant covariates were used to estimate HRs of incident events. The validity of the proportional hazards assumption was evaluated by using Schoenfeld residuals, and the assumptions were met. Absolute risk reduction (ARR) was calculated by multiplying the absolute risk (AR) in the reference group by the multivariable-adjusted HR reduction in the comparison group. The confounders were selected on the basis of

established risk factors for mortality, previously published associations in the KIHD, or on associations with exposures or outcomes in the present analysis. Model 1 included age (years) and examination year. The multivariable model 2 included model 1 and BMI (in kg/m^2); smoking (pack-years); education (years); income (in euros); leisure-time physical activity (kilocalories per day); intake of alcohol (grams per week); serum long-chain n–3 PUFAs (percentage); hypertension (yes or no); family history of CVD, cancer, or diabetes (yes or no); use of hypercholesterolemia, hypertension, or diabetes medications at baseline or during follow-up (yes or no); and intakes of SFAs (percentage of energy), MUFAs (percentage of energy), *trans* fatty acids (percentage of energy), fiber (grams per day), and fruit, berries, and vegetables (grams per day). We also further adjusted model 2 for potential effect mediators: serum LDL and HDL cholesterol, triglycerides, and C-reactive protein. All of the quantitative variables were entered in the models as continuous variables. The cohort mean was used to replace missing values in covariates (<3%). Significance of the interactions on a multiplicative scale was assessed by stratified analysis and likelihood ratio tests by 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. Potential nonlinear associations were assessed semiparametrically by using restricted cubic splines. Restricted cubic spline analysis was also used to evaluate the relation of dietary intakes of LA and AA with the serum concentrations. All *P* values were 2-tailed ($\alpha = 0.05$). Data were analyzed by using SPSS 21.0 for Windows (IBM Corporation.) and Stata version 13.1 for spline analysis (StataCorp).

RESULTS

Of the serum n–6 PUFAs, LA was the most abundant, accounting for 80.5% of serum n–6 PUFAs, followed by AA (14.5% of the n–6 PUFAs) (**Supplemental Table 1**). LA and AA

TABLE 1
Baseline characteristics of the 2480 men from the Kuopio Ischaemic Heart Disease Risk Factor Study in 1984–1989[1]

	All ($n = 2480$)	Disease history ($n = 1019$)	No disease history ($n = 1461$)
Age, y	53.0 ± 5.2	54.4 ± 4.3	52.0 ± 5.5*
Serum long-chain n–3 PUFAs, %	4.7 ± 1.6	4.6 ± 1.6	4.7 ± 1.0
Serum α-linolenic acid, %	0.73 ± 0.24	0.75 ± 0.23	0.72 ± 0.25*
BMI, kg/m^2	26.9 ± 3.5	27.4 ± 3.8	26.6 ± 3.3*
Leisure-time physical activity, kcal/d	141 ± 176	149 ± 199	135 ± 158
Income, €/y	13,183 ± 8828	11,548 ± 7700	14,332 ± 9374*
Education, y	8.6 ± 3.4	8.2 ± 3.1	9.0 ± 3.5*
Alcohol, g/wk	74 ± 130	75 ± 144	73 ± 119
Smoking, %	31.6	32.8	30.7
History of cardiovascular disease, %	37.3	90.0	0*
Family history of cardiovascular disease, %	82.1	87.3	78.4*
Hypercholesterolemia medication at baseline, %	0.6	1.3	0.1*
Hypercholesterolemia medication during follow-up, %	51.4	53.1	50.2
History of cancer, %	1.9	4.7	0*
Family history of cancer, %	24.4	23.8	24.8
Diabetes, %	5.8	14.1	0*
Family history of diabetes, %	28.3	31.6	26.0*
Diabetes medication at baseline, %	1.2	2.9	0*
Diabetes medication during follow-up, %	23.3	29.3	19.1*
Hypertension, %	60.6	71.0	53.5*
Hypertension medication at baseline, %	22.7	40.0	10.5*
Hypertension medication during follow-up, %	76.9	79.2	75.4*
Dietary intakes			
Energy, kcal/d	2438 ± 622	2375 ± 602	2481 ± 631*
SFAs, % of energy	18.2 ± 4.1	18.2 ± 4.4	18.1 ± 3.9
MUFAs, % of energy	11.7 ± 2.2	11.8 ± 2.3	11.7 ± 2.2
PUFAs, % of energy	4.5 ± 1.4	4.5 ± 1.4	4.5 ± 1.4
Linoleic acid	3.3 ± 1.3	3.3 ± 1.3	3.3 ± 1.3
Arachidonic acid	0.07 ± 0.03	0.07 ± 0.03	0.07 ± 0.03
α-Linolenic acid	0.57 ± 0.23	0.58 ± 0.23	0.56 ± 0.22*
EPA + DHA	0.17 ± 0.18	0.17 ± 0.18	0.17 ± 0.19
trans Fatty acids, % of energy	1.1 ± 0.4	1.1 ± 0.4	1.0 ± 0.4*
Vegetable oils, g/d	2 ± 4	2 ± 3	2 ± 4
Vegetable margarine, g/d	18 ± 17	18 ± 18	17 ± 17
Fiber, g/d	25 ± 7	25 ± 7	25 ± 7
Fruit, berries, and vegetables,[2] g/d	252 ± 157	243 ± 155	259 ± 158*
Red meat, g/d	139 ± 75	137 ± 75	140 ± 76

[1] Values are means ± SDs unless otherwise indicated. *P-trend across quintiles: <0.05. P-trend values were assessed by using linear regression (continuous variables) or chi-square test (bivariate relations).

[2] Excluding potatoes.

TABLE 2
Risk of death from any disease in quintiles of serum n–6 PUFAs among 2480 men from the Kuopio Ischaemic Heart Disease Risk Factor Study[1]

	Serum fatty acid quintile						Per 1-SD change			
	1 ($n = 496$)	2 ($n = 496$)	3 ($n = 496$)	4 ($n = 496$)	5 ($n = 496$)	*P*-trend	All ($n = 2480$)	Disease history[2] ($n = 1019$)	No disease history ($n = 1461$)	*P*-interaction
LA, %	20.48	24.06	26.39	28.75	32.19					
Cases, *n*	290	242	209	210	192		1143	597	546	
Person-years	9949	11,012	11,430	11,412	11,762		55,565	21,019	34,535	
Model 1	1	0.73	0.61	0.63	0.56 (0.47, 0.67)[3]	<0.001	0.80 (0.76, 0.85)	0.81 (0.74, 0.88)	0.82 (0.76, 0.90)	0.96
Model 2	1	0.69	0.63	0.69	0.57 (0.46, 0.71)	<0.001	0.81 (0.76, 0.87)	0.81 (0.73, 0.90)	0.86 (0.77, 0.95)	0.47
GLA, %	0.16	0.22	0.27	0.33	0.43					
Cases, *n*	233	229	241	233	207					
Person-years	11,146	10,720	11,168	11,105	11,425					
Model 1	1	0.98	1.04	1.06	0.96 (0.79, 1.15)	0.87	0.99 (0.93, 1.05)	0.97 (0.90, 1.06)	1.01 (0.93, 1.10)	0.55
Model 2	1	1.04	1.04	1.03	0.90 (0.74, 1.09)	0.25	0.96 (0.91, 1.02)	0.93 (0.86, 1.02)	0.98 (0.90, 1.06)	0.67
DGLA, %	1.00	1.20	1.32	1.46	1.68					
Cases, *n*	244	241	208	220	230					
Person-years	10,896	10,795	11,267	11,509	11,098					
Model 1	1	1.01	0.88	0.84	1.01 (0.84, 1.20)	0.52	0.98 (0.92, 1.05)	0.97 (0.88, 1.07)	0.99 (0.91, 1.08)	0.82
Model 2	1	1.00	0.95	0.88	0.95 (0.78, 1.15)	0.33	0.97 (0.91, 1.04)	0.96 (0.87, 1.06)	0.98 (0.90, 1.08)	0.57
AA, %	3.54	4.20	4.70	5.25	6.06					
Cases, *n*	276	247	213	197	210					
Person-years	10,453	10,944	11,401	11,573	11,192					
Model 1	1	0.88	0.72	0.68	0.76 (0.63, 0.90)	<0.001	0.87 (0.82, 0.92)	0.90 (0.83, 0.97)	0.88 (0.81, 0.96)	0.76
Model 2	1	0.87	0.73	0.73	0.80 (0.65, 0.98)	0.01	0.88 (0.82, 0.94)	0.89 (0.81, 0.98)	0.91 (0.82, 1.00)	0.79

[1] Values are medians unless otherwise indicated. Model 1 adjusted for age and examination year; model 2 adjusted as for model 1 and BMI (kg/m^2); family history of type 2 diabetes (yes or no); smoking (pack-years); education (years); income (€); leisure-time physical activity (kilocalories per week); intake of alcohol (grams per week); serum long-chain n–3 PUFAs (percentage); hypertension (yes or no); family history of cardiovascular disease, cancer, or diabetes (yes or no); use of hypercholesterolemia, hypertension, or diabetes medications at baseline or during follow-up (yes or no); and intakes of SFAs (percentage of energy), MUFAs (percentage of energy), *trans* fatty acids (percentage of energy), fiber (grams per day), and fruit, berries, and vegetables (grams per day). AA, arachidonic acid; DGLA, dihomo-γ-linolenic acid; GLA, γ-linolenic acid; LA, linoleic acid.

[2] Disease was defined as cardiovascular disease ($n = 926$), cancer ($n = 48$), or type 2 diabetes ($n = 148$).

[3] HRs with 95% CIs in parentheses (all such values). Obtained from Cox proportional hazards regression models. The significance of the interactions on a multiplicative scale was assessed by stratified analysis and likelihood ratio tests by using a cross-product term.

concentrations were slightly higher among those without a history of disease. Only serum GLA and DGLA showed a moderate correlation with each other ($r = 0.54$); all other intercorrelations were weak ($r \leq 0.19$) (Supplemental Table 1). In the subset of 801 men for whom data from the KIHD 4-y and 11-y re-examinations also were available (Supplemental Figure 2), Spearman correlation coefficients between baseline, 4-y, and 11-y serum n–6 PUFA values were all $\geq$0.5 (**Supplemental Table 2**).

Baseline characteristics for the whole study population and for those with or without history of CVD, cancer, or diabetes are presented in **Table 1**. Men with a history of disease were older, had a higher BMI, and less education and income and lower intakes of energy, fruit, berries, and vegetables.

In univariate analyses, serum LA and AA showed similar, beneficial associations with several mortality risk factors at baseline (**Supplemental Tables 3** and **4**). For example, higher serum LA or AA concentrations were related to younger age, lower BMI, less smoking, or lower likelihood of having diabetes or hypertension or of using diabetes or hypertension medications during follow-up. In contrast, higher serum LA was related to lower serum long-chain n–3 PUFA concentration and to lower alcohol intake, whereas higher serum AA was related to higher serum long-chain n–3 PUFA concentration and higher alcohol intake. The associations were also quite different with dietary factors (Supplemental Tables 3 and 6). A higher LA concentration was related to, for example, higher intakes of vegetable margarines and fruit, berries, and vegetables, whereas a higher AA concentration was related to a higher intake of red meat. Those with higher serum GLA or DGLA concentrations were, for example, more likely to be younger, have a lower serum long-chain n–3 PUFA concentration, use diabetes medication during follow-up, have a lower PUFA intake, and be less likely to smoke (**Supplemental Tables 5** and **6**).

Estimated dietary LA intake showed a nonlinear association with serum LA concentration; the association was relatively linear with intakes of $\leq$4% of energy, with a smaller increase in serum concentrations with higher intakes (P value for overall association < 0.001; for nonlinearity, $P < 0.001$; **Supplemental Figure 3**). Similarly, a higher AA intake was associated with higher serum AA (P-overall < 0.001, P-nonlinearity $= 0.03$; Supplemental Figure 3). We did not have information on GLA and DGLA intakes. LA intake showed a weak, inverse association with serum DGLA ($\beta = -0.012$; 95% CI: -0.003, -0.021), but no association with serum GLA ($\beta = -0.002$; 95% CI: -0.006, 0.001) or AA ($\beta = -0.026$; 95% CI: -0.057, 0.005).

During the mean $\pm$ SD follow-up of 22.4 $\pm$ 7.8 y (55,565 person-years), 1143 deaths due to disease occurred in the whole study population (incidence rate: 2.1 events/100 person-years). Of these, 575 (50.3%) were CVD deaths, 317 (27.7%) were cancer deaths, and 251 (22.0%) were deaths due to other causes. The most common non-CVD or noncancer causes of death were deaths due to dementia and Alzheimer disease (6.1% of all disease deaths), respiratory-related causes (3.7%), and liver disease (2.8%). The incidence rate of death was higher among men with history of major diseases (mean $\pm$ SD follow-up: 20.6 $\pm$ 8.5 y; 2.8 events/100 person-years) than among men without such

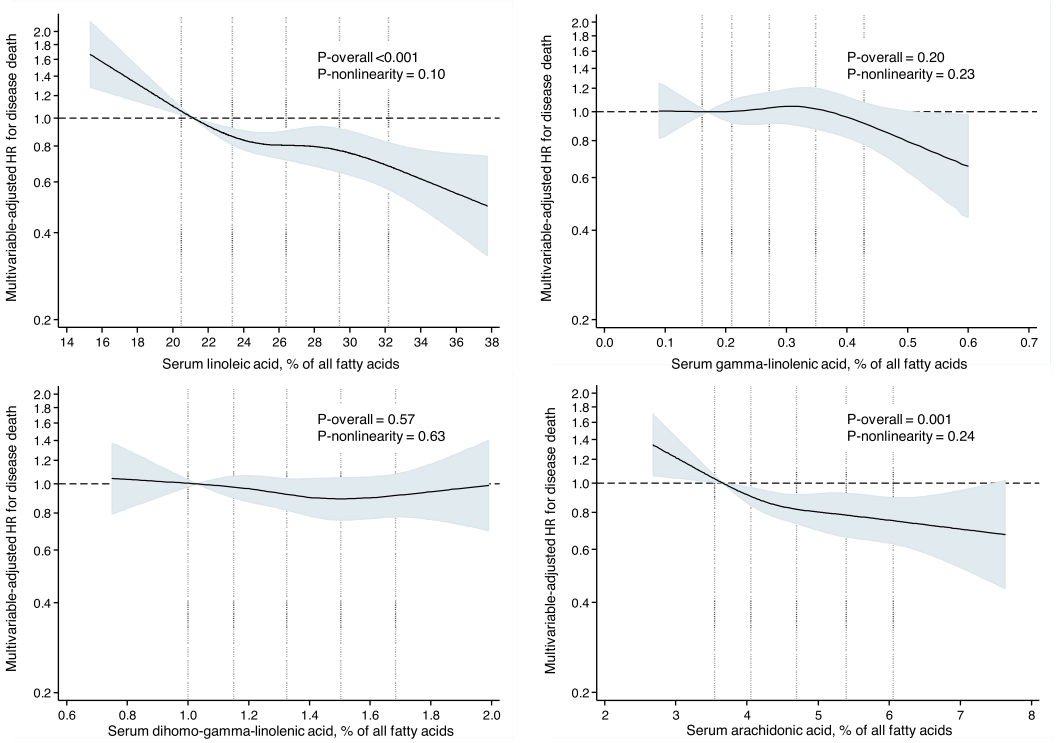

FIGURE 1 Multivariable-adjusted HRs of serum n–6 PUFAs with risk of disease death among 2480 men, evaluated by restricted cubic splines from Cox proportional hazards models. The models were adjusted for age; examination year; BMI (kg/m^2); family history of type 2 diabetes (yes or no); smoking (pack-years); education (years); income (euros); leisure-time physical activity (kilocalories per day); intake of alcohol (grams per day); serum long-chain n–3 PUFAs (percentage); hypertension (yes or no); family history of cardiovascular disease, cancer, or diabetes (yes or no); use of hypercholesterolemia, hypertension, or diabetes medications at baseline or during follow-up (yes or no); and intakes of SFAs (percentage of energy), MUFAs (percentage of energy), *trans* fatty acids (percentage of energy), fiber (grams per day), and fruit, berries, and vegetables (grams per day). The solid lines represent the central risk estimates, and the shaded areas represent 95% CIs, relative to the reference level (12.5th percentile). The dotted vertical lines correspond to the 10th, 25th, 50th, 75th and 90th percentiles of fatty acid concentrations.

history (mean ± SD follow-up: 23.6 ± 6.9 y; 1.6 events/100 person-years).

The associations of the serum n–6 PUFAs with the risk of any death due to disease are shown in **Table 2**. Both serum LA and AA were associated with a lower risk of total mortality, but GLA and DGLA were not associated. Those in the highest compared with the lowest LA quintile had multivariable-adjusted 43% (95% CI: 29%, 64%) lower relative risk of death and those in the highest compared with the lowest AA quintile had a 20% (95% CI: 2%, 35%) lower relative risk (model 2; Table 2) (the AR in the lowest LA quintile was 58.5% and in the lowest AA quintile 55.6%, the ARR was 25.4% in the highest LA quintile and 11.2% in the highest AA quintile). Each 1-SD increase in serum LA (4.7% of total fatty acids) was associated with a multivariable-adjusted 19% (95% CI: 13%, 24%) lower relative risk of disease death, and each 1-SD increase in serum AA (1.0% of total fatty acids) was associated with a 12% (95% CI: 6%, 18%) lower risk (Table 2). The associations were generally similar among men with or without history of disease (Table 2). Restricted cubic splines analysis showed relatively linear associations of serum LA and AA with a lower risk of death (for overall associations: $P < 0.001$ and $P = 0.001$, respectively) and little evidence for nonlinearity ($P = 0.10$ and $P = 0.24$, respectively) (**Figure 1**).

When we investigated the associations with the major causes of death, CVD and cancer, serum LA showed an inverse association with CVD mortality, with a multivariable-adjusted 46% (95% CI: 26%, 60%) lower relative risk in the highest compared with the lowest quintile (AR in the reference group: 31.5%; ARR: 14.7%) (**Table 3, Supplemental Figure 4**). In addition, serum AA showed a trend toward a lower risk of CVD death (Table 3). GLA or DGLA was not associated with a risk of CVD death (Table 3). None of the n–6 PUFAs were associated with risk of cancer death (**Table 4,** Supplemental Figure 4). The associations were again generally similar in the analyses stratified by disease status (Tables 3 and 4).

When we further explored the associations with specific causes of death, serum LA showed an inverse association with the risk of death due to coronary artery disease (CAD; extreme-quintile HR: 0.47; 95% CI: 0.33, 0.68) and non-CVD or noncancer death (HR: 0.48; 95% CI: 0.30, 0.76) (**Supplemental Tables 7** and **8**, Supplemental Figure 4). Serum AA showed a borderline significant inverse association with the risk of death due to CAD (extreme-quintile HR: 0.71; 95% CI: 0.50, 1.01) and a trend toward a lower risk of non-CVD or noncancer death (P-trend = 0.02) (Supplemental Tables 7 and 8). Serum GLA and DGLA showed no association with these outcomes, and again we did not find evidence for effect modification by history of major diseases (Supplemental Tables 7 and 8).

The associations of serum LA and AA were modestly attenuated after further adjustment for potential effect mediators: serum LDL and HDL cholesterol, triglycerides, and C-reactive

TABLE 3
Risk of cardiovascular disease death in quintiles of serum n–6 PUFAs among 2480 men from the Kuopio Ischaemic Heart Disease Risk Factor Study[1]

	Serum fatty acid quintile						Per 1-SD change			
	1 ($n = 496$)	2 ($n = 496$)	3 ($n = 496$)	4 ($n = 496$)	5 ($n = 496$)	*P*-trend	All ($n = 2480$)	Disease history[2] ($n = 1019$)	No disease history ($n = 1461$)	*P*-interaction
LA, %	20.48	24.06	26.39	28.75	32.19					
Cases, *n*	156	122	102	101	94		575	334	241	
Person-years	9949	11,012	11,430	11,412	11,762		55,565	21,019	34,535	
Model 1	1	0.69	0.55	0.57	0.51 (0.40, 0.66)[3]	<0.001	0.78 (0.71, 0.85)	0.79 (0.70, 0.88)	0.81 (0.71, 0.92)	0.95
Model 2	1	0.67	0.62	0.67	0.54 (0.40, 0.74)	<0.001	0.81 (0.73, 0.89)	0.80 (0.70, 0.91)	0.88 (0.75, 1.02)	0.72
GLA, %	0.16	0.22	0.27	0.33	0.43					
Cases, *n*	106	119	123	122	105					
Person-years	11,146	10,720	11,168	11,105	11,425					
Model 1	1	1.12	1.16	1.23	1.07 (0.82, 1.40)	0.57	1.03 (0.95, 1.12)	0.99 (0.89, 1.11)	1.07 (0.95, 1.22)	0.38
Model 2	1	1.14	1.12	1.14	0.99 (0.75, 1.30)	0.83	0.99 (0.91, 1.07)	0.93 (0.83, 1.04)	1.03 (0.90, 1.17)	0.46
DGLA, %	1.00	1.20	1.32	1.46	1.68					
Cases, *n*	115	124	117	99	120					
Person-years	10,896	10,795	11,267	11,509	11,098					
Model 1	1	1.10	1.05	0.80	1.11 (0.86, 1.44)	0.95	1.02 (0.93, 1.11)	0.99 (0.87, 1.12)	1.06 (0.94, 1.20)	0.41
Model 2	1	1.11	1.14	0.84	1.06 (0.80, 1.38)	0.70	1.00 (0.91, 1.10)	0.97 (0.85, 1.10)	1.04 (0.91, 1.18)	0.28
AA, %	3.54	4.20	4.70	5.25	6.06					
Cases, *n*	140	113	117	96	109					
Person-years	10,453	10,944	11,401	11,573	11,192					
Model 1	1	0.80	0.79	0.66	0.78 (0.60, 1.00)	0.02	0.88 (0.81, 0.96)	0.91 (0.82, 1.02)	0.91 (0.80, 1.04)	0.98
Model 2	1	0.79	0.80	0.71	0.80 (0.60, 1.06)	0.09	0.88 (0.80, 0.97)	0.89 (0.79, 1.02)	0.93 (0.80, 1.08)	0.82

[1] Values are medians unless otherwise indicated. Model 1 adjusted for age and examination year; model 2 adjusted as for model 1 and BMI (kg/m^2); family history of type 2 diabetes (yes or no); smoking (pack-years); education (years); income (€); leisure-time physical activity (kilocalories per week); intake of alcohol (grams per week); serum long-chain n–3 PUFAs (percentage); hypertension (yes or no); family history of cardiovascular disease, cancer, or diabetes (yes or no); use of hypercholesterolemia, hypertension, or diabetes medications at baseline or during follow-up (yes or no); and intakes of SFAs (percentage of energy), MUFAs (percentage of energy), *trans* fatty acids (percentage of energy), fiber (grams per day), and fruit, berries, and vegetables (grams per day). AA, arachidonic acid; DGLA, dihomo-γ-linolenic acid; GLA, γ-linolenic acid; LA, linoleic acid.

[2] Disease was defined as cardiovascular disease ($n = 926$), cancer ($n = 48$), or type 2 diabetes ($n = 148$).

[3] HRs with 95% CIs in parentheses (all such values). Obtained from Cox proportional hazards regression models. The significance of the interactions on a multiplicative scale was assessed by stratified analysis and likelihood ratio tests by using a cross-product term.

protein. For example, each 1-SD increase in serum LA was associated with a 16% (HR: 0.84; 95% CI: 0.77, 0.91) lower risk of death due to any disease and each 1-SD increase in serum AA with an 8% (HR: 0.92; 95% CI: 0.85, 0.99) lower risk. In the case of CVD death, each 1-SD increase in LA was associated with a 14% (HR: 0.86; 95% CI: 0.77, 0.97) lower risk and each 1-SD increase in AA with a 5% (HR: 0.95; 95% CI: 0.85, 1.05) lower risk (other data not shown). Because the long follow-up may attenuate the associations with an exposure that is measured only at the study baseline, we also evaluated the associations in analyses limited to the first half (11 y) of follow-up. The associations were generally similar to those with the longer follow-up (**Supplemental Table 9**).

DISCUSSION

In this prospective cohort study in middle-aged and older men from eastern Finland, higher serum concentrations of the major n–6 PUFA LA showed an inverse association with the risk of death due to disease and with CVD, CAD, and non-CVD or non-cancer mortality. Similar, although weaker, inverse associations were observed with serum AA. Both were correlated with their dietary intakes but not with each other. Serum GLA and DGLA were not associated with risk of death, and none of the n–6 PUFAs were associated with cancer mortality. The results were generally similar among those with or without a baseline history of major chronic disease.

There is an ongoing debate about the health benefits of replacing saturated fat with n–6 PUFAs. Some arguments may stem from the findings of the meta-analyses of the dietary fat modification trials that seemed to give little support for the cardiovascular benefits of replacing saturated fat with n–6 PUFAs (2–6). In some of these trials, the replacement of saturated fat sources with vegetable margarines or oils high in n–6 PUFAs failed to reduce CVD or total mortality (2–6). However, the majority of the trials were conducted in the 1960s and 1970s, when n–6 PUFA–rich margarines were also major sources of industrially produced *trans* fatty acids, a major risk factor for all-cause and CAD mortality (17). Many of the trials also had serious limitations, such as low compliance or a low number of events, or methodologic issues, such as single-blinding or lack of appropriate randomization (6, 18), which prevent drawing firm conclusions on the basis of these trials.

Many observational studies do, however, support cardiovascular benefits of higher LA intake, and the benefits do not appear to depend on whether LA replaces saturated fat or carbohydrates in the diet (19). Similar findings have been observed with objective biomarkers of intake, circulating or adipose tissue LA, in some (20–23), although not all (24–27), studies. These studies included participants without CVD at baseline, but our findings suggest that the associations are also similar among men with a history of major chronic disease (of whom 90% had established CVD). Reverse causation is a cause for concern, especially when the study includes participants with established disease, because these individuals may have changed their diet because of the

TABLE 4
Risk of cancer death in quintiles of serum n–6 PUFAs among 2480 men from the Kuopio Ischaemic Heart Disease Risk Factor Study[1]

	Serum fatty acid quintile						Per 1-SD change			
	1 ($n = 496$)	2 ($n = 496$)	3 ($n = 496$)	4 ($n = 496$)	5 ($n = 496$)	*P*-trend	All ($n = 2480$)	Disease history[2] ($n = 1019$)	No disease history ($n = 1461$)	*P*-interaction
LA, %	20.48	24.06	26.39	28.75	32.19					
Cases, *n*	67	66	62	64	58		317	137	180	
Person-years	9949	11,012	11,430	11,412	11,762		55,565	21,019	34,535	
Model 1	1	0.86	0.78	0.82	0.72 (0.51, 1.03)[3]	0.08	0.90 (0.80, 1.01)	0.91 (0.76, 1.09)	0.90 (0.77, 1.04)	0.85
Model 2	1	0.78	0.73	0.83	0.71 (0.47, 1.07)	0.15	0.90 (0.78, 1.03)	0.92 (0.75, 1.13)	0.92 (0.77, 1.10)	0.62
GLA, %	0.16	0.22	0.27	0.33	0.43					
Cases, *n*	64	67	69	58	59					
Person-years	11,146	10,720	11,168	11,105	11,425					
Model 1	1	1.04	1.07	0.95	0.97 (0.68, 1.38)	0.71	0.96 (0.85, 1.07)	0.95 (0.79, 1.13)	0.97 (0.84, 1.13)	0.69
Model 2	1	1.16	1.14	0.97	0.93 (0.65, 1.33)	0.41	0.94 (0.84, 1.05)	0.89 (0.75, 1.06)	0.97 (0.84, 1.13)	0.74
DGLA, %	1.00	1.20	1.32	1.46	1.68					
Cases, *n*	70	69	49	57	72					
Person-years	10,896	10,795	11,267	11,509	11,098					
Model 1	1	1.02	0.72	0.76	1.09 (0.79, 1.52)	0.96	0.96 (0.85, 1.08)	1.04 (0.85, 1.27)	0.91 (0.78, 1.05)	0.25
Model 2	1	0.99	0.77	0.79	1.02 (0.72, 1.46)	0.84	0.95 (0.83, 1.08)	1.01 (0.82, 1.24)	0.92 (0.78, 1.08)	0.28
AA, %	3.54	4.20	4.70	5.25	6.06					
Cases, *n*	68	75	55	66	53					
Person-years	10,453	10,944	11,401	11,573	11,192					
Model 1	1	1.07	0.74	0.91	0.77 (0.54, 1.10)	0.09	0.88 (0.78, 0.99)	0.85 (0.71, 1.01)	0.91 (0.79, 1.06)	0.50
Model 2	1	1.05	0.75	1.01	0.89 (0.59, 1.33)	0.52	0.92 (0.81, 1.05)	0.93 (0.75, 1.14)	0.94 (0.79, 1.13)	0.64

[1] Values are medians unless otherwise indicated. Model 1 adjusted for age and examination year; model 2 adjusted as for model 1 and BMI (kg/m^2); family history of type 2 diabetes (yes or no); smoking (pack-years); education (years); income (€); leisure-time physical activity (kilocalories per week); intake of alcohol (grams per week); serum long-chain n–3 PUFAs (percentage); hypertension (yes or no); family history of cardiovascular disease, cancer, or diabetes (yes or no); use of hypercholesterolemia, hypertension, or diabetes medications at baseline or during follow-up (yes or no); and intakes of SFAs (percentage of energy), MUFAs (percentage of energy), *trans* fatty acids (percentage of energy), fiber (grams per day), and fruit, berries, and vegetables (grams per day). AA, arachidonic acid; DGLA, dihomo-γ-linolenic acid; GLA, γ-linolenic acid; LA, linoleic acid.

[2] Disease was defined as cardiovascular disease ($n = 926$), cancer ($n = 48$), or type 2 diabetes ($n = 148$).

[3] HRs with 95% CIs in parentheses (all such values). Obtained from Cox proportional hazards regression models. The significance of the interactions on a multiplicative scale was assessed by stratified analysis and likelihood ratio tests by using a cross-product term.

disease. Because those with a history of disease are at a higher risk of new events or death than healthy participants, these changes in diet may result in spurious findings that indicate that a better diet (e.g., higher LA intake) is associated with increased risk of incident events or death. However, in our study, the inverse associations of LA with any deaths and with cardiovascular mortality actually appeared to be slightly stronger among those with an established disease. Our findings thus support the cardiovascular benefits of higher LA intake in both the primary and secondary prevention of CVD.

In addition to the inverse association with CVD risk in observational studies, a higher LA intake has had a beneficial impact on, for example, serum lipids (28), liver fat accumulation (29), and glucose metabolism (30). Although some animal studies have suggested that high LA intake induces obesity (31), human studies give little support for these findings (29, 32); and in the current study, LA was inversely related to BMI cross-sectionally. However, a high n–6 PUFA intake has also been suggested to increase the risk of non-CVD outcomes—for example, because of their proinflammatory properties (7). LA and especially AA are indeed substrates to several proinflammatory substances, but both are also substrates to compounds with anti-inflammatory or pro-resolving properties, such as lipoxins, epoxy fatty acids, and nitrated LA (33–36). In experimental studies, even very high intakes of LA or AA have not increased inflammation markers (37–39). High n–6 PUFA intakes could also theoretically reduce the endogenous production of long-chain n–3 PUFAs and subsequently the anti-inflammatory and other beneficial effects of long-chain n–3 PUFAs, because of the shared metabolic pathways of n–3 and n–6 PUFAs. However, typical dietary LA intakes do not seem to affect long-chain n–3 PUFA status (40). Our findings are thus well in line with several previous studies that observed an inverse association of dietary or biomarker LA with total mortality (20–22, 25, 26, 41).

n–6 PUFAs could also contribute to cancer risk, because some AA-derived proinflammatory eicosanoids can affect cell proliferation, apoptosis, angiogenesis, and migration (36, 42). However, observational studies of n–6 PUFA intake and cancer incidence have produced inconsistent findings, with some studies finding a higher risk and some studies a lower risk (42). Only a few studies have investigated the association with cancer mortality, and have either found an inverse association with dietary LA (41) or no association with circulating n–6 PUFAs (22, 27). We found no evidence for increased cancer mortality risk with higher n–6 PUFA exposure, but in contrast, observed an inverse association of both LA and AA with mortality also due to causes other than CVD and cancer. These findings, together with previous observations (22), indicate that a higher intake of n–6 PUFA provides cardiovascular benefits without increasing the risk of non-CVD mortality.

The use of objective biomarkers of intake has the benefit of reducing the measurement error inherent in subjective dietary assessment methods. However, for the biomarker to be useful, it needs to correlate with dietary intakes. LA intake correlates with LA in different tissues and blood compartments (43, 44), and an inverse association with mortality has been observed with

LA measured in adipose tissue (26), plasma phospholipids (22), cholesterol esters (21), and total serum (20). It is less clear which tissues are preferable for measurement of other n–6 PUFAs. AA intake has usually shown no or only a weak correlation with the biomarker for AA (22, 26, 43). Similarly, AA has not been associated with mortality in studies that used adipose tissue (26), erythrocytes (27), plasma phospholipids (22, 24), or cholesterol esters (21) as the tissue being measured. In contrast, we found both a correlation between dietary and serum AA and similar, although weaker, inverse associations with mortality than with serum LA. There was no association of dietary LA with serum AA or between serum LA and AA, which is consistent with other studies (22, 26, 45). This suggests that endogenous conversion of LA to AA is tightly regulated and that the findings with AA are not due to its correlations with LA. However, our findings of the inverse associations of serum AA with mortality risk highlight the need for further investigations of the health effects of AA in different blood compartments and tissues.

After conversion from LA, GLA is rapidly elongated to DGLA, which can then be converted to AA (36). As in the case with AA, GLA and especially DGLA are precursors to both pro- and anti-inflammatory compounds (36, 46). Some compounds derived from DGLA also possess antiproliferative properties (36, 46). Few prospective studies have investigated the associations of GLA or DGLA with mortality, and, no associations have been observed (21, 22, 26), suggesting that these minor, mainly endogenously produced n–6 PUFAs do not have a major independent role in mortality risk.

The strengths of our study are the use of objective biomarkers for n–6 PUFA exposure, extensive examinations, adjustment for potential confounders, large numbers of incident events, and no loss to follow-up. A major weakness is the single baseline fatty acid measurement for all men. Because dietary habits can change during a long follow-up period, this would add random error and thus attenuate the associations. However, in the subgroup of men with repeated measurements, we observed rather strong correlations ($\geq$0.5) for all n–6 PUFAs, even 11 y after baseline. The associations between n–6 PUFAs and incident events were also generally similar in the analyses with the shorter, 11-y follow-up. Higher serum LA was associated with more favorable lifestyle and dietary factors, so residual confounding by unmeasured or imprecisely measured factors cannot be excluded. Our study also included only white middle-aged and older men, and the findings may not be generalizable to women and other races/ethnicities.

In conclusion, we found an inverse association of both serum LA and AA with the risk of total, CVD, and non-CVD or non-cancer mortality and no association with cancer mortality. The minor n–6 PUFAs, GLA and DGLA, showed no association with mortality risk. The findings for LA confirm the results of previous observational studies and suggest overall benefits with a higher LA intake with little concern for risk. Thus, these results support the current recommendations to increase the intake of LA for CVD prevention (18). The findings with AA need to be replicated in other cohorts.

The authors' responsibilities were as follows—JKV, SV, JM, and T-PT: acquired the data and designed and conducted the research; JKV: analyzed the data, drafted the manuscript, and had primary responsibility for the final content; JHYW, SV, JM, and T-PT: critically revised the manuscript for important intellectual content; and all authors: read and approved the final manuscript. JHYW reported research support from Unilever Ltd. for other projects on fatty acid biomarkers. The other authors reported no conflicts of interest.

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