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Plasma eicosapentaenoic acid is negatively associated with all-cause mortality among men and women in a population-based prospective study

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ABSTRACT

Omega-3 polyunsaturated fatty acids (PUFAs) have anti-inflammatory properties, whereas omega-6 PUFAs appear to have proinflammatory properties. We aimed to assess plasma omega-3 and omega-6 PUFA status in relation to all-cause mortality in an Australian community–based study. We hypothesized that omega-3 PUFA would be inversely associated, and omega-6 PUFA positively associated with all-cause mortality. Plasma phospholipid omega-3 (eicosapentaenoic acid [EPA], docosapentaenoic acid [DPA], docosahexaenoic acid, α-linolenic acid, and total) and omega-6 PUFAs (linoleic acid, arachidonic acid, and total) were measured among 1008 adults (44% men) in 1996. Plasma PUFA composition was quantified using gas chromatography. During 17-year follow-up, 98 men and 81 women died. After adjustment for potential confounding factors, plasma EPA was inversely associated with all-cause mortality overall (adjusted hazard ratio [HR] per 1-SD increase, 0.81; 95% confidence interval [CI], 0.68-0.95), in men (HR, 0.78; 95% CI, 0.62-0.98), and in women (HR, 0.78; 95% CI, 0.65-0.94), separately. Inverse associations with mortality among men were also seen for DPA (HR, 0.76; 95% CI, 0.60-0.97) and α-linolenic acid (HR, 0.73; 95% CI, 0.57-0.94). No omega-6 PUFAs were significantly associated with mortality. Our findings of reduced all-cause mortality in men and women who have high EPA in plasma, and in men with high plasma DPA and α-linolenic acid, partially support our hypothesis that omega-3 PUFAs help reduce mortality but provide no evidence that omega-6 PUFAs may increase mortality.

1. Introduction

Studies of dietary intake of fatty acids in relation to health have shown that high intakes of omega-3 polyunsaturated fatty acids (PUFAs), especially the long-chain omega-3 PUFAs, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), reduce risk of cardiovascular disease (CVD) [1,2] and a range of other inflammation-related chronic diseases such as

Abbreviations: AA, arachidonic acid; BMI, body mass index; CI, confidence interval; CVD, cardiovascular disease; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid (22:5n-3); EPA, eicosapentaenoic acid; HR, hazard ratio; IQR, interquartile range; LA, linoleic acid; METS, metabolic equivalents; PUFA, polyunsaturated fatty acid; RCS, restricted cubic spline.

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diabetes, obesity, and rheumatoid arthritis, possibly as a result of anti-inflammatory properties of omega-3 PUFAs [3-5]. Omega-6 PUFAs, on the other hand, may have proinflammatory effects due to eicosanoids produced during the metabolism of arachidonic acid (AA) converted from linoleic acid (LA), the major dietary omega-6 PUFA [6]. Therefore, they may increase risk of CVD [7,8] and other chronic diseases [9]. Omega-6 PUFAs may also promote cancer through promoting tumor growth and antiapoptotic effects [10]. However, high intakes of omega-6 PUFAs have also shown cardioprotective effects by reducing cholesterol and lowering blood pressure [6,11].

Despite many investigations of the potential health effects of omega-3 and omega-6 PUFAs [1,6,8], the vast majority have been based on dietary intake. The problems associated with the measurement of dietary intake are well recognized, and specifically for fatty acids, the growing availability of a wide range of manufactured and processed foods coupled with variations in the fats and oils used by the food industry makes characterization of the PUFA composition of foods increasingly difficult [12,13]. Biomarkers such as plasma fatty acids provide more reliable evidence about associations between omega-3 or omega-6 PUFAs and health outcomes [14-16]. Indeed, some studies have used biomarkers of omega-3 and omega-6 PUFA intake, but most examined incident rather than fatal health outcomes [17-19]. The results from the limited studies that have examined biomarkers of these PUFAs and mortality were inconclusive [20-26], and this may be because most were conducted among high-risk clinical populations, such as patients with established CVD [1]. To address some of these gaps in knowledge about dietary PUFA and major chronic diseases in the general population, we prospectively assessed plasma phospholipid omega-3 and omega-6 PUFA status in relation to all-cause mortality in a 17-year community-based study in Australia. In view of the known anti-inflammatory and proinflammatory effects of omega-3 and omega-6 PUFAs, respectively, we hypothesized that plasma omega-3 PUFAs would be negatively and omega-6 PUFAs would be positively associated with all-cause mortality.

2. Methods and materials

2.1. Study population

Participants were drawn from the Nambour Skin Cancer Study, a cohort study in a representative community sample, which has been fully described elsewhere [27,28]. Briefly, 2095 Nambour residents aged 20 to 69 years were originally randomly selected from the electoral roll (enrollment is compulsory by law) in 1986 for a study of skin cancer. Between 1992 and 1996, of the 2095 residents, 1621 took part in a randomized controlled trial of skin cancer prevention through sunscreen use and β-carotene supplementation [27,28]. Trial participants completed questionnaires about personal factors including level of education, smoking and alcohol intake [29], serious medical conditions (including high blood pressure, angina, myocardial infarction, stroke, or cancer), and use of specific medications. Height and weight were measured in field clinics using standardized procedures, and carotenodermia was measured on the palm by a jaundice meter as an indicator of adherence to β-carotene supplement treatment. Participants were eligible for the present study if they provided blood samples during a follow-up clinic in August 1996 and were alive on January 1, 1997. The study was approved by the ethics committee of the QIMR Berghofer Medical Research Institute, and all participants provided written informed consent.

2.2. Plasma phospholipid fatty acids

Before blood collection, those taking part were requested to eat only a light breakfast (eg, toast or cereal, no cooked breakfast). Nonfasting venous 30 mL blood samples were collected by standard venipuncture techniques performed by experienced phlebotomists, and samples were processed at collection and stored in approximately 1-mL aliquots at –70°C. Measurements of plasma phospholipid fatty acids were conducted by Flinders Medical Centre, Adelaide, Australia, using procedures described in detail previously [30]. Plasma was extracted in chloroform/methanol, thin-layer chromatography was used to separate phospholipid fractions, and fatty acid methyl esters were quantified using gas chromatography (Hewlett-Packard, California, USA 6890 with a 50-m capillary column). Phosphatidylcholine diheptadecanoyl (C17:0 phospholipids) was used as an internal standard. Fatty acid methyl esters were identified based on the retention time to authentic lipid standards (GLC-463; Nuchek Prep Inc, Elysian, MN, USA) and quantified by comparison to the internal standard using ChemStation software (Agilent, Santa Clara, CA, USA). Specific PUFAs analyzed for this study were EPA, docosapentaenoic acid (DPA), DHA, α-linolenic acid, total long-chain omega-3, LA, AA, and total omega-6, expressed in μg/mL. Plasma phospholipid has been found to be a good biomarker of PUFA status in the general population [14,31,32].

2.3. Outcome

Study outcome was risk of death from all causes. Mortality was monitored to the end of August 2014 through the National Death Index of Australia and the Queensland Registry of Births, Deaths, and Marriages.

2.4. Statistical analyses

Descriptive statistics were used to characterize study participants, specifically percentage distribution for categorical and ordinal variables, and means ± SD and medians for continuous variables that were normally distributed or interquartile ranges (IQRs) otherwise. Bivariate comparison of participants' characteristics by sex or vital status was carried out using χ^2 tests for categorical variables, and *t* test and Mann-Whitney *U* test for normally and nonnormally distributed variables, respectively.

We examined the associations between plasma phospholipid PUFAs and subsequent mortality using continuous and categorical PUFA variables. Categorical PUFA variables were created by classifying participants into sex-specific ranked thirds based on each PUFA type. Hazard ratios (HRs) with 95%

Table 1 – Baseline characteristics of participants.

	All (N = 1008)	Men (n = 444)	Women (n = 564)	P [a]
	Means ± SD			
Age (y)	50 ± 12	50 ± 13	49 ± 12	.46 [a]
BMI (kg/m^2)	26.6 ± 4.2	27.0 ± 3.6	26.4 ± 4.7	.014 [a]
Blood cholesterol (mmol/L)	5.2 ± 1.0	5.0 ± 1.0	5.3 ± 1.0	<.001 [a]
Proxy serum β-carotene [b]	6.0 ± 1.4	5.9 ± 1.3	6.1 ± 1.4	.035 [a]
	Medians (IQR)			
Physical activity (METS)	5.3 (16.5)	7.1 (21.0)	4.5 (14.1)	.014 [c]
Alcohol consumption (g/d)	2.6 (10.8)	5.6 (14.8)	1.3 (7.0)	<.001 [c]
	n (%)			
Education				
High school	533 (53)	170 (38)	363 (64)	<.001 [d]
Trade/certificate/diploma	409 (40)	232 (52)	177 (31)	
Bachelor degree or higher	66 (7)	42 (9)	24 (4)	
Cigarette consumption				
Lifelong nonsmoker	561 (56)	191 (43)	371 (66)	<.001 [d]
Ex-smoker	349 (35)	203 (46)	146 (26)	
Current smoker	98 (10)	50 (11)	48 (9)	
History of serious medical condition [e]	372 (37)	165 (37)	207 (37)	.88 [d]
Taking dietary supplement [f]	551 (55)	199 (45)	352 (62)	<.001 [d]
Died during follow-up	179 (18)	98 (22)	81 (14)	.002 [d]

[a] *P* value from *t* test (normally distributed data).
[b] Using jaundice-meter reading from palm.
[c] *P* value from Mann-Whitney *U* test (nonnormally distributed data).
[d] *P* value from χ^2 test (categorical or ordinal data).
[e] Include high blood pressure, angina, myocardial infarction, stroke, or cancer.
[f] Include any type of supplements. Fish oil supplements were taken by n = 19 (2%) participants (men, n = 9 [2%]; women, n = 10 [2%]; survived, n = 13 [2%]; deceased, n = 6 [3%]).

confidence intervals (CIs) were calculated using Cox proportional hazards models. Hazard ratios for continuous variables were calculated in 1-SD increments. For ordinal-value groups, HRs compared each of the 2 higher PUFA groups to the lowest, the reference group. To test for linear trends, we assigned an ordinal number ranging from 1 (for the lowest third of PUFA level) to 3 (for the highest third) for each participant's plasma PUFA level and modeled this value as a continuous variable. Time to death was calculated from January 1, 1997, to date of death. Participants were censored if no death was recorded by August 31, 2014. The initial multivariable model controlled for age, sex, and smoking history. The final adjusted model was further controlled for the presence of a history of serious health conditions, blood cholesterol level (mmol/L), and level of carotenodermia. These variables were selected based on previous studies of the association between PUFAs and mortality or if they changed HR by more than 10%. There was no additional confounding factors by education (4 categories), physical activity (using metabolic equivalents [METS]), body mass index (BMI; kg/m^2 using measured height and weight), alcohol consumption (g/d), or overall dietary supplement use (yes/no). The proportional hazards assumption and the functional form of continuous covariates were assessed using the ASSESS option of PHREG in SAS. Restricted cubic spline (RCS) regression using the %RCS_reg macro [33] was used to test nonlinear trends by analyzing PUFAs as continuous variables in the Cox proportional hazard models [33,34], with 3 knots located at the 5th, 50th, and 95th percentiles [35]. Sex-stratified analyses were conducted based on reported sex differences in serum PUFA levels [20,36], as well as overall analyses. All statistical analyses were performed using SAS version 9.2 (SAS Institute Inc, Cary, NC, USA), and $P < .05$ was considered statistically significant.

3. Results

3.1. *Characteristics of participants*

Of the original 1621 trial participants, plasma PUFAs were measured in 1193 (74%) at baseline in 1996, and those who had missing information on smoking, physical activity, and BMI were excluded from analyses, leaving 1008 in the present study. Included participants were more likely to have a history of serious medical conditions compared with those excluded from the analyses, but they were no different in terms of age, sex, randomized trial treatment allocation, education, smoking status, or BMI.

Study participants had a mean (SD) age of 50 (12) years at baseline, and 44% were men (Table 1). Men had received more education, smoked more cigarettes, and consumed more alcohol than did women but took fewer dietary supplements. Women had significantly higher plasma PUFA levels, with the exception of EPA and DPA (Table 2). During the 17-year follow-up, 98 men (22%) and 81 women (14%) died: they tended to be older, more likely to report a serious medical condition, and to

Table 2 – Plasma phospholipid omega-3 and omega-6 PUFA status [a] overall and by sex.

	All participants	Men (n = 444)	Women (n = 564)	P
	Medians (IQR)			
Total long-chain n-3 [b]	67.61 (25.94)	64.80 (23.94)	69.72 (26.71)	<.001 [c]
EPA	11.14 (6.81)	10.87 (6.68)	11.47 (6.74)	.09 [c]
DPA	13.87 (4.85)	14.09 (4.74)	13.76 (4.86)	.51 [c]
DHA	41.89 (17.48)	39.07 (15.32)	43.89 (19.67)	<.001 [c]
α-Linolenic acid	2.06 (1.38)	1.92 (1.20)	2.25 (1.51)	<.001 [c]
Total n-6	441.12 (108.47)	422.15 (94.79)	458.10 (113.23)	<.001 [d]
LA	249.03 (71.6)	239.75 (69.51)	257.25 (70.09)	<.001 [d]
AA	125.55 (42.08)	120.49 (39.37)	130.59 (43.97)	<.001 [c]

[a] Values are in μg/mL.
[b] Sum of EPA, DPA, and DHA.
[c] P value from Mann-Whitney U test.
[d] P value from t test.

have higher plasma levels of total long-chain omega-3 (specifically DHA) at baseline (Table 3). In addition, women who died had higher baseline levels of total cholesterol than did survivors, whereas men who died had higher baseline AA compared with survivors.

3.2. *Association between plasma PUFAs and all-cause mortality: all participants*

In models adjusted for age, sex, and smoking status, there were no significant associations between total long-chain

Table 3 – Characteristics of participants by vital status.

	Men (n = 444)			Women (n = 564)		
	Alive (n = 346)	Died (n = 98)	P	Alive (n = 483)	Died (n = 81)	P
	Means ± SD [a]					
Age (y)	46 ± 11	64 ± 9	<.001	47 ± 11	62 ± 10	<.001
BMI (kg/m^2)	27 ± 4	27 ± 4	.98	26 ± 5	26.6 ± 5	.54
Jaundice measure [b]	5.9 ± 1.3	6.1 ± 1.3	.08	6.1 ± 1.4	6.2 ± 1.4	.56
Serum total cholesterol (mmol/L)	4.9 ± 1.0	5.1 ± 1.1	.10	5.3 ± 1.0	5.7 ± 1.0	<.001
	Medians (IQR)					
Physical activity (METS)	5.9 (20.0)	10.6 (23.5)	.012 [c]	5.0 (14.0)	3.0 (15.0)	.17 [c]
Total long-chain omega-3 [d] (μg/mL)	62.61 (22.57)	69.80 (24.20)	.001 [c]	68.21 (26.23)	77.89 (23.73)	.001 [c]
EPA (μg/mL)	10.74 (6.75)	11.73 (6.35)	.56 [c]	11.35 (6.83)	12.11 (7.09)	.15 [c]
DPA (μg/mL)	13.99 (4.90)	14.24 (4.26)	.46 [c]	13.56 (4.44)	14.89 (6.32)	.014 [c]
DHA (μg/mL)	37.06 (14.48)	46.22 (16.24)	<.001 [c]	43.38 (18.85)	50.31 (20.63)	.002 [c]
α-Linolenic acid (μg/mL)	1.92 (1.22)	1.84 (1.11)	.99 [c]	2.18 (1.49)	2.62 (1.42)	.031 [c]
Total omega-6 (μg/mL)	423.94 (95.98)	404.79 (96.00)	.09 [a]	454.99 (112.35)	466.60 (101.47)	.15 [a]
LA (μg/mL)	243.22 (68.87)	230.18 (75.11)	.34 [a]	261.65 (71.61)	259.24 (67.36)	.21 [a]
AA (μg/mL)	122.92 (41.28)	114.51 (29.25)	.031 [c]	129.35 (42.43)	137.46 (52.08)	.70 [c]
	No. (%) [e]					
Education						
High school	128 (37)	42 (43)	.15	312 (65)	51 (63)	.70
Trade/certificate/diploma	180 (52)	52 (53)		149 (31)	28 (35)	
Bachelor degree or higher	38 (11)	4 (5)		22 (5)	2 (2)	
Cigarette consumption						
Lifelong nonsmoker	157 (45)	34 (35)	.017	315 (65)	55 (68)	.86
Ex-smoker	146 (42)	57 (58)		127 (26)	19 (23)	
Current smoker	43 (12)	7 (7)		41 (8)	7 (9)	
History of a serious medical condition [f]	102 (29)	63 (64)	<.001	153 (32)	54 (67)	<.001
Taking dietary supplements [g]	153 (44)	46 (47)	.36	306 (63)	46 (57)	.26

METS, metabolic equivalents
[a] P value from t test.
[b] Using jaundice-meter reading from palm.
[c] P value from Mann-Whitney U test.
[d] Sum of EPA, DPA, and DHA.
[e] P value from χ^2 test.
[f] History of a serious medical condition includes high blood pressure/hypertension, angina, myocardial infarction, stroke, or cancer.
[g] Include any type of supplements. Fish oil supplements were taken by n = 19 (2%) participants (men, n = 9 [2%]; women, n = 10 [2%]; survived, n = 13 [2%]; deceased, n = 6 [3%]).

Table 4 – Risk of all-cause mortality in relation to plasma omega-3 and omega-6 PUFAs overall and by sex.

Total long-chain n-3 [a]	All		Men		Women	
	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P
Minimally adjusted [b]	1.02 (0.88-1.18)	.82	1.05 (0.86-1.28)	.64	1.00 (0.91-1.23)	.97
Multivariable model [c]	0.96 (0.82-1.12)	.62	0.99 (0.80-1.22)	.90	0.95 (0.76-1.19)	.66
EPA						
Minimally adjusted [b]	0.85 (0.73-1.00)	.05	0.87 (0.71-1.08)	.20	0.84 (0.67-1.07)	.17
Multivariable model [c]	0.81 (0.68-0.95)	.012	0.78 (0.62-0.98)	.036	0.78 (0.65-0.94)	.007
DPA						
Minimally adjusted [b]	0.88 (0.75-1.03)	.11	0.80 (0.64-1.00)	.046	0.99 (0.79-1.23)	.90
Multivariable model [c]	0.90 (0.77-1.05)	.08	0.76 (0.60-0.97)	.028	0.98 (0.79-1.22)	.86
DHA						
Minimally adjusted [b]	1.13 (0.98-1.29)	.10	1.18 (0.98-1.41)	.09	1.07 (0.87-1.31)	.52
Multivariable model [c]	1.07 (0.92-1.25)	.35	1.12 (0.92-1.36)	.25	1.02 (0.82-1.28)	.83
α-Linolenic acid						
Minimally adjusted [b]	0.88 (0.74-1.04)	.13	0.78 (0.61-1.00)	.046	0.97 (0.78-1.21)	.79
Multivariable model [c]	0.86 (0.72-1.01)	.07	0.73 (0.57-0.94)	.015	0.97 (0.78-1.22)	.82
Total n-6						
Minimally adjusted [b]	0.92 (0.79-1.08)	.32	0.85 (0.67-1.06)	.15	1.03 (0.83-1.28)	.77
Multivariable model [c]	0.87 (0.73-1.04)	.13	0.80 (0.92-1.04)	.10	0.96 (0.76-1.21)	.71
LA						
Minimally adjusted [b]	0.92 (0.79-1.08)	.32	0.85 (0.69-1.05)	.14	1.06 (0.85-1.32)	.59
Multivariable model [c]	0.87 (0.73-1.04)	.13	0.82 (0.65-1.03)	.09	1.04 (0.83-1.30)	.74
AA						
Minimally adjusted [b]	0.91 (0.78-1.06)	.21	0.91 (0.74-1.13)	.41	0.92 (0.74-1.14)	.43
Multivariable model [c]	0.85 (0.72-1.01)	.06	0.91 (0.72-1.15)	.43	0.83 (0.66-1.05)	.12

Hazard ratio is per 1-SD increase in the relevant fatty acid concentrations.
Total participants, N = 1008 (men, n = 444; women, n = 564).
[a] Sum of EPA, DPA, and DHA.
[b] Adjusted for age (ordinal, in quartiles), sex, and smoking status.
[c] Adjusted for minimally adjusted model plus blood cholesterol, jaundice measure (proxy serum β-carotene level), and history of serious medical condition.

omega-3 PUFAs and mortality; however, after full adjustment, plasma EPA was significantly inversely associated with all-cause mortality: HR, 0.81 (95% CI, 0.68-0.95; Table 4): that is, each 1-SD increase in EPA was associated with a 19% decrease in mortality. Lower risks of all-cause mortality were also observed for high DPA and α-linolenic acid, although these were not significant. Among the omega-6 PUFAs, higher plasma AA tended to be inversely associated with mortality but not significantly. When HRs were estimated using tertile models, the direction or magnitude of the associations was similar to the linear models (Supplemental Table 1). There was no evidence of nonlinear associations between any plasma PUFAs and all-cause mortality ($P > .05$, data not shown), nor was there any evidence of violation of the proportional hazards assumption or the functional form of continuous covariates.

3.3. *Plasma PUFAs and all-cause mortality: men*

Analyses were repeated for men and women separately because statistically significant interactions were observed with DHA, α-linolenic acid, and total omega-6 PUFAs ($P < .1$). The multivariable models showed that 1-SD increases in EPA and α-linolenic acid were significantly associated with a 22% decrease (HR, 0.78; 95% CI, 0.62-0.98) and a 27% decrease (HR, 0.73; 95% CI, 0.57-0.94) in deaths, respectively (Table 4). Similarly, a 1-SD increase in DPA was associated with a 24% decrease in mortality (HR, 0.76; 95% CI, 0.60-0.97). Although higher plasma DHA was associated with increased mortality, lower plasma total omega-6 PUFAs, LA, and AA tended to be inversely associated but not significantly. The tertile models showed generally similar associations with the linear models, with the exception that risk of mortality was significantly higher in men with the highest plasma DHA (Supplemental Table 2). There were no significant nonlinear associations between omega-3 or omega-6 PUFAs and mortality among men.

3.4. *Plasma PUFAs and all-cause mortality: women*

Among women, plasma EPA was also inversely associated with mortality (HR, 0.78; 95% CI, 0.65-0.94; Table 4). There was some evidence of a nonlinear association between plasma α-linolenic acid and all-cause mortality from the RCS regression analysis ($P = .039$, results not shown); however, no overall statistical significant association was observed. No consistent associations or trends, linear or nonlinear, were observed between other individual or total omega-3 PUFAs or omega-6 PUFAs and all-cause mortality among women (Supplemental Table 3).

4. Discussion

In this prospective population-based study of Australian adults, we showed that certain omega-3 PUFAs in plasma, namely EPA and, among men, DPA and α-linolenic acid, were

consistently inversely associated with all-cause mortality. No other consistent significant associations were observed between omega-3 and omega-6 fatty acids and all-cause mortality. Indeed, almost all our estimated HRs for plasma omega-6 PUFAs were less than 1.0, although none was statistically significant. Thus, our findings partly support our hypothesis that omega-3 PUFAs reduce all-cause mortality but do not support the hypothesized positive association between omega-6 PUFAs and all-cause mortality.

To date, most studies have investigated cardiovascular health outcomes in relation to dietary intakes of omega-3 and omega-6 PUFAs rather than objective biomarkers of intake, and most have studied selected high-risk groups rather than representative populations. Findings from the few previous studies that have examined biomarkers of omega-3 and all-cause mortality showed that higher plasma EPA was associated with lower all-cause mortality [21,22,25], similar to our findings. In addition, one of these studies that was carried out among healthy older US men and women also showed DPA to be inversely associated with lower all-cause mortality [21]. However, the results of previous studies of DHA have been inconsistent [21-23,25]. Although 2 previous cohort studies found a significant inverse association between serum DHA and all-cause mortality among older-age groups [21,25], results of 1 nested case-control and 2 other cohort studies were null [20,22,23]. In the current study, higher plasma α-linolenic acid appeared to protect against mortality only among men, whereas several earlier population-based studies did not find any association [20,23-25,37].

Previous studies that have examined biomarkers of omega-6 PUFAs and all-cause mortality in the general population are also sparse [20,23,25,26,37]. Similar to our finding, no association with LA was seen in a Dutch-nested case-control study [20], whereas in contrast, inverse associations were reported by 3 cohort studies [25,26,37]. For AA, none of the population-based studies reported a significant association with mortality [20,23,26], also in line with our results. Although the findings were inconsistent, no studies reported harmful effects of omega-6 PUFAs [20,23,25,26,37].

The plasma composition of PUFA reflects dietary intake of PUFA, endogenous synthesis, and metabolism. Although knowledge of health effects of DPA is still scarce, long-chain omega-3 PUFAs are well known to have anti-inflammatory properties [5,38-40] and to be inversely associated with CVD events [39,41]. Although conversion is limited, α-linolenic acid can be converted to EPA and DHA [42,43] and exert anti-inflammatory effects even before its conversion to long-chain omega-3 PUFAs [44]. Therefore, omega-3 PUFAs are thought to aid the prevention of a range of inflammation-associated diseases such as CVD and cancer and potentially reduce the risk of mortality [3,4,45].

Linoleic acid is the major omega-6 PUFAs in the human diet and is converted to AA in the body [46]. In contrast to omega-3 PUFAs, omega-6 PUFAs are believed to increase inflammation through the production of eicosanoids via metabolism of AA [8,47] but to have protective effects against CVD events because of their ability to reduce serum cholesterol and lower blood pressure [6]. Omega-6 PUFAs also may promote tumor growth and antiapoptotic effects suggesting possible procancer effects [10]. In summary, the net effect of omega-6 PUFAs on health is uncertain [7-9,47-49].

In the present study, men had significantly lower plasma DHA, α-linolenic acid, total long-chain omega-3 PUFAs, LA, AA, and total omega-6 PUFAs compared with women, consistent with previous findings [17,40,50-53]. Hormonal interaction (ie, estrogen) is thought to be one possible reason that women have a greater ability than men to synthesize DHA from α-linolenic acid [42,52]. We also observed some sex-specific associations with mortality: inverse associations with DPA and α-linolenic acid and, to some extent, LA among men but not women. Previous studies have also found sex-specific differences in the associations between plasma omega-3 and omega-6 PUFAs and all-cause mortality, although these were somewhat different in that inverse associations between serum LA and EPA and all-cause mortality were found only among men [22,25]. The mechanisms of these sex differences may be due to metabolism, diet, or other reasons that are not well understood [25].

Limitations of this study include that plasma phospholipid of omega-3 and omega-6 PUFAs was measured only once at baseline, when it is known that levels of these PUFAs may vary with seasons or availability of foods that contain omega-3 or omega-6 PUFAs. However, multiple measurements of omega-3 PUFAs for a 6-month period have been reported as being consistent, and so we believe that our single measurements of plasma levels of omega-3 PUFAs should be reasonably accurate markers of habitual intake [54]. Our participants had a narrower range of omega-3 and omega-6 PUFA levels compared with people studied in Japan and the United Kingdom [17,55]. This low variability of PUFA exposure may have contributed to some of the nonsignificant associations observed in our study, although the relatively small number of events, especially among women in our study, also reduced the power of our study. Our results using nonfasting plasma samples may have differed from results obtained if we had used fasting samples, although likely only by a small degree because we have previously shown that nonfasting plasma samples reflect usual dietary intakes [56]. Indeed, nonfasting blood sample may better reflect "usual" status than fasting blood because most people are not usually in a fasting state [57]. Because this was an observational study, residual confounding factors may have affected our results. Regarding study strengths, our results were based on a population-based longitudinal study of plasma biomarkers, and they add to the scarce evidence that currently exists about the association between plasma omega-3 and omega-6 PUFAs and mortality in the general population.

From this prospective study of Australian adults, we conclude that people with relatively high levels of plasma EPA have a reduced risk of all-cause mortality. In addition, men with high levels of plasma DPA and α-linolenic acid may have lower all-cause mortality than other men. These findings partially support the hypothesis that omega-3 PUFAs reduce all-cause mortality but do not support the hypothesis that omega-6 PUFAs raise the risk of mortality. Large population-based longitudinal studies are needed to further investigate associations between biomarkers of individual omega-3 and omega-6 PUFA intakes and mortality in view of the continuing lack of consistency in current evidence.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.nutres.2016.09.006.

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