

# n-3 Fatty acids and 5-y risks of death and cardiovascular disease events in patients with coronary artery disease<sup>1-3</sup>

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## ABSTRACT

**Background:** Data on the association of n-3 fatty acid content in serum lipids with mortality in patients with coronary artery disease (CAD) are limited.

**Objective:** We hypothesized that a high proportion of n-3 fatty acids in serum lipids would be associated with reduced risks of death and coronary events in patients with established CAD.

**Design:** We measured dietary intakes via food records and the fatty acid composition of serum cholesteryl esters (CEs) in 285 men and 130 women with CAD ( $\bar{x}$  age: 61 y; range: 33–74 y). The patients participating in the EUROASPIRE (European Action on Secondary Prevention through Intervention to Reduce Events) study were followed up for 5 y.

**Results:** During the follow-up, 36 patients died, 21 had myocardial infarctions, and 12 had strokes. The relative risks (RRs) of death adjusted for cardiovascular disease risk factors for subjects in the highest tertile of fatty acids in CEs compared with those in the lowest tertile were 0.33 (95% CI: 0.11, 0.96) for  $\alpha$ -linolenic acid, 0.33 (0.12, 0.93) for eicosapentaenoic acid, and 0.31 (0.11, 0.87) for docosahexaenoic acid (*P* for trend = 0.063, 0.056, and 0.026, respectively). A high proportion of eicosapentaenoic acid in CEs was associated with a low risk of CAD death. Compared with no consumption, consumption of fish tended to be associated with a lower risk of death [1–57 g/d, RR = 0.50 (0.20, 1.28); > 57 g/d, RR = 0.37 (0.14, 1.00); *P* for trend = 0.059].

**Conclusion:** High proportions of n-3 fatty acids in serum lipids are associated with a substantially reduced risk of death. *Am J Clin Nutr* 2003;78:65–71.

**KEY WORDS** Diet, n-3 fatty acids, mortality, coronary artery disease, risk, cohort studies

## INTRODUCTION

Dietary fat quality has been shown to be associated with the development of coronary artery disease (CAD). Possible protective effects of long chain n-3 fatty acids derived mainly from fish have been of particular interest. High intakes of the long chain n-3 fatty acids eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) were found to be associated with reduced CAD mortality in ecologic population studies (1, 2), prospective cohort studies (3–6), and dietary intervention studies (7–10).

Biomarkers of the intake of long chain n-3 fatty acids, ie, their content in plasma or adipose tissue, have been inconsistently associated with the risk of CAD events. In several case-

control and cohort studies, a high content of n-3 fatty acids was associated with reduced risks of acute myocardial infarction (AMI) (11–15), primary cardiac arrest (16), and sudden cardiac death (17). However, some studies found no association between AMI and biomarkers of the intake of long-chain n-3 fatty acids (18–21).

The long chain n-3 fatty acids may reduce the risk of cardiovascular diseases (CVDs) through several possible mechanisms. The long-chain n-3 fatty acids are hypotriglyceridemic (22), improve endothelial function in arteries (23), and reduce the expression of vascular adhesion molecules (24). Furthermore, antiarrhythmic effects of n-3 fatty acids have been shown in cell-culture, animal, and human studies (25, 26). However, there have been concerns about possible adverse effects of high doses of long-chain n-3 fatty acids on LDL-cholesterol concentrations (22), the susceptibility of LDL to oxidation (27), and the activity of plasminogen activator inhibitor-1 (28, 29).

In intervention studies, a high intake of  $\alpha$ -linolenic acid (ALA) was associated with a reduced risk of fatal and nonfatal CAD events in patients with AMI (10, 30). In observational prospective studies, ALA intake was shown to be associated with a reduced risk of fatal ischemic events in women without prior CAD (31) but not in elderly subjects (32). In several studies, the ALA content of serum or adipose tissue was not associated with the risk of CAD (12, 15, 20), although one study suggested a reduced risk (18).

In prospective cohort studies of patients with CAD, the findings concerning the relations of dietary fat and serum lipid fatty acid profile with the risks of death and recurrent CAD events have been inconsistent. The aim of the present study was to evaluate the associations of diet and serum lipid fatty acid composition with mortality and cardiovascular events during a 5-y follow-up of patients with clinically established CAD (33).

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## SUBJECTS AND METHODS

### Patients

The EUROASPIRE (European Action on Secondary Prevention through Intervention to Reduce Events) study was a study of the practice of secondary prevention of CAD that was conducted in 9 European countries (33). The Finnish cohort of the EUROASPIRE study comprised 415 patients (285 men, 130 women) with clinically established CAD who had been admitted to the Kuopio University Hospital in 1991–1994 and who were aged <71 y at the time of hospital admission. Following the EUROASPIRE study protocol, we used hospital discharge lists and cardiac surgery and coronary angiography registers to retrospectively identify consecutive patients belonging to the following 4 diagnostic categories: 1) patients having their first elective or emergency coronary artery bypass grafting (CABG), 2) patients having their first elective or emergency percutaneous transluminal coronary angioplasty (PTCA) but with no previous CABG, 3) patients having their first or a recurrent AMI but with no previous CABG or PTCA, 4) patients admitted with symptoms compatible with acute myocardial ischemia (AMIS) but in whom the diagnosis of AMI could not be confirmed (patients with unstable angina pectoris) and who had no previous CABG, PTCA, or AMI.

The aim was to get  $\approx 100$  patients from each diagnostic category to participate in an interview and examination to be carried out  $\geq 6$  mo after hospitalization. To allow for mortality and non-participation, 125 patients each were identified from the CABG, PTCA, and AMIS categories, and 156 patients were identified from the AMI category because of higher expected mortality in that category. The examination, which formed the baseline for the present study, was conducted in 1995. The number of patients from each category who participated in the study was as follows: CABG, 109; PTCA, 106; AMI, 101; and AMIS, 99. The overall participation rate was 82%. The median time interval between hospital admission and examination was 20 mo (range: 10–48 mo). The Finnish center was the only one of the EUROASPIRE centers to carry out detailed dietary studies and measurements of the fatty acid composition of serum lipid fractions.

The study was approved by the Ethics Committee of the University of Kuopio and the Kuopio University Hospital. All patients gave their informed consent.

### Patient interview and examination

Patient interviews were conducted with the use of a structured questionnaire that covered the following topics: demographic details, including years of school education; smoking habits; and use of drugs. Height and weight were measured while the subjects wore light clothing but no shoes. Body mass index was calculated as weight (kg)/height<sup>2</sup> (m). Waist circumference was measured midway between the lower rib margin and the iliac crest. Hip circumference was measured at the point yielding the maximum circumference over the buttocks. Blood pressure was measured from the subjects' right arm with the use of an automatic digital sphygmomanometer (Takeda UA 731; A&D Co Ltd, Abingdon, United Kingdom) while the subjects were in a sitting position and after they had rested for 5 min. Nonsmoking status was validated by performing a breath carbon monoxide measurement ( $\leq 10$  parts per million) (Bedfont Scientific EC 50, Sittingbourne, United Kingdom). Patients were classified as diabetic if their diagnosis of diabetes had previously been confirmed by a physician or if their plasma glucose concentration was  $\geq 7$  mmol/L.

### Food records

The patients completed a 4-d food record (3 weekdays and 1 weekend day) at home and estimated the amounts of foods consumed by comparing them with portion sizes listed in a booklet (34). The patients returned the food records at the interview, and all the records were checked by a clinical nutritionist; if necessary, missing information was completed. Food intakes were converted into nutrient intakes by using the MICRO-NUTRICA dietary analysis program (version 2.0; Finnish Social Insurance Institution, Turku, Finland), which is based on the national database of the Finnish Social Insurance Institution.

### Laboratory measurements

Blood samples were collected between 0800 and 1000 after the subjects had fasted for 12 h. Serum and blood samples were stored at  $-70^{\circ}\text{C}$  until analyzed, except that fresh serum was used for analyses of serum total and lipoprotein lipids.

Standardized enzymatic methods were used for the analysis of serum lipids. Lipoproteins were separated by ultracentrifugation for 18 h at  $4^{\circ}\text{C}$ ,  $144\,000 \times g$ , and a density of 1.006 kg/L to remove VLDL. LDL was precipitated from the infranatant fluid with dextran sulfate–magnesium chloride (35). HDL cholesterol was analyzed from the remaining supernatant fluid. LDL cholesterol was calculated by subtracting the amount of HDL cholesterol from the amount of cholesterol in the infranatant fluid containing both HDL and LDL cholesterol. Cholesterol in the whole serum and in separated lipoproteins and serum triacylglycerols was analyzed by using commercial kits (kits 237574 and 701904; Boehringer GmbH, Mannheim, Germany) and a Kone Specific Clinical Analyzer (Kone Ltd, Espoo, Finland). Plasma glucose was analyzed by using an amperometric enzymatic method (Glucose Auto & Stat GA 110 analyzer; Daiichi Co, Kyoto, Japan).

Lipids were extracted from the serum sample (100  $\mu\text{L}$ ) with chloroform:methanol (2:1, by vol) (36). Lipid fractions [cholesteryl esters (CEs) and phospholipids] were separated with an aminopropyl column. Fatty acids of lipid fractions were transmethylated with 14% boron trifluoride in methanol at  $100^{\circ}\text{C}$  for 1 h. Finally, fatty acid methyl esters were analyzed with a gas chromatograph (Hewlett-Packard 5890 series II; Hewlett-Packard Co, Waldbronn, Germany) equipped with an FFAP column (length, 25 m; inside diameter, 2 mm; film thickness, 0.3  $\mu\text{m}$ ; Hewlett-Packard). Helium was used as the carrier gas. Fatty acids are presented as molar percentages of total fatty acids.

### Endpoint ascertainment

The censoring date was the date of the earliest event or the end of the follow-up period (30 April 2001 for deaths and 31 December 2000 for hospitalizations). The endpoints included deaths from all causes, CVD, and CAD; nonfatal AMI; nonfatal stroke; CABG; and PTCA. Deaths from CAD included codes I20–I25 from the *International Classification of Diseases*, 10th revision, and deaths from CVD included codes I20–28, I60–69, G45, and G46. Deaths were ascertained by computer linkage of Finnish social security numbers to the national death register (Statistics Finland, Helsinki). Copies of death certificates were also obtained from the register. Data on AMIs, strokes, and revascularization procedures were obtained from the national hospital discharge registers of the National Research and Development Centre for Welfare and Health on the basis of social security numbers. Medical records were also obtained, and the diagnosis or revascularization procedure was ascertained.

**TABLE 1**  
Baseline characteristics

	Patients who died (n = 28 M, 8 F)	Patients who survived (n = 257 M, 122 F)
Age (y)	63.8 ± 8.3 <sup>1</sup>	60.7 ± 8.0 <sup>2</sup>
Serum cholesterol (mmol/L)		
Total	6.61 ± 1.26	6.07 ± 1.17 <sup>3</sup>
LDL	4.69 ± 1.04	4.22 ± 1.04 <sup>4</sup>
HDL	1.20 ± 0.34	1.23 ± 0.29
Serum total triacylglycerol (mmol/L)	2.23 ± 1.09	1.92 ± 1.54 <sup>5</sup>
Plasma glucose (mmol/L)	6.09 ± 1.44	5.97 ± 1.78
Systolic blood pressure (mm Hg)	145 ± 27	140 ± 22
Diastolic blood pressure (mm Hg)	85 ± 16	82 ± 12
BMI (kg/m <sup>2</sup> )	28.2 ± 4.2	28.1 ± 4.0
Waist-to-hip ratio	0.95 ± 0.08	0.93 ± 0.08
Diabetes (diagnosis or plasma glucose ≥ 7 mmol/L) [n (%)]	8 (22)	62 (16)
Lipid-lowering drugs [n (%)]	10 (28)	152 (40)
Education < 12 y [n (%)]	35 (97)	327 (86)
Smoking [n (%)]	5 (14)	48 (13)

<sup>1</sup> $\bar{x} \pm SD$ .<sup>2,3</sup>Significantly different from patients who died (Mann-Whitney *U* test): <sup>2</sup>*P* = 0.009, <sup>3</sup>*P* = 0.024.<sup>3,4</sup>Significantly different from patients who died (ANOVA with adjustment for sex and age): <sup>3</sup>*P* = 0.002, <sup>4</sup>*P* = 0.010.

### Statistical analyses

Statistical analyses were performed with SPSS for WINDOWS, version 10 (SPSS Inc, Chicago). For each patient, person-years of follow-up were calculated. Nutrient intakes were adjusted for energy intake by using the residual method (37). Before further analyses, the normality of distribution of variables was checked with the Kolmogorov-Smirnov test. A logarithmic transformation was performed for variables that were not normally distributed. If the transformation did not alter the distribution to normality, non-parametric tests were used. Differences in baseline characteristics were analyzed by using analysis of variance with adjustment for sex and age, the Mann-Whitney *U* test, or the chi-square test, as appropriate. Spearman's correlation coefficients for correlations between fish intake and serum lipid fatty acids were calculated. The relative risks of different endpoints were calculated by using the Cox proportional hazards model. In Cox models with combined endpoints, the first endpoint that occurred was used. Nutrient intakes were entered in the models as continuous variables, and risks associated with 1-SD increments in intake were estimated. The proportions of fatty acids in serum lipids were classified into tertiles, and risks in tertiles (with Bonferroni-corrected CIs) and *P* values for overall trends were calculated. The analyses were adjusted for sex, age, diagnostic category (CABG or PTCA compared with AMI or AMIS), education (< 12 compared with ≥ 12 y), serum cholesterol concentration, serum triacylglycerol concentration, body mass index, and diabetes, and models that included nutrient or food intakes were also adjusted for energy intake.

### RESULTS

During the 5-y follow-up, 36 patients died; 21 of these deaths were related to the cardiovascular system, and 18 were due to CAD. There were 5 deaths from cancers, 4 from pulmonary diseases, and 6 from miscellaneous other causes. Among the patients,

**TABLE 2**  
Nutrient intakes<sup>1</sup>

	Patients who died (n = 34)	Patients who survived (n = 367)
Energy (kJ/d)	6945 ± 1937	7272 ± 2159
Fat (% of energy)	34.8 ± 7.1	32.5 ± 6.5 <sup>2</sup>
Saturated fat (% of energy)	14.9 ± 5.5	12.7 ± 3.6 <sup>3</sup>
Monounsaturated fat (% of energy)	11.6 ± 2.3	11.2 ± 2.7
Polyunsaturated fat (% of energy)	5.5 ± 1.7	5.8 ± 1.8
Cholesterol (mg/d)	228 ± 108	217 ± 90
Protein (% of energy)	16.7 ± 2.9	17.4 ± 3.0
Carbohydrates (% of energy)	44.9 ± 7.0	46.5 ± 6.8
Fiber (g/d)	19.1 ± 7.8	21.6 ± 8.4 <sup>4</sup>
Alcohol (% of energy)	2.2 ± 5.1	2.2 ± 4.5

<sup>1</sup> $\bar{x} \pm SD$ .<sup>2-4</sup>Nearly significantly different from patients who died (ANOVA with adjustment for sex and age): <sup>2</sup>*P* = 0.107, <sup>3</sup>*P* = 0.074, <sup>4</sup>*P* = 0.064.

21 had nonfatal AMIs and 39 had CABG or PTCA during the follow-up. Altogether, 12 strokes were recorded.

The patients who died were significantly older at baseline than those who survived and had significantly higher serum total- and LDL-cholesterol concentrations at baseline than did those who survived (Table 1). Serum total triacylglycerol concentrations were also significantly higher in the patients who died than in those who survived. The patients who died tended to have higher intakes of fat and saturated fat and a lower intake of fiber than did the patients who survived (Table 2).

The risk of death from all causes was significantly higher with higher intakes of saturated fat and higher Keys scores (38) (Table 3). However, there was no association between the intake of saturated fat and the risks of the combined endpoint of CAD death or AMI; of the combined endpoint of CVD death, AMI, or stroke; or of revascularization procedures. Fish intake was divided into 3 categories: no intake (0 g/d) and below and above median consumption (57 g/d). Fish intake tended to be associated with low risks of death and of the combined endpoint of CVD death, AMI, or stroke (Table 4).

The middle tertile of palmitic acid in CEs tended to be associated with a low risk of CAD death and AMI combined (Table 5). A high proportion of oleic acid in CEs tended to be associated with a high risk of death. A high proportion of linoleic acid in CEs tended to be associated with a low risk of the combined endpoint of CVD death, AMI, or stroke. A high proportion of arachidonic acid in CEs tended to be associated with a low risk of the combined endpoint of CVD death, AMI, or stroke.

High proportions of ALA, EPA, and DHA in CEs either tended to be associated or were associated with a low risk of death (*P* values for trend = 0.063, 0.056, and 0.026, respectively) (Table 5). However, proportions of ALA were not associated with the risks of the other endpoints. A high proportion of EPA in CEs was associated with a low risk of CAD death. DHA also tended to protect against the combined endpoints of fatal and nonfatal CAD events and of fatal and nonfatal CVD events. Fish intake correlated with proportions of EPA (*r* = 0.568, *P* < 0.01) and DHA (*r* = 0.545, *P* < 0.01) in serum CEs.

High proportions of EPA and DHA in serum phospholipids also tended to be associated with a low risk of death [the risk ratios for the highest tertiles of EPA and DHA were 0.31 (95% CI: 0.11, 0.93) (*P* for trend = 0.055) and 0.41 (95% CI: 0.15, 1.10)

**TABLE 3**Relative risks (RRs) and 95% CIs of death and cardiovascular disease (CVD) events per 1-SD increment in nutrient intake<sup>1</sup>

	Death ( <i>n</i> = 34/400) <sup>2</sup>		CAD death ( <i>n</i> = 16/400)		CAD death or AMI ( <i>n</i> = 34/400)		CVD death, AMI, or stroke ( <i>n</i> = 44/400)		Revascularization ( <i>n</i> = 38/400)	
	RR (95% CI)	<i>P</i>	RR (95% CI)	<i>P</i>	RR (95% CI)	<i>P</i>	RR (95% CI)	<i>P</i>	RR (95% CI)	<i>P</i>
Fat	1.38 (0.98, 1.95)	0.065	1.03 (0.63, 1.70)	0.902	1.05 (0.73, 1.52)	0.799	1.22 (0.89, 1.67)	0.216	1.31 (0.94, 1.82)	0.113
Saturated fat	1.57 (1.13, 2.17)	0.007	1.01 (0.61, 1.69)	0.966	1.00 (0.68, 1.46)	0.993	1.23 (0.89, 1.68)	0.211	1.19 (0.85, 1.66)	0.304
Polyunsaturated fat	0.92 (0.64, 1.31)	0.631	0.92 (0.55, 1.54)	0.758	1.08 (0.78, 1.51)	0.642	1.08 (0.82, 1.42)	0.571	1.10 (0.83, 1.44)	0.516
Cholesterol	1.07 (0.76, 1.50)	0.698	0.76 (0.45, 1.28)	0.302	0.97 (0.68, 1.40)	0.887	1.10 (0.81, 1.50)	0.583	1.23 (0.91, 1.66)	0.184
Fiber	0.81 (0.55, 1.19)	0.287	1.11 (0.68, 1.81)	0.684	1.10 (0.75, 1.61)	0.618	1.02 (0.73, 1.43)	0.899	0.85 (0.59, 1.23)	0.389
Alcohol	1.07 (0.74, 1.55)	0.710	0.96 (0.56, 1.66)	0.885	1.08 (0.73, 1.61)	0.692	0.99 (0.69, 1.41)	0.939	1.01 (0.69, 1.49)	0.942
Keys score <sup>3</sup>	1.51 (1.12, 2.04)	0.006	1.00 (0.61, 1.62)	0.990	1.00 (0.71, 1.41)	0.982	1.19 (0.89, 1.60)	0.250	1.21 (0.88, 1.66)	0.247

<sup>1</sup>Models were adjusted for age, sex, diagnostic category [coronary artery bypass grafting or percutaneous transluminal coronary angioplasty compared with acute myocardial infarction (AMI) or acute myocardial ischemia], energy intake, serum cholesterol, serum triacylglycerol, diabetes (diagnosis or plasma glucose concentration  $\geq 7$  mmol/L), BMI, and education ( $< 12$  compared with  $\geq 12$  y). The intakes of fat, saturated fat, polyunsaturated fat, cholesterol, fiber, and alcohol were adjusted for energy intake by using the residual method (37). CAD, coronary artery disease.

<sup>2</sup>Number of cases/total *n*.

<sup>3</sup>Calculated from the intakes of saturated fat, polyunsaturated fat, and cholesterol (38).

(*P* for trend = 0.085)]. However, ALA in phospholipids was not associated with the risk of death [the risk ratio for the highest tertile was 1.06 (95% CI: 0.38, 3.01) (*P* for trend = 0.463)].

## DISCUSSION

The main finding of the present study is that proportions of ALA, EPA, and DHA in serum CEs are associated with a reduction in the risk of all-cause mortality. The associations between EPA and DHA and the risk of death were confirmed by the reduced risk observed in the subjects who ate fish or who had high proportions of EPA and DHA in serum phospholipids. The associations of *n*-3 fatty acids with combined fatal and nonfatal CVD events were, however, not significant.

In accord with our findings, the protective effect of long chain *n*-3 fatty acids against fatal endpoints was observed in previous studies (4–6, 8, 9, 39), and it has been suggested that the association is stronger for sudden deaths (16, 17, 26, 40). A reduced mortality was observed after only 3 mo of *n*-3 fatty acid supplementation (1 g/d) in the GISSI (Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico) study, which is the only clinical trial in AMI patients focusing purely on the effect of *n*-3 fatty acids on mortality and cardiac endpoints (41). Furthermore, a recent meta-analysis of randomized controlled trials concluded that dietary intakes and supplements of *n*-3 fatty acids reduce the risk of fatal AMI, sudden death, and overall mortality (7). In

addition to their own independent effects, a high intake of fish or a high proportion of *n*-3 fatty acids in serum lipids may also reflect a healthier diet and lifestyle in observational studies.

The known effects of *n*-3 fatty acids on serum lipids (22) are not likely to be the major risk-lowering mechanism (9, 10). A growing body of evidence indicates that EPA and DHA have antiarrhythmic effects (25). Addition of *n*-3 fatty acids electrically stabilizes cardiac myocytes by inhibiting sodium and calcium channels (42). *n*-3 Fatty acids are also suggested to lower myocardial oxygen consumption, thus ensuring energy for maintenance of transmembrane potentials and possibly leading to reduced susceptibility to arrhythmia (43). In a dog model of sudden cardiac death, EPA and, even more efficiently, DHA prevented ischemia-induced ventricular fibrillation (44). In clinical studies, *n*-3 fatty acids increased heart rate variability, which reflects a higher ventricular fibrillation threshold, thus reducing vulnerability to arrhythmia (45).

Acute vascular events can be triggered by endothelial dysfunction that reflects an imbalance between vasoconstriction and vasodilatation (46). Improvements in the functioning of systemic large arteries were observed in hypercholesterolemic subjects after dietary supplementation with 4 g marine *n*-3 fatty acids/d (23). The expression of vascular adhesion molecules is reduced after consumption of DHA, and a greater number of double bonds rather than the type of unsaturation (ie, *n*-6 compared with *n*-3) has been suggested to be the critical feature in inhibiting endothelial activation (24). Furthermore, *n*-3 fatty acids

**TABLE 4**Relative risks and 95% CIs of death and cardiovascular disease (CVD) events by category of fish intake (no intake and below and above the median intake)<sup>1</sup>

	0 g/d ( <i>n</i> = 103)	1–57 g/d ( <i>n</i> = 147)	>57 g/d ( <i>n</i> = 150)	<i>P</i> for trend
Death	1 [14]	0.50 (0.20, 1.28) [11]	0.37 (0.14, 1.00) [9]	0.059
CAD death	1 [5]	1.59 (0.39, 6.49) [5]	1.04 (0.25, 4.31) [6]	0.731
CAD death or AMI	1 [10]	1.00 (0.38, 2.66) [14]	0.49 (0.17, 1.41) [10]	0.209
CVD death, AMI, or stroke	1 [16]	0.64 (0.28, 1.47) [15]	0.45 (0.19, 1.09) [13]	0.121
Revascularization	1 [7]	1.89 (0.68, 5.25) [17]	1.09 (0.37, 3.17) [14]	0.226

<sup>1</sup>Number of cases in brackets. The model was adjusted for age, sex, diagnostic category [coronary artery bypass grafting or percutaneous transluminal coronary angioplasty compared with acute myocardial infarction (AMI) or acute myocardial ischemia], energy intake, serum cholesterol, serum triacylglycerol, diabetes (diagnosis or plasma glucose concentration  $\geq 7$  mmol/L), BMI, and education ( $< 12$  compared with  $\geq 12$  y). CAD, coronary artery disease.

**TABLE 5**Relative risks (RRs) and 95% CIs of death and cardiovascular disease (CVD) events by tertile of fatty acids in serum cholesteryl esters<sup>†</sup>

	Tertile 1 (n = 133)	Tertile 2 (n = 133)	Tertile 3 (n = 132)	P for trend
Palmitic acid (mol%)	<12.81	12.81–13.87	>13.87	
Death	1 [13]	0.58 (0.22, 1.55) [9]	0.76 (0.30, 1.90) [13]	0.460
CAD death	1 [9]	0.30 (0.08, 1.20) [4]	0.34 (0.09, 1.26) [5]	0.072
CAD death or AMI	1 [14]	0.31 (0.10, 0.94) [6]	0.71 (0.29, 1.76) [13]	0.060
CVD death, AMI, or stroke	1 [15]	0.60 (0.25, 1.48) [11]	1.02 (0.45, 2.32) [18]	0.336
Revascularization	1 [15]	0.55 (0.22, 1.38) [10]	0.77 (0.32, 1.85) [14]	0.347
Oleic acid (mol%)	<20.05	20.06–22.31	>22.31	
Death	1 [6]	1.99 (0.65, 6.13) [13]	2.63 (0.87, 7.97) [16]	0.149
CAD death	1 [5]	1.02 (0.25, 4.14) [6]	1.37 (0.35, 5.42) [7]	0.834
CAD death or AMI	1 [9]	0.96 (0.34, 2.73) [11]	1.57 (0.57, 4.39) [13]	0.440
CVD death, AMI, or stroke	1 [14]	0.63 (0.25, 1.59) [11]	1.44 (0.63, 3.30) [19]	0.105
Revascularization	1 [13]	1.12 (0.47, 2.69) [15]	0.95 (0.37, 2.45) [11]	0.907
Linoleic acid (mol%)	<46.74	46.74–50.69	>50.69	
Death	1 [17]	0.55 (0.22, 1.42) [9]	0.67 (0.26, 1.76) [9]	0.337
CAD death	1 [6]	0.93 (0.24, 3.70) [5]	1.77 (0.48, 6.53) [7]	0.496
CAD death or AMI	1 [15]	0.56 (0.21, 1.52) [8]	0.82 (0.31, 2.16) [10]	0.435
CVD death, AMI, or stroke	1 [22]	0.48 (0.20, 1.14) [10]	0.67 (0.29, 1.54) [12]	0.147
Revascularization	1 [14]	0.88 (0.36, 2.15) [12]	0.98 (0.40, 2.40) [13]	0.939
Arachidonic acid (mol%)	<4.73	4.73–6.02	>6.02	
Death	1 [17]	0.51 (0.20, 1.31) [9]	0.66 (0.26, 1.70) [9]	0.250
CAD death	1 [10]	0.43 (0.11, 1.69) [4]	0.49 (0.12, 1.91) [4]	0.278
CAD death or AMI	1 [17]	0.50 (0.19, 1.33) [9]	0.45 (0.16, 1.28) [7]	0.131
CVD death, AMI, or stroke	1 [21]	0.67 (0.30, 1.46) [15]	0.39 (0.15, 1.02) [8]	0.081
Revascularization	1 [13]	0.79 (0.31, 2.01) [11]	1.12 (0.46, 2.72) [15]	0.669
α-Linolenic acid (mol%)	<0.77	0.77–0.89	>0.89	
Death	1 [14]	0.76 (0.32, 1.82) [14]	0.33 (0.11, 0.96) [7]	0.063
CAD death	1 [6]	1.12 (0.33, 3.82) [8]	0.44 (0.10, 1.93) [4]	0.304
CAD death or AMI	1 [9]	1.10 (0.39, 3.09) [11]	0.95 (0.35, 2.57) [13]	0.940
CVD death, AMI, or stroke	1 [13]	1.04 (0.44, 2.43) [16]	0.81 (0.34, 1.93) [15]	0.780
Revascularization	1 [9]	1.61 (0.61, 4.28) [14]	1.56 (0.60, 4.09) [16]	0.495
Eicosapentaenoic acid (mol%)	<1.34	1.34–2.11	>2.11	
Death	1 [16]	0.68 (0.29, 1.61) [12]	0.33 (0.12, 0.93) [7]	0.056
CAD death	1 [10]	0.23 (0.05, 1.03) [3]	0.31 (0.08, 1.14) [5]	0.034
CAD death or AMI	1 [12]	0.66 (0.25, 1.77) [11]	0.50 (0.18, 1.38) [10]	0.307
CVD death, AMI, or stroke	1 [14]	0.92 (0.39, 2.15) [15]	0.76 (0.33, 1.79) [15]	0.766
Revascularization	1 [15]	0.49 (0.19, 1.29) [9]	0.71 (0.30, 1.68) [15]	0.251
Docosahexaenoic acid (mol%)	<0.59	0.59–0.79	>0.79	
Death	1 [17]	0.51 (0.21, 1.22) [11]	0.31 (0.11, 0.87) [7]	0.026
CAD death	1 [7]	0.66 (0.18, 2.39) [6]	0.48 (0.12, 1.93) [5]	0.490
CAD death or AMI	1 [12]	0.61 (0.24, 1.58) [12]	0.41 (0.14, 1.18) [9]	0.162
CVD death, AMI, or stroke	1 [17]	0.52 (0.22, 1.22) [13]	0.55 (0.24, 1.29) [14]	0.158
Revascularization	1 [12]	0.77 (0.30, 1.96) [12]	0.80 (0.32, 2.00) [15]	0.795

<sup>†</sup>Number of cases in brackets. The models were adjusted for age, sex, diagnostic category [coronary artery bypass grafting or percutaneous transluminal coronary angioplasty compared with acute myocardial infarction (AMI) or acute myocardial ischemia], energy intake, serum cholesterol, serum triacylglycerol, diabetes (diagnosis or plasma glucose concentration  $\geq 7$  mmol/L), BMI, and education (<12 compared with  $\geq 12$  y). CAD, coronary artery disease.

have a minor blood pressure-lowering effect and decrease platelet aggregation (46, 47).

There is less evidence for the beneficial effect of ALA than there is for EPA and DHA. In the present study, proportions of ALA in CEs, but not in phospholipids, were associated with a reduced risk of death. The fatty acid profile of phospholipids does not reflect dietary fat quality as directly as does the fatty acid profile of CEs, which may explain the controversy. A Mediterranean-type diet with enrichment of ALA intake significantly reduced the risk of cardiac events in survivors of AMI (10). However, other dietary changes, eg, decreases in the intakes of saturated fat and cholesterol, may have also affected the result. In women with prior AMIs, ALA intake tended to be associated with a reduction in fatal ischemic events in the Nurses' Health Study (31). On the contrary, ALA intake was not associated with the risk of incident CAD in


elderly subjects (32). ALA may directly reduce the risk of arrhythmia (25, 44) and has beneficial effects on serum lipid profiles (22). Even though the proportion of dietary ALA that is converted to EPA and DHA in the body is small, the effects of ALA may be mediated via that route (48).

In the present study, oleic acid in CEs tended to be associated with an increased risk of death. The results of previous studies have been inconclusive: they have shown either a reduced risk (49) or an increased risk (50). These inconsistent results may be explained by the fact that the intakes of saturated and monounsaturated fat correlate strongly in the Western diet. In the present study, a high proportion of arachidonic acid in CEs tended to be associated with a reduced risk of fatal and nonfatal CVD events combined. The results of previous studies, however, have been inconsistent: some studies reported a positive association

(11, 13, 51), but one study reported an inverse association (20). In comparison with n-3 fatty acids, arachidonic acid could adversely affect eicosanoid metabolism (46).

In the present study, an increase in dietary saturated fat intake was associated with an increase in the risk of death. On the contrary, the middle tertile of palmitic acid in CEs was associated with a decreased risk of CAD death and AMI combined. These inconsistent results may have resulted from insufficient variability in saturated fat intake, which made it infeasible to show relations between intake and disease risk. Another possible explanation is that the small size of the study cohort limited the power of the statistical analyses. Virtually all the patients were taking cardiovascular drugs, and this could have confounded the observed associations. Twenty-eight percent of the patients who died and 40% of the patients who survived took lipid-lowering drugs. However, this difference in percentages was not significant, and the Cox models were adjusted for serum cholesterol and triacylglycerol concentrations.

The patients constituted a representative sample of CAD patients who lived in a geographically defined area, had either undergone a myocardial revascularization procedure or been hospitalized for an acute CAD event, and were in a stable phase of the disease at the time of the baseline examination. Dietary intakes and serum lipid fatty acid profiles were measured only at baseline, and it is possible that dietary changes may have occurred during the follow-up period. Even if major dietary changes occurred after the baseline examination, they probably would have weakened the observed associations. Despite all these limitations, our findings on the associations of dietary intakes and serum lipid contents of n-3 fatty acids with the risks of mortality and morbidity were consistent with each other. Proportions of EPA and DHA in serum CEs correlated strongly with fish intake. Furthermore, we previously reported that proportions of EPA in serum CEs were inversely related to serum triacylglycerol concentrations and positively related to serum HDL concentrations in a subset of the cohort used in the present study who did not take lipid-lowering medications (52).

In conclusion, ALA, EPA, and DHA are nutritional factors that could potentially reduce the risk of death in patients with CAD. Furthermore, this benefit can be obtained through the intake of foods and without the intake of supplements. 

All the authors were responsible for the study design and contributed to the editing of the manuscript. SL supervised the medical examinations. ATE was responsible for dietary monitoring, analyzing serum lipid fatty acid compositions, analyzing the data, and writing the manuscript. None of the authors had any conflicts of financial or personal interest with the financial sponsor of this research.

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