Absolute versus relative measures of plasma fatty acids and health outcomes

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Title: Absolute vs. relative measures of plasma fatty acids and health outcomes: example of phospholipid omega-3 and omega-6 fatty acids and all-cause mortality in women

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Abbreviation used:

AA: arachidonic acid; BMI: body mass index; DHA: docosahexaenoic acid; DPA: docosapentaenoic acid (22:5 n-3); EPA: eicosapentaenoic acid; FA: Fatty acid; FAME: fatty acid methyl esters; HR: hazard ratio.

Abstract

Purpose: In a well-characterised community-based prospective study, to assess systematically the differences in associations of plasma omega-3 and omega-6 fatty acid (FA) status with all-cause mortality when plasma FA status is expressed in absolute concentrations versus relative levels.

Methods: In a community sample of 564 women aged 25–75 years in Queensland, Australia, baseline plasma phospholipid FA levels were measured using gas chromatography. Specific FAs analysed were eicosapentaenoic acid, docosapentaenoic acid, docosahexaenoic acid, total long-chain omega-3 FAs, linoleic acid, arachidonic acid, and total omega-6 FAs. Levels of each FA were expressed in absolute amounts ($\mu\text{g/mL}$) and relative levels (% of total FAs) and divided into thirds. Deaths were monitored for 17-years and hazard ratios and 95% confidence intervals calculated to assess risk of death according to absolute *vs.* relative plasma FA levels.

Results: In total 81 (14%) women died during follow-up. Agreement between absolute and relative measures of plasma FAs was higher in omega-3 than omega-6 FAs. The results of multivariate analyses for risk of all-cause mortality were generally similar with risk tending to inverse associations with plasma phospholipid omega-3 FAs and no association with omega-6 FAs. Sensitivity analyses examining effects of age and presence of serious medical conditions on risk of mortality did not alter findings.

Conclusions: The directions and magnitude of associations with mortality of absolute *vs.* relative FA levels were comparable. However, plasma FA expressed as absolute concentrations may be preferred for ease of comparison and since relative units can be deduced from absolute units.

Introduction

Intakes of different types and amounts of dietary fatty acids (FAs) appear to influence the risk of cardiovascular disease [1,2] and several types of cancer [3-5]. Early observational studies estimated amounts of FAs consumed using dietary intake methods that rely on memory are prone to bias. Current studies therefore increasingly use biomarkers to assess FA intake.

Plasma phospholipids are the commonly used biomarkers of FA intake [6,7]. Plasma levels reflect short- to moderate-term dietary intake of FAs and are considered acceptable indicators in epidemiological studies [6-9]. However to date, there has been no agreement in the published literature about whether absolute amounts (e.g. $\mu\text{g/mL}$) or relative levels (e.g. % of total FAs) of phospholipid FAs in plasma provide the more informative measure in relation to health outcomes. This lack of agreement makes it difficult to compare the findings across studies especially if the results expressed as absolute levels differ from those expressed as relative values. The same percentage of a FA may correspond to different absolute amounts of the FA across individuals, or in reverse, the proportion of one FA can differ between people although they have the same absolute amounts of that FA. Several studies have previously compared the two measures of serum or plasma phospholipid FA among people with and without health conditions [10-13]. Two studies showed significant differences in the FA status using the two measures [10,11]. Because of this finding, Schwertner and Mosser [10] recommended use of absolute concentrations of FA status as these showed the differences between cases and controls that were predicted a priori whereas using relative levels of FAs (wt%) were much weaker. Similarly, two cross-sectional studies that compared the two FA measures in relation to blood lipids found conflicting directions of association when FAs were expressed as a concentration of FA ($\mu\text{mol/L}$ [12] or mg/dL [14]) or a proportion (% of total FAs [12] or $\text{mol\%}$ [14]). However, these two studies reached the

opposite conclusion in relation to the recommended expression of FAs [12,14]. In the absence of an accepted standardised measure, it is not surprising that recommendations are not uniform [15-19].

Since the choice of FA measure may change the associations between FA status and health outcomes, and since the issue of duality remains unresolved [8,15], we have addressed these questions directly using prospective data. Specifically, we have investigated and compared the associations of plasma omega-3 and omega-6 FA on mortality in women when FAs were measured: 1) as the concentration of plasma-bound FAs (absolute concentrations) ($\mu\text{g/mL}$); and 2) as the plasma % compositions (relative amount, % of total FAs). We hypothesised that the magnitude and direction of association between FAs and all-cause mortality would be similar between the two measures.

Methods

Study population

The study cohort comprised Australian women aged 25–79 years who were participants in the Nambour Skin Cancer Study, a random community sample which has been fully described elsewhere [20,21]. Briefly, 2095 adult Nambour residents were randomly selected from the Australian Electoral Roll in 1986 for a study on skin cancer. Between 1992 and 1996, 1621 of the 2095 took part in a randomised controlled trial of sunscreen use, beta-carotene supplementation and skin cancer prevention [20,21], of whom 911 were women and eligible for the present study. During the trial, participants completed questionnaires about their socio-demographic factors, lifestyle behaviours, existing medical conditions and also completed a validated food frequency questionnaire [22]. Weight and height were measured in field clinics using standardized procedures. Women were eligible for the present study if

they provided blood samples during a follow-up clinic in August 1996 and were still alive on the 1st of January, 1997. The study was approved by the Ethics committee of the QIMR Berghofer Medical Research Institute, and all participants provided written informed consent.

Plasma phospholipid fatty acids

Prior to blood collection, those taking part were requested to eat a light breakfast (e.g. toast or cereal, no cooked breakfast). Non-fasting 30 mL venous blood samples were collected and samples were processed at collection and stored in approximately 1 ml aliquots at -70°C . Measurements of plasma phospholipids FAs were conducted by Flinders Medical Centre, Adelaide, Australia using procedures described in detail previously [23]. Plasma was extracted in chloroform:methanol, thin-layer chromatography was used to separate phospholipid fractions, and fatty acid methyl esters (FAME) were quantified using gas chromatography (Hewlett-Packard 6890 with a 50-m capillary column). Phosphatidylcholine diheptadecanoyl (C17:0 phospholipids) was used as an internal standard. FAME were identified based on the retention time to authentic lipid standards (GLC-463, Nuchek Prep Inc. Elysian, MN) and quantified by comparison to the internal standard using ChemStation software (Agilent, CA, USA). Specific FAs analysed were eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA, 22:5 n-3), docosahexaenoic acid (DHA), α -linolenic acid, total long-chain omega-3, linoleic acid, arachidonic acid (AA) and total omega-6 FAs. This study focused on these FAs *a priori* since they are the major omega-3 and omega-6 FAs that are extensively studied and widely considered to have health effects [1-5]. The relative amount of FA is expressed as a % of total plasma phospholipid FAs analysed (series of omega-3, omega-6, omega-7, omega-9, saturated- and trans fatty acids).

Outcome

Outcome was 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. Cause of death was based on the 10th revision of the International Classification of Diseases.

Statistical Analysis

Difference in personal characteristics and plasma FA levels between women who died during follow-up and those who survived were assessed using χ^2 tests for categorical variables, ANOVA for age, body mass index (BMI) and serum cholesterol and Mann-Whitney U-test for alcohol consumption and FA levels. To assess extent of agreement between the two measures in terms of ranking women by plasma FA level, we classified women into ranked thirds based on each FA type (Supplemental Table 1). The proportion of individuals classified in the same tertile by the two measures (exact agreement), the proportion who deviated by one tertile, and the proportion of gross discrepancy (disagreement by two tertiles) were calculated.

We analysed associations between plasma phospholipid FAs and subsequent mortality using Cox proportional hazards models. Hazard ratios (HR) with 95% confidence intervals (CI) were calculated comparing each of the two higher FA groups to the lowest. To test for linear trends, we assigned an ordinal number ranging from 1 (for the lowest third of FA level) to 3 (for the highest third) for each participant's plasma FA level and modelled this value as a continuous variable. We also examined FAs as continuous variables, with the interval used to estimate the HR being one standard deviation (SD) for each FA. Time to death was calculated from 1st January 1997 to date of death and women were censored if alive on 31st August 2014. All models were controlled for age (categorised into 4 equal groups), smoking status

and serum cholesterol level (mmol/L) as the serum cholesterol concentrations relate to plasma FA levels [12]. The proportional hazards assumption and functional form of continuous covariates were assessed using the ASSESS option of PHREG in SAS. We used the Aikake Information Criterion (AIC) as our metric to compare the quality of the models. A lower AIC value identified the model with the better balance between goodness of fit and model complexity. All statistical analyses were performed using SAS version 9.2 (SAS Institute Inc., Cary, North Carolina, USA), and $p < 0.05$ was considered statistically significant.

Results

Of the 911 female original trial participants, 24 died, 127 withdrew from active participation, 41 did not attend the 1996 study clinic and 62 did not give blood. An additional 93 were excluded due to missing information on cholesterol ($n=1$), education ($n=57$), physical activity ($n=10$), height and weight ($n=66$), leaving 564 participants. A comparison of the 564 women included in the present study with the 347 who were not, showed that the latter group had greater BMI and consumed less alcohol but otherwise were no different in terms of age, randomised trial treatment allocation, education, occupation, smoking, levels of physical activity, energy intake, alcohol consumption or history of serious medical conditions to those included.

Study participants had a mean age of 49 years (SD 12) at baseline, the majority ($n=370$, 66%) were life-long non-smokers, and only $n=10$ (2%) of women were taking fish oil supplements (Table 1). The 81 women (14%) who died during the 17-year follow-up were older and consumed less alcohol, and more likely to report an existing medical condition, and to have higher serum cholesterol compared to those who survived. The two major causes of death were cardiovascular disease ($n=40$) and cancer ($n=29$).

149

150 The median values and interquartile range of both expressions of FAs are presented in Table
151 S1 of the online supplementary material. Distribution of FA profiles differed according to
152 vital status on follow-up (Table 2), but these differences varied depending on which measure
153 of plasma FA level was used. When plasma phospholipid FA was expressed in absolute
154 amounts, the concentration of DPA and α -linolenic acid were significantly higher in women
155 who died compared with those who survived but there were no statistically significant
156 differences when FAs were expressed as relative levels. On the other hand, plasma
157 phospholipid total omega-6 FA and AA were significantly higher when expressed in relative
158 levels but not absolute amounts. Among those who died, plasma phospholipid long-chain
159 omega-3 and DHA were consistently higher but EPA and linoleic acid were not significantly
160 different to those alive at end of study. Since both absolute and relative amount of plasma
161 phospholipid omega-3 FAs were tended to be higher among deceased women, we examined
162 dietary intake of fish, the main source of these FAs, between survived and deceased women.
163 While deceased women consumed more fish (median intake: survived 17.4g/day vs. deceased
164 21.4g/day; $p=0.048$), dietary intake of total omega-3 FAs did not differ between the two
165 groups (median intake: survived 0.98g/day vs. deceased 1.03g/day; $p=0.57$). Total meat
166 intake as well as intakes of omega-6 FAs were also compared between survived and deceased
167 women; however, none of them were significantly different (median total meat intake:
168 survived 175.6g/day vs. deceased 168.3g/day; $p=0.16$; median total omega-6 FA intake:
169 survived 8.08 g/day vs. deceased 8.15g/day; $p=0.70$, mean AA intake: survived 0.08g/day vs.
170 deceased 0.08g/day; $p=0.43$).

171

172 As expected, absolute concentration of FAs showed stronger associations with blood
173 cholesterol than relative measures (Table S2 of the online supplementary material). Both

expressions of FAs were significantly correlated with blood cholesterol with one exception, namely relative expression of α -linolenic acid did not reach statistical significance ($r=0.08$, $p=0.051$). While the association between absolute measures of total omega-6 FAs, linoleic acid, and AA and cholesterol were positive, the direction of associations using the relative measures was negative.

For the majority of women, allocation into categories of omega-3 FA status was the same whether FAs were expressed as absolute or relative measures: ranging from 61% (DPA) to 79% (α -linolenic acid) (**Figure 1**). For omega-6 FAs, exact category allocation was somewhat lower than omega-3 FAs and ranged from 40% (total omega-6 FAs) to 61% (AA). The extreme disagreement (e.g. the highest third using absolute but categorised in the lowest thirds using relative levels) was lower for omega-3 FAs (ranging from 0% (EPA and α -linolenic acid) to 3% (DPA)), than for omega-6 FAs, and the highest disagreement was observed with total omega-6 FAs (19%). Further, all extreme disagreements showed symmetric patterns; that is, the percentage categorised in the highest third using absolute but categorised in the lowest third in relative and the percentage of categorised in the opposite pattern of disagreement appeared to be the same. Disagreements by only one category also showed a symmetrical pattern. Higher Spearman rank correlation coefficients were observed in omega-3 FAs: total long-chain omega-3 0.75; EPA 0.91; DPA 0.73; DHA 0.82; α -linolenic acid 0.92; than omega-6 FAs: total omega-6 FAs 0.14; linoleic acid 0.51; and AA 0.69. All correlation coefficients were statistically significant at $p<0.001$.

Although precise HRs for total mortality differed somewhat between absolute and relative measures of plasma omega-3 and omega-6 FAs, observed associations were generally similar in the direction and magnitude of the associations, and were null, except for the relative

expression of EPA in the linear model (HR 0.76, 95% CI 0.59–0.98) (Table 3). The model evaluation using AIC also showed that there was no evidence that either of the expressions showed a consistent and reproducible association with all-cause mortality over the other (Figure S1 of the online supplemental material). Since FA desaturation has been reported to decrease with ageing [8,24], a stratified analysis by <60 years vs. ≥60 years was undertaken; however, the estimated HRs were also generally similar, particularly for both measures of plasma omega-3 FAs (Table S3 of the online supplementary material).

Sensitivity analyses excluding those who reported being diagnosed with a history of serious medical conditions (high blood pressure, stroke, angina, heart attack and cancer excluding skin cancers but including melanoma) also showed similar associations between absolute and relative measures of plasma omega-3 and omega-6 FAs (Table 4). In both measures, higher plasma EPA was negatively associated with all-cause mortality [Absolute measure: T1 vs. T3 HR 0.38 (95% CI 0.13–1.07), *p-trend*=0.06; linear model HR 0.60 (95% CI 0.36–0.99). Relative measure: T1 vs. T3 HR 0.37 (95% CI 0.13–1.04), *p-trend*=0.054; linear model HR 0.57 (95% CI 0.35–0.93)]. Similarly, other plasma omega-3 FAs, including total long-chain omega-3 FAs, in both measures were tended to be negatively associated with all-cause mortality. Absolute and relative measures of plasma omega-6 FAs, in contrast, were not associated with all-cause mortality (all estimated HRs were around 1.0).

Discussion

Some investigators have strong views about which of the two common measures of FA status is the more appropriate for use in studies of their relationship to health outcomes [14,16,17]. However, we observed similar results after using both absolute and relative measures in blood to study the relation of FAs to total mortality in a cohort of women drawn from the

general Australian population, despite some differences in precise categorisation of FA levels using the two measures. In particular, plasma phospholipid omega-6 FAs showed lower correlations and higher disagreement between absolute and relative measurements which was similar to the previous study [15]. However, disagreement patterns for all FAs (whether by one category or extreme categories) were symmetric, which means that the disagreements were not biased in relation to any specific FA type.

To date, very few other studies have compared the absolute amount and relative level of FAs in relation to health outcomes. Four reported some significant differences in FA status depending on the choice of FA expression used [10-12,14]. Schwertner and Mosser [10] compared FA status among male patients with and without coronary artery disease (total N=30) and they found the differences in FA composition between cases and controls were much greater when they used absolute amounts (mg/L) compared with relative levels (wt%). While they recommended the use of absolute concentrations, they also suggested using both FA measures so that comparisons could be made. In contrast, one case-control study described fatty acid status using phospholipid and erythrocytes among those with and without recurrent major depression [13]. Different types of fatty acid showed significant differences between cases and controls: for example, when phospholipid FA status was expressed as an absolute measure ($\mu\text{mol/L}$), α -linolenic acid and AA were significantly higher among cases compared with controls, and no differences in DPA and DHA were observed. When FA status was expressed in relative (% total), DPA, DHA, and AA were significantly higher among controls but there were no differences in α -linolenic acid. Two recently published cross-sectional studies compared the association between circulating FAs expressed in absolute and proportion of total FAs in relation to blood lipids as disease biomarkers [12,14].

Similar to our findings, both studies reported statistically significant negative associations between AA [14] and linoleic acid [12] and total cholesterol when FA was measured as proportion but the direction was reversed when FA was expressed as absolute concentration. Since omega-6 FAs are known to reduce blood cholesterol level, Bradbury and colleagues [14] supported the relative measure of FAs (mol%) as the better method to show the metabolic relationship with cholesterol concentrations. In contrast, Sergeant and college [12] recommend using absolute concentration of the FA. It is possible that studies using the proportion of total FA may have been ‘misinterpreted’ in relation to disease biomarkers or risk because, for example, omega-6 FAs are also known to have pro-inflammatory effects. Further, people with different absolute concentration of the FA should not be grouped with others purely because they have the same proportion of the FA [12].

Only one previous nested case-control study, the EPIC-Norfolk Study, has presented both absolute ($\mu\text{mol/L}$) and relative levels (mol%) of plasma FAs in relation to a health outcome, namely the incidence of coronary heart disease [25]. While they did not present individual plasma phospholipid omega-3 and omega-6 FA status in relation to coronary heart disease or blood cholesterol, the results using quartile or continuous plasma total omega-3 and omega-6 FAs were comparable between the two FA measures, which is in agreement with our results. Further studies would be helpful to elucidate whether biomarker of FA expressed in concentration and proportion of total FAs show similar associations with health outcomes in larger longitudinal studies.

One of the main reasons supporting the use of absolute FA concentrations is that the measurement is not dependent on the levels of other FAs, unlike relative FA concentrations [10,16,17]. For example, an increasing percent of a FA may result from i) increasing, ii)

decreasing, or iii) not changing the percent of other FAs [9]. This interdependency means that the results expressed as a percent of total FAs may not be comparable across the group of people whose absolute values are significantly different. Further, expressing FAs as absolute amounts would enable comparisons between studies [10,16], and comparison of changes in concentration over time within the same group. In contrast, the relative proportion of total FAs may be more appropriate in presenting biomarkers of FAs and making assessments in relation to health outcomes [8,14,18] given the interrelationship between plasma total cholesterol, plasma lipoproteins, and plasma FA levels [8]. Therefore, absolute concentration of FAs may even be misleading and the proportion of FAs is thus more informative [18]. However, the estimated risk of all-cause mortality was similar between data from absolute and relative plasma phospholipid FA status. Presenting data primarily in absolute concentration units may be preferred as it facilitates comparisons across studies: no information is lost and ratios or relative units can always be deduced from absolute concentrations as long as sufficient FA concentrations are reported or mathematically modelled [12]. Although it may appear mathematically reasoned, there could also be biological relationships with health outcomes that are a function of absolute concentration of FAs [12].

The strengths of the present study are the representativeness of women participants of women in the general population, and, unlike previous studies, its long-term prospective design. Although our sample size was larger than most of the earlier studies [10-12], the numbers of deaths were relatively small and thus power to detect a significant association with total mortality may have been somewhat limited. Another limitation is that we used non-fasting blood samples. Although our previous study has shown non-fasting plasma samples reflect usual dietary intakes [26], the findings may differ from those based on fasting samples.

299 However, plasma phospholipid FA status (unlike whole blood FA status) reflects dietary
300 intake over a few days rather than recent dietary intake [8,9]. Therefore, our results are
301 unlikely to differ substantially due to the non-fasting state. Additionally, routine lipid assay
302 studies reported no differences or even possible superior results when using non-fasting blood
303 [27,28]. The benefits of fasting vs. non-fasting blood sampling has not been proven and
304 cohort studies are increasingly moving towards non-fasting blood collection [13,29]. The
305 findings of this study may be valuable in light of this development. Finally, possible selection
306 bias may have occurred in this study as the women not included had a greater BMI and
307 consumed less alcohol, though notwithstanding any change in the associations that might
308 have occurred if these women had been included, this would not have affected comparability
309 of the results for two FA expressions.

310
311 In conclusion, we have shown in a community-based prospective study of mortality that there
312 is no substantial difference in associations with plasma phospholipid FAs, whether expressed
313 in absolute or relative units. Presenting data primarily in absolute concentrations facilitates
314 the comparison across the studies without any loss of information, and relative units can
315 always be deduced from this approach if sufficient FA concentrations are reported. For these
316 reasons, FA status expressed in absolute amounts may be preferred.

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320 conducted research, MCBH managed the data; KM analysed the data, JPJU assisted data
321 interpretation, DDS assisted with the statistical analysis and data interpretation, all authors
322 wrote paper, and KM had primary responsibility for final content. All authors read and
323 approved the final manuscript.

324

325 **Ethical standards**

326 Ethical approval for the study was obtained from the Ethics committee of the QIMR
327 Berghofer Medical Research Institute. All participants provided written informed consent in
328 accordance with the Declaration of Helsinki.

329

330 **Conflict of interest**

331 The authors declare that they have no conflict of interest.

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Figure Legend

Figure 1 Comparison of ranked thirds of plasma phospholipid omega-3 and omega-6 fatty acids measured in absolute (µg/mL) and relative (%) amounts in 564 Australian women

AA=arachidonic acid; Abl: absolute; ALA= α -linolenic acid; EPA=eicosapentaenoic acid;

DHA=docosahexaenoic acid; DPA=docosapentaenoic acid; LA=linoleic acid; Rel: relative

Long-chain omega-3 polyunsaturated fatty acids: sum of eicosapentaenoic acid, docosapentaenoic acid (22:5 n-3) and docosahexaenoic acid.

Extreme disagreement, Rel high tertile = Abs low tertile: the highest thirds using relative but categorised in the lowest thirds using absolute levels.

One category disagreement, Abs tertile < Rel tertile: categorised into one below using absolute than using relative levels.

Exact agreement: allocation into tertile categories were the same between absolute and relative levels.

One category disagreement, Abs tertile > Rel tertile: categorized into one above using absolute than using relative levels.

Extreme disagreement, Abs high tertile = Rel low tertile: the highest thirds using absolute but categorised in the lowest thirds using relative levels.

Table 1 Baseline characteristics of participants and according to vital status on follow-up

	No. (%)			<i>p</i> -value ^a
	All (N=564)	Survived (n=483)	Deceased (n=81)	
Age, years (mean±SD)	49±12	47±11	62±10	<0.001
BMI, kg/m ² (mean±SD)	26.4±4.7	26.3 (4.7)	26.6 (4.7)	0.54
Education				
High school	363 (64)	312 (64)	51 (63)	0.70
Trade/Certificate/Diploma	177 (31)	149 (31)	28 (35)	
Bachelor degree or higher	24 (4)	22 (5)	2 (2)	
Cigarette consumption				
Life-long non-smoker	370 (66)	315 (65)	55 (68)	0.86
Ex-smoker	146 (26)	127 (26)	19 (23)	
Current smoker	48 (9)	41 (8)	7 (9)	
Physical activity ^b				
None	182 (32)	153 (32)	29 (36)	0.61
Low	129 (23)	115 (24)	14 (17)	
Medium	132 (23)	113 (23)	19 (23)	
High	121 (21)	102 (21)	19 (23)	
History of serious medical condition ^c	207 (37)	153 (32)	54 (67)	<0.001
Alcohol consumption, g/day [median (min, max)]	1.3 (0.0, 158.2)	1.4 (0.00, 49.6)	0.3 (0.0, 158.2)	0.008 ^d
Serum cholesterol, mmol/L (mean±SD)	5.3±1.0	5.2±1.0	5.7±1.0	<0.001
Taking dietary supplement/s ^e	352 (62)	306 (63)	46 (57)	0.26

^a *p*-value from Chi-square test (categorical test) or ANOVA (continuous data).

^b low= 0.1 hour to 1.5 hours, medium = 1.6 hours to 4.0 hours, 4.1 hours or more

^c Include high blood pressure/hypertension, angina, heart attack, stroke or cancer.

^d *p*-value from Mann-Whitney U-test.

^e Include any type of supplements. Fish oil supplements were taken by n=10 (2%) of women (survived: n=7; deceased: n=3).

Table 2 Median values for plasma phospholipid fatty acid status measured in absolute (µg/mL) and relative (%) according to vital status on follow-up

		Median (IQR)		<i>p</i> -value
	All (N=564)	Survived (n=483)	Deceased (n=81)	
Long-chain n-3 fatty acids^a				
μg/mL	69.72 (26.71)	68.21 (26.23)	77.89 (23.73)	<0.001 ^b
%	5.41 (1.53)	5.33 (1.50)	5.67 (1.37)	0.011 ^b
EPA				
μg/mL	11.47 (6.74)	11.35 (6.83)	12.11 (7.09)	0.15 ^b
%	0.85 (0.40)	0.84 (0.40)	0.88 (0.35)	0.41 ^b
DPA				
μg/mL	13.76 (4.86)	13.56 (4.44)	14.89 (6.32)	0.003 ^c
%	1.06 (0.29)	1.05 (0.28)	1.09 (0.33)	0.12 ^c
DHA				
μg/mL	43.89 (19.67)	43.38 (18.85)	50.31 (20.63)	0.002 ^b
%	3.42 (1.25)	3.38 (1.19)	3.72 (1.32)	0.025 ^c
ALA				
μg/mL	2.25 (1.51)	2.18 (1.49)	2.62 (1.42)	0.031 ^b
%	0.17 (0.10)	0.17 (0.10)	0.19 (0.10)	0.24 ^c
Total N-6 fatty acids				
μg/mL	458.10 (113.23)	454.99 (112.35)	466.60 (101.47)	0.15 ^c
%	35.28 (3.13)	35.34 (3.10)	34.85 (2.97)	0.011 ^c
LA				
μg/mL	257.25 (70.09)	256.92 (71.61)	259.24 (67.36)	0.21 ^c
%	19.98 (3.97)	20.07 (3.81)	19.27 (5.03)	0.27 ^c
AA				
μg/mL	130.59 (43.97)	129.35 (42.43)	137.46 (52.08)	0.70 ^b
%	10.06 (2.36)	10.14 (2.29)	9.63 (2.57)	0.038 ^c

AA=arachidonic acid; ALA= α -linolenic acid; EPA=eicosapentaenoic acid; DHA=docosahexaenoic acid; DPA=docosapentaenoic acid; IQR: inter quartile range; LA=linoleic acid.

^a Long-chain omega-3 polyunsaturated fatty acids: sum of EPA, DPA, and DHA.

^b *p*-value from Mann-Whitney U-test.

^c *p*-value from ANOVA.

Table 3 HR and 95% CI for all-cause mortality by ranked thirds of plasma phospholipid fatty acid status expressed in absolute (µg/ml) and relative (%) amounts in 564 Australian women^a

	Absolute (µg/ml)	Relative (%)
Long-chain n-3 fatty acids^b		
Low	1.00	1.00
Middle	0.96 (0.51, 1.80)	1.31 (0.73, 2.35)
High	1.05 (0.57, 1.92)	0.97 (0.54, 1.72)
<i>p</i> -value	0.84	0.77
Linear model ^c	0.97 (0.78, 1.20)	0.92 (0.75, 1.13)
EPA		
Low	1.00	1.00
Middle	0.71 (0.40, 1.25)	0.98 (0.56, 1.74)
High	0.60 (0.34, 1.06)	0.73 (0.42, 1.29)
<i>p</i> -value	0.09	0.24
Linear model ^c	0.82 (0.64, 1.05)	0.76 (0.59, 0.98)
DPA		
Low	1.00	1.00
Middle	0.50 (0.27, 0.92)	0.73 (0.41, 1.29)
High	0.64 (0.37, 1.10)	0.79 (0.47, 1.34)
<i>p</i> -value	0.20	0.43
Linear model ^c	0.95 (0.76, 1.20)	0.90 (0.72, 1.13)
DHA		
Low	1.00	1.00
Middle	0.93 (0.51, 1.70)	0.94 (0.51, 1.71)
High	1.05 (0.59, 1.85)	1.22 (0.71, 2.09)
<i>p</i> -value	0.82	0.42
Linear model ^c	1.05 (0.85, 1.30)	1.01 (0.83, 1.23)
ALA		
Low	1.00	1.00
Middle	1.25 (0.69, 2.27)	1.08 (0.61, 1.90)
High	1.42 (0.82, 2.54)	1.19 (0.71, 2.01)
<i>p</i> -trend	0.23	0.50
Linear model ^c	0.95 (0.77, 1.19)	0.95 (0.77, 1.19)
Total n-6 fatty acids		
Low	1.00	1.00
Middle	1.20 (0.66, 2.17)	0.90 (0.54, 1.51)
High	1.19 (0.65, 2.18)	1.02 (0.58, 1.78)
<i>p</i> -value	0.60	1.00
Linear model ^c	1.00 (0.78, 1.26)	0.93 (0.75, 1.15)
LA		
Low	1.00	1.00
Middle	1.22 (0.71, 2.11)	0.67 (0.39, 1.15)
High	0.99 (0.56, 1.74)	1.00 (0.59, 1.69)
<i>p</i> -value	0.94	0.84
Linear model ^c	1.04 (0.82, 1.30)	1.02 (0.82, 1.28)
AA		
Low	1.00	1.00
Middle	0.57 (0.32, 1.02)	0.69 (0.40, 1.19)
High	0.79 (0.47, 1.32)	0.64 (0.38, 1.08)

<i>p</i> -value	0.40	0.09
Linear model ^c	0.88 (0.70, 1.11)	0.81 (0.65, 1.01)

AA=arachidonic acid; ALA= α -linolenic acid; EPA=eicosapentaenoic acid;
DHA=docosahexaenoic acid; DPA=docosapentaenoic acid (22:5 n-3); LA=linoleic acid.

^a Adjusted for age (categorical), smoking status, and blood cholesterol.

^b Sum of EPA, DPA and DHA.

^c Linear model HR is per 1 SD increase in the relevant FA concentrations.

p-value for trend across tertile.

Table 4 Hazard ratio (HR) and its confidence intervals (95% CI) of all-cause mortality among 357 Australian women without history of serious medical conditions^{a,b}

	Absolute (µg/mL)	Relative (%)
Long-chain n-3 PUFA^c		
Low	1.00	1.00
Middle	1.61 (0.59, 4.39)	1.42 (0.59, 3.42)
High	0.92 (0.30, 2.80)	0.41 (0.13, 1.28)
<i>p</i> -value	0.78	0.12
Linear model ^d	0.72 (0.46, 1.14)	0.69 (0.46, 1.04)
EPA		
Low	1.00	1.00
Middle	0.69 (0.27, 1.79)	1.04 (0.41, 2.61)
High	0.38 (0.13, 1.07)	0.37 (0.13, 1.04)
<i>p</i> -value	0.06	0.054
Linear model ^d	0.60 (0.36, 0.99)	0.57 (0.35, 0.93)
DPA		
Low	1.00	1.00
Middle	0.39 (0.13, 1.19)	0.35 (0.11, 1.08)
High	0.56 (0.21, 1.49)	0.61 (0.23, 1.60)
<i>p</i> -value	0.36	0.59
Linear model ^d	0.81 (0.50, 1.30)	0.79 (0.52, 1.20)
DHA		
Low	1.00	1.00
Middle	1.50 (0.59, 3.85)	1.58 (0.64, 3.89)
High	0.89 (0.31, 2.55)	0.79 (0.28, 2.19)
<i>p</i> -value	0.79	0.66
Linear model ^d	0.81 (0.54, 1.21)	0.78 (0.54, 1.14)
ALA		
Low	1.00	1.00
Middle	1.46 (0.54, 3.96)	0.80 (0.30, 2.13)
High	0.83 (0.27, 2.55)	0.73 (0.29, 1.79)
<i>p</i> -value	0.64	0.49
Linear model ^d	0.77 (0.51, 1.18)	0.74 (0.48, 1.15)
Total n-6		
Low	1.00	1.00
Middle	0.84 (0.29, 2.38)	1.77 (0.68, 4.62)
High	1.23 (0.46, 3.28)	1.72 (0.62, 4.79)
<i>p</i> -value	0.64	0.30
Linear model ^d	1.14 (0.74, 1.77)	1.02 (0.72, 1.46)
LA		
Low	1.00	1.00
Middle	0.65 (0.24, 1.76)	0.56 (0.22, 1.45)
High	0.90 (0.35, 2.32)	0.77 (0.31, 1.91)
<i>p</i> -value	0.86	0.50
Linear model ^d	1.13 (0.74, 1.73)	1.03 (0.69, 1.54)
AA		
Low	1.00	1.00
Middle	0.25 (0.07, 0.91)	0.67 (0.24, 1.85)
High	1.02 (0.44, 2.33)	0.91 (0.38, 2.20)
<i>p</i> -value	0.89	0.84

Linear model ^d	1.02 (0.69, 1.51)	0.94 (0.65, 1.36)
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AA=arachidonic acid; ALA= α -linolenic acid; EPA=eicosapentaenoic acid;
DHA=docosahexaenoic acid; DPA=docosapentaenoic acid; LA=linoleic acid.

^a Adjusted for age (categorical), smoking status, and blood cholesterol.

^b History of serious medical conditions include high blood pressure, heart attack, stroke, angina, cancer (except keratinocyte skin cancers).

^c Sum of EPA, DPA and DHA.

^d Linear model HR is per 1 SD increase in the relevant FA concentrations.

p-value for trend across tertile.