Higher ratio of plasma omega-6/omega-3 fatty acids is associated with greater risk of all-cause, cancer, and cardiovascular mortality: a population-based cohort study in UK Biobank

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Abstract

Background

Circulating omega-3 and omega-6 polyunsaturated fatty acids (PUFAs) have been associated with various chronic diseases and mortality, but results are conflicting. Few studies examined the role of a balanced omega-6/omega-3 ratio in mortality.

Methods

We investigated plasma omega-3 and omega-6 PUFAs and their ratio in relation to all-cause and cause-specific mortality in a large prospective cohort, the UK Biobank. Of 117,546 participants who had complete information on circulating PUFAs, 4,733 died during follow-up, including 2,585 from cancer and 1,017 from cardiovascular disease (CVD). Associations were estimated by multivariable Cox proportional hazards regression with adjustment for relevant risk factors.

Results

Results: Risk for all three mortality outcomes increased as the ratio of omega-6/omega-3 PUFAs increased (all $P_{trend} < 0.001$). Comparing the highest to the lowest quintiles, individuals had 42% (95% CI, 28-57%) higher total mortality, 31% (95% CI, 13-50%) higher cancer mortality, and 40% (95% CI, 12-75%) higher CVD mortality. Moreover, omega-3 and omega-6 PUFAs in plasma were all inversely associated with all-cause, cancer, and CVD mortality, with omega-3 showing stronger effects.

Conclusions

Using a population-based cohort in UK Biobank, our study revealed a strong association between the ratio of circulating omega-6/omega-3 PUFAs and the risk of all-cause, cancer, and CVD mortality.

Keywords

Polyunsaturated fatty acids, Omega-3 fatty acids, Omega-6 fatty acids, Mortality, Prospective studies

Background

Cancer and cardiovascular disease (CVD) are the two leading causes of non-communicable disease mortality globally[1]. Substantial epidemiologic evidence has linked the dietary or circulating levels of omega-3 and omega-6 polyunsaturated fatty acids (PUFAs) to the risks of all-cause, cancer, and CVD mortality (**Additional file 1: Table S1**). However, the results are inconsistent, especially for omega-6 PUFAs. Most large observational studies support the protective effects of omega-3 PUFAs. In addition to total and individual omega-3 PUFAs, the omega-3 index, defined as the percentage of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in total fatty acids in red blood cells, was shown to be a validated biomarker of the dietary intake and tissue levels of long-chain omega-3 PUFAs, and was proposed to be a risk factor for CVD and related mortality[2,3]. While the omega-3 index has been observed to be inversely associated with all cause-mortality, its association patterns with CVD and cancer mortality are less clear[4-6]. Most importantly, results from clinical trials of omega-3 PUFA supplementation have been inconsistent[7,8]. On the other hand, while some studies revealed inverse associations of omega-6 PUFAs with all-cause mortality[9-11], others reported null results[4,12-15]. The role of omega-6 PUFAs in cancer and CVD mortality is less studied, and the patterns are similarly conflicting[4,9-11]. Interpreting previous observational results is challenging due to the limitations of small sample sizes, insufficient adjustments for confounding, and unique sample characteristics. Moreover, many studies rely on self-reported dietary intake or fish oil supplementation status, which are subject to large variability and reporting bias[16]. Despite the tremendous interest and research effort, the roles of omega-3 and omega-6 PUFAs in all-cause and cause-specific mortality remain controversial.

It has been suggested that the high omega-6/omega-3 ratio in Western diets, 20:1 or even higher, as compared to an estimated 1:1 during the most time of human evolution, contributes to many chronic diseases, including CVD, cancer, and autoimmune disorders[17,18]. However, while many previous studies have examined total or individual omega-3 and omega-6 PUFAs, fewer investigated the role of their imbalance, as measured by the omega-6/omega-3 ratio, in mortality[4,14,15,19]. In a prospective cohort study of postmenopausal women, the omega-6/omega-3 ratio in red blood cells was associated with an increased risk of all-cause mortality but not with cancer or CVD mortality[4]. Similar positive associations with all-cause mortality were observed in smaller cohorts investigating the ratio in serum or dietary intake[14,19]. However, prospective studies in two independent cohorts from China and US did not find a linear positive association between the omega-6/omega-3 ratio in diet and all-cause mortality[15]. To address these gaps in our understanding of the roles of omega-3 and omega-6 PUFAs and their imbalance in all-cause and cause-specific mortality, we perform a prospective study in a large population-based cohort (N = 117,546) from UK Biobank, using objective measurements of PUFA levels in plasma.

Methods

Study population

The UK Biobank study is a prospective, population-based cohort study in the United Kingdom[20]. Between 2006 and 2010, 502,536 prospective participants, aged 40-69, in 22 assessment Centers throughout the UK were recruited for the study. The population information was collected through a self-completed touch-screen questionnaire; brief computer-assistant interview; physical and functional measures; and blood, urine, and saliva collection during the assessment visit. Participants with incomplete data on the plasma omega-6/omega-3 ratio (n=383,692) and

105 those who withdrew from the study (n=1,298) were excluded from this study, leaving 117,546
106 participants, 4,733 died during follow-up, including 2,585 from cancer and 1,017 from CVD.
107
108 ***Ascertainment of exposure***
109 Omega-3 and omega-6 PUFAs were measured by nuclear magnetic resonance (NMR) in plasma
110 samples collected between 2007 and 2010[20-22].
111
112 ***Ascertainment of outcome***
113 The date and cause of death were identified through the death registries of the National Health
114 Service (NHS) Information Centre for participants from England and Wales and the NHS Centre
115 Register Scotland for participants from Scotland[20]. At the time of the analysis (3 May 2022),
116 mortality data were available up to 14 February 2018 for England and Wales and 31 December
117 2016 for Scotland. Therefore, follow-up time was calculated as the time between the date of
118 entering the assessment Centre and this date, or the date of death, whichever happened first. The
119 underlying cause of death was assigned and coded in vital registries according to the ICD-10
120 (International Classification of Diseases, 10th revision). CVD mortality was defined using codes
121 I00-I99, and cancer mortality was defined using codes C00-D48.
122
123 ***Ascertainment of covariates***
124 The baseline questionnaire included detailed information on several possible confounding
125 variables: demographic factors (age, sex, assessment Centre, ethnicity), socioeconomic status
126 (Townsend Deprivation Index), lifestyle habits (alcohol assumption, smoking status, body mass
127 index (BMI), physical activity) and other supplementation (Fish-oil supplementation).

128

129 ***Statistical analysis***

130 We summarized and compared the characteristics of the participants across quintiles of the
131 omega-6/omega-3 ratio at baseline using descriptive statistics. Pearson's Chi-squared test and
132 ANOVA test were used to compare the demographic characteristics across quintiles, respectively,
133 for categorical variables and continuous variables. To investigate associations of the ratio with
134 cause-specific and all-cause mortality, we used multivariable Cox proportional hazards
135 regression models to calculate hazard ratios[23] and their 95% confidence intervals (CI). The
136 proportional hazards assumption was not violated based on Schoenfeld residuals. We analyzed
137 the ratio as continuous and categorical variables (i.e., quintiles). For all trend tests, we used the
138 median value of each quintile as a continuous variable in the models. Potential nonlinear
139 associations were assessed semi-parametrically using restricted cubic splines (4 knots were used
140 in regression splines)[24].

141

142 Based on previous literature and biological plausibility[11,25,26], we chose the following variables
143 as covariates in the multivariate models: age (years; continuous), sex (male, female), race (White,
144 Black, Asian, Others), Townsend deprivation index (continuous), assessment Centre, BMI
145 (kg/m2; continuous), smoking status (never, previous, current), alcohol intake status (never,
146 previous, current), physical activity (low, moderate, high).

147

148 In secondary analyses to assess potential differences in associations across different population
149 subgroups, we repeated the above-described analyses stratified by age (< vs. ≥ the median age of
150 58 years), sex (male/female), Townsend deprivation index (< vs. ≥ the population median of -2),

BMI ((< vs. ≥ 25), current smoking status (yes vs. no), and physical activity (low and moderate vs. high).

We also conducted several sensitivity analyses. First, to assess whether the associations of the omega-6/omega-3 ratio with mortality outcomes were primarily driven by omega-3 fatty acids or omega-6 fatty acids, we assessed both the separate and the joint associations of omega-3 fatty acids to total fatty acids percentage (omega-3%) and omega-6 fatty acids to total fatty acids percentage (omega-6%) with the three mortality outcomes. We also performed a joint analysis with categories of the omega-3% and omega-6% quintiles, using participants in both the lowest omega-3% and omega-6% quintiles as the reference category. An interaction term between omega-3% and omega-6% was included in the multivariable Cox proportional hazards model, and a Wald test was used to assess its significance. The correlation between omega-3% and omega-6% was assessed by the Spearman correlation. Second, to address the potential residual confounding by fish oil supplementation, we further adjusted for the supplementation status from the baseline questionnaire. Third, to investigate the effects of missing values, we imputed missing values (<1% for most factors, up to 19% for physical activity) by chained equations and performed synthesis analyses on the imputed datasets[27]. Fourth, to assess whether the observed associations are attenuated by reverse causation, we excluded those who died in the first year of follow-up. Last, to assess the representativeness of the participants included in our study, we compared the baseline characteristics between the participants with and without exposure information. All P-values were two-sided. We considered a P-value ≤0.05 or a 95% confidence interval (CI) excluding 1.0 for HRs as statistically significant. We conducted all analyses using R, version 4.0.3.

Results

Baseline characteristics

In the analytic cohort of 117,546 participants, over a mean of 8.9 years of follow-up, 4,733 died, including 2,585 from cancer and 1,017 from CVD. The baseline characteristics of the participants across quintiles of the ratio of omega-6/omega-3 were summarized in **Table 1**. Study participants were, on average, 57 years old and 91% White. Those in the higher ratio quintiles were more likely to be younger, male, and current smokers, but less likely to take fish oil supplementation.

Main results

The associations of the omega-6/omega-3 ratio with all-cause and cause-specific mortality risks were presented in **Table 2**. A higher ratio was strongly associated with higher mortality from all causes, cancer, and CVD ($P_{trend} < 0.001$ for all three). In the fully adjusted models that considered the ratio as a continuous variable, every unit increase in the ratio corresponded to 2%, 1%, and 2% higher risk in all-cause, cancer, and CVD mortality, respectively. When comparisons were made between the highest and the lowest quintile of the omega-6/omega-3 ratio, there were 42%, 31%, and 40% increased risk for all-cause, cancer, and CVD mortality, respectively.

Stratified analysis

The fully adjusted associations of the omega-6/omega-3 ratio with all-cause mortality revealed that compared to the lowest quintile, the highest quintile has strong, statistically significant

associations with elevated risk within all categories of sex, TDI, BMI, smoking status, and physical activity **(Figure 1, Additional file 2: Table S2)**, except in those aged less than 58 years old. The estimated associations with all-cause mortality were stronger in current smokers and those with a higher TDI (P for interaction < 0.01 and = 0.02, respectively; **Figure 1**). For cancer and CVD mortality, they also tended to be stronger among current smokers (P for interaction = 0.01 and 0.05, respectively). No significant interactions were found for other risk factors (**Figure 2**).

Restricted cubic spline analysis

Restricted cubic spline analysis suggested significant positive associations of the omega-6/omega-3 ratio with all-cause, cancer, and CVD mortality ($P < 0.01$ for all three outcomes, **Figure 3**). Potential nonlinearity in these positive associations was identified for all-cause mortality ($P = 0.011$) and CVD mortality ($P = 0.011$) but not for cancer mortality ($P > 0.05$). The strength of the relationship between the ratio and all-cause mortality appears to remain at a relatively low level before it starts to increase quickly after the ratio exceeds 8. A similar trend with higher uncertainties was observed for CVD mortality.

Omega-3, Omega-6, and Joint analysis

We further performed analyses to assess whether the associations of the omega-6/omega-3 ratio with mortality outcomes were primarily driven by omega-3 or omega-6 fatty acids. The correlation between the omega-3% and omega-6% was relatively low with $r = 0.12$ ($P < 0.01$). Across all models, both the omega-3% and omega-6% were inversely associated with all three mortality outcomes ($P_{trend} < 0.001$ for both exposures and all three outcomes, **Additional file 2:**

Table S3 and Table S4). Notably, their associations remained significant when they were included in the same models. On the other hand, the effect sizes of the inverse associations were always bigger for the omega-3%. For example, when comparing those in the highest omega-3% quintile to the lowest quintile, the fully adjusted HRs (95% CI) for all-cause, cancer, and CVD mortality were, respectively, 0.62 (0.55, 0.68), 0.68 (0.59, 0.79), and 0.62 (0.49, 0.78) **(Additional file 2: Table S3)**. The corresponding HRs for the omega-6% were 0.81 (0.72, 0.90), 0.78 (0.67, 0.91), and 0.87 (0.67, 1.12) **(Additional file 2: Table S4)**. Furthermore, in another joint analysis of the omega-3% and omega-6%, the lowest risk for all three mortality outcomes was observed among those in the joint highest categories of the two fatty acids **(Additional file 2: Table S5**). For example, when comparing those in the highest quintiles of the two fatty acids to the group with the joint lowest group, the HRs (95% CI) for all-cause, cancer, and CVD mortality were, respectively, 0.46 (95% CI, 0.32, 0.66), 0.41 (95% CI, 0.25, 0.68), and 0.77 (95% CI, 0.36, 1.64).

Other sensitivity analyses

After further adjustment of the fish oil supplementation status, the associations between the ratio of omega-6/omega-3 and mortality outcomes were slightly attenuated yet did not alter the main findings **(Additional file 2: Table S6**). Our primary analysis excluded participants with missing information (i.e., a complete-case analysis). We performed a sensitivity analysis using the multiply-imputed datasets, and there were no substantial changes (**Additional file 2: Table S7**). Moreover, the exclusion of participants who died during their first-year follow-up did not materially alter the results **(Additional file 2: Table S8)**. The baseline characteristics are

comparable between participants with or without exposure information **(Additional file 2: Table S9)**.

Discussion

In this prospective population-based study of UK individuals, we showed that a higher ratio of plasma omega-6/omega-3 fatty acids was positively associated with the risk of all-cause, cancer, and CVD mortality. These associations were independent of most risk factors examined, including age, sex, BMI, and physical activity, but they were all stronger in current smokers. These relationships were linear for cancer mortality but not for all-cause and CVD mortality. For those two outcomes, the risk of mortality first decreased at lower ratios and then increased, with an inflection point around the ratio of 8. Moreover, omega-3 and omega-6 PUFAs in plasma were consistently and inversely associated with all-cause, cancer, and CVD mortality, with omega-3 showing stronger effects.

To date, studies that examined the relationship between the ratio of omega-6/omega-3 PUFAs and mortality in the general population are sparse[4,14,15,19]. Similar to our finding, in a 2017 report from a prospective women cohort study (n=6,501; 1,875 all-cause deaths, 617 CVD deaths, 462 cancer deaths)[4], the adjusted HR for all-cause mortality was 1.10 (1.02-1.19) per 1-SD increase of omega-6/omega-3 ratio in red blood cells, however, the effects were not significant for cancer and CVD deaths. Another study supporting our results was conducted on elderly Japanese individuals (n=1,054; 422 deaths). It found that the ratio of an omega-3 fatty acid, eicosapentaenoic acid, to an omega-6 fatty acid, arachidonic acid (ARA), is inversely associated with all-cause mortality, with an HR of 0.71 (95% 0.53–0.96) comparing the highest to the lowest tertile[14]. Findings of previous studies based on dietary intake were null[15,19]. A prospective

cohort involving 145 hemodialysis patients enrolled in Southern California during 2001-2007 (42 all-cause deaths) showed that the estimated HR (95% CI) for all-cause mortality among those in the lowest relative to the highest quartiles of dietary omega-6/omega-3 ratio was 0.37 (0.14-1.08)[19]. Although the effect was not significant, it still shows the protective trend of a lower omega-6/omega-3 ratio against premature death, when taking the sample size and study population into consideration. In a 2019 report based on two population-based prospective cohorts in China (n=14,117, 1,007 all-cause deaths) and the US (n=36,032, 4,826 all-cause deaths), the effect of the omega-6/omega-3 ratio intake was not significant (HR (95% CI) is 0.95 (0.80-1.14) for China and 0.99 (0.89-1.11) for US, respectively)[15]. These discrepancies may be explained by the usage of circulating biomarkers or dietary intakes for calculating the omega-6/omega-3 ratio. The estimated dietary intakes may be inaccurate due to recall bias or outdated food databases[16]. The circulating level is a more objective measurement of PUFA status and thus provides a more reliable picture of the effects of omega-3 and omega-6 PUFAs on mortality.

A large number of existing observational studies documented the reverse association of circulating levels and intake of omega-3 PUFAs with mortality[5,6,26,28-33], which is in line with our finding that individuals in the highest quintile of the omega-6/omega-3 ratio had approximately 40% higher risk for all-cause and CVD mortality and 30% higher risk for cancer mortality, when compared to those in the lowest quintile. Few previous studies have examined the association in generally healthy populations[6,26,28,30]. In a 2016 meta-analysis[30] of seven epidemiologic studies for dietary omega-3 PUFAs intake and four studies for circulating levels, the estimated relative risk for all-cause mortality was 0.91 (95% CI: 0.84-0.98) when comparing the highest to the lowest categories of omega-3 PUFA intake. In two 2018 reports of population-based studies in

the US, the circulating level and dietary intake of omega-3 PUFAs were significantly associated with lower total mortality[6,26]. In a 2021 meta-analysis of 17 epidemiologic studies, comparing the highest to the lowest quintiles of circulating EPA and DHA, the estimated HRs for all-cause, CVD, and cancer mortality were 0.84 (0.79-0.89), 0.80 (0.73-0.88) and 0.87 (0.78-0.96), respectively[28]. Moreover, inverse associations of omega-3 PUFAs biomarkers with total mortality were found in patients with myocardial infarction[31,32], type 2 diabetes[12], and other diseases[5,29,33,34]; inverse associations of omega-3 PUFAs with CVD mortality were also reported in these patient groups[5,12,31]. However, there are also reports of a null relationship between omega-3 PUFAs and mortality[13,14,35]. One possible explanation for the discrepancies could be the limited statistical power due to the small sample size and the small number of events. Moreover, the inconsistency may be due to unique sample characteristics; one study only involved elder patients[14], and one only involved women[35].

A smaller number of studies have evaluated the associations of omega-6 PUFAs with mortality. Our findings in the large UK prospective cohort are in accordance with the results of several previous studies that increased circulating levels of omega-6 PUFAs were associated with decreased all-cause mortality[9,11,15,36]. Moreover, a 2019 meta-analysis involving 18 cohort studies and 12 case-control studies showed that an omega-6 PUFA, linoleic acid, was inversely associated with CVD mortality; the HR per interquintile range was 0.78 (95% CI, 0.70-0.85)[10]. Other studies, however, did not support such reverse associations[4,12-14,35]. Although the findings were inconsistent, no previous studies reported harmful effects of omega-6 PUFAs on mortality[4,11-15,35-38]. Our studies support the protective effects of omega-6 PUFAs on all-cause

and cause-specific mortality. Further research is needed to investigate the health impacts of omega-6 PUFAs in laboratory studies, epidemiological investigations, and clinical trials.

Strengths of our current study include the use of objective PUFA biomarkers in plasma instead of the estimated intakes from dietary questionnaires, which increases the accuracy of exposure assessment. Moreover, the prospective population-based study design, large sample size, long duration of follow-up, and detailed information on potential confounding variables, substantially mitigate the possible complications from reverse causality and confounding bias. In several sensitivity analyses, most of the documented associations remain materially unchanged, indicating the robustness of our results.

Several potential limitations deserve attention. First, plasma omega-3 and omega-6 PUFAs were measured only once at baseline. Their levels may vary with diet or other lifestyle factors, which could cause misclassification over follow-up. However, some studies demonstrated that multiple measurements of omega-3 PUFAs have been consistent for a 6-month period[39]. Moreover, the 13-year within-person correlation for circulating omega-6 PUFAs was comparable to such correlations for other major CVD risk factors[40]. Thus, the single measurement of PUFAs at baseline, although not perfect, provides us with adequate information to investigate the relative long-term effects. Second, although we adjusted for many potential confounders in the model, we cannot rule out the imprecisely measured and unmeasured factors. Third, we did not analyze the individual omega-3 and omega-6 PUFAs due to their unavailability in the NMR-based metabolomics data. Future studies should investigate the effects of specific individual PUFAs on all-cause and cause-specific mortality. Last, although we included individuals of different

ancestries in the analysis, over 90% of the participants were of European ancestry. The generalizability of our findings across ancestries requires future verification.

Conclusions

In this large prospective cohort study, we documented robust positive associations of the plasma omega-6/omega-3 fatty acids ratio with the risk of all-cause, cancer, and CVD mortality. Moreover, we found that plasma omega-3 and omega-6 PUFAs were independently and inversely associated with the three mortality outcomes, with omega-3 fatty acids showing stronger protective effects. Our findings support the active management of a high circulating level of omega-3 fatty acids and a low omega-6/omega-3 ratio to prevent premature death.

Declarations

Ethics approval and consent to participate

The UK Biobank received ethical approval from the research ethics committee (reference ID: 11/NW/0382). Written informed consent was obtained from participants.

Consent for publication

Not applicable.

Availability of data and materials

The datasets analyzed during the current study are available from the UK Biobank through an application process (www.ukbiobank.ac.uk/).

Competing interests

The authors declare no competing interests.

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Authors' contributions

YZ performed data analysis, prepared visualizations, and wrote the original draft of the manuscript. YS contributed to the data analysis. YS (yeshen@uga.edu) and KY (kaixiong.ye@uga.edu) contributed equally to this project and should be considered co-corresponding authors. They jointly designed and supervised the project. JTB contributed to the interpretations of results. All authors critically edited the manuscript for important intellectual content. The corresponding author (KY) attests that all listed authors meet the authorship criteria and that no others meeting the criteria were omitted.

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Abbreviations

95% CI	95% confidence interval
ANOVA	Analysis of variance
BMI	Body mass index
CVD	Cardiovascular disease
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid
HR	Hazard ratio
Omega-3%	Omega-3 fatty acids to total fatty acids percentage
Omega-6%	Omega-6 fatty acids to total fatty acids percentage
PUFAs	Polyunsaturated fatty acids
Ref	Reference
SD	Standard deviation

Supplementary Information

Additional file 1: Table S1. Literature review.

Additional file 2: Table S2. Risk estimates of ratio of plasma omega-6 to omega-3 PUFAs with all-cause, cancer and CVD mortality, stratified by potential risk factors, in the UK Biobank Study. **Table S3.** Associations of plasma omega-3 PUFAs percentage with all-cause, cancer, and CVD mortality risk in the UK Biobank Study. **Table S4.** Associations of plasma omega-6 PUFAs percentage with all-cause, cancer, and CVD mortality risk in the UK Biobank Study. **Table S5.** Fully adjusted joint associations of plasma omega-3 PUFAs percentage and omega-6 PUFAs percentage with all-cause and cause-specific mortality in the UK Biobank Study. **Table S6.** Associations of ratio of omega-6/omega-3 PUFAs with all-cause, cancer, and CVD mortality risk in the UK Biobank, covariates including fish oil supplementation status. **Table S7.** Associations of ratio of omega-6/omega-3 PUFAs with all-cause, cancer, and CVD mortality risk in the UK Biobank Study, with multiple imputation for missing data. **Table S8.** Associations of ratio of omega-6/omega-3 PUFAs with all-cause, cancer, and CVD mortality risk in the UK Biobank Study, excluding those who died in the first follow-up year. **Table S9.** Baseline characteristics of participants with missing exposure information and not included in the study.

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Tables

Table 1. Selected participant characteristics at baseline across quintiles of the plasma omega-6/omega-3 PUFAs ratio (n=117,546).

	Omega-6/omega-3 ratio quintiles					
Characteristics[a]	**1** (median = 5.8) (*n* = 23,504)	**2** (median = 7.5) (*n* = 23,504)	**3** (median = 9.0) (*n* = 23,510)	**4** (median = 10.8) (*n* = 23,516)	**5** (median = 14.6) (*n* = 23,512)	*P*
Age (years)	59.2 (7.2)	57.8 (7.7)	56.4 (8.0)	55.3 (8.2)	53.9 (8.2)	<0.001[b]
Sex (male%)	39.5	43.6	46.5	48.2	51.4	<0.001[c]
Ethnicity(n%)						
White	21,193 (90.6)	21,324 (91.1)	21,407 (91.5)	21,332 (91.2)	21,273 (90.9)	0.047[c]
Black	144 (0.6)	124 (0.5)	127 (0.5)	130 (0.6)	146 (0.6)	
Asian	888 (3.8)	867 (3.7)	866 (3.7)	879 (3.8)	902 (3.9)	
Others	1,175 (5.0)	1,092 (4.7)	994 (4.2)	1,054 (4.5)	1,086 (4.6)	
Missing (n)	104	97	116	121	105	
BMI	27.3 (4.4)	27.6 (4.6)	27.6 (4.8)	27.5 (4.9)	27.2 (5.1)	<0.001[b]
Missing (n)	77	90	84	78	102	
Smoking status (n%)						<0.001[c]
Never	12,890 (55.1)	12,672 (54.2)	12,875 (55.0)	12,788 (54.7)	12,605 (53.9)	
Previous	8,971 (38.4)	8,686 (37.1)	8,201 (35.0)	7,813 (33.4)	7,018 (30.0)	
Current	1,519 (6.5)	2,025 (8.7)	2,324 (9.9)	2,786 (11.9)	3,768 (16.1)	
Missing (n)	124	121	110	129	121	
Alcohol status (n%)						<0.001[c]
Never	895 (3.8)	892 (3.8)	953 (4.1)	1059(4.5)	1343 (5.7)	
Previous	736 (3.1)	752 (3.2)	778 (3.3)	841 (3.6)	1161 (5.0)	
Current	21,815 (93.0)	21,817 (93.0)	21,720 (92.6)	21,552 (91.9)	20,943 (89.3)	
Missing (n)	58	43	59	64	65	
Physical activity (n%)						<0.001[c]
High	7,634 (40.1)	7,492 (39.3)	7,518 (39.5)	7,669 (40.5)	7,984 (42.2)	
Moderate	7,988 (41.9)	7,909 (41.5)	7,800 (41.0)	7,624 (40.3)	7,287 (38.5)	
Low	3,430 (18.0)	3,645 (19.1)	3,715 (19.5)	3,643 (19.2)	3,640 (19.2)	
Missing (n)	4,452	4,458	4,447	4,580	4,601	
Fish oil supplementation (Yes%)	48.6	38.1	30.4	23.8	15.9	<0.001[c]

Missing (n)	73	64	97	106	97	
Omega-3 percentage	6.7 (1.4)	5.0 (0.5)	4.2 (0.4)	3.6 (0.4)	2.6 (0.5)	<0.001[b]
Omega-6 percentage	36.1 (3.7)	37.3 (3.5)	38.1 (3.3)	38.9 (3.2)	40.1 (3.1)	<0.001[b]

[a] All variables measured at baseline are presented as mean (SD) unless otherwise specified.

[b] From the ANOVA test for continuous variables.

[c] From the Pearson's Chi-squared test for categorical variables.

Table 2. Associations[a] of the plasma omega-6/omega-3 PUFAs ratio with all-cause, cancer, and CVD mortality risk in the UK Biobank

Omega ratio variable forms	**Causes of death**								
	All-cause			**Cancer**			**Cardiovascular diseases**		
	Number of deaths	Partially adjusted associations[b] HR (95% CI)	Fully adjusted associations[c] HR (95% CI)	Number of deaths	Partially adjusted associations[b] HR (95% CI)	Fully adjusted associations[c] HR (95% CI)	Number of deaths	Partially adjusted associations[b] HR (95% CI)	Fully adjusted associations[c] HR (95% CI)
Continuous	4,733	1.02 (1.02-1.03)	1.02 (1.02-1.03)	2,585	1.02 (1.01-1.03)	1.01 (1.00-1.02)	1,017	1.02 (1.01-1.03)	1.02 (1.01-1.03)
Quintiles (median)									
1 (5.8)	900	1.00 (ref)	1.00 (ref)	512	1.00 (ref)	1.00 (ref)	202	1.00 (ref)	1.00 (ref)
2 (7.5)	896	1.07 (0.97-1.17)	1.02 (0.91-1.13)	539	1.15 (1.02-1.30)	1.11 (0.97-1.27)	176	0.92 (0.75-1.13)	0.87 (0.69-1.11)
3 (9.0)	868	1.09 (0.99-1.19)	1.03 (0.92-1.14)	469	1.06 (0.94-1.21)	1.01 (0.87-1.16)	187	0.99 (0.81-1.21)	0.91 (0.72-1.16)
4 (10.8)	980	1.31 (1.20-1.44)	1.26 (1.14-1.40)	529	1.30 (1.15-1.47)	1.26 (1.10-1.45)	208	1.17 (0.96-1.42)	1.17 (0.93-1.47)
5 (14.6)	1,089	1.55 (1.42-1.70)	1.42 (1.28-1.57)	533	1.42 (1.25-1.60)	1.31 (1.13-1.50)	244	1.42 (1.17-1.72)	1.40 (1.12-1.75)
P_{trend}		*<0.001*	*<0.001*		*<0.001*	*<0.001*		*<0.001*	*<0.001*

Abbreviations: CI, confidence interval; HR, hazards ratio; ref, reference.

[a] From Cox proportional hazards regression.

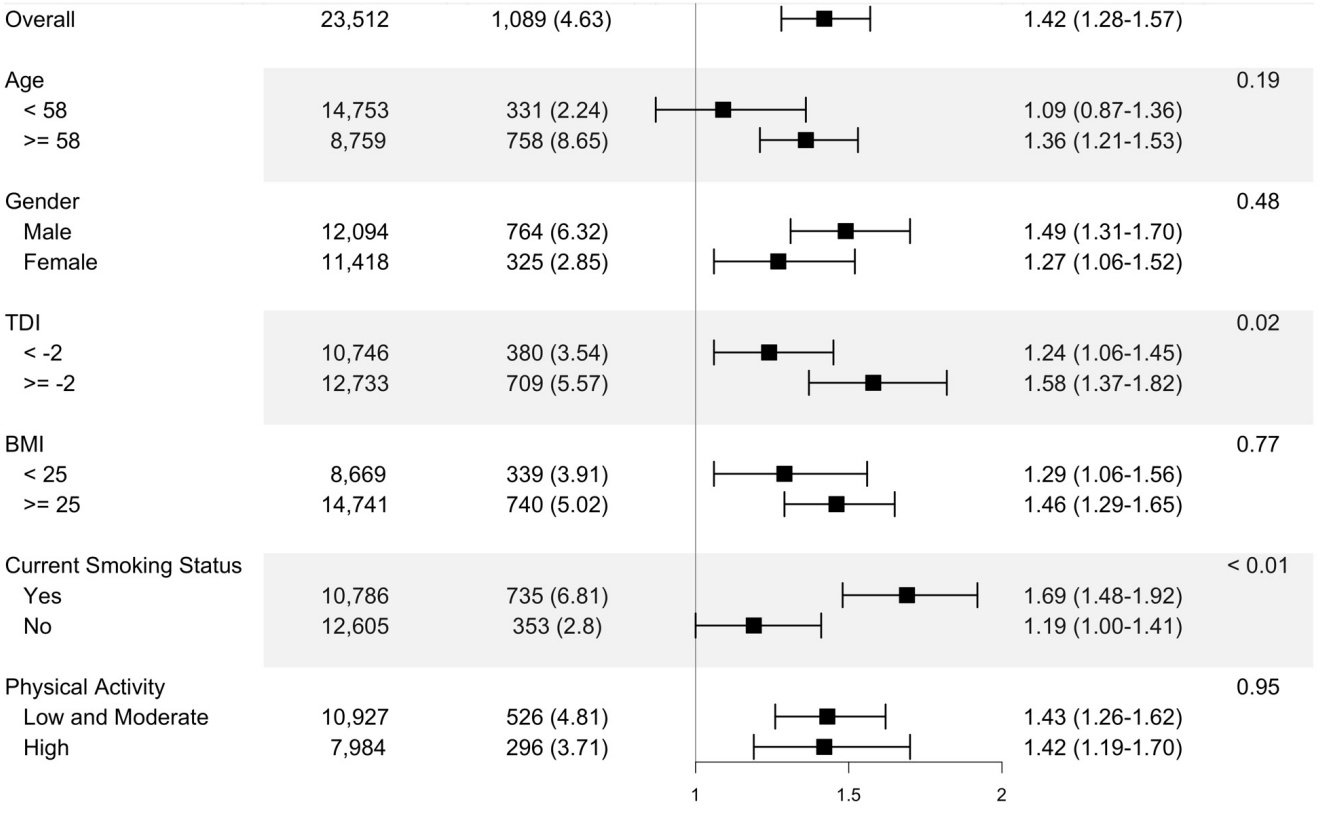
[b] Adjusted for age (years; continuous), sex (male, female), race (White, Black, Asian, Others), Townsend deprivation index (continuous), assessment Centre.
[c] Adjusted for age (years; continuous), sex (male, female), race (White, Black, Asian, Others), Townsend deprivation index (continuous), assessment Centre, BMI (kg/m2; continuous), smoking status (never, previous, current), alcohol intake status (never, previous, current), physical activity (low, moderate, high).

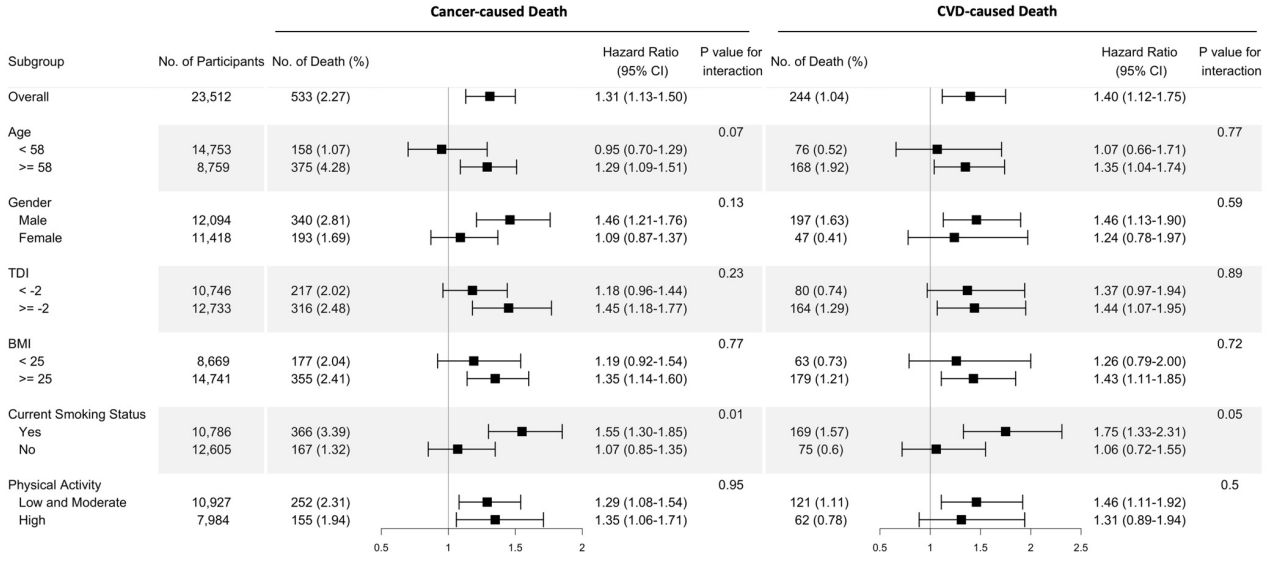
Figure Legends

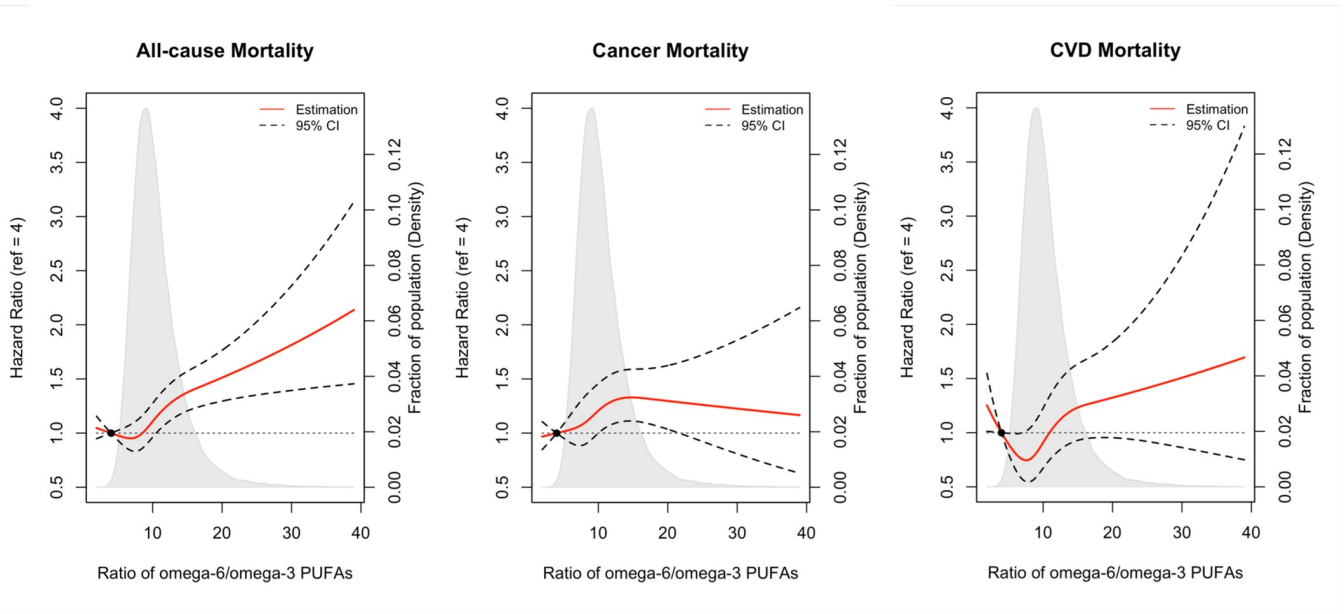
Figure 1. Risk estimates of all-cause mortality for the highest compared with the lowest quintile of the ratio of plasma omega-6 to omega-3 PUFAs, stratified by potential risk factors. Results were adjusted for age (years; continuous), sex (male, female), race (White, Black, Asian, Others), Townsend deprivation index (continuous), assessment Centre, BMI (kg/m2; continuous), smoking status (never, previous, current), alcohol intake status (never, previous, current), physical activity (low, moderate, high).

Figure 2. Risk estimates of cause-specific mortality for the highest compared with the lowest quintile of the ratio of plasma omega-6 to omega-3 PUFAs, stratified by potential risk factors. Results were adjusted for age (years; continuous), sex (male, female), race (White, Black, Asian, Others), Townsend deprivation index (continuous), assessment Centre, BMI (kg/m2; continuous), smoking status (never, previous, current), alcohol intake status (never, previous, current), physical activity (low, moderate, high).

Figure 3. Associations of the ratio of omega-6/omega-3 PUFAs with all-cause and cause-specific mortality evaluated using restricted cubic splines. Hazard ratios and omega ratios are presented in the vertical and horizontal axis, respectively. The best estimates and their confidence intervals are presented as solid red lines and dotted black lines, respectively. The ratio 4 was selected as a reference level, and the x-axis depicts the ratio from 0 to 40. Potential nonlinearity was identified for all-cause mortality ($p = 0.011$) and CVD-caused mortality ($p = 0.011$), but not for cancer-caused mortality ($p > 0.05$). All HRs are adjusted for age (years; continuous), sex (male, female), race (White, Black, Asian, Others), Townsend deprivation index (continuous), assessment Centre, BMI (kg/m2; continuous), smoking status (never, previous, current), alcohol intake status (never, previous, current), physical activity (low, moderate, high).


Subgroup
No. of Participants
No. of Death (%)
Hazard Ratio (95% CI)
P value for interaction
Overall
23,512
1,089 (4.63)
1.42 (1.28-1.57)
Age
0.19
< 58
14,753
331 (2.24)
1.09 (0.87-1.36)
>= 58
8,759
758 (8.65)
1.36 (1.21-1.53)
Gender
0.48
Male
12,094
764 (6.32)
1.49 (1.31-1.70)
Female
11,418
325 (2.85)
1.27 (1.06-1.52)
TDI
0.02
< -2
10,746
380 (3.54)
1.24 (1.06-1.45)
>= -2
12,733
709 (5.57)
1.58 (1.37-1.82)
BMI
0.77
< 25
8,669
339 (3.91)
1.29 (1.06-1.56)
>= 25
14,741
740 (5.02)
1.46 (1.29-1.65)
Current Smoking Status
< 0.01
Yes
10,786
735 (6.81)
1.69 (1.48-1.92)
No
12,605
353 (2.8)
1.19 (1.00-1.41)
Physical Activity
0.95
Low and Moderate
10,927
526 (4.81)
1.43 (1.26-1.62)
High
7,984
296 (3.71)
1.42 (1.19-1.70)
1
1.5
2


Cancer-caused Death
CVD-caused Death
Subgroup
No. of Participants
No. of Death (%)
Hazard Ratio (95% CI)
P value for interaction
No. of Death (%)
Hazard Ratio (95% CI)
P value for interaction
Overall 23,512 533 (2.27) 1.31 (1.13-1.50) 244 (1.04) 1.40 (1.12-1.75)
Age 0.07 0.77
< 58 14,753 158 (1.07) 0.95 (0.70-1.29) 76 (0.52) 1.07 (0.66-1.71)
>= 58 8,759 375 (4.28) 1.29 (1.09-1.51) 168 (1.92) 1.35 (1.04-1.74)
Gender 0.13 0.59
Male 12,094 340 (2.81) 1.46 (1.21-1.76) 197 (1.63) 1.46 (1.13-1.90)
Female 11,418 193 (1.69) 1.09 (0.87-1.37) 47 (0.41) 1.24 (0.78-1.97)
TDI 0.23 0.89
< -2 10,746 217 (2.02) 1.18 (0.96-1.44) 80 (0.74) 1.37 (0.97-1.94)
>= -2 12,733 316 (2.48) 1.45 (1.18-1.77) 164 (1.29) 1.44 (1.07-1.95)
BMI 0.77 0.72
< 25 8,669 177 (2.04) 1.19 (0.92-1.54) 63 (0.73) 1.26 (0.79-2.00)
>= 25 14,741 355 (2.41) 1.35 (1.14-1.60) 179 (1.21) 1.43 (1.11-1.85)
Current Smoking Status 0.01 0.05
Yes 10,786 366 (3.39) 1.55 (1.30-1.85) 169 (1.57) 1.75 (1.33-2.31)
No 12,605 167 (1.32) 1.07 (0.85-1.35) 75 (0.6) 1.06 (0.72-1.55)
Physical Activity 0.95 0.5
Low and Moderate 10,927 252 (2.31) 1.29 (1.08-1.54) 121 (1.11) 1.46 (1.11-1.92)
High 7,984 155 (1.94) 1.35 (1.06-1.71) 62 (0.78) 1.31 (0.89-1.94)
0.5
1
1.5
2
0.5
1
1.5
2
2.5


All-cause Mortality
Cancer Mortality
CVD Mortality
Estimation
95% CI
Hazard Ratio (ref = 4)
Fraction of population (Density)
Ratio of omega-6/omega-3 PUFAs
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
0.00
0.02
0.04
0.06
0.08
0.10
0.12
10
20
30
40