Blood n-3 fatty acid levels and total and cause-specific mortality from 17 prospective studies

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The health effects of omega-3 fatty acids have been controversial. Here we report the results of a de novo pooled analysis conducted with data from 17 prospective cohort studies examining the associations between blood omega-3 fatty acid levels and risk for all-cause mortality. Over a median of 16 years of follow-up, 15,720 deaths occurred among 42,466 individuals. We found that, after multivariable adjustment for relevant risk factors, risk for death from all causes was significantly lower (by 15–18%, at least \( p < 0.003 \)) in the highest vs the lowest quintile for circulating long chain (20–22 carbon) omega-3 fatty acids (eicosapentaenoic, docosapentaenoic, and docosahexaenoic acids). Similar relationships were seen for death from cardiovascular disease, cancer and other causes. No associations were seen with the 18-carbon omega-3, alpha-linolenic acid. These findings suggest that higher circulating levels of marine n-3 PUFA are associated with a lower risk of premature death.
The n-3 polyunsaturated fatty acid (PUFA) family has been the subject of intense investigation ever since their inverse associations with risk for acute myocardial infarction were reported in Greenland Eskimos in the 1970s.

The PUFAs in this family include the 18-carbon, plant-derived alpha-linolenic acid (ALA), as well as the 20–22-carbon, long-chain (LC, mostly seafood-derived) eicosapentaenoic (EPA), docosapentaenoic (DPA), and docosahexaenoic (DHA) acids.

The efficacy of the LC n-3 PUFAs in reducing risk for cardiovascular disease (CVD) remains controversial as findings from different randomized controlled trials (RCTs) have been conflicting. Nevertheless, a 2019 meta-analysis of RCTs reported significant reductions in risk for myocardial infarction, coronary heart disease (CHD) events and mortality, and CVD mortality in patients randomized to supplemental LC n-3 PUFAs.

Another meta-analysis of observational studies found that higher levels of circulating LC n-3 PUFA levels were significantly associated with a lower risk for CHD death.

However, no meta-analysis has yet examined the relationship between LC n-3 PUFAs blood levels and risk for all-cause mortality. Indeed, the only meta-analyses to report a beneficial association with all-cause mortality were based on the self-reported intake of fish.

Fish contain many nutrients besides just LC n-3 PUFAs, self-reported fish intake is memory dependent, food databases can be out of date, and fish meals often replace less healthful choices. As a result, studies that link LC n-3 PUFAs and health outcomes based on self-reported fish intake have potential limitations. A more reliable and objective measure of LC n-3 PUFA consumption is their level in the blood, which is primarily determined by the consumption of preformed LC n-3 PUFAs (although synthesis from dietary ALA can make a small contribution).

Here, we show significant inverse associations for all mortality endpoints with the LC n-3 PUFA levels. Hence, chronically higher tissue levels of these FAs operating through a variety of potential mechanisms may slow the aging process.

**Results**

**Population.** The pooled analyses included circulating n-3 PUFA measurements on 42,466 individuals, 15,720 (37%) of whom died during follow-up (Table 1). At baseline, the average age was 65 years (range of mean ages across cohorts was 50–81 years), 55% were women (range of 0–100% across cohorts) and the median follow-up time was 16 years (range of 5–32 years across cohorts).

Whites constituted 87% of the sample. Circulating levels of the n-3 PUFAs (and of the n-6 PUFAs linoleic and arachidonic acids, which were included as covariates) are shown in Supplementary Fig. 1 and in Supplementary Table 2. Supplementary Table 3 shows the number of cause-specific deaths from participating cohorts. Overall, approximately 30% of the deaths were attributed to CVD, 30% to cancer, and the remaining 39% to all other causes.

**Total mortality.** Comparing the medians of the first and fifth quintiles (i.e., approximately the 90th and the 10th percentiles), higher EPA, DPA, DHA, and EPA + DHA levels were associated with between 9% and 13% lower risk of all-cause mortality (Table 2). (The fatty acid levels associated with these percentiles...
for each cohort and sample type are shown in Supplementary Table 4). The HR for total mortality for EPA + DHA was 0.87 (95% CI: 0.83–0.90) (Fig. 1). In contrast, ALA was not significantly associated with all-cause mortality [HR 0.99 (0.96–1.02)]. In an across quintiles analysis, significant trends were observed for EPA, DPA, DHA, and EPA + DHA (all < 0.01); and comparing the top to the bottom quintile, each was associated with 15–18% lower risk of death (Table 3). There was little evidence for nonlinearity in these inverse associations for all each LC n-3 PUFAs except for EPA (p = 0.002 for the nonlinearity; Fig. 2). The relationship of EPA with mortality was most pronounced at lower levels and then appeared to plateau at higher levels. ALA was generally unassociated with total mortality, except for a borderline association in the top quintile [HR 0.94 (0.89–0.99); P-trend = 0.13], and there was no evidence for nonlinearity (Supplementary Fig. 2).

**Cause-specific mortality.** Comparing the 90th to the 10th percentile, each of the LCn-3 PUFAs was significantly associated with lower risk of death for CVD mortality (EPA: 0.85, 0.79–0.91; DPA: 0.86, 0.80–0.92; DHA: 0.88, 0.83–0.96; EPA + DHA: 0.88, 0.83–0.93), cancer mortality (EPA: 0.89, 0.83–0.96; DPA: 0.93, 0.86–1.00; DHA: 0.90, 0.84–0.95; EPA + DHA: 0.88, 0.82–0.93), and other mortality (EPA: 0.92, 0.87–0.97; DPA: 0.88, 0.82–0.94; DHA: 0.90, 0.84–0.95; EPA + DHA: 0.88, 0.82–0.93). For ALA, there was no significant association with CVD mortality (0.97, 0.89–1.05) but a trend towards lower risk of cancer mortality (0.93, 0.86–1.00).

### Table 2 Associations of circulating n-3 PUFA biomarkers with risk of total and cause-specific mortality in 17 cohorts: Fatty Acids and Outcomes Research Consortium.

| Fatty acid | All-cause mortality (HR (95% CI)) | CVD mortality (HR (95% CI)) | Cancer mortality (HR (95% CI)) | Other mortality (HR (95% CI)) |
|------------|----------------------------------|----------------------------|--------------------------------|-----------------------------|
|            | (17 cohorts; 15,720 deaths)     | (15 cohorts; 4571 deaths)  | (15 cohorts; 4284 deaths)    | (14 cohorts; 6022 deaths)   |
| ALA        | 0.99 (0.96–1.02)                | 1.01 (0.95–1.07)            | 1.02 (0.96–1.08)              | 0.99 (0.95–1.04)            |
| EPA        | 0.91 (0.88–0.94)                | 0.88 (0.83–0.94)            | 0.91 (0.85–0.96)              | 0.92 (0.87–0.97)            |
| DPA        | 0.87 (0.84–0.91)                | 0.91 (0.84–0.99)            | 0.87 (0.81–0.95)              | 0.88 (0.82–0.94)            |
| DHA        | 0.89 (0.85–0.92)                | 0.86 (0.80–0.92)            | 0.93 (0.86–1.00)              | 0.90 (0.84–0.95)            |
| EPA + DHA  | 0.87 (0.83–0.90)                | 0.85 (0.79–0.91)            | 0.89 (0.83–0.96)              | 0.88 (0.82–0.93)            |

Hazard ratios (HRs) and 95% CIs expressed per cohort-specific inter-quintiles range comparing the midpoint of the top and bottom quintiles (see Supplementary Table 4 for cohort-specific n-3 PUFA values). All HRs are adjusted for age, sex, race, field center, body-mass index, education, occupation, marital status, smoking, physical activity, alcohol intake, prevalent diabetes, hypertension, and dyslipidemia, self-reported general health, and the sum of circulating n-6 PUFA (linoleic plus arachidonic acids). See Supplementary Table 4 for the 10th and 90th percentile values from each cohort for each PUFA of interest and the average PUFA values per lipid pool. Abbreviations: ALA alpha-linolenic acid, CI confidence interval, CVD cardiovascular disease, DHA docosahexaenoic acid, DPA docosapentaenoic acid, EPA eicosapentaenoic acid, HR hazard ratio.
with a lower risk for death from CVD, cancer, and all other causes combined [except for DHA and cancer mortality, HR 0.93 (0.86–1.00)] (Table 2). ALA was not significantly associated with any cause-specific mortality. Evaluating the trend across quintiles, EPA, DHA, and EPA + DHA were inversely associated with CVD death, EPA and DPA were inversely associated with cancer death, and each of the LC n-3 PUFAs was inversely associated with other death. Comparing the top to the bottom quintile, EPA, DPA, DHA, and EPA + DHA were each significantly, inversely associated with CVD, cancer, and other mortality (Table 3).

### Heterogeneity and sensitivity analyses

Inter-cohort heterogeneity was at least moderate ($I^2 > 50\%$) in the pooled analyses of all-cause mortality for all n-3 PUFAs except ALA ($I^2 = 26\%$) and EPA ($I^2 = 41\%$), while heterogeneity for cause-specific mortality ranged from little to moderate (0–56\%) (Supplementary Table 5). There was little evidence of differential associations with mortality by PUFA lipid compartment after accounting for multiple testing (5 PUFAs × 4 outcomes; Bonferroni correction 0.05/20 = 0.0025, Supplementary Table 6). Likewise, associations of n-3 PUFAs with total mortality were similar across strata based on age, sex, race, and fish oil use (Supplementary Table 7), with no significant differences after accounting for multiple testing (5 PUFAs × 4 strata results; Bonferroni correction 0.05/20 = 0.0025). Overall findings did not change with the removal of participants taking fish oil (Supplementary Table 7) or in the drop-one-cohort analyses.

### Discussion

In this meta-analysis utilizing a harmonized analytical strategy with individual-level data from 17 cohorts, we examined the associations between circulating levels of the n-3 PUFAs and mortality. We found that, after controlling for other major risk factors, LC n-3 PUFAs (but not ALA) were associated with a 15–18% lower risk of total mortality comparing the top to the bottom quintiles. These relationships were generally linear for DPA, DHA, and EPA + DHA, but not for EPA. For this PUFA there was a steeper risk reduction across the lower blood levels but little additional difference in risk at higher blood levels. Inverse correlations were also generally observed between LC n-3 PUFA levels and CVD, cancer, and other causes of death.

This pooled analysis including over 40,000 participants and over 15,000 deaths greatly expands upon the findings of prior individual cohort studies that examined associations of circulating levels of n-3 PUFAs and all-cause mortality. Relatively few studies have evaluated self-reported dietary fish (or estimated n-3 PUFA) intake in relation to total mortality, but those that have typically support our observations here. Interestingly, reported use of fish oil supplements was linked to a
lower risk for death from any cause in a study from the UK including over 427,000 individuals. Associations with total and cause-specific mortality were not significant for the plant-derived n-3 PUFA ALA. Prior biomarker-based meta-analyses reported inverse associations of ALA with CHD death, but relationships with total or CVD mortality were not examined. Whether our finding of no association on CVD mortality was because ALA has no role to play in fatal strokes (included in the CVD mortality metric) or because of differences in the cohorts included in these prior meta-analyses vs. the present one is not clear. Circulating ALA levels are less dependable markers of intake compared with the LC n-3 PUFAs and provide doses of EPA and DHA that produce higher blood levels. An intake of about 250 mg EPA + DHA per day as recommended in the Dietary Guidelines for Americans may raise circulating levels into the ranges observed here for some but not all adults.

Although circulating marine n-3 PUFA levels have not been measured in all of the major intervention trials, the doses of EPA + DHA used in most trials (<1 g/day) may not have resulted in marked differences in levels between treated and control patients. For example, in the Vitamin D and Omega-3 Trial (VITAL) trial, treatment with 840 mg of EPA + DHA per day increased plasma phospholipid EPA + DHA levels from 2.7 to 4.1%, a 55% increase. This relatively small difference in LC n-3 PUFA levels between the placebo and active treatment groups could be one of the potential reasons for the failure of some RCTs to detect an effect of n-3 PUFAs on mortality risk is sparse, as well as randomized controlled clinical trials of n-3 PUFAs (although the most recent trial has not yet been included in meta-analyses). Compared with CVD, evidence for a link between n-3 PUFAs and cancer mortality risk is sparse, with no significant relationship for self-reported estimates of fish or n-3 PUFA consumption. Meta-analyses of RCTs with n-3 PUFA supplementation also have not observed effects on cancer, although short-term durations of such trials (generally up to 5 years) would likely preclude any ability to detect an effect on cancer. The difference between these findings and what we observed may arise from the use of biomarker levels instead of self-reported fish intake. Biomarkers are potentially truer reflections of long-term exposure, making it easier to detect subtle relationships. In addition, circulating LC n-3 PUFA levels reflect endogenous metabolism, especially for EPA which is not correlated with estimated dietary EPA intake but may have important biologic effects. Finally, since neurodegenerative diseases are a major non-CVD, non-cancer cause of death, a report that higher fish intake was associated with reduced mortality from this cause is consistent with our observations here.

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Another 2-year trial in elderly post-MI patients from Norway reasonable reproducibility has been reported for n-3 PUFA bio-
changes over time could lead to misclassi-
PUFAs and covariates were measured once at baseline, and
population characteristics, differences in laboratory assessment of
were not rare, the hazard ratios (HRs) reported here (instanta-
field method, moderate heterogeneity remained
between studies that may be due to unmeasured background
PUFAs and of outcomes, chance, or any combination of these.
PUFAs and covariates were measured once at baseline, and
changes over time could lead to misclassification, which could
bias the results in uncertain directions. On the other hand, rea-
sonable reproducibility has been reported for n-3 PUFA bio-
marker concentrations over time. Because analytical methods,
even within the same lipid fraction, were not standardiz-
and n-3 PUFA levels were measured in multiple fractions, we assessed
cohort-specific n-3 PUFA percentiles rather than absolute per-
centages of total fatty acids in each fraction. Since FA levels were
reported as a percent of total FAs in each lipid compartment,
levels of one FA could affect levels of another. Indeed, in the
plasma or RBC PL and CE pools, higher levels of the LC n-3
PUFAs (which were the focus of this study) are linked with lower
levels of the n-6 PUFAs but not of saturated or mono-unsaturated
FA. Since we adjusted for differences in linoleic and arach-
idonic levels in our analyses, this concern was accounted for.
Each lipid pool used in this study reflects LC n-3 PUFA intake
during relatively different and overlapping time periods generally
from months to weeks following this hierarchy: RBC ≥ Plasma
PL ≥ Plasma CE ≥ total plasma. In addition, we cannot rule
out the potential for residual confounding. That is, higher LC n-3
PUFA levels may simply be markers of a “healthy lifestyle,” and
the fatty acids themselves may not be playing any physiological
role in postponing death but would be biomarkers of a suite of
other healthy behaviors (dietary/exercise/non-smoking, etc.), or
endogenous metabolic processes, that might, in a multiplicity of
ways, manifest in greater longevity. Although we adjusted for
many major risk factors (age, income, marital status, smoking,
hyperlipidemia, hypertension, etc.), residual confounding by
other factors is always possible. However, the magnitude of the
observed effect of the meta-analysis of circulating LC n-3 PUFAs
and total mortality reported herein is consistent with the known
associations with CHD mortality and sudden cardiac death.

Finally, as the attribution of cause of death is never as unam-
biguous as death itself, some uncertainty must attend to the
cause-specific analyses reported here. In summary, in a global
pooled analysis of prospective studies, LC n-3 PUFA levels
were inversely associated with risk for death from all causes and
from CVD, cancer, and other causes.

Methods

Study design and population: FORCE Consortium. The study was conducted within FORCE, a consortium of observational studies with fatty acid biomarker data and ascertained chronic disease events. For the current project, 48 pro-
spective studies in the consortium as of December 2018 were invited to participate. Of these, seven did not have relevant data (e.g., no mortality outcomes or no circulating PUFA levels at baseline), two included only participants with prevalent (incident investigated) or prevalent (pre-existing or incident investigated) outcomes and reported mortality after at least 5 separate invitations to participate over a 9-month period. The study sample comprised data from 17 studies across 10 countries with available data on circu-
lating PUFA levels at baseline and mortality during follow-up. The details of each individual study are presented in Supplementary Table 1. All participating studies followed a prespecified standardized analysis protocol with harmonized inclusions and exclusions, exposures, outcomes, covariates, and analytical methods including
assessment of missing covariate data and statistical models. In each study, new
analyses of individual data were performed according to the protocol, and study-
specific results were collected using a standardized electronic form. Information
regarding harmonization for any outcomes included (that required it prior to study initiation) is shown in Supplementary Table 1.

Individual cohorts conducted their studies in accordance with the criteria set by the Declaration of Helsinki, and informed consent was obtained from all participants. The review boards or ethics committees from each cohort were as follows: 60YO (Ethical Committee at the Karolinska Institut); AGES-R (Icelandic Heart Association and the Intramural Research Program of the National Institute on Aging); CCCC (National Taiwan University Research Ethics Committee); CHS (Tufts University Research Ethics Committee); CSHA (Laval University and the Research Center of the Centre Hospitalier Affile Universitaire); EPIC-Norfolk (Norfolk and Norwich Research Ethics Committee); FHS (Boston University Institutional Review Board); Hisayama (Kyushu University Certified Institutional Review Board); HPFS (Human Subjects Review Committee of the Harvard School of Public Health); KIHD (Research Ethics Committee of the University of Kuopio); MCCS (Cancer Council Victoria Human Research Ethics Committee); MESA (University of Washington Human Subjects Division); MetSIM (Ethics Committee of the University of Eastern Finland and Kuopio University Hospital); NHS (Human Research Committee at the Brigham and Women’s Hospital); 3C (Consultative Committee for the Protection of Persons participating in Biomedical Research at Kremlin-Bicêtre University Hospital); ULSAM (Swedish Ethical Review Authority); and WHIMS (Fred Hutchinson Cancer Research Center Institutional Review Board).

Study participants in the included cohorts (a) were >18 years old, (b) had no major medical diagnoses (prior myocardial infarction, prior stroke, severe active cancer, severe renal disease, severe liver or lung disease), (c) were not taking supplements that did not change within a year, (d) were at least 5 separate invitations to participate over a 9-month period. The exception to (c) was the inclusion of the Age, Genes, Environment Susceptibility Study (Reykjavik) (AGES-R) from Iceland in which 68% of participants reported taking cod liver oil. This factor was adjusted for in the AGES-R analysis, and participants in AGES-R taking cod liver oil were also excluded in a sensitivity analysis.

Fatty acid measurements. Participating studies measured PUFAs in at least one blood compartment, including plasma phospholipids, cholesterol esters, ery-
throcytes, and whole plasma. All PUFA levels were reported as a percent of total fatty acids. Detailed information regarding PUFA measurement methods for each study is in Supplementary Table 1.

Outcome assessment. The primary endpoint of this study was total mortality (death from any cause). Additional endpoints of interest were deaths from CVD, cancer, and all other causes. Detailed information on the definitions of the outcomes used in each cohort is included in Supplementary Table 1.

Covariates. Prespecified covariates included age (continuous), sex (men/women), race (binary: White/non-White), field center (categories), body-mass index (con-
tinuous), education (less than high school graduate, high school graduate, at least some college or vocational school), occupation (if available), marital status (mar-
rried, never married, widowed, divorced), smoking (current, former, never), phy-
ical activity (kcal/week, METS/hour, or hours/day), alcohol intake (drinks or servings/day, g/day or ml/day), prevalent diabetes mellitus (treated or physician-diagnosed), prevalent hypertension (treated or physician-diagnosed), prevalent myocardial infarction (treated or physician-diagnosed), self-reported general health (if available) and circulating n-6 PUFA levels (i.e., the sum of linoleic and arachidonic acids). If individual cohorts could not categorize these covariates exactly according
to these definitions, then study-specific categories were used as surrogates. Missing variables were handled as detailed in the Online Supplementary Materials.

**Statistical analysis and pooling.** Study-specific analyses were harmonized across cohorts. They were carried out using Cox proportional hazards models using robust variance estimates to calculate the multivariable-adjusted HRs in each study, with follow-up from the date of biomarker measurement to date of death, loss to follow-up, or end of follow-up. Associations and relevant statistical interactions were also assessed in prespecified strata within each cohort by age (<60 vs. ≥60), sex, and race (White vs. non-White). To allow comparison and pooling of results from different biomarker compartments, n-3 PUFA levels were standardized to the study-specific inter-quartiles range defined as the range between the medians of the top and bottom quintile categories (i.e., about the 90th and 10th percentiles). In addition, each cohort computed HRs across study-specific quintiles, with the lowest quintile as the reference. Pooling by quintiles instead of absolute fatty acid values were necessary because values differ by lipid compartment. Nevertheless, such an approach was reasonable given the observed correlations among different lipid compartments. For example, the Pearson correlations between EPA + DHA levels (i.e., percent of total fatty acids) in RBC and CE, PL, and whole plasma are 0.83, 0.88, and 0.93, respectively (unpublished data from Harris lab based on 49 samples analyzed in all four compartments).

**Meta-analysis.** Cohort-specific HRs were pooled by inverse-variance weighted meta-analysis. Heterogeneity was assessed by the I² statistic and Q-test. Heterogeneity was further explored by meta-analyzing prespecified subgroups. Sensitivity analyses included (1) the removal of those subjects from AGES-R who reported fish oil use, and (2) re-analysis after the removal of each cohort one at a time. The potential for a nonlinear association of each n-3 PUFA with all-cause mortality was analyzed in all four compartments (i.e., about the 90th and 10th percentiles). In addition, each cohort computed HRs across study-specific quintiles, with the lowest quintile as the reference. Pooling by quintiles instead of absolute fatty acid values were necessary because values differ by lipid compartment. Nevertheless, such an approach was reasonable given the observed correlations among different lipid compartments. For example, the Pearson correlations between EPA + DHA levels (i.e., percent of total fatty acids) in RBC and CE, PL, and whole plasma are 0.83, 0.88, and 0.93, respectively (unpublished data from Harris lab based on 49 samples analyzed in all four compartments).

**Reporting summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability**

Policies for data sharing vary between the cohorts depending on their original human subjects’ approvals and existing procedures. For approved data-sharing requests, types of data that may be shared can include demographics, exposures, covariates, and outcomes. Please contact each individual principal investigator for cohort-specific data requests (See Supplementary Table 1).

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