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Plasma Phospholipid Long-Chain Omega-3 Fatty Acids and Total and Cause-Specific Mortality in Older Adults: the Cardiovascular Health Study

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Abstract

Background—Long-chain n-3 polyunsaturated fatty acids (n3-PUFA), including eicosapentaenoic acid (EPA/20:5n-3), docosapentaenoic acid (DPA/22:5n-3), and docosahexaenoic acid (DHA/22:6n-3), experimentally reduce cardiovascular risk. Yet, effects on

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Dr. Mozaffarian: Conception and design, obtained funding, data collection, statistical analysis, data interpretation, manuscript drafting, manuscript critical revision, and approval of final submitted manuscript.
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cause-specific and total mortality and potential dose-responses remain controversial. Most observational studies have assessed self-reported dietary intakes, rather than objective biomarkers; while most randomized trials have tested effects of adding supplements to background dietary intake and evaluated secondary prevention, limiting inference for dietary n3-PUFA or primary prevention.

Objective—We investigated associations of plasma phospholipid EPA, DPA, DHA, and total n-3 PUFA with total and cause-specific mortality among generally healthy older adults not taking fish oil supplements.

Design—Prospective cohort, 1992–2008.

Setting—Four U.S. communities.

Participants—2,692 U.S. adults age 75±5 years, free of prevalent coronary heart disease (CHD), stroke, or heart failure.

Measurements—Phospholipid fatty acids and cardiovascular risk factors were measured in 1992 using standardized methods. Relationships with total and cause-specific mortality through 2008, and incident total (fatal+nonfatal) CHD and stroke, were assessed using Cox proportional-hazards.

Results—During 30,829 person-years, 1,625 deaths (including 570 cardiovascular deaths), 359 fatal and 371 nonfatal CHD events, and 130 fatal and 276 nonfatal strokes occurred. After multivariable-adjustment, n3-PUFA biomarkers associated with lower total mortality, with extreme-quintile hazard ratios (95% CI) of 0.83 for EPA (0.71–0.98), 0.77 for DPA (0.66–0.90), 0.80 for DHA (0.67–0.94), and 0.73 for total n3-PUFA (0.61–0.86) (P-trend≤0.008 each). Lower risk was largely attributable to fewer cardiovascular, rather than noncardiovascular, deaths, in particular fewer arrhythmic cardiac deaths (total n3-PUFA: hazard ratio=0.52, 95%CI=0.31–0.86; P-trend=0.008). Based on relations with total mortality, individuals in the highest quintile of phospholipid n3-PUFA, versus the lowest, experienced 2.22 greater years of life (95%CI=0.75–3.13) after age 65.

Limitations—Temporal changes in fatty acid levels and misclassification of death causes may cause underestimated associations; and unmeasured/imperfectly measured covariates, residual confounding.

Conclusions—Circulating individual and total n3-PUFA are associated with lower total mortality, especially CHD death, in older adults.

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INTRODUCTION

Experiments and clinical studies demonstrate physiologic benefits of long-chain n-3 polyunsaturated fatty acids (n3-PUFA), which include eicosapentaenoic acid (EPA/20:5n-3), docosapentaenoic acid (DPA/22:5n-3), and docosahexaenoic acid (DHA/22:6n-3)(1). Yet, while observational studies have found inverse associations between dietary n3-PUFA and death from coronary heart disease (CHD)(1, 2), randomized trials of n3-PUFA supplementation have shown mixed results(3). Consequently, effects of n3-PUFA on cardiovascular diseases (CVD) and total and cause-specific mortality remain controversial. Understanding the influence of n3-PUFA on CVD or mortality, whether such effects vary for EPA, DPA, or DHA, and their potential dose-response is crucial for both advancement of science and dietary guidance.

Most observational studies of n3-PUFA have assessed self-reported dietary intakes, rather than objective biomarkers, which could lead to measurement errors or bias. Conversely, most randomized trials have tested the effects of n3-PUFA supplements among patients with established CVD or multiple risk factors, limiting inference for primary prevention. In addition, the trials tested n3-PUFA supplements provided on top of background dietary intake, which could reduce efficacy if the dose-response for n3-PUFA were nonlinear. In particular, a potential threshold effect(4, 5) could explain the observed benefits of moderate consumption compared with little to no consumption in observational studies, but small to no effects of higher supplement doses added to already moderate background dietary consumption in trials. Differences could also owe to stronger effects of n3-PUFA on CHD death, often evaluated in observational studies(4, 5), versus composite endpoints of total CHD or CVD events in trials.

It is also unclear whether potential cardiovascular benefits of n3-PUFA translate into lower total mortality, or whether n3-PUFA influence other, noncardiovascular causes of death. Competing risks from noncardiovascular conditions (e.g., cancer, lung disease) may be unaffected by n3-PUFA(6), minimizing effects on total mortality, particular later in life. In meta-analyses of trials, n3-PUFA supplementation produces nonsignificant trends toward lower total mortality(3). Yet, these trials typically evaluated higher-dose fish oil supplements in high-risk patients, many of whom were already consuming fish. In generally healthy populations, a few prospective cohorts have reported nonsignificant inverse trends between self-reported dietary n3-PUFA and total mortality(7–9). Self-reported diet also cannot reliably distinguish between specific long-chain n3-PUFA (i.e., EPA, DPA, or DHA), which may have partly differing physiologic effects(10).

Circulating n3-PUFA biomarkers objectively reflect dietary consumption and also biologically relevant processes, e.g. absorption, incorporation, or metabolism, that influence tissue levels. Metabolic influences appear especially relevant for DPA, which is elongated from and retroconverted to EPA(10). Biomarkers also permit direct evaluation of individual n-3 fatty acids, which may have differing effects on certain biologic pathways or clinical endpoints(10). Yet, to our understanding, no prior studies have evaluated how circulating n3-PUFA biomarkers relate to total mortality and diverse CVD subtypes in generally healthy populations.

To address each of these gaps in knowledge, we prospectively designed and implemented the current investigation of n3-PUFA biomarkers, including EPA, DPA, and DHA, and risk of CVD (CHD, stroke) and total and cause-specific mortality in a large, community-based cohort of older U.S. adults. Based on mechanistic studies and physiologic effects(1, 10), we hypothesized that n3-PUFA would associate with lower cardiovascular mortality, especially CHD death, but not noncardiovascular mortality. We also hypothesized that among individual n3-PUFA, DHA would most strongly associate with arrhythmic CHD death; and EPA and DPA, with nonfatal CHD.

METHODS

Design and Population

The Cardiovascular Health Study is a multicenter U.S. prospective cohort of older adults. In 1989–90, 5,201 ambulatory, non-institutionalized adults age 65+ were randomly selected and enrolled from Medicare eligibility lists in 4 communities (Forsyth County, North Carolina; Sacramento County, California; Washington County, Maryland; Allegheny County, Pennsylvania); 687 additional black participants were similarly recruited and enrolled in 1992–93. Among all eligible adults contacted, 57% agreed to participate, who were slightly healthier than those who declined. Annual study-clinic evaluations were performed by trained personnel and included physical examination, diagnostic testing, blood sampling, and questionnaires on health status, medical history, and lifestyle. Each center’s institutional review committee approved the study; all participants provided informed written consent.

Study Measures

Among 5,565 participants alive at the 1992–93 study visit, we measured fatty acids in 3,941 using stored blood samples from this visit, considered the baseline year for this analysis. See Supplementary Appendix for details of cohort sampling and fatty acid measurements, which have been described(11). The assessment of EPA, DPA, and DHA and incident CVD and mortality was a prespecified aim of the research supporting the fatty acid measurements. After excluding 1,113 participants with prevalent CVD and 136 taking fish oil supplements at time of blood-sampling, 2,692 participants were included in this analysis. At the same 1992–93 visit, cardiovascular risk factors, anthropometrics, blood pressure, and laboratory measures were measured using standardized procedures, and alcohol use and physical activity by validated questionnaires(12–17). Dietary habits were assessed 3 years earlier (1989–90) using a validated semi-quantitative food frequency questionnaire(18), from which dietary EPA+DHA was estimated as previously described(19).

Endpoints

Participants were followed by means of annual study-clinic examinations with interim phone contacts through 2000, and biannual telephone contacts thereafter. Vital status follow-up was 100% complete; <1% of all person-time was otherwise missing and censored early. All-cause and cause-specific mortality, as well as all suspected cases of incident (fatal or nonfatal) CHD and stroke, were assessed and adjudicated by a centralized events committee using available data from interviews, next-of-kin, death certificates, and medical records including diagnostic tests and consultations. Algorithms and methods for follow-up, confirmation, and classification of deaths, CHD, and stroke have been described(20–22). CVD mortality included deaths due to CHD, stroke, other atherosclerotic disease, and other CVD. Non-CVD mortality included deaths due to cancer, pulmonary diseases, infection, dementia, fractures/trauma, and other causes. Arrhythmic CHD deaths were also adjudicated(21), with sensitivity and specificity of 93% and 95% as compared with Hinkle classification.

Statistical Analysis

We evaluated n3-PUFA levels in quintiles as indicator variables. For testing trend, quintiles were assessed as a continuous variable after assigning participants the median value in each quintile. Cox proportional-hazards (stcox command) estimated the hazard ratio, with time-at-risk until first event, other deaths in cause-specific mortality analyses, or the latest date of adjudicated follow-up. The proportional-hazards assumption was not violated based on Schoenfeld residuals. Covariates were selected on biologic interest, well-established relations with mortality in older adults, or associations with exposures (Supplementary Table 1). Missing covariates (most factors=0.18–0.72%; dietary factors=7.79–12.30%) were imputed by best-subset-regression (impute command) using multiple demographic/risk variables; results excluding missing values were comparable. Potential nonlinear associations were evaluated semi-parametrically using restricted cubic splines (mkspline command)(23). We estimated absolute years of remaining life gained or lost according to quintiles of n3-PUFA using both semi-parametric and parametric approaches(24–26) (Supplementary Appendix).

Sensitivity analyses adjusted for regression dilution bias in n3-PUFA(27–29) and measurement error in covariates(30)(Supplementary Appendix); limited to mid-follow-up (8 years) to minimize misclassification of exposures and covariates over time; and excluded deaths within the first 2 years to minimize effects of unrecognized subclinical disease on fatty acid levels. Statistical significance was defined as two-tailed-alpha=0.05. Exploratory analyses evaluated whether age, sex, or education modified relationships of EPA, DPA, and DHA with total mortality, with Bonferroni-corrected two-tailed-alpha=0.0056 (3 effect modifiers x 3 exposures = 9 exploratory comparisons). Analyses were performed using Stata12.0 (StataCorp, College Station, Texas) and SAS9.2 (SAS, Cary, North Carolina).

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RESULTS

At baseline, 63.7% of participants were women, and mean±SD age was 74±5 years. Most (87.8%) were white; ~1 in 8 (11.7%) were African-American. In unadjusted comparisons, plasma phospholipid EPA, DPA, and DHA had dissimilar relationships with several baseline characteristics that might be potential confounders, such as age, sex, race, education, and alcohol use (Supplementary Table 1). As seen in other cohorts(31), fish consumption associated with EPA and DHA, but not DPA, levels. EPA and DHA (Spearman r=0.43) and EPA and DPA (r=0.51) were modestly intercorrelated; DPA and DHA, less so (r=0.13).

During 30,829 person-years, 1,625 deaths occurred (5.3 per 100 person-years). After adjustment for demographic, cardiovascular, lifestyle, and dietary factors including fish intake, both individual and combined levels of EPA, DPA, and DHA were associated with lower total mortality (Table 1). Across quintiles, individuals with higher EPA, DPA, and DHA levels had 17, 23, and 20% lower risk, respectively; and with higher total n3-PUFA levels, 27% lower risk (P-trend≤0.008 each). Further adjustment for other dietary factors or use of aspirin, lipid-lowering drugs, or other medications had no appreciable effects (not shown).

Among cause-specific deaths, all three n3-PUFA were associated with lower CVD mortality; and their combined levels, with 35% lower risk across quintiles (P-trend<0.001) (Table 2). Among CVD subtypes, DHA appeared most strongly related to CHD death (40% lower risk), especially arrhythmic CHD death (45% lower risk); while DPA, to stroke death (47% lower risk).

As hypothesized, n3-PUFA concentrations were generally unassociated with non-CVD mortality (Supplementary Table 2). Exceptions included inverse associations between DPA and cancer mortality (P-trend=0.032), and total n3-PUFA and infectious deaths (P-trend=0.010).

EPA, DHA, and total n3-PUFA were each associated with lower incidence of total (fatal plus nonfatal) CHD (Table 2). For both DHA and total n3-PUFA, this appeared predominantly driven by lower risk of fatal CHD. Neither EPA nor DPA significantly associated with fatal CHD, nor DPA and DHA with nonfatal MI; nonsignificant trends toward modestly lower risk could not be excluded. DHA and total n3-PUFA demonstrated nominal inverse associations with incident ischemic stroke. Significant associations were not seen for total or hemorrhagic stroke.

In semi-parametric analyses, associations of circulating EPA, DPA, and DHA with total mortality appeared generally linear (Figure 1). A possible threshold effect for EPA was visually suggested but not statistically significant (P-nonlinearity=0.14). To understand how diet related to circulating biomarker levels, we evaluated the dose-response relation between estimated dietary EPA+DHA consumption and phospholipid EPA+DHA (Figure 2). The association was strongly nonlinear (P-nonlinearity<0.001), with steepest dose-responses up to dietary intakes of about 400mg/d, and then smaller increases in circulating levels thereafter.

Relations of n3-PUFA with mortality and CVD were similar when excluding deaths during the first 2 years; or censoring at mid-follow-up (not shown). Adjustment for regression dilution bias in n3-PUFA levels strengthened all risk estimates, with wider CI's (Supplementary Table 3). After additional multivariable measurement error correction for covariates, associations of EPA, DPA, DHA, and total n3-PUFA with total mortality, CVD mortality, and CHD mortality were each further strengthened (Supplementary Table 4). For arrhythmic CHD death, 69% lower risk was evident across total n3-PUFA quintiles (multivariable measurement-error corrected HR=0.31, 95%CI=0.12–0.78, P-trend=0.009). In comparison, for non-arrhythmic CHD death, the corresponding HR was 0.60 (95%CI=0.22–1.59, P-trend=0.13). After multivariable measurement error correction, the magnitude of association of total n3-PUFA with ischemic stroke was unchanged and, due to greater uncertainty, no longer statistically significant.

Simultaneous adjustment for EPA, DPA, and DHA levels partly attenuated the inverse associations of EPA and DPA, but not of DHA, with total and CHD mortality (Supplementary Table 5). In comparison, simultaneous adjustment partly attenuated the inverse associations of DPA and DHA, but not of EPA, with nonfatal MI. There was little evidence that relationships of EPA, DPA, or DHA with total mortality varied by age, gender, or education (Bonferroni-corrected p=NS each).

To inform potential personal and public health relevance of these associations, we calculated the multivariable-adjusted differences in remaining years of life, after age 65 years, among persons with higher or lower n3-PUFA levels. Compared to individuals with lower levels, those with higher levels had significantly greater longevity after age 65 (Table 3). For total n3-PUFA, representative individuals in the highest quintile lived an average of 2.22 more years (95%CI: 0.75–3.13) after age 65. Findings were similar for other representative individuals with varying baseline characteristics (Table 4).

DISCUSSION

In this prospective study of older adults, circulating individual and total n3-PUFA were associated with lower total mortality, with 27% lower risk across total n3-PUFA quintiles. Associations appeared strongest for cardiovascular deaths, especially arrhythmic CHD deaths, with nearly 50% lower risk across quintiles. The observed mortality differences corresponded to ~2.2 greater years of remaining life after age 65 in people with higher versus lower n3-PUFA levels.

Because these biomarkers were measured among older adults, our findings suggest that dietary −3 PUFA late in life may be of benefit in reducing total mortality. Alternatively, these associations could reflect an influence of life-long dietary habits. Specificity for CVD events, especially arrhythmic CHD death, as well as magnitudes of the latter association argue against residual confounding as the sole explanation for our results. Cardiovascular benefits of n3-PUFA are supported by in vitro studies, animal models, and placebo-controlled trials demonstrating physiologic benefits(1). Effects include reduced heart rate, lower blood pressure, improved myocardial efficiency and diastolic function, lower hepatic triglyceride production, and possibly improved autonomic and endothelial function, anti-thrombotic effects, and anti-arrhythmic effects(1). n3-PUFA also give rise to recently identified resolvins, protectins, maresins, and monoepoxides, synthesized by cyclooxygenase, lipoxygenase, and cytochrome-P450 pathways, which appear crucial for restoring homeostasis following tissue injury/inflammation(32, 33). Although many of these physiologic effects are modest, their combined benefits could plausibly reduce mortality, especially related to CVD.

Whereas associations of circulating DPA and DHA with mortality appeared relatively linear, relationships of dietary versus circulating n3-PUFA did not, with steepest dose-responses up to ~400mg/d consumption. Other circulating nutrients show similar dietary dose-responses, with concentrations increasing steeply at lower consumption levels and then relatively saturating thereafter(34, 35). A meta-analysis of cohort studies and randomized trials found a significant, nonlinear threshold relationship between dietary n3-PUFA and CHD mortality, with greatest benefits up to ~250mg/d(5). In light of these prior studies, the present findings utilizing n3-PUFA biomarkers suggest that circulating n3-PUFA – especially DHA – may linearly reduce CHD death within ranges determined by dietary intakes, and that previously observed nonlinear (threshold) relations of dietary n3-PUFA with CHD death may partly relate to nonlinear dose-response of circulating levels to dietary consumption. The observed dose-response between dietary and circulating n3-PUFA represents an average, and individual variation will exist. Nonetheless, the present findings support an average target dietary range of 250–400mg/d EPA+DHA. Relatively few interventions substantially alter total mortality later in life, and these results highlight potential benefits of modest n3-PUFA consumption, compared with little or none, for primary prevention in older adults.

Compared with self-reported diet, circulating biomarkers provide objective measures of exposure, allow evaluation of individual fatty acids, and account for potential nondietary processes that might influence disease risk. Nondietary processes might be most relevant for DPA, which was uncorrelated with dietary fish intake and at least partly derives from metabolic interconversion with EPA(10). Conversely, diet clearly influences circulating EPA and DHA, which were correlated with fish consumption and each other.

Adjustment for self-reported fish consumption did not substantially alter results, concordant with prior analyses of circulating n–3 biomarkers and CVD outcomes(11, 36, 37). If circulating n-3 levels are a key casual mediator of cardiovascular effects of fish consumption, then self-reported fish consumption and its correlates should not confound the associations. In addition, these results might suggest that other nondietary, metabolic influences on circulating n3-PUFA levels are also relevant for disease risk.

A strength of this investigation was ability to evaluate each long-chain n3-PUFA separately. DHA most strongly associated with fatal CHD and arrhythmic CHD death. In light of known higher myocardial concentrations of DHA(38) and prior studies demonstrating inverse associations of circulating DHA, but not EPA or DPA, with incident atrial fibrillation(37, 39), our results suggest that DHA might be especially relevant for cardiac arrhythmias(10). Conversely, only EPA was significantly associated with nonfatal MI. In a large randomized trial, combined treatment of EPA plus a statin, compared to statin treatment alone, reduced nonfatal coronary events(40); and recent prospective studies observed that circulating EPA and/or DPA more strongly associated with nonfatal cardiac outcomes than did DHA(11, 31). In the present investigation, the mutually-adjusted results support greater specificity of DHA for fatal CHD, and of EPA for nonfatal MI. Yet, circulating concentrations of these fatty acids are causally interrelated due to common dietary sources and/or metabolic interconversion, so biologic relevance of mutually-adjusted results should be interpreted cautiously. We also found DHA, but not EPA, associated with less ischemic stroke, an intriguing finding given experimental studies suggesting that DHA reduces brain hypoxic injury and apoptosis(41). However, this association was no longer statistically significant after multivariable measurement error correction, and in randomized trials with durations ranging from ~ 1 to 5 years, fish oil supplements have not reduced stroke(3). Our overall findings, together with other mechanistic studies, support the hypothesis that each long-chain n3-PUFA may have partially differing, complementary effects on pathways of cardiovascular risk(10).

Our findings do not support major effects of circulating n3-PUFA on mortality from non-CVD conditions later in life. Evidence for effects of n3-PUFA on incidence of cancers, dementia, or chronic inflammatory conditions has been inconsistent(42–44). The present results do not exclude potential benefits on incidence or severity of these conditions, or on mortality due to more specific subtypes of these diseases. The observed inverse association of DPA with cancer mortality warrants further study; higher DPA levels could have independent beneficial effects or be a marker of healthier underlying physiology and metabolism. The observed lower risk of infectious deaths was unexpected, but supported by protective effects against infection in animal studies(45, 46); and beneficial effects in some, although not all, trials of n3-PUFA in severe acute lung injury(47–49). Our results support need for evaluation of n3-PUFA in less severe infections, such as community-acquired pneumonia, in older adults. Due to absence of *a priori* hypotheses related to cancer or infection in this analysis, these findings should be considered exploratory.

Few observational studies have evaluated fish or n3-PUFA consumption and total mortality in generally healthy populations(7–9). Most observed only nonsignificant inverse trends, perhaps limited by smaller numbers of events or reliance on self-reported diet. A few prior reports, although not all(50), found inverse associations of n3-PUFA biomarkers with total mortality in CHD patients(51–53) or hospitalized patients(54); associations with cause-specific mortality were generally not reported. To our knowledge, no prior study has evaluated how objectively measured n3-PUFA biomarkers relate to total mortality in generally healthy populations such as ours.

Four large randomized trials among patients with established CVD or at high-risk demonstrated that fish or fish oil supplementation reduced coronary events(3). However, more recent trials have not confirmed these findings(3). A meta-analysis found that n3-PUFA supplementation reduced cardiac death (relative risk=0.91, 95%CI=0.85–0.98), but not all-cause mortality (0.96, 0.91–1.02) (3). Such meta-analyses have not accounted for differences in background dietary fish consumption nor potential nonlinear effects of supplemental n3-PUFA. Our dose-response analysis of diet and circulating levels suggests that dietary or supplemental n3-PUFA may be most beneficial for people with little to no consumption.

No controlled trials have reported effects of n3-PUFA on total mortality in generally healthy populations; one primary prevention trial is enrolling(55). Based on nonlinear effects in observational studies(5), adding a supplement to background consumption of ~150mg/d EPA+DHA (the approximate mean consumption in the US and many European countries) would be calculated to produce ~15% lower CHD mortality (95%CI=8–21%). In comparison, increasing from a baseline of low intake to at least modest consumption (>250mg/d) – or similarly, as in our present investigation, comparing low to high circulating levels – would predict larger benefits, consistent our observations. In our analysis, we evaluated biomarkers of n3-PUFA, measured late in life, that would be generally derived from dietary seafood and perhaps partly from endogenous metabolism (e.g., DPA), rather than from supplements. Ranges of dietary exposure, and absolute levels in the reference group, were also generally much lower than would be seen in a supplement trial.

Our analysis has strengths. Information on demographics, risk factors, and lifestyle were prospectively collected in a well-established multicenter cohort with little loss to follow-up.

We adjusted for multiple covariates, minimizing confounding. The cohort focused on older adults, in whom mortality and competing causes of death are common. Circulating biomarkers provided objective measures of individual fatty acids. Total and cause-specific mortality were prospectively adjudicated using medical records, and large numbers of events provided statistical power. Population-based enrollment from several U.S. communities increased generalizability. Compared with randomized trials, our investigation allowed evaluation of generally healthy adults, of a larger number of mortality and CVD events, of n3-PUFA exposures related to usual dietary habits rather than supplements, of different n-3 fatty acid separately, and of a wide range of dose-response (very low to high).

Limitations should be considered. Fatty acid levels were measured at baseline, and dietary and metabolic fluctuations over time would increase exposure misclassification during follow-up, causing underestimation of true relationships with mortality. Although events were centrally adjudicated, some deaths may have been misclassified; such errors would likely be random with respect to baseline circulating n3-PUFA levels, again causing attenuation of true relationships. This cohort included older men and women, and results may not be generalizable to younger populations. Relatively few hemorrhagic strokes occurred, limiting statistical power for this endpoint. The observational design cannot exclude residual confounding by unknown or unmeasured factors. Yet, results were robust to adjustment for multiple major risk factors. Also, varying relationships were present between each fatty acid and different potential confounders. For example, DPA was unassociated with education or fish intake, limiting potential confounding for this fatty acid due to these factors or their correlates.

In summary, our findings suggest that circulating n3-PUFA levels are linked to lower total mortality among generally healthy adults later in life, with potentially greatest associations with cardiovascular events and especially arrhythmic cardiac death.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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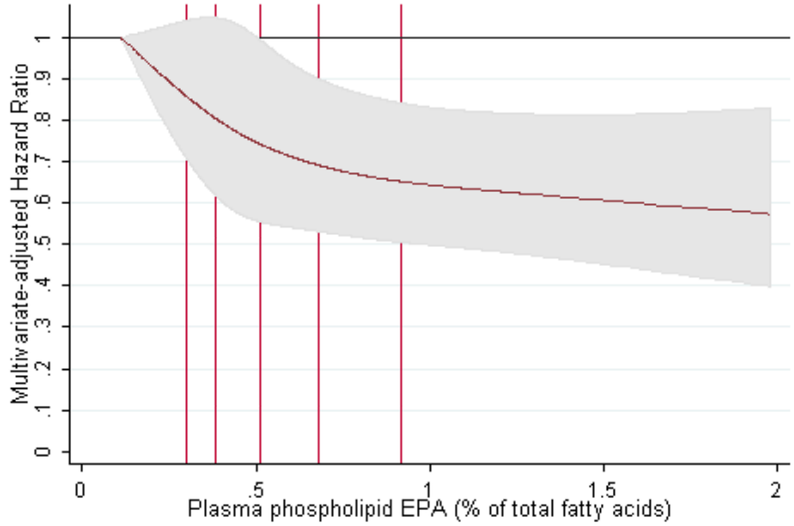
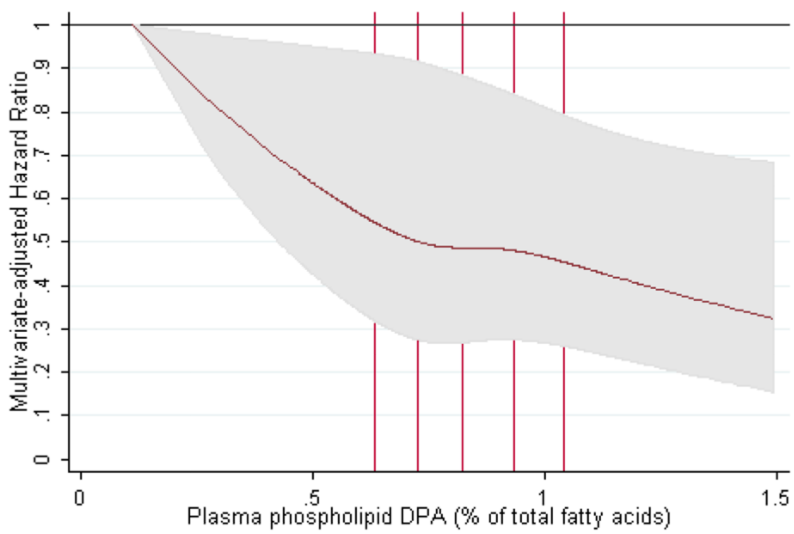


p-value for nonlinearity = 0.14
p-value for overall trend = 0.001



p-value for nonlinearity = 0.43
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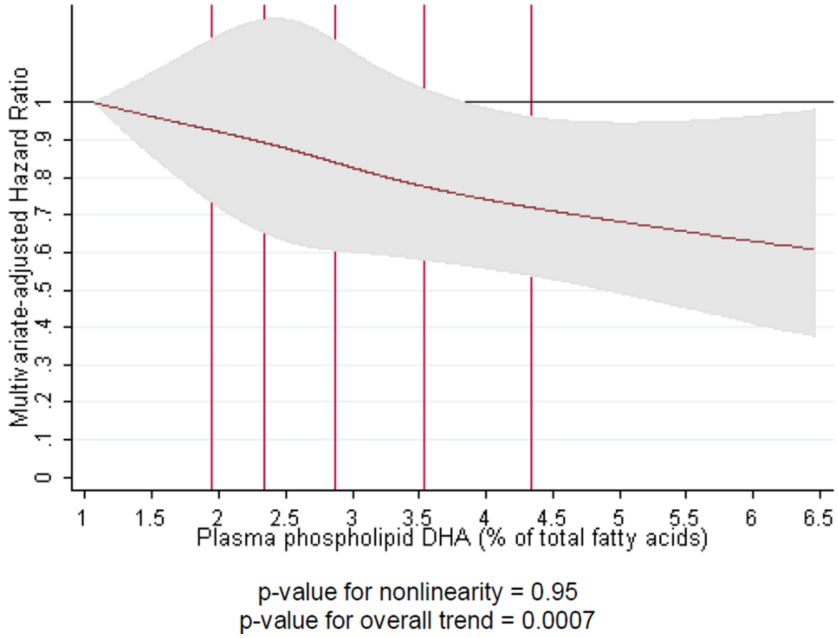

Figure 1.
Multivariable-adjusted relationship of plasma phospholipid eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA) with total mortality among 2,692 older US adults, evaluated using restricted cubic splines. The solid line and shaded area represent the central risk estimate and 95% CIs, respectively. The red vertical lines correspond to the 10th, 25th, 50th, 75th, and 90th percentiles for each fatty acid. Adjusted for age (years), sex, race (white, nonwhite), education (<high school, high school, some college, college graduate), enrollment site (4 sites), fatty acid measurement batch (1994–96, 2007–10), smoking (never, former, current), prevalent diabetes (yes, no), prevalent atrial fibrillation (yes, no), prevalent drug-treated hypertension (yes, no), leisure-time physical activity (kcal/wk), body mass index (kg/m^2), waist circumference (cm), and alcohol use (6 categories).

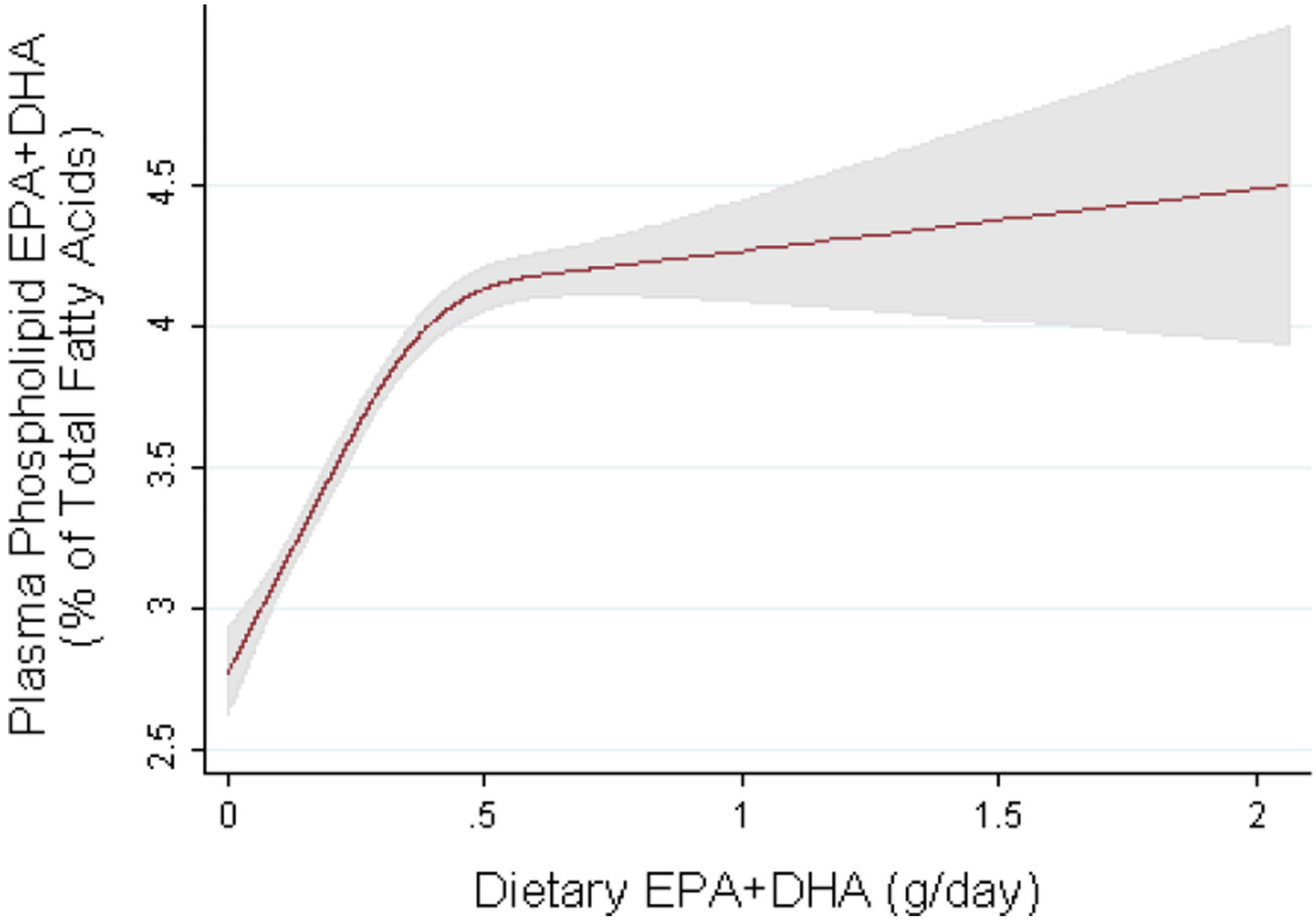

Figure 2.
Relationship between dietary eicosapentaenoic acid (EPA) plus docosahexaenoic acid (DHA) consumption and plasma phospholipid EPA+DHA concentrations among 2,692 older US adults, evaluated using restricted cubic splines, and adjusted for age, sex, race, and education. Because the dietary questionnaire estimated only EPA+DHA (and not DPA), for comparability we evaluated circulating EPA+DHA (rather than EPA+DPA+DHA). Median circulating levels of EPA+DHA in the highest quintile were ~5 percent of total fatty acids. The solid line and shaded area represent the central estimate and 95% CIs, respectively. There was strong evidence for both an overall trend (P<0.001) and nonlinearity of this relationship (P<0.001).

Table 1

Prospective Association of Plasma Phospholipid EPA, DPA and DHA with Total Mortality among 2,692 US Adults.

	Quintiles of Phospholipid Fatty Acids					
	I	**II**	**III**	**IV**	**V**	**P for Trend**
EPA						
% of total FA, median	0.30	0.41	0.51	0.64	0.92	
Deaths (person-years)	371 (5,779)	354 (5,884)	314 (6,307)	290 (6,478)	296 (6,381)	
Hazard ratio (95% CI)						
Age- and sex-adjusted	1.00 (reference)	0.97 (0.84–1.12)	0.83 (0.71–0.96)	0.76 (0.65–0.88)	0.79 (0.68–0.92)	<0.001
Multivariate *	1.00 (reference)	0.99 (0.86–1.15)	0.87 (0.74–1.01)	0.78 (0.67–0.92)	0.80 (0.68–0.95)	0.001
Multivariate+diet adjusted †	1.00 (reference)	1.00 (0.86–1.16)	0.88 (0.75–1.02)	0.80 (0.68–0.94)	0.83 (0.71–0.98)	0.005
DPA						
% of total FA, median	0.63	0.75	0.82	0.91	1.04	
Deaths (person-years)	353 (5,963)	307 (6,209)	330 (6,262)	332 (6,083)	303 (6,312)	
Hazard ratio (95% CI)						
Age- and sex-adjusted	1.00 (reference)	0.78 (0.67–0.91)	0.80 (0.69–0.93)	0.83 (0.71–0.96)	0.75 (0.64–0.87)	0.002
Multivariate *	1.00 (reference)	0.77 (0.66–0.90)	0.82 (0.71–0.96)	0.82 (0.71–0.96)	0.76 (0.65–0.89)	0.004
Multivariate+diet adjusted †	1.00 (reference)	0.77 (0.66–0.90)	0.82 (0.71–0.96)	0.83 (0.71–0.97)	0.77 (0.66–0.90)	0.008
DHA						
% of total FA, median	1.95	2.44	2.87	3.36	4.34	
Deaths (person-years)	349 (5,999)	326 (6,095)	343 (6,168)	317 (6,179)	290 (6,389)	
Hazard ratio (95% CI)						
Age- and sex-adjusted	1.00 (reference)	0.95 (0.81–1.10)	0.92 (0.80–1.07)	0.89 (0.77–1.04)	0.76 (0.65–0.88)	<0.001
Multivariate *	1.00 (reference)	0.98 (0.84–1.14)	0.95 (0.81–1.10)	0.89 (0.76–1.04)	0.77 (0.65–0.91)	<0.001
Multivariate+diet adjusted †	1.00 (reference)	0.99 (0.85–1.15)	0.96 (0.82–1.11)	0.92 (0.78–1.08)	0.80 (0.67–0.94)	0.006
Total n3-PUFA						
% of total FA, median	3.17	3.72	4.21	4.80	6.04	
Deaths (person-years)	347 (5,879)	343 (6,158)	340 (6,077)	309 (6,242)	286 (6,473)	
Hazard ratio (95% CI)						
Age- and sex-adjusted	1.00 (reference)	0.87 (0.75–1.01)	0.95 (0.82–1.10)	0.83 (0.72–0.97)	0.69 (0.59–0.81)	<0.001

	Quintiles of Phospholipid Fatty Acids					P for Trend
	I	II	III	IV	V	
Multivariate *	1.00 (reference)	0.90 (0.78–1.05)	0.93 (0.80–1.08)	0.85 (0.72–0.99)	0.70 (0.59–0.83)	<0.001
Multivariate+diet adjusted †	1.00 (reference)	0.91 (0.78–1.05)	0.94 (0.80–1.09)	0.87 (0.74–1.02)	0.73 (0.61–0.86)	<0.001

*Adjusted for age (years), sex, race (white, nonwhite), education(<high school, high school, some college, college graduate), enrollment site (4 sites), fatty acid measurement batch (1994–96, 2007–10), smoking (never, former, current), prevalent diabetes (yes, no), prevalent atrial fibrillation (yes, no), prevalent drug-treated hypertension (yes, no), leisure-time physical activity (mcal/week), body mass index (kg/m^2), waist circumference (cm), and alcohol use (6 categories).

†Further adjusted for dietary factors including consumption of tuna or other broiled or baked fish (servings/week), fried fish (servings/week), red meat (servings/week), fruits (servings/day), vegetables (servings/day), and dietary fiber (g/day).

EPA=eicosapentaenoic acid. DPA=docosapentaenoic acid. DHA=docosahexaenoic acid. PUFA=polyunsaturated fatty acids.

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Table 2

Prospective Association of Plasma Phospholipid EPA, DPA and DHA Levels with Cardiovascular Mortality and Incident Cardiovascular Diseases among 2,692 US Adults.

	Hazard Ratio (95% CI) According to Quintiles of Phospholipid Fatty Acids					P for Trend
	I	II	III	IV	V	
Total Cardiovascular Mortality (570 deaths) *						
EPA	1.00 (reference)	1.01 (0.79–1.30)	0.87 (0.67–1.14)	0.81 (0.62–1.06)	0.72 (0.54–0.96)	0.009
DPA	1.00 (reference)	0.73 (0.56–0.95)	0.82 (0.63–1.06)	0.80 (0.62–1.03)	0.68 (0.52–0.89)	0.021
DHA	1.00 (reference)	1.09 (0.84–1.41)	1.01 (0.78–1.30)	0.92 (0.70–1.20)	0.66 (0.49–0.89)	0.002
Total n3-PUFA	1.00 (reference)	0.92 (0.71–1.19)	1.05 (0.82–1.35)	0.74 (0.56–0.98)	0.65 (0.48–0.87)	<0.001
Coronary Heart Disease Mortality (359 deaths)						
EPA	1.00 (reference)	0.98 (0.71–1.36)	0.94 (0.68–1.31)	0.90 (0.64–1.26)	0.77 (0.54–1.11)	0.121
DPA	1.00 (reference)	0.69 (0.49–0.97)	0.99 (0.72–1.37)	0.82 (0.59–1.15)	0.79 (0.56–1.11)	0.36
DHA	1.00 (reference)	0.98 (0.71–1.36)	0.96 (0.69–1.32)	0.77 (0.55–1.08)	0.60 (0.41–0.87)	0.003
Total n3-PUFA	1.00 (reference)	0.88 (0.64–1.22)	1.03 (0.75–1.41)	0.62 (0.43–0.89)	0.60 (0.42–0.87)	0.002
Arrhythmic (194 deaths) †						
EPA	1.00 (reference)	0.96 (0.62–1.50)	0.83 (0.53–1.31)	0.82 (0.52–1.29)	0.76 (0.47–1.23)	0.22
DPA	1.00 (reference)	0.79 (0.49–1.27)	1.32 (0.85–2.04)	0.83 (0.52–1.34)	0.79 (0.49–1.30)	0.39
DHA	1.00 (reference)	0.97 (0.62–1.51)	0.85 (0.54–1.33)	0.92 (0.59–1.44)	0.55 (0.33–0.93)	0.028
Total n3-PUFA	1.00 (reference)	0.79 (0.50–1.24)	1.07 (0.70–1.63)	0.68 (0.42–1.10)	0.52 (0.31–0.86)	0.008
Non-Arrhythmic (165 deaths) †						
EPA	1.00 (reference)	1.03 (0.63–1.69)	1.11 (0.68–1.80)	1.00 (0.61–1.65)	0.80 (0.46–1.38)	0.34
DPA	1.00 (reference)	0.60 (0.37–0.99)	0.70 (0.43–1.15)	0.81 (0.50–1.30)	0.80 (0.49–1.30)	0.70
DHA	1.00 (reference)	0.99 (0.62–1.59)	1.08 (0.68–1.70)	0.59 (0.34–1.01)	0.65 (0.37–1.12)	0.038
Total n3-PUFA	1.00 (reference)	1.00 (0.63–1.59)	0.97 (0.61–1.56)	0.54 (0.31–0.95)	0.72 (0.42–1.22)	0.074
Stroke Mortality (130 deaths)						
EPA	1.00 (reference)	1.05 (0.63–1.75)	0.77 (0.44–1.34)	0.67 (0.38–1.21)	0.84 (0.47–1.48)	0.34
DPA	1.00 (reference)	0.56 (0.33–0.96)	0.57 (0.33–0.96)	0.68 (0.41–1.13)	0.53 (0.31–0.92)	0.056
DHA	1.00 (reference)	1.30 (0.76–2.22)	1.14 (0.66–1.96)	1.01 (0.57–1.78)	0.62 (0.32–1.20)	0.082
Total n3-PUFA	1.00 (reference)	0.92 (0.53–1.58)	1.11 (0.66–1.88)	0.84 (0.48–1.48)	0.60 (0.32–1.12)	0.092
Total Fatal and Nonfatal Coronary Heart Disease (630 cases) ‡						

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	Hazard Ratio (95% CI) According to Quintiles of Phospholipid Fatty Acids					P for Trend
	I	II	III	IV	V	
EPA	1.00 (reference)	1.04 (0.82–1.34)	0.91 (0.71–1.18)	0.98 (0.76–1.26)	0.76 (0.58–1.00)	0.032
DPA	1.00 (reference)	0.72 (0.56–0.93)	0.88 (0.69–1.13)	0.82 (0.64–1.05)	0.82 (0.63–1.05)	0.28
DHA	1.00 (reference)	0.94 (0.73–1.20)	1.06 (0.83–1.35)	0.83 (0.64–1.08)	0.72 (0.55–0.95)	0.010
Total n3-PUFA	1.00 (reference)	0.88 (0.69–1.13)	1.06 (0.83–1.35)	0.74 (0.57–0.96)	0.72 (0.55–0.95)	0.009
Nonfatal Myocardial Infarction (371 cases)						
EPA	1.00 (reference)	1.14 (0.83–1.57)	0.84 (0.60–1.19)	1.01 (0.73–1.41)	0.72 (0.51–1.04)	0.038
DPA	1.00 (reference)	0.77 (0.56–1.07)	0.81 (0.59–1.13)	0.75 (0.54–1.05)	0.86 (0.63–1.19)	0.44
DHA	1.00 (reference)	0.84 (0.60–1.17)	1.05 (0.76–1.44)	0.92 (0.66–1.28)	0.79 (0.56–1.13)	0.28
Total n3-PUFA	1.00 (reference)	0.82 (0.59–1.15)	1.04 (0.76–1.43)	0.78 (0.56–1.10)	0.83 (0.59–1.18)	0.32
Total Fatal and Nonfatal Stroke (406 cases) §						
EPA	1.00 (reference)	1.01 (0.74–1.37)	0.95 (0.69–1.29)	0.91 (0.66–1.25)	1.05 (0.76–1.45)	0.85
DPA	1.00 (reference)	0.71 (0.53–0.97)	0.70 (0.52–0.95)	0.85 (0.64–1.15)	0.74 (0.55–1.01)	0.180
DHA	1.00 (reference)	1.08 (0.80–1.46)	1.08 (0.80–1.45)	0.74 (0.53–1.03)	0.84 (0.59–1.18)	0.092
Total n3-PUFA	1.00 (reference)	0.97 (0.72–1.32)	0.91 (0.67–1.23)	0.93 (0.68–1.28)	0.75 (0.53–1.06)	0.098
Ischemic Stroke (319 cases)						
EPA	1.00 (reference)	0.99 (0.70–1.41)	0.94 (0.66–1.34)	0.83 (0.58–1.20)	1.09 (0.76–1.57)	0.74
DPA	1.00 (reference)	0.77 (0.55–1.08)	0.73 (0.52–1.04)	0.78 (0.56–1.10)	0.78 (0.55–1.10)	0.22
DHA	1.00 (reference)	1.01 (0.72–1.41)	1.00 (0.72–1.40)	0.73 (0.51–1.06)	0.74 (0.50–1.10)	0.052
Total n3-PUFA	1.00 (reference)	0.88 (0.63–1.23)	0.77 (0.54–1.08)	0.93 (0.66–1.31)	0.63 (0.43–0.94)	0.043
Hemorrhagic Stroke (65 cases)						
EPA	1.00 (reference)	1.14 (0.56–2.32)	1.00 (0.47–2.14)	0.90 (0.41–1.99)	0.70 (0.30–1.67)	0.32
DPA	1.00 (reference)	0.58 (0.28–1.23)	0.33 (0.14–0.80)	0.75 (0.37–1.51)	0.66 (0.32–1.35)	0.39
DHA	1.00 (reference)	1.41 (0.64–3.09)	1.61 (0.75–3.46)	0.63 (0.24–1.66)	1.24 (0.52–2.94)	0.90
Total n3-PUFA	1.00 (reference)	1.03 (0.45–2.35)	1.81 (0.86–3.82)	0.74 (0.29–1.88)	1.23 (0.53–2.89)	0.86

Hazard ratios (95%CI) were adjusted for the covariates in the multivariate+diet model in Table 1: age (years), sex, race (white, nonwhite), education(<high school, high school, some college, college graduate), enrollment site (4 sites), fatty acid measurement batch (1994–96, 2007–10), smoking (never, former, current), prevalent diabetes (yes, no), prevalent atrial fibrillation (yes, no), prevalent drug-treated hypertension (yes, no), leisure-time physical activity (mcal/week), body mass index (kg/m^2), waist circumference (cm), and alcohol use (6 categories), and consumption of tuna or other broiled or baked fish (servings/week), fried fish (servings/week), red meat (servings/week), fruits (servings/day), vegetables (servings/day), and dietary fiber (g/day).

* Including 359 coronary heart disease deaths, 130 stroke deaths, 32 other atherosclerotic deaths (e.g., due to abdominal aortic aneurysm, mesenteric ischemia/infarctions, or peripheral vascular disease), and 49 other cardiovascular deaths (e.g., due to aortic stenosis, nonischemic cardiomyopathy, or venous thromboembolism).

[†]Subsets of coronary heart disease mortality, with adjudication on whether the underlying event was arrhythmic or non-arrhythmic.

[‡]Including 371 nonfatal myocardial infarctions and 259 coronary heart disease deaths. Analyses of incident coronary heart disease deaths included an additional 100 deaths that occurred with additional follow-up after an incident nonfatal myocardial infarction.

[§]Including 319 ischemic strokes, 65 hemorrhagic strokes, and 22 strokes for which clinical information was insufficient for subtype classification.

EPA=eicosapentaenoic acid. DPA=docosapentaenoic acid. DHA=docosahexaenoic acid. PUFA=polyunsaturated fatty acids.

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Table 3

Estimated Remaining Years of Life Gained, After Age 65, According to Quintiles of Plasma Phospholipid EPA, DPA and DHA among 2,692 US Adults.

	Years of Life Gained (95% CI) in Each Quintile of Phospholipid Fatty Acids *				
	I	**II**	**III**	**IV**	**V**
EPA	0.00 (reference)	0.03 (−0.86, 1.09)	0.96 (−0.02, 1.96)	1.55 (0.55, 2.31)	1.39 (0.23, 2.47)
DPA	0.00 (reference)	1.70 (0.47, 2.66)	1.28 (0.24, 2.15)	1.27 (0.31, 2.01)	1.82 (0.69, 2.82)
DHA	0.00 (reference)	0.66 (−0.20, 1.50)	0.28 (−0.58, 1.14)	0.67 (−0.34, 1.48)	1.64 (0.42, 2.47)
Total n3-PUFA	0.00 (reference)	0.63 (−0.21, 1.71)	0.46 (−0.62, 1.51)	1.06 (−0.05, 2.05)	2.22 (0.75, 3.13)

*Values are the estimated years of life gained after age 65, compared to the lowest quintile as the reference, based on semi-parametric survival models with adjustment for the covariates in the multivariate model in Table 1. Parametric estimates utilizing log-normal, log-logistic, Weibull, or Gamma survival distribution were very similar. The results in this table would be representative of a participant entering the study at age 65, with average (mean) values for each of the continuous covariates, including body mass index (26.7 kg/ m^2), waist circumference (96.8 cm), and leisure-time physical activity (1070 kcal/wk); and falling into the most representative category (mode) for each of the categorical covariates, including sex (female), race (white), education (<high school), enrollment site (Forsyth County, North Carolina), fatty acid measurement batch (2007–10), smoking (never), prevalent diabetes (no), prevalent atrial fibrillation (no), prevalent drug-treated hypertension (no), and alcohol use (none).

EPA=eicosapentaenoic acid. DPA=docosapentaenoic acid. DHA=docosahexaenoic acid. PUFA=polyunsaturated fatty acids.

Table 4

Estimated Remaining Years of Life Gained, After Age 65, Among Different Representative Older Adults According to Plasma Phospholipid Total n3-PUFA.

Individual Characteristics	Years of Life Gained (95% CI) in the Highest vs. Lowest Quintile of Total n3-PUFA*
Female, white, education < high school †	2.22 (0.75, 3.13) †
Male, white, education < high school ‡	2.33 (1.01, 3.40) ‡
Male, white, college-educated	2.33 (0.95, 3.33)
Male, non-white, college-educated	2.31 (0.88, 3.24)
Male, non-white, college-educated, diabetic	2.33 (1.01, 3.52)
Male, non-white, college-educated, diabetic, current smoker	2.20 (0.92, 3.30)

*Values are the multivariable-adjusted estimated years of life gained after age 65 in the highest quintile of total n-3 PUFA, compared to the lowest quintile as the reference, based on semi-parametric survival models (see Table 3).

†These results are representative of a participant entering the study at age 65, with average (mean) values for each of the continuous covariates of body mass index (26.7 kg/ m^2), waist circumference (96.8 cm), and leisure-time physical activity (1070 kcal/wk); and falling into the most representative category (mode) for each of the categorical covariates of sex (female), race (white), education (<high school), enrollment site (Forsyth County, North Carolina), fatty acid measurement batch (2007–10), smoking (never), prevalent diabetes (no), prevalent atrial fibrillation (no), prevalent drug-treated hypertension (no), and alcohol use (none).

‡We also calculated the life-years gained for representative variations of the above individual, for example if the same individual were instead male (row two in the table); male and college-educated (row three); male, college educated, and nonwhite (row four); male, college-educated, nonwhite, and diabetic (row five); and male, college-educated, nonwhite, diabetic, and a current smoker (row six)

PUFA=polyunsaturated fatty acids.