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Plasma Phospholipid Long-Chain ω-3 Fatty Acids and Total and Cause-Specific Mortality in Older Adults

A Cohort Study

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Background: Long-chain ω-3 polyunsaturated fatty acids (ω3-PUFAs), including eicosapentaenoic acid (EPA) (20:5ω-3), docosapentaenoic acid (DPA) (22:5ω-3), and docosahexaenoic acid (DHA) (22:6ω-3), have been shown to reduce cardiovascular risk, but effects on cause-specific and total mortality and potential dose-responses remain controversial. Most observational studies have assessed self-reported dietary intake and most randomized trials have tested effects of adding supplements to dietary intake and evaluated secondary prevention, thus limiting inference for dietary ω3-PUFAs or primary prevention.

Objective: To investigate associations of plasma phospholipid EPA, DPA, DHA, and total ω3-PUFA levels with total and cause-specific mortality among healthy older adults not receiving supplements.

Design: Prospective cohort study.

Setting: 4 U.S. communities.

Participants: 2692 U.S. adults aged 74 years (±5 years) without prevalent coronary heart disease (CHD), stroke, or heart failure at baseline.

Measurements: Phospholipid fatty acid levels and cardiovascular risk factors were measured in 1992. Relationships with total and cause-specific mortality and incident fatal or nonfatal CHD and stroke through 2008 were assessed.

Results: During 30 829 person-years, 1625 deaths (including 570 cardiovascular deaths), 359 fatal and 371 nonfatal CHD events, and 130 fatal and 276 nonfatal strokes occurred. After adjustment, higher plasma levels of ω3-PUFA biomarkers were associated with lower total mortality, with extreme-quintile hazard ratios of 0.83 for EPA (95% CI, 0.71 to 0.98; P for trend = 0.005), 0.77 for DPA (CI, 0.66 to 0.90; P for trend = 0.008), 0.80 for DHA (CI, 0.67 to 0.94; P for trend = 0.006), and 0.73 for total ω3-PUFAs (CI, 0.61 to 0.86; P for trend < 0.001). Lower risk was largely attributable to fewer cardiovascular than noncardiovascular deaths. Individuals in the highest quintile of phospholipid ω3-PUFA level lived an average of 2.22 more years (CI, 0.75 to 3.13 years) after age 65 years than did those in the lowest quintile.

Limitation: Temporal changes in fatty acid levels and misclassification of causes of death may have resulted in underestimated associations, and unmeasured or imperfectly measured covariates may have caused residual confounding.

Conclusion: Higher circulating individual and total ω3-PUFA levels are associated with lower total mortality, especially CHD death, in older adults.

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Experiments and clinical studies have shown physiologic benefits of long-chain ω-3 polyunsaturated fatty acids (ω3-PUFAs), which include eicosapentaenoic acid (EPA) (20:5ω-3), docosapentaenoic acid (DPA) (22:5ω-3), and docosahexaenoic acid (DHA) (22:6ω-3) (1). Yet, although observational studies have found inverse associations between dietary ω3-PUFA level and death from coronary heart disease (CHD) (1, 2), randomized trials of ω3-PUFA supplementation have had mixed results (3). Consequently, effects of ω3-PUFAs on cardiovascular disease (CVD) and total and cause-specific mortality remain controversial. Understanding the influence of ω3-PUFAs on CVD and mortality; whether such effects vary for EPA, DPA, or DHA; and their potential dose-response is crucial for scientific advancement and dietary guidance.

Most observational studies of ω3-PUFAs have assessed self-reported dietary intake rather than objective biomarkers, which may have led to measurement errors or bias. Conversely, most randomized trials have tested the effects of ω3-PUFA supplements among patients with established CVD or multiple risk factors, thus limiting inference for primary prevention. In addition, the trials evaluated ω3-PUFA supplements that were added to background dietary intake, which could reduce efficacy if the dose-response for ω3-PUFAs is nonlinear. In particular, a potential threshold effect (4, 5) could explain why moderate consumption is associated with benefits when compared with little or no consumption in observational studies, whereas adding higher supplement doses to already moderate background dietary intake produces smaller or no effects in trials. Differences could also be due to stronger effects of ω3-PUFAs on CHD death, which is often evaluated in observational studies (4, 5), versus composite end points of total CHD or total CVD events in trials.

Whether potential cardiovascular benefits of ω3-PUFAs translate into lower total mortality or whether ω3-PUFAs influence noncardiovascular causes of death is also unclear. Competing risks from noncardiovascular conditions (for example, cancer or lung disease) may be unaffected by ω3-PUFAs (6), thus minimizing effects on total mortality, particularly later in life. In meta-analyses of trials, ω3-PUFA supplementation produced non–statistically significant trends toward lower total mortality (3). However, these trials typically evaluated higher-dose fish oil supplements in high-risk patients, many of whom were

Context

The effects of dietary long-chain ω-3 polyunsaturated fatty acids (ω3-PUFAs) on total and cause-specific mortality are uncertain, as are the potential benefits for the primary prevention of cardiovascular disease.

Contribution

In this cohort of individuals aged 65 years or older without known cardiovascular disease at baseline, higher baseline levels of specific individual and total ω3-PUFAs were associated with decreased total mortality, primarily due to fewer cardiovascular events.

Caution

Fatty acid levels were obtained at baseline.

Implication

Further study is required to evaluate whether certain ω3-PUFAs are beneficial for the primary prevention of cardiovascular disease and decreased mortality.

—The Editors

already consuming fish. Several prospective cohort studies of generally healthy populations have found non–statistically significant inverse trends between self-reported dietary ω3-PUFA intake and total mortality (7–9). Self-reported diet also cannot reliably distinguish among specific long-chain ω3-PUFAs (EPA, DPA, or DHA), which may partially differ in physiologic effects (10).

Circulating ω3-PUFA biomarkers objectively reflect dietary consumption and biologically relevant processes (for example, absorption, incorporation, or metabolism) that influence tissue levels. Metabolic influences seem especially relevant for DPA, which is elongated from and retroconverted to EPA (10). Biomarkers also permit direct evaluation of individual ω-3 fatty acids, which may have different effects on certain biological pathways or clinical end points (10). However, to our knowledge no prior studies have evaluated how circulating ω3-PUFA biomarkers relate to total mortality and diverse CVD subtypes in generally healthy populations.

To address these gaps, we prospectively designed and implemented an investigation of ω3-PUFA biomarkers, including EPA, DPA, and DHA, and risk for CVD (CHD or stroke) and total and cause-specific mortality in a large, community-based cohort of older U.S. adults. We hypothesized, on the basis of mechanistic studies and physiologic effects (1, 10), that ω3-PUFA levels would be associated with decreases in cardiovascular mortality (especially CHD death) but not noncardiovascular mortality. We also hypothesized that among individual ω3-PUFAs, DHA would be most strongly associated with arrhythmic CHD death, and EPA and DPA would be most strongly associated with nonfatal CHD.

METHODS

Design and Population

The CHS (Cardiovascular Health Study) is a multicenter prospective cohort of older U.S. adults. In 1989–1990, 5201 ambulatory, noninstitutionalized adults aged 65 years or older were randomly selected and enrolled from Medicare eligibility lists in 4 communities: Forsyth County, North Carolina; Sacramento County, California; Washington County, Maryland; and Allegheny County, Pennsylvania. An additional 687 black participants were similarly recruited and enrolled in 1992–1993. Among all eligible adults contacted, 57% agreed to participate; these adults were slightly healthier than those who declined. Trained personnel performed annual study clinic evaluations, which 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, and all participants provided informed written consent.

Study Measures

We measured fatty acids in 3941 of the 5565 living cohort participants who had blood samples taken at the 1992–1993 study visit, which we considered the baseline for this analysis. Details of cohort sampling and fatty acid measurements have been described elsewhere (11) and are provided in the **Appendix** (available at www.annals.org). The assessment of EPA, DPA, and DHA levels and incident CVD and mortality was a prespecified aim of the research. After exclusion of 1113 participants with prevalent CVD and 136 receiving fish oil supplements at the time of blood sampling, 2692 participants were included in this analysis. At the 1992–1993 visit, cardiovascular risk factors, anthropometric values, blood pressure, and laboratory values were measured using standardized procedures, and alcohol use and physical activity were assessed using validated questionnaires (12–17). Dietary habits were assessed 3 years earlier (1989–1990) using a validated semiquantitative food-frequency questionnaire (18), from which dietary EPA plus DHA consumption was estimated as previously described (19).

End Points

Participants were followed by means of alternating study clinic examinations and telephone contacts every 6 months through 2000 and biannual telephone contacts thereafter. Vital status follow-up was 100% complete; less than 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). Cardiovascular disease 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 or trauma, and other causes. Arrhythmic CHD deaths were also adjudicated (21); sensitivity and specificity in comparison to a Hinkle classification were found to be 93% and 95%, respectively.

Statistical Analysis

We evaluated ω3-PUFA levels in quintiles as indicator variables. To evaluate trends, we assessed quintiles as a continuous variable after participants were assigned the median value in each quintile. We estimated the hazard ratio using a Cox proportional hazards model (stcox command in Stata, release 12.0 [StataCorp, College Station, Texas]) 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 on the basis of Schoenfeld residuals. Covariates were selected on the basis of biological interest, well-established relations with mortality in older adults, or associations with exposures (**Appendix Table 1**, available at www.annals.org). We imputed missing covariates (0.18% to 0.72% for most factors and 7.79% to 12.30% for dietary factors) by best-subset regression (impute command in Stata) using multiple demographic and risk variables; results were similar when missing values were excluded. Potential nonlinear associations were evaluated semiparametrically using restricted cubic splines (mkspline command in Stata) (23). We estimated absolute years of remaining life gained or lost for each quintile of ω3-PUFAs by using both semiparametric and parametric approaches (24–26) (**Appendix**).

Sensitivity analyses were adjusted for regression dilution bias in ω3-PUFA levels (27–29) and measurement error in covariates (30) (**Appendix**), were 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. We defined statistical significance as a 2-tailed α level of 0.05. Exploratory analyses evaluated whether age, sex, or education affected relationships of EPA, DPA, and DHA levels with total mortality, with a Bonferroni-corrected, 2-tailed α level of 0.0056 (9 exploratory comparisons). Analyses were performed using Stata, release 12.0, and SAS, version 9.2 (SAS Institute, Cary, North Carolina).

Role of the Funding Source

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RESULTS

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

During 30 829 person-years, 1625 deaths occurred (5.3/100 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 (P for trend = 0.005, 0.008, and 0.006, respectively), and those with higher total ω3-PUFA levels had 27% lower risk (P for trend < 0.001). Further adjustment for other dietary factors or use of aspirin, lipid-lowering drugs, or other medications had no appreciable effects (data not shown).

For cause-specific deaths, all 3 ω3-PUFAs were associated with lower CVD mortality and their combined levels were associated with 35% lower risk across quintiles (P for trend < 0.001) (**Table 2**). Among CVD subtypes, DHA seemed most strongly related to CHD death (40% lower risk), especially arrhythmic CHD death (45% lower risk), whereas DPA was most strongly related to stroke death (47% lower risk).

As hypothesized, ω3-PUFA concentrations were generally unassociated with non-CVD mortality (**Appendix Table 2**, available at www.annals.org). Exceptions included inverse associations between DPA level and cancer mortality (P for trend = 0.032) and between total ω3-PUFA level and deaths from infection (P for trend = 0.010).

Levels of EPA, DHA, and total ω3-PUFA were each associated with lower incidence of total (fatal plus nonfatal) CHD (**Table 2**). For both DHA and total ω3-PUFA levels, this seemed to be predominantly driven by lower risk for fatal CHD. Neither EPA nor DPA was statistically significantly associated with fatal CHD, and DPA and DHA were not associated with nonfatal myocardial infarction (MI). Non–statistically significant trends toward modestly lower risk could not be excluded. Levels of DHA and total ω3-PUFA showed nominal inverse associations with incident ischemic stroke. Statistically significant associations were not seen for total or hemorrhagic stroke.

In semiparametric analyses, associations of circulating EPA, DPA, and DHA levels with total mortality seemed

Table 1. **Prospective Association of Plasma Phospholipid EPA, DPA, and DHA Levels With Total Mortality**

Variable	Quintile of Phospholipid FA Level					*P* Value for Trend
	I	II	III	IV	V	
EPA						
Median proportion of total FAs	0.30	0.41	0.51	0.64	0.92	–
Deaths (person-years), *n*	371 (5779)	354 (5884)	314 (6307)	290 (6478)	296 (6381)	–
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-adjusted*	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- and 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						
Median proportion of total FAs	0.63	0.75	0.82	0.91	1.04	–
Deaths (person-years), *n*	353 (5963)	307 (6209)	330 (6262)	332 (6083)	303 (6312)	–
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-adjusted*	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- and 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						
Median proportion of total FAs	1.95	2.44	2.87	3.36	4.34	–
Deaths (person-years), *n*	349 (5999)	326 (6095)	343 (6168)	317 (6179)	290 (6389)	–
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-adjusted*	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- and 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 ω3-PUFAs						
Median proportion of total FAs	3.17	3.72	4.21	4.80	6.04	–
Deaths (person-years), *n*	347 (5879)	343 (6158)	340 (6077)	309 (6242)	286 (6473)	–
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
Multivariate-adjusted*	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- and 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

DHA = docosahexaenoic acid; DPA = docosapentaenoic acid; EPA = eicosapentaenoic acid; FA = fatty acid; PUFA = polyunsaturated fatty acid.
* Adjusted for age (years), sex, race (white or nonwhite), education (less than high school, high school, some college, or college graduate), enrollment site (4 sites), FA measurement batch (1994–1996 or 2007–2010), smoking status (never, former, or current), prevalent diabetes (yes or no), prevalent atrial fibrillation (yes or no), prevalent drug-treated hypertension (yes or no), leisure-time physical activity (mcal/wk), 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/wk), fried fish (servings/wk), red meat (servings/wk), fruits (servings/d), vegetables (servings/d), and dietary fiber (g/d).

generally linear (**Figure 1**). A possible threshold effect for EPA was visually suggested but not statistically significant (*P* for nonlinearity = 0.142). To understand how diet was related to circulating biomarker levels, we evaluated the dose–response relation between estimated dietary EPA plus DHA consumption and phospholipid EPA plus DHA level (**Figure 2**). The association was strongly nonlinear (*P* for nonlinearity < 0.001), with steepest dose-responses up to dietary intakes of about 400 mg/d and smaller increases in circulating levels thereafter.

Relations of ω3-PUFA level with mortality and CVD were similar when deaths were excluded during the first 2 years or censored at mid–follow-up (data not shown). Adjustment for regression dilution bias in ω3-PUFA level strengthened all risk estimates and widened CIs (**Appendix Table 3**, available at www.annals.org). After additional multivariate measurement error correction for covariates, associations of EPA, DPA, DHA, and total ω3-PUFA levels with total mortality, CVD mortality, and CHD mortality were each strengthened (**Appendix Table 4**, available at www.annals.org). For arrhythmic CHD death, 69% lower risk was evident across total ω3-PUFA quintiles (multivariate measurement error–corrected hazard ratio, 0.31 [95% CI, 0.12 to 0.78]; *P* for trend = 0.009). In comparison, for nonarrhythmic CHD death, the corresponding hazard ratio was 0.60 (CI, 0.22 to 1.59; *P* for trend = 0.134). After multivariate measurement error correction, the magnitude of association of total ω3-PUFA level with ischemic stroke was unchanged and, due to greater uncertainty, no longer statistically significant.

Simultaneous adjustment for EPA, DPA, and DHA levels attenuated the inverse associations of DPA and DHA levels with total mortality and the association of EPA level with nonfatal MI (**Appendix Table 5**, available at www.annals.org). The association between DHA level and CHD mortality was not substantially altered by adjustment for EPA and DPA levels, whereas the association between EPA level and total mortality was no longer present after adjustment for DPA and DHA levels. There was little evidence that relationships of EPA, DPA, or DHA levels with total mortality varied by age, sex, or education (Bonferroni-corrected *P* > 0.0056 for each).

Table 2. **Prospective Association of Plasma Phospholipid EPA, DPA, and DHA Levels With Cardiovascular Mortality and Incident Cardiovascular Diseases**

Variable	Hazard Ratio (95% CI), by Quintile of Phospholipid FA Level*					*P* Value 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 ω3-PUFAs	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
Total CHD 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 ω3-PUFAs	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 CHD mortality (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 ω3-PUFAs	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
Nonarrhythmic CHD mortality (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 ω3-PUFAs	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 ω3-PUFAs	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 CHD (630 cases)§						
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 ω3-PUFAs	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 ω3-PUFAs	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 ω3-PUFAs	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 ω3-PUFAs	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

Continued on following page

Table 2—Continued

Variable	Hazard Ratio (95% CI), by Quintile of Phospholipid FA Level*					*P* Value for Trend
	I	II	III	IV	V	
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 ω3-PUFAs	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

CHD = coronary heart disease; DHA = docosahexaenoic acid; DPA = docosapentaenoic acid; EPA = eicosapentaenoic acid; FA = fatty acid; PUFA = polyunsaturated fatty acid.

* Hazard ratios and 95% CIs were adjusted for the covariates in the multivariate- and diet-adjusted model in **Table 1**: age (years); sex; race (white or nonwhite); education (less than high school, high school, some college, or college graduate); enrollment site (4 sites); FA measurement batch (1994–1996 or 2007–2010); smoking status (never, former, or current); prevalent diabetes (yes or no); prevalent atrial fibrillation (yes or no); prevalent drug-treated hypertension (yes or no); leisure-time physical activity (mcal/wk); body mass index (kg/m^2); waist circumference (cm); alcohol use (6 categories); and consumption of tuna or other broiled or baked fish (servings/wk), fried fish (servings/wk), red meat (servings/wk), fruits (servings/d), vegetables (servings/d), and dietary fiber (g/d).

† Includes 359 CHD 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 CHD mortality, with adjudication on whether the underlying event was arrhythmic or nonarrhythmic.

§ Includes 371 nonfatal myocardial infarctions and 259 CHD deaths. Analyses of incident CHD deaths included an additional 100 deaths that occurred with additional follow-up after an incident nonfatal myocardial infarction.

‖ Includes 319 ischemic strokes, 65 hemorrhagic strokes, and 22 strokes for which clinical information was insufficient for subtype classification.

To inform potential personal and public health relevance of these associations, we calculated the multivariate-adjusted differences in remaining years of life after age 65 years among persons with higher or lower ω3-PUFA levels. Individuals with higher levels had significantly greater longevity after age 65 years than those with lower levels (**Table 3**). For total ω3-PUFA level, representative persons in the highest quintile lived an average of 2.22 more years (CI, 0.75 to 3.13 years) after age 65 years. Differences in life expectancy for other representative persons with varying baseline characteristics were similar (**Table 4**).

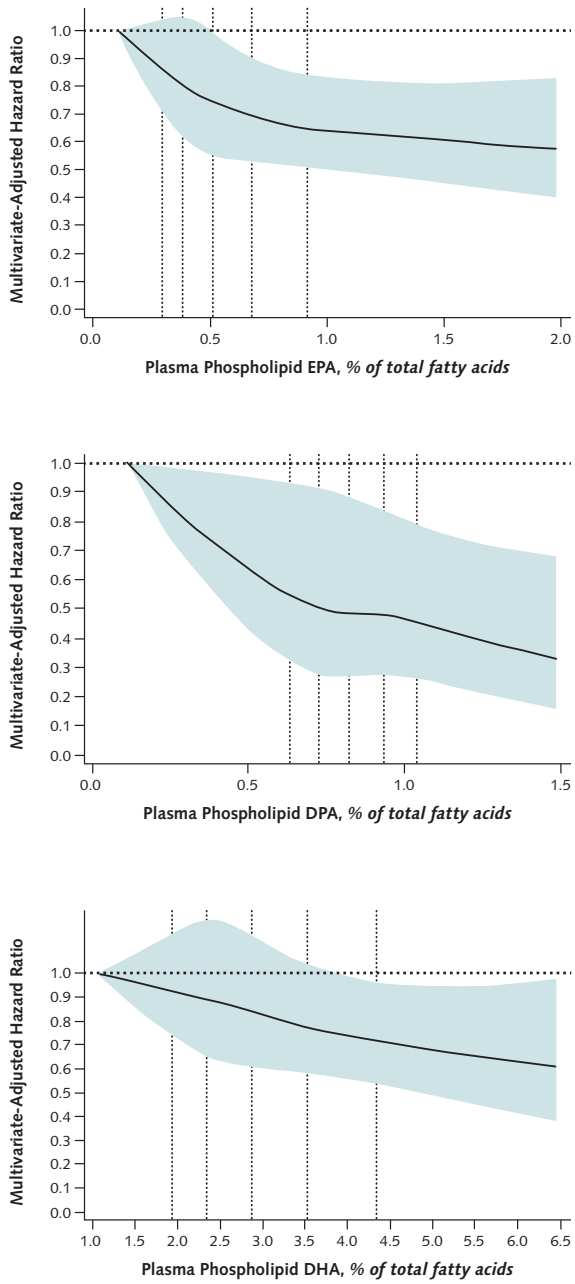
DISCUSSION

In this prospective study of older adults, circulating individual and total ω3-PUFA levels were associated with lower total mortality, with a 27% lower risk across total ω3-PUFA quintiles. Associations seemed strongest for cardiovascular deaths, especially arrhythmic CHD deaths, with nearly 50% lower risk across quintiles. The observed mortality differences corresponded to approximately 2.2 more years of remaining life after age 65 years in persons with higher ω3-PUFA levels than in those with lower levels.

Because these biomarkers were measured among older adults, our findings suggest that dietary ω3-PUFAs late in life may reduce total mortality. Alternatively, these associations could reflect an influence of life-long dietary habits. Specificity for CVD events, especially arrhythmic CHD death, and magnitudes of the latter association argue against residual confounding as the sole explanation for our results. Cardiovascular benefits of ω3-PUFAs are supported by in vitro studies, animal models, and placebo-controlled trials showing physiologic benefits (1). Effects include reduced heart rate, lower blood pressure, improved myocardial efficiency and diastolic function, and lower hepatic triglyceride production and may also include improved autonomic and endothelial function, antithrombotic effects, and antiarrhythmic effects (1). In addition, ω3-PUFAs are precursors to recently identified resolvins, protectins, maresins, and monoepoxides, which are synthesized by cyclooxygenase, lipoxygenase, and cytochrome-P450 pathways and seem crucial for restoring homeostasis after tissue injury or inflammation (32, 33). Although many of these physiologic effects are modest, their combined benefits could plausibly reduce mortality, especially deaths related to CVD.

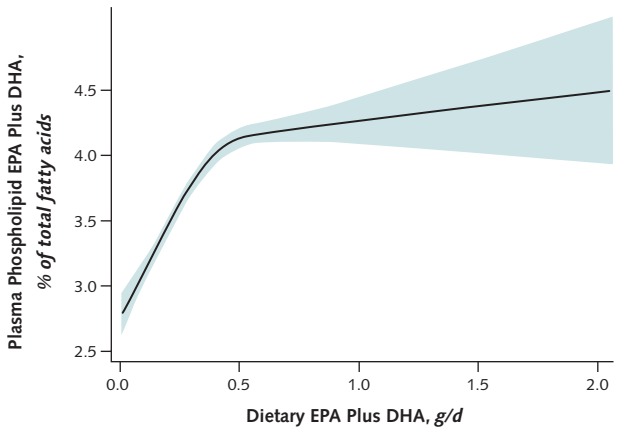
Although associations of circulating DPA and DHA levels with mortality seemed relatively linear, relationships of dietary versus circulating ω3-PUFA levels did not, with steepest dose-responses up to consumption of approximately 400 mg/d. Other circulating nutrients show similar dietary dose-responses, with concentrations increasing steeply at lower consumption levels and relatively saturating thereafter (34, 35). A meta-analysis of cohort studies and randomized trials found a statistically significant, nonlinear threshold relationship between dietary ω3-PUFA level and CHD mortality, with greatest benefits up to consumption of approximately 250 mg/d (5). In light of these prior studies, the present findings for ω3-PUFA biomarkers suggest that circulating ω3-PUFAs (especially DHA) may linearly reduce CHD death within ranges determined by dietary intake and that previously observed nonlinear (threshold) relations of dietary ω3-PUFA level with CHD death may partly relate to a nonlinear dose-response of circulating levels to dietary consumption. The observed dose-response between dietary and circulating ω3-PUFAs represents an average, and individual variation will exist. Nonetheless, the present findings support an average target dietary range of 250 to 400 mg of EPA plus DHA per day. Relatively few interventions substantially alter total mortality later in life, and these results highlight potential benefits

Figure 1. **Multivariate-adjusted relationship of plasma phospholipid EPA, DPA, and DHA levels with total mortality, evaluated using restricted cubic splines.**



The solid lines and shaded areas represent the central risk estimate and 95% CI, respectively, for each fatty acid. The dotted vertical lines correspond to the 10th, 25th, 50th, 75th, and 90th percentiles for each fatty acid. Adjusted for age (years), sex, race (white or nonwhite), education (less than high school, high school, some college, or college graduate), enrollment site (4 sites), fatty acid measurement batch (1994–1996 or 2007–2010), smoking (never, former, or current), prevalent diabetes (yes or no), prevalent atrial fibrillation (yes or no), prevalent drug-treated hypertension (yes or no), leisure-time physical activity (kcal/wk), body mass index (kg/m^2), waist circumference (cm), and alcohol use (6 categories). DHA = docosahexaenoic acid; DPA = docosapentaenoic acid; EPA = eicosapentaenoic acid.

Figure 2. **Relationship between dietary EPA plus DHA consumption and plasma phospholipid EPA plus DHA concentrations, evaluated using restricted cubic splines and adjusted for age, sex, race, and education.**



Because the dietary questionnaire assessed only EPA plus DHA (and not DPA), for comparability we evaluated circulating EPA plus DHA (rather than EPA plus DPA plus DHA) level. Median circulating levels of EPA plus DHA in the highest quintile were approximately 5% of total fatty acids. The solid line and shaded area represent the central estimate and 95% CI, respectively. There was strong evidence for both an overall trend ($P < 0.001$) and nonlinearity of this relationship ($P < 0.001$). DHA = docosahexaenoic acid; DPA = docosapentaenoic acid; EPA = eicosapentaenoic acid.

of modest ω3-PUFA consumption, compared with little or none, for primary prevention in older adults.

In contrast to 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 not correlated with dietary fish intake and at least partly derives from metabolic interconversion with EPA (10). Conversely, diet clearly influences circulating EPA and DHA levels, which were correlated with fish consumption and each other.

Adjustment for self-reported fish consumption did not substantially alter the results, and this is concordant with prior analyses of circulating ω3-PUFA biomarkers and CVD outcomes (11, 36, 37). If circulating ω3-PUFA levels are a key causal mediator of cardiovascular effects of fish consumption, 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 ω3-PUFA levels are also relevant for disease risk.

A strength of this investigation was its ability to evaluate each long-chain ω3-PUFA separately. Docosahexaenoic acid was most strongly associated with fatal CHD and arrhythmic CHD death. In light of known higher myocardial concentrations of DHA (38) and prior studies showing

Table 3. **Estimated Years of Remaining Life Gained After Age 65 y, by Quintile of Plasma Phospholipid EPA, DPA, and DHA Level**

Variable	Remaining Life Gained (95% CI), y*				
	I	II	III	IV	V
EPA	0.00 (reference)	0.03 (−0.86 to 1.09)	0.96 (−0.02 to 1.96)	1.55 (0.55 to 2.31)	1.39 (0.23 to 2.47)
DPA	0.00 (reference)	1.70 (0.47 to 2.66)	1.28 (0.24 to 2.15)	1.27 (0.31 to 2.01)	1.82 (0.69 to 2.82)
DHA	0.00 (reference)	0.66 (−0.20 to 1.50)	0.28 (−0.58 to 1.14)	0.67 (−0.34 to 1.48)	1.64 (0.42 to 2.47)
Total ω3-PUFAs	0.00 (reference)	0.63 (−0.21 to 1.71)	0.46 (−0.62 to 1.51)	1.06 (−0.05 to 2.05)	2.22 (0.75 to 3.13)

DHA = docosahexaenoic acid; DPA = docosapentaenoic acid; EPA = eicosapentaenoic acid; PUFA = polyunsaturated fatty acid.
* Values are the estimated years of life gained after age 65 y, with the lowest quintile as the reference, based on semiparametric survival models with adjustment for the covariates in the multivariate-adjusted model in **Table 1**. Parametric estimates using log-normal, log-logistic, Weibull, or γ survival distribution were similar. The results in this table would be representative of a participant entering the study at age 65 y with the mean values for each of the continuous covariates (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 (sex [female], race [white], education [less than high school], enrollment site [Forsyth County, North Carolina], fatty acid measurement batch [2007–2010], smoking status [never], prevalent diabetes [no], prevalent atrial fibrillation [no], prevalent drug-treated hypertension [no], and alcohol use [none]).

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 statistically significantly associated with nonfatal MI. In a large randomized trial, treatment consisting of EPA combined with a statin reduced nonfatal coronary events compared with statin treatment alone (40), and recent prospective studies found that circulating EPA or DPA level was more strongly associated with nonfatal cardiac outcomes than was DHA level (11, 31). In the present investigation, the mutually adjusted results support greater specificity of DHA level for fatal CHD and of EPA level for nonfatal MI. Yet, circulating concentrations of these fatty acids are causally interrelated due to common dietary sources, metabolic interconversion, or both, so biological relevance of mutually adjusted results should be interpreted cautiously. We also found that DHA, but not EPA, was associated with less ischemic stroke—an intriguing finding, given experimental studies suggesting that DHA reduces hypoxic brain injury and apoptosis (41). However, this association was no longer statistically significant after multivariate measurement error correction, and in randomized trials with durations ranging from approximately 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 ω3-PUFA may have partially differing, complementary effects on pathways of cardiovascular risk (10).

Table 4. **Estimated Years of Remaining Life Gained After Age 65 y Among Representative Older Adults, by Plasma Phospholipid Total ω3-PUFA Level**

Characteristics	Remaining Life Gained (95% CI), y*
Female, white, less than high school education	2.22 (0.75–3.13)†
Male, white, less than high school education	2.33 (1.01–3.40)
Male, white, college-educated	2.33 (0.95–3.33)
Male, nonwhite, college-educated	2.31 (0.88–3.24)
Male, nonwhite, college-educated, diabetic	2.33 (1.01–3.52)
Male, nonwhite, college-educated, diabetic, current smoker	2.20 (0.92–3.30)

PUFA = polyunsaturated fatty acid.
* Values are the multivariate-adjusted estimated years of life gained after age 65 y in the highest quintile of total ω3-PUFAs compared with the lowest quintile and are based on semiparametric survival models (see **Table 3**).
† This result is representative of a participant entering the study at age 65 y with average (mean) values for each of the continuous covariates (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 (sex [female], race [white], education [less than high school], enrollment site [Forsyth County, North Carolina], fatty acid measurement batch [2007–2010], smoking status [never], prevalent diabetes [no], prevalent atrial fibrillation [no], prevalent drug-treated hypertension [no], and alcohol use [none]).

Our findings do not support major effects of circulating ω3-PUFA levels on mortality from non-CVD conditions later in life. Evidence for effects of ω3-PUFA on risk for cancer, 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 level with cancer mortality warrants further study; higher DPA levels could have independent beneficial effects or be a marker for healthier underlying physiology and metabolism. The observed lower risk for death from infection was unexpected but supported by protective effects against infection in animal studies (45, 46) and beneficial effects in some but not all trials of ω3-PUFAs in severe acute lung injury (47–49). Our results support the need for evaluation of ω3-PUFAs in less severe infections, such as community-acquired pneumonia, in older adults. Because of the 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 ω3-PUFA consumption and total mortality in generally healthy populations (7–9). Most found only non–statistically significant inverse trends and may have been limited by smaller numbers of events or reliance on self-reported diet. Several but not all prior reports (50) found inverse associations of ω3-PUFA biomarkers with total mortality in patients with CHD (51–53) and in hospitalized patients (54); associations with cause-specific mortality were generally not re-

ported. To our knowledge, no prior study has evaluated how objectively measured ω3-PUFA biomarkers relate to total mortality in generally healthy populations, such as ours.

Four large randomized trials among patients who either have or are at high risk for CVD showed that fish consumption or fish oil supplementation reduced coronary events (3). However, more recent trials have not confirmed these findings (3). A meta-analysis found that ω3-PUFA supplementation reduced cardiac death (relative risk, 0.91 [CI, 0.85 to 0.98]) but not all-cause mortality (relative risk, 0.96 [CI, 0.91 to 1.02]) (3). Such meta-analyses have not accounted for differences in background dietary fish consumption or potential nonlinear effects of supplemental ω3-PUFAs. Our dose–response analysis of diet and circulating levels suggests that dietary or supplemental ω3-PUFAs may be most beneficial for persons with little or no consumption.

No controlled trials have reported effects of ω3-PUFAs on total mortality in generally healthy populations; 1 primary prevention trial is enrolling (55). Adding a supplement to background consumption of approximately 150 mg of EPA plus DHA per day (the approximate mean consumption in the United States and many European countries) would be calculated, on the basis of nonlinear effects in observational studies (5), to produce a reduction in CHD mortality of approximately 15% (CI, 8% to 21%). Such a modest effect, while clinically revelant, may be challenging to detect in new trials. In comparison, increasing from a baseline of low intake to at least moderate consumption (>250 mg/d)—or similarly, as in our present investigation, comparing low and high circulating levels—would be predicted to have larger benefits, consistent with our observations. In our analysis, we evaluated biomarkers of ω3-PUFAs, measured late in life, that would be generally derived from dietary seafood and perhaps partly from endogenous metabolism (for example, 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 demographic characteristics, risk factors, and lifestyle was prospectively collected in a well-established multicenter cohort with little loss to follow-up. We adjusted for multiple covariates, thus 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. In contrast to randomized trials, our investigation allowed evaluation of generally healthy adults, a larger number of mortality and CVD events, ω3-PUFA exposures related to usual dietary habits rather than supplements, different ω-3 fatty acids separately, and a wide range of dose-response (very low to high).

Our study also has limitations. Fatty acid levels were measured at baseline, and dietary and metabolic fluctuations over time would increase exposure misclassification during follow-up, thus causing underestimation of true relationships with mortality. Although events were centrally adjudicated, some deaths may have been misclassified; such errors would probably be random with respect to baseline circulating ω3-PUFA levels, again causing attenuation of true relationships. The 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 end point. The observational design precludes the exclusion of 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 ω3-PUFA levels are linked to lower total mortality among generally healthy adults later in life, with the potentially greatest associations with cardiovascular events and, especially, arrhythmic cardiac death.

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Reproducible Research Statement: *Study protocol:* Available from Dr. Mozaffarian (e-mail, dmozaffa@hsph.harvard.edu). *Statistical code:* Not available. *Data set:* Not available from the authors. Interested readers can

review the CHS procedures for outside investigators to obtain and analyze data (www.chs-nhlbi.org/CHS_DistribPolicy.htm).

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Appendix: Supplementary Materials

Study Measures

We measured fatty acids in 3941 of the 5565 living cohort participants who had blood samples taken at the 1992–1993 study visit, which we considered the baseline for this analysis. Blood was drawn after a 12-hour fast, stored at −70 °C, and shipped on dry ice for long-term storage at −80 °C. The remaining 1624 participants did not originally provide blood samples or had insufficient stored blood available. Fatty acid was measured in 2 periods: 3441 measurements were taken in 2007–2010, and 500 measurements were taken in 1994–1996 as part of a prior nested case–control study of incident MI (56). All measurements were done by the same laboratory using identical techniques (see next section). We confirmed minimal laboratory drift by taking repeated measurements in 1994–1996 and 2007 on the same 1992–1993 blood samples in a subset of 163 participants. Intraclass correlations were excellent: 0.91 for EPA, 0.92 for DPA, and 0.92 for DHA. For the present analysis, we excluded 1113 participants with prevalent CVD (MI, angina, coronary revascularization, stroke, transient ischemic attack, or heart failure) and 136 participants receiving fish oil supplements at the time of blood sampling, resulting in 2692 participants included in this analysis. All measurements of fatty acids, other risk factors, and clinical end points were assessed similarly in all participants on the basis of the 1992–1993 visit, except for dietary habits that were assessed in 1989–1990 at the time of original enrollment (see Bias Due to Measurement Error in Exposure and Covariates).

Fatty Acid Measurements

Plasma phospholipid fatty acids were measured using previously described methods (11). Total lipids were extracted from plasma by using the Folch method, and phospholipids were separated from neutral lipids by 1-dimensional thin-layer chromatography. Fatty acid methyl esters were prepared by direct transesterification and separated using gas chromatography to quantify 45 distinct fatty acid peaks. Identification, precision, and accuracy were continuously evaluated using both model mixtures of known fatty acid methyl esters and established in-house control pools, with identification confirmed by gas chromatography–mass spectrometry at the U.S. Department of Agriculture in Peoria, Illinois. Interassay coefficients of variation were 2.1%, 1.5%, and 1.6% for EPA, DPA, and DHA, respectively.

Bias Due to Measurement Error in Exposure and Covariates

For a given individual, circulating levels of EPA, DPA, and DHA vary over time because of dietary changes and normal biological fluctuations, as would be expected for any physiologic measure or biomarker, such as ω3-PUFA level, blood pressure, or blood cholesterol level. When future risk is based on a single measurement of any of these factors at baseline, this normal variation over time causes the risk estimate to be attenuated toward the null (regression dilution bias) (27–29). To evaluate the potential for regression dilution bias due to changes in exposure over time, we measured serial fatty acid in blood samples collected at baseline (1992–1993), 6 years of follow-up (1998–1999), and 13 years of follow-up (2005–2006) in a subset of 100 CHS participants. Within-person correlations of fatty acid levels from baseline to 13 years were 0.50 for EPA, 0.52 for DPA, 0.60 for DHA, and 0.60 for total ω3-PUFAs. These correlations were similar or superior to those over similar periods for widely used epidemiologic measures, such as blood pressure and cholesterol level (27, 28). Thus, our single baseline measure provided a reasonable but not perfect estimate of long-term ω3-PUFA levels in this cohort. In these CHS participants, the median consumption of fish (excluding fried fish sandwiches) in 1989–1990 was 1.54 servings/wk (interquartile range, 0.60 to 1.96 servings/wk). Fish consumption was reassessed in 1996–1997, at which time median consumption was 1.38 servings/wk (interquartile range, 0.54 to 2.04 servings/wk). Thus, there was little evidence that average fish consumption systematically changed during this period.

We performed sensitivity analyses in which we adjusted for regression dilution bias in ω3-PUFA levels (27–29). When only accounting for random within-person variation in exposure over time, such methods correct the risk estimate, widen the CI, and leave the statistical significance (*P* value) unchanged. However, random within-person variation in covariates can also occur over time, which could increase residual confounding and cause bias in unpredictable directions. Therefore, we also performed multivariate measurement error correction for major time-varying covariates, including dietary habits, physical activity, and alcohol use, that can alter the risk estimate, CI, and statistical significance by reducing residual confounding. We first derived the within-person variance–covariance matrix (30) by using repeatedly measured exposures and covariates in subsets of CHS participants, including EPA, DPA, and DHA levels (serial measurements at

baseline, 6 years, and 13 years); physical activity (baseline, 3 years, and 6 years); alcohol use (baseline, 3 years, and 6 years); and dietary habits (baseline and 7 years, plus an imputed third serial measurement), including total energy intake and consumption of fish, red meats, fruits, vegetables, and dietary fiber. Each variable was transformed to approximate normality. Cox proportional hazards models were used to estimate the corrected regression coefficient (β^*) and its variance–covariance matrix, including the corrected SE (SE*), for each exposure–disease association. The coefficients were corrected for both regression dilution bias and measurement error in covariates according to the methods of Rosner and colleagues (30). For parsimony, we used a simplified covariance matrix that did not include additional terms representing uncertainty in the within-person correlations of serial measurements over time after first confirming little influence of accounting for such uncertainty on the final corrected risk estimates and their 95% CIs. These methods provide multivariate measurement error correction for continuous risk estimates.

Although multivariate methods exist for misclassification error correction for multiple-category exposures (57, 58) (for example, fatty acid quintiles evaluated as indicators), extensions of existing methods and, more important, statistical software that take into account the complexity of these data are currently unavailable. Consequently, to obtain an approximate sensitivity analysis on the extent to which bias due to covariate misclassification in this setting might have affected the relative risk estimates in each quintile of fatty acid level, we first calculated the ratio of the observed and corrected continuous risk estimates [β/β^*] for each main exposure of interest. This multivariate-corrected continuous regression dilution ratio was used to correct each categorical risk estimate coefficient by using methods corresponding to those for univariate regression dilution ratios (27–29). We similarly used the ratio of the continuous coefficients of variation [(SE/β) / (SE*/β^*)] to approximate a correction of the SE and corresponding 95% CI for each categorical risk estimate, as well as to estimate the corrected P value for trend across quintiles by correcting the original Z score for trend by this same ratio.

Both univariate and multivariate corrections are valid when the association between exposure and outcome is linear. We evaluated evidence for nonlinearity of each association by testing nonlinear terms in restricted cubic spline models. There was little evidence for nonlinearity, except for borderline evidence for nonlinear relations of total ω3-PUFA level with arrhythmic death (P for nonlinearity = 0.041) and of DPA level with stroke mortality (P for nonlinearity = 0.038) and incident stroke (P for nonlinearity = 0.047). Thus, measurement error–corrected results for these associations should be interpreted cautiously. In addition, the multivariate measurement error correction methods were proposed for use in Cox regression models under the assumption of rare events (<5% risk during follow-up), partly because measures of reproducibility are subject to survivor bias when events are not rare. We confirmed that findings were similar in analyses restricted to participants younger than the median age at baseline (74 years) in whom the rare-disease assumption was met (data not shown).

Finally, we performed analyses limited to the midpoint of follow-up (8 years) to minimize misclassification in exposures and covariates with longer durations of follow-up.

Determining Years of Life Lost or Gained

We estimated the absolute years of remaining life gained or lost according to quintile of ω3-PUFA exposure by using both semiparametric and parametric approaches. We used the semiparametric multivariate-adjusted Cox proportional hazards model to generate left-truncated survival estimates (24) for a typical participant, based on average levels of all covariates starting at age 65 years. Life expectancies for each quintile were then calculated between ages 65 and 100 years using estimated age-specific survival probabilities, with 95% CIs determined by bootstrapping with 200 replicates (25). These results were compared with those obtained using the parametric accelerated failure time model (26), which evaluated several parametric distributions, including log-normal, log-logistic, Weibull, and γ distributions. Most of these parameters fit the observed data well on the basis of minimization of the −2 log likelihood, and findings for differences in estimated life expectancy obtained by using well-fit parametric distributions did not materially differ from the semiparametric results. The multivariate-adjusted results can be considered representative of a person aged 65 years at baseline who has the mean value for each of the continuous covariates and the mode (the most common value) for each of the categorical covariates in the model. We also calculated differences in life-years for other representative individuals, such as nonwhite persons, current smokers, and persons with diabetes.

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Appendix Table 1. **Baseline Characteristics, by Plasma Phospholipid EPA, DPA, and DHA Levels**

Characteristic	EPA Quintile				
	I	II	III	IV	V
Mean proportion of total fatty acids (SD)	0.29 (0.06)	0.41 (0.03)	0.52 (0.03)	0.64 (0.04)	1.08 (0.54)
Median proportion of total fatty acids (range)	0.30 (0.11–0.36)	0.41 (0.36–0.46)	0.51 (0.46–0.57)	0.64 (0.57–0.73)	0.92 (0.73–8.52)
Participants, *n*	539	539	539	537	538
Mean age (SD), *y*	74.6 (5.5)	74.3 (5.2)	74.0 (5.2)	73.4 (4.8)	73.5 (4.7)*
Male, %	43	40	33	31	34*
White, %	88	89	87	86	89
Education beyond high school, %	39	43	44	50	56*
Current smoker, %	12	11	9	7	9
Diabetes mellitus, %	13	16	13	12	11
Atrial fibrillation, %	5	4	5	4	5
Treated hypertension, %	37	41	40	42	38
Mean BMI (SD), kg/m^2	26.1 (4.6)	26.5 (4.7)	27.1 (4.7)	27.1 (4.5)	26.5 (4.4)
Mean waist circumference (SD), *cm*	96.1 (13)	96.7 (13)	97.7 (14)	97.9 (13)	95.6 (13)
Mean alcohol consumption (SD), *drinks/wk*	0.9 (2.9)	1.6 (3.9)	1.8 (4.6)	2.9 (11)	3.7 (6.8)*
Mean physical activity (SD), *mcal/wk*	0.9 (1.3)	1.2 (1.6)	1.1 (1.5)	1.0 (1.3)	1.2 (1.5)
Mean consumption of tuna and other broiled/baked fish (SD), *servings/wk*	1.2 (1.2)	1.5 (1.3)	1.6 (1.4)	1.7 (1.3)	2.1 (1.4)*
Mean fried fish consumption (SD), *servings/wk*	0.5 (0.7)	0.5 (0.7)	0.5 (0.6)	0.5 (0.5)	0.5 (0.6)
Mean red meat consumption (SD), *servings/wk*	2.9 (2.4)	3.0 (2.5)	2.6 (2.2)	2.7 (2.3)	2.5 (2.1)*
Mean fruit consumption (SD), *servings/d*	2.0 (1.0)	2.0 (1.1)	2.1 (1.0)	2.2 (1.0)	2.3 (1.1)*
Mean vegetable consumption (SD), *servings/d*	2.3 (1.2)	2.4 (1.2)	2.5 (1.3)	2.6 (1.2)	2.7 (1.3)*
Mean dietary fiber consumption (SD), *g/d*	29.1 (12)	29.6 (12)	29.2 (12)	30.0 (12)	30.4 (12)

Appendix Table 1—**Continued**

DPA Quintile				
I	II	III	IV	V
0.61 (0.07)	0.74 (0.02)	0.82 (0.02)	0.91 (0.03)	1.08 (0.11)
0.63 (0.11–0.70)	0.75 (0.70–0.78)	0.82 (0.78–0.87)	0.91 (0.87–0.96)	1.04 (0.96–1.63)
542	535	545	537	533
73.5 (4.9)	73.8 (5.2)	74.3 (5.4)	74.3 (5.0)	73.8 (5.0)
34	37	38	34	38
89	87	87	88	87
43	49	45	45	49
12	10	9	9	7*
14	16	11	12	11
5	4	4	5	4
41	41	38	39	39
26.5 (4.9)	27.6 (4.8)	26.5 (4.6)	26.6 (4.4)	26.2 (4.2)
96.0 (14)	98.9 (14)	96.8 (13)	96.7 (13)	95.7 (12)
2.0 (4.6)	2.1 (4.9)	2.3 (5.1)	2.2 (5.3)	2.4 (11)
1.0 (1.5)	1.1 (1.5)	1.1 (1.5)	1.1 (1.4)	1.1 (1.3)
1.5 (1.3)	1.6 (1.4)	1.6 (1.4)	1.6 (1.3)	1.7 (1.4)
0.6 (0.7)	0.5 (0.7)	0.5 (0.6)	0.5 (0.5)	0.5 (0.6)*
2.7 (2.3)	2.7 (2.1)	2.9 (2.5)	2.7 (2.5)	2.7 (2.2)
2.1 (1.0)	2.1 (1.0)	2.1 (1.0)	2.1 (1.1)	2.2 (1.0)
2.4 (1.2)	2.6 (1.3)	2.5 (1.2)	2.4 (1.2)	2.6 (1.3)
28.8 (12)	30.3 (13)	29.2 (12)	29.4 (12)	30.8 (12)

Appendix Table 1—**Continued**

DHA Quintile				
I	II	III	IV	V
1.90 (0.24)	2.44 (0.12)	2.87 (0.12)	3.38 (0.19)	4.54 (0.73)
1.95 (1.07–2.22)	2.44 (2.22–2.66)	2.87 (2.66–3.09)	3.36 (3.09–3.75)	4.34 (3.76–8.17)
540	537	539	539	537
74.0 (5.3)	73.7 (5.0)	74.1 (5.0)	73.9 (5.1)	74.0 (5.1)
40	36	35	37	33
95	94	90	83	77*
39	45	43	49	55*
14	9	7	8	8*
12	12	14	14	12
4	6	4	4	5
36	36	41	45	40
26.1 (4.5)	26.9 (4.4)	26.9 (4.5)	26.9 (4.7)	26.6 (4.7)
96.1 (13)	97.6 (13)	97.6 (13)	97.0 (13)	95.7 (14)
2.5 (6.0)	2.3 (11)	1.9 (4.5)	2.1 (4.9)	2.0 (4.8)
1.1 (1.5)	1.0 (1.4)	1.1 (1.5)	1.0 (1.4)	1.1 (1.4)
1.0 (1.1)	1.3 (1.2)	1.5 (1.3)	1.8 (1.4)	2.3 (1.5)*
0.4 (0.5)	0.5 (0.7)	0.5 (0.6)	0.5 (0.6)	0.5 (0.6)
3.1 (2.6)	2.9 (2.3)	2.9 (2.4)	2.6 (2.2)	2.3 (2.0)*
2.0 (1.0)	2.0 (1.0)	2.2 (1.1)	2.1 (1.0)	2.3 (1.0)*
2.3 (1.2)	2.5 (1.3)	2.4 (1.2)	2.5 (1.3)	2.7 (1.2)*
29.3 (13)	29.0 (12)	29.8 (12)	29.8 (12)	30.5 (12)

BMI = body mass index; DHA = docosahexaenoic acid; DPA = docosapentaenoic acid; EPA = eicosapentaenoic acid.
* P for trend < 0.01 across quintiles in linear regression for continuous variables and logistic regression for binary variables.

Appendix Table 2. **Prospective Association of Plasma Phospholipid EPA, DPA, and DHA Levels With Noncardiovascular Mortality**

Variable	Hazard Ratio (95% CI), by Quintile of Phospholipid FA Level*					*P* Value for Trend
	I	II	III	IV	V	
Cancer (356 deaths)						
EPA	1.00 (reference)	1.19 (0.86–1.65)	0.81 (0.57–1.16)	0.89 (0.63–1.27)	1.16 (0.82–1.64)	0.61
DPA	1.00 (reference)	0.88 (0.64–1.20)	0.73 (0.52–1.01)	0.78 (0.56–1.08)	0.70 (0.50–0.98)	0.032
DHA	1.00 (reference)	1.02 (0.73–1.42)	1.12 (0.80–1.56)	1.08 (0.76–1.54)	1.33 (0.93–1.90)	0.104
Total ω3-PUFAs	1.00 (reference)	0.86 (0.62–1.20)	0.94 (0.68–1.32)	0.98 (0.70–1.37)	1.04 (0.73–1.48)	0.60
Dementia (247 deaths)						
EPA	1.00 (reference)	1.04 (0.70–1.54)	1.21 (0.81–1.78)	0.95 (0.63–1.44)	0.87 (0.57–1.33)	0.38
DPA	1.00 (reference)	0.81 (0.53–1.24)	0.89 (0.59–1.36)	1.11 (0.75–1.64)	0.95 (0.63–1.43)	0.72
DHA	1.00 (reference)	0.92 (0.61–1.38)	0.90 (0.61–1.33)	0.87 (0.57–1.31)	0.72 (0.47–1.12)	0.144
Total ω3-PUFAs	1.00 (reference)	1.23 (0.82–1.85)	1.07 (0.70–1.63)	1.00 (0.65–1.55)	0.88 (0.56–1.38)	0.27
Infection (111 deaths)†						
EPA	1.00 (reference)	1.11 (0.65–1.90)	0.89 (0.50–1.58)	0.61 (0.32–1.16)	0.64 (0.33–1.24)	0.065
DPA	1.00 (reference)	0.58 (0.32–1.06)	0.69 (0.39–1.22)	0.58 (0.32–1.05)	0.66 (0.37–1.17)	0.20
DHA	1.00 (reference)	0.79 (0.45–1.40)	0.67 (0.37–1.19)	0.72 (0.39–1.30)	0.53 (0.27–1.02)	0.070
Total ω3-PUFAs	1.00 (reference)	0.55 (0.30–0.99)	0.68 (0.39–1.19)	0.74 (0.42–1.30)	0.33 (0.16–0.67)	0.010
Respiratory (94 deaths)‡						
EPA	1.00 (reference)	1.27 (0.71–2.24)	1.04 (0.57–1.91)	0.54 (0.25–1.19)	0.97 (0.48–1.97)	0.48
DPA	1.00 (reference)	0.35 (0.17–0.71)	0.58 (0.32–1.04)	0.69 (0.38–1.23)	0.55 (0.29–1.05)	0.20
DHA	1.00 (reference)	1.12 (0.60–2.10)	1.20 (0.64–2.25)	1.48 (0.78–2.80)	0.83 (0.36–1.92)	0.99
Total ω3-PUFAs	1.00 (reference)	0.77 (0.42–1.41)	0.79 (0.42–1.49)	1.34 (0.72–2.47)	0.66 (0.29–1.51)	0.75
Trauma/fracture (84 deaths)						
EPA	1.00 (reference)	0.66 (0.35–1.25)	0.67 (0.34–1.30)	0.67 (0.33–1.35)	0.74 (0.37–1.47)	0.48
DPA	1.00 (reference)	0.83 (0.42–1.65)	1.38 (0.75–2.52)	0.56 (0.26–1.23)	0.78 (0.38–1.60)	0.35
DHA	1.00 (reference)	0.97 (0.51–1.84)	0.88 (0.47–1.68)	0.95 (0.48–1.87)	0.66 (0.29–1.47)	0.34
Total ω3-PUFAs	1.00 (reference)	0.94 (0.50–1.77)	1.03 (0.54–1.95)	0.90 (0.45–1.80)	0.65 (0.29–1.46)	0.32
Other (160 deaths)§						
EPA	1.00 (reference)	0.77 (0.48–1.25)	0.77 (0.47–1.25)	0.79 (0.49–1.29)	0.78 (0.47–1.31)	0.48
DPA	1.00 (reference)	1.25 (0.73–2.14)	1.12 (0.65–1.94)	1.24 (0.72–2.13)	1.48 (0.88–2.50)	0.163
DHA	1.00 (reference)	0.81 (0.50–1.31)	0.72 (0.44–1.17)	0.60 (0.36–1.02)	0.73 (0.44–1.21)	0.21
Total ω3-PUFAs	1.00 (reference)	0.91 (0.57–1.46)	0.57 (0.33–0.97)	0.79 (0.48–1.29)	0.67 (0.40–1.13)	0.139

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

* Hazard ratios and 95% CIs were adjusted for the covariates in the multivariate- and diet-adjusted model in **Table 1**: age (years); sex; race (white or nonwhite); education (less than high school, high school, some college, or college graduate); enrollment site (4 sites); FA measurement batch (1994–1996 or 2007–2010); smoking status (never, former, or current); prevalent diabetes (yes or no); prevalent atrial fibrillation (yes or no); prevalent drug-treated hypertension (yes or no); leisure-time physical activity (mcal/wk); body mass index (kg/m^2); waist circumference (cm); alcohol use (6 categories); and consumption of tuna or other broiled or baked fish (servings/wk), fried fish (servings/wk), red meat (servings/wk), fruits (servings/d), vegetables (servings/d), and dietary fiber (g/d).

† Includes deaths due to pneumonia, sepsis, and other infection.

‡ Includes deaths due to chronic pulmonary diseases.

§ Includes deaths due to liver disease, gastrointestinal disease, renal failure, amyotrophic lateral sclerosis, Parkinson disease, bladder disease, metabolic conditions, amyloid, failure to thrive, the myelodysplastic syndrome, and other musculoskeletal diseases.

Appendix Table 3. **Prospective Association of Plasma Phospholipid EPA, DPA, and DHA Levels With Total Mortality, Cardiovascular Mortality, and Incident Cardiovascular Diseases, With Adjustment for Regression Dilution Bias in EPA, DPA, and DHA Levels***

Variable	Hazard Ratio (95% CI), by Quintile of Phospholipid FA Level					*P* Value for Trend
	I	II	III	IV	V	
Total mortality (1625 deaths)						
EPA	1.00 (reference)	1.00 (0.74–1.35)	0.77 (0.56–1.04)	0.64 (0.46–0.88)	0.69 (0.50–0.96)	0.005
DPA	1.00 (reference)	0.60 (0.45–0.82)	0.68 (0.52–0.92)	0.70 (0.52–0.94)	0.60 (0.45–0.82)	0.008
DHA	1.00 (reference)	0.98 (0.76–1.26)	0.93 (0.72–1.19)	0.87 (0.66–1.14)	0.69 (0.51–0.90)	0.006
Total ω3-PUFAs	1.00 (reference)	0.85 (0.66–1.08)	0.90 (0.69–1.15)	0.79 (0.61–1.03)	0.59 (0.44–0.78)	<0.001
Total cardiovascular mortality (570 deaths)						
EPA	1.00 (reference)	1.02 (0.62–1.69)	0.76 (0.45–1.30)	0.66 (0.38–1.12)	0.52 (0.29–0.92)	0.009
DPA	1.00 (reference)	0.55 (0.33–0.91)	0.68 (0.41–1.12)	0.65 (0.40–1.06)	0.48 (0.28–0.80)	0.021
DHA	1.00 (reference)	1.15 (0.75–1.77)	1.02 (0.66–1.55)	0.87 (0.55–1.36)	0.50 (0.30–0.82)	0.002
Total ω3-PUFAs	1.00 (reference)	0.87 (0.57–1.34)	1.08 (0.72–1.65)	0.61 (0.38–0.97)	0.49 (0.29–0.79)	<0.001
CHD mortality (359 deaths)						
EPA	1.00 (reference)	0.96 (0.50–1.85)	0.88 (0.46–1.72)	0.81 (0.41–1.59)	0.59 (0.29–1.23)	0.121
DPA	1.00 (reference)	0.49 (0.25–0.94)	0.98 (0.53–1.83)	0.68 (0.36–1.31)	0.64 (0.33–1.22)	0.36
DHA	1.00 (reference)	0.97 (0.57–1.67)	0.93 (0.54–1.59)	0.65 (0.37–1.14)	0.43 (0.23–0.79)	0.003
Total ω3-PUFAs	1.00 (reference)	0.81 (0.48–1.39)	1.05 (0.62–1.77)	0.45 (0.24–0.82)	0.43 (0.24–0.79)	0.002
Arrhythmic CHD mortality (194 deaths)†						
EPA	1.00 (reference)	0.92 (0.38–2.25)	0.69 (0.28–1.72)	0.67 (0.27–1.66)	0.58 (0.22–1.51)	0.22
DPA	1.00 (reference)	0.64 (0.25–1.58)	1.71 (0.73–3.94)	0.70 (0.28–1.76)	0.64 (0.25–1.66)	0.39
DHA	1.00 (reference)	0.95 (0.45–1.99)	0.76 (0.36–1.61)	0.87 (0.42–1.84)	0.37 (0.16–0.89)	0.028
Total ω3-PUFAs	1.00 (reference)	0.68 (0.31–1.43)	1.12 (0.55–2.26)	0.53 (0.24–1.17)	0.34 (0.14–0.78)	0.008
Nonarrhythmic CHD mortality (165 deaths)†						
EPA	1.00 (reference)	1.06 (0.40–2.86)	1.23 (0.46–3.24)	1.00 (0.37–2.72)	0.64 (0.21–1.90)	0.34
DPA	1.00 (reference)	0.37 (0.15–0.98)	0.50 (0.20–1.31)	0.67 (0.26–1.66)	0.65 (0.25–1.66)	0.70
DHA	1.00 (reference)	0.98 (0.45–2.17)	1.14 (0.53–2.42)	0.42 (0.17–1.02)	0.49 (0.19–1.21)	0.038
Total ω3-PUFAs	1.00 (reference)	1.00 (0.46–2.17)	0.95 (0.44–2.10)	0.36 (0.14–0.92)	0.58 (0.24–1.39)	0.074
Stroke mortality (130 deaths)						
EPA	1.00 (reference)	1.10 (0.40–3.06)	0.59 (0.19–1.80)	0.45 (0.14–1.46)	0.71 (0.22–2.19)	0.34
DPA	1.00 (reference)	0.33 (0.12–0.92)	0.34 (0.12–0.92)	0.48 (0.18–1.26)	0.29 (0.11–0.85)	0.056
DHA	1.00 (reference)	1.55 (0.63–3.78)	1.24 (0.50–3.07)	1.02 (0.39–2.61)	0.45 (0.15–1.36)	0.082
Total ω3-PUFAs	1.00 (reference)	0.87 (0.35–2.14)	1.19 (0.50–2.86)	0.75 (0.29–1.92)	0.43 (0.15–1.21)	0.092
Total fatal and nonfatal CHD (630 cases)‡						
EPA	1.00 (reference)	1.08 (0.67–1.80)	0.83 (0.50–1.39)	0.96 (0.58–1.59)	0.58 (0.34–1.00)	0.032
DPA	1.00 (reference)	0.53 (0.33–0.87)	0.78 (0.49–1.26)	0.68 (0.42–1.10)	0.68 (0.41–1.10)	0.28
DHA	1.00 (reference)	0.90 (0.59–1.36)	1.10 (0.73–1.65)	0.73 (0.48–1.14)	0.58 (0.37–0.92)	0.010
Total ω3-PUFAs	1.00 (reference)	0.81 (0.54–1.23)	1.10 (0.73–1.65)	0.61 (0.39–0.93)	0.58 (0.37–0.92)	0.009
Nonfatal myocardial infarction (371 cases)						
EPA	1.00 (reference)	1.30 (0.69–2.46)	0.71 (0.36–1.42)	1.02 (0.53–1.99)	0.52 (0.26–1.08)	0.038
DPA	1.00 (reference)	0.60 (0.33–1.14)	0.67 (0.36–1.26)	0.58 (0.31–1.10)	0.75 (0.41–1.40)	0.44
DHA	1.00 (reference)	0.75 (0.43–1.30)	1.08 (0.63–1.84)	0.87 (0.50–1.51)	0.68 (0.38–1.23)	0.28
Total ω3-PUFAs	1.00 (reference)	0.72 (0.42–1.26)	1.07 (0.63–1.82)	0.66 (0.38–1.17)	0.73 (0.42–1.32)	0.32
Total fatal and nonfatal stroke (406 cases)§						
EPA	1.00 (reference)	1.02 (0.55–1.88)	0.90 (0.48–1.66)	0.83 (0.44–1.56)	1.10 (0.58–2.10)	0.85
DPA	1.00 (reference)	0.52 (0.29–0.94)	0.50 (0.28–0.91)	0.73 (0.42–1.31)	0.56 (0.32–1.02)	0.180
DHA	1.00 (reference)	1.14 (0.69–1.88)	1.14 (0.69–1.86)	0.61 (0.35–1.05)	0.75 (0.42–1.32)	0.092
Total ω3-PUFAs	1.00 (reference)	0.95 (0.58–1.59)	0.85 (0.51–1.41)	0.89 (0.53–1.51)	0.62 (0.35–1.10)	0.098
Ischemic stroke (319 cases)						
EPA	1.00 (reference)	0.98 (0.49–1.99)	0.88 (0.44–1.80)	0.69 (0.34–1.44)	1.19 (0.58–2.46)	0.74
DPA	1.00 (reference)	0.60 (0.32–1.16)	0.55 (0.28–1.08)	0.62 (0.33–1.20)	0.62 (0.32–1.20)	0.22
DHA	1.00 (reference)	1.02 (0.58–1.77)	1.00 (0.58–1.75)	0.59 (0.33–1.10)	0.61 (0.31–1.17)	0.052
Total ω3-PUFAs	1.00 (reference)	0.81 (0.46–1.41)	0.65 (0.36–1.14)	0.89 (0.50–1.57)	0.46 (0.24–0.90)	0.043

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Appendix Table 3—**Continued**

Variable	Hazard Ratio (95% CI), by Quintile of Phospholipid FA Level					*P* Value for Trend
	I	II	III	IV	V	
Hemorrhagic stroke (65 cases)						
EPA	1.00 (reference)	1.30 (0.31–5.38)	1.00 (0.22–4.58)	0.81 (0.17–3.96)	0.49 (0.09–2.79)	0.32
DPA	1.00 (reference)	0.35 (0.09–1.49)	0.12 (0.02–0.65)	0.58 (0.15–2.21)	0.45 (0.11–1.78)	0.39
DHA	1.00 (reference)	1.77 (0.48–6.56)	2.21 (0.62–7.92)	0.46 (0.09–2.33)	1.43 (0.34–6.03)	0.90
Total ω3-PUFAs	1.00 (reference)	1.05 (0.26–4.15)	2.69 (0.78–9.33)	0.61 (0.13–2.86)	1.41 (0.35–5.86)	0.86

CHD = coronary heart disease; DHA = docosahexaenoic acid; DPA = docosapentaenoic acid; EPA = eicosapentaenoic acid; FA = fatty acid; PUFA = polyunsaturated fatty acid.

* Based on regression dilution ratios (within-person correlations of serial measures over 13 y) of 0.50 for EPA, 0.52 for DPA, 0.60 for DHA, and 0.60 for total ω3-PUFAs. The regression dilution ratio was used to correct the β-coefficient and SE for each risk estimate to account for underestimation of the strength of true associations due to changes in exposure over time (regression dilution bias) (27–29). Such methods correct the risk estimate, widen the CI, and leave the statistical significance (*P* value) unchanged. Hazard ratios and 95% CIs were adjusted for the covariates in the multivariate- and diet-adjusted model in **Table 1**: age (years); sex; race (white or nonwhite); education (less than high school, high school, some college, or college graduate); enrollment site (4 sites); FA measurement batch (1994–1996 or 2007–2010); smoking status (never, former, or current); prevalent diabetes (yes or no); prevalent atrial fibrillation (yes or no); prevalent drug-treated hypertension (yes or no); leisure-time physical activity (mcal/wk); body mass index (kg/m^2); waist circumference (cm); alcohol use (6 categories); and consumption of tuna or other broiled or baked fish (servings/wk), fried fish (servings/wk), red meat (servings/wk), fruits (servings/d), vegetables (servings/d), and dietary fiber (g/d).

† Subsets of CHD mortality, with adjudication on whether the underlying event was arrhythmic or nonarrhythmic.

‡ Includes 371 nonfatal myocardial infarctions and 259 CHD deaths. Analyses of incident CHD deaths included an additional 100 deaths that occurred with additional follow-up after an incident nonfatal myocardial infarction.

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

Appendix Table 4. **Prospective Association of Plasma Phospholipid EPA, DPA, and DHA Levels With Total Mortality, Cardiovascular Mortality, and Incident Cardiovascular Diseases, With Adjustment for Regression Dilution Bias in EPA, DPA, and DHA Levels and Measurement Error in Covariates, Including Dietary Habits, Physical Activity, and Alcohol Use***

Variable	Hazard Ratio (95% CI), by Quintile of Phospholipid FA Level					*P* Value for Trend
	I	II	III	IV	V	
Total mortality (1625 deaths)						
EPA	1.00 (reference)	1.00 (0.59–1.72)	0.73 (0.42–1.29)	0.59 (0.33–1.05)	0.65 (0.36–1.17)	0.068
DPA	1.00 (reference)	0.51 (0.33–0.80)	0.60 (0.38–0.94)	0.62 (0.40–0.97)	0.50 (0.32–0.79)	0.017
DHA	1.00 (reference)	0.98 (0.67–1.43)	0.93 (0.63–1.35)	0.86 (0.58–1.28)	0.67 (0.44–1.03)	0.055
Total ω3-PUFAs	1.00 (reference)	0.88 (0.66–1.16)	0.92 (0.69–1.21)	0.83 (0.62–1.11)	0.65 (0.48–0.89)	0.008
Total cardiovascular mortality (570 deaths)						
EPA	1.00 (reference)	1.03 (0.41–2.60)	0.66 (0.25–1.71)	0.52 (0.20–1.38)	0.36 (0.13–1.04)	0.025
DPA	1.00 (reference)	0.40 (0.18–0.85)	0.56 (0.26–1.18)	0.52 (0.24–1.10)	0.32 (0.15–0.72)	0.021
DHA	1.00 (reference)	1.19 (0.62–2.26)	1.01 (0.53–1.92)	0.84 (0.43–1.64)	0.43 (0.20–0.91)	0.012
Total ω3-PUFAs	1.00 (reference)	0.88 (0.55–1.40)	1.08 (0.68–1.71)	0.62 (0.38–1.04)	0.50 (0.29–0.85)	0.004
CHD mortality (359 deaths)						
EPA	1.00 (reference)	0.95 (0.28–3.14)	0.83 (0.24–2.80)	0.70 (0.20–2.42)	0.43 (0.11–1.62)	0.171
DPA	1.00 (reference)	0.32 (0.12–0.87)	0.98 (0.38–2.53)	0.55 (0.21–1.45)	0.48 (0.18–1.33)	0.34
DHA	1.00 (reference)	0.96 (0.43–2.16)	0.90 (0.41–2.01)	0.55 (0.24–1.30)	0.32 (0.12–0.81)	0.007
Total ω3-PUFAs	1.00 (reference)	0.81 (0.45–1.47)	1.05 (0.59–1.86)	0.45 (0.23–0.87)	0.43 (0.22–0.84)	0.004
Arrhythmic CHD mortality (194 deaths)†						
EPA	1.00 (reference)	0.88 (0.18–4.34)	0.52 (0.10–2.76)	0.50 (0.09–2.61)	0.38 (0.06–2.20)	0.23
DPA	1.00 (reference)	0.43 (0.11–1.78)	2.60 (0.71–9.48)	0.53 (0.13–2.16)	0.45 (0.11–1.91)	0.31
DHA	1.00 (reference)	0.93 (0.31–2.81)	0.66 (0.22–2.04)	0.81 (0.27–2.48)	0.24 (0.07–0.87)	0.032
Total ω3-PUFAs	1.00 (reference)	0.65 (0.29–1.49)	1.13 (0.53–2.43)	0.50 (0.21–1.20)	0.31 (0.12–0.78)	0.009
Nonarrhythmic CHD mortality (165 deaths)†						
EPA	1.00 (reference)	1.09 (0.18–6.71)	1.31 (0.22–7.93)	1.00 (0.16–6.38)	0.54 (0.07–4.11)	0.49
DPA	1.00 (reference)	0.30 (0.07–1.26)	0.43 (0.10–1.79)	0.60 (0.15–2.37)	0.59 (0.14–2.40)	0.75
DHA	1.00 (reference)	0.99 (0.30–3.23)	1.17 (0.37–3.69)	0.33 (0.09–1.29)	0.40 (0.10–1.60)	0.088
Total ω3-PUFAs	1.00 (reference)	1.00 (0.43–2.35)	0.96 (0.40–2.28)	0.39 (0.14–1.10)	0.60 (0.22–1.59)	0.134
Stroke mortality (130 deaths)						
EPA	1.00 (reference)	1.12 (0.17–7.20)	0.55 (0.07–4.16)	0.41 (0.05–3.37)	0.66 (0.08–5.28)	0.55
DPA	1.00 (reference)	0.21 (0.04–1.03)	0.22 (0.05–1.05)	0.36 (0.08–1.58)	0.19 (0.04–0.95)	0.083
DHA	1.00 (reference)	1.46 (0.39–5.49)	1.21 (0.32–4.61)	1.02 (0.25–4.13)	0.50 (0.10–2.58)	0.31
Total ω3-PUFAs	1.00 (reference)	0.89 (0.33–2.36)	1.16 (0.45–2.99)	0.79 (0.28–2.20)	0.49 (0.16–1.54)	0.199
Total fatal and nonfatal CHD (630 cases)‡						
EPA	1.00 (reference)	1.17 (0.47–2.88)	0.73 (0.29–1.86)	0.93 (0.37–2.33)	0.39 (0.14–1.05)	0.040
DPA	1.00 (reference)	0.30 (0.14–0.63)	0.63 (0.30–1.29)	0.47 (0.23–0.98)	0.47 (0.22–0.98)	0.169
DHA	1.00 (reference)	0.86 (0.47–1.60)	1.15 (0.63–2.10)	0.65 (0.34–1.23)	0.46 (0.23–0.91)	0.015
Total ω3-PUFAs	1.00 (reference)	0.80 (0.50–1.26)	1.11 (0.71–1.74)	0.59 (0.36–0.95)	0.56 (0.34–0.94)	0.012
Nonfatal myocardial infarction (371 cases)						
EPA	1.00 (reference)	1.67 (0.53–5.30)	0.52 (0.15–1.82)	1.06 (0.32–3.49)	0.29 (0.08–1.08)	0.030
DPA	1.00 (reference)	0.61 (0.23–1.58)	0.67 (0.26–1.74)	0.57 (0.22–1.51)	0.75 (0.29–1.91)	0.61
DHA	1.00 (reference)	0.60 (0.26–1.37)	1.16 (0.52–2.57)	0.78 (0.34–1.77)	0.50 (0.21–1.21)	0.198
Total ω3-PUFAs	1.00 (reference)	0.66 (0.36–1.23)	1.09 (0.60–1.97)	0.59 (0.31–1.12)	0.68 (0.35–1.30)	0.26
Total fatal and nonfatal stroke (406 cases)§						
EPA	1.00 (reference)	0.98 (2.97–0.33)	1.10 (3.40–0.35)	1.18 (3.74–0.37)	0.92 (2.97–0.29)	0.93
DPA	1.00 (reference)	0.53 (0.22–1.30)	0.51 (0.21–1.26)	0.74 (0.32–1.76)	0.57 (0.23–1.40)	0.39
DHA	1.00 (reference)	1.13 (0.54–2.36)	1.13 (0.54–2.34)	0.62 (0.27–1.40)	0.75 (0.32–1.74)	0.27
Total ω3-PUFAs	1.00 (reference)	0.97 (0.56–1.67)	0.89 (0.51–1.55)	0.92 (0.52–1.63)	0.71 (0.38–1.33)	0.28
Ischemic stroke (319 cases)						
EPA	1.00 (reference)	–‖	–‖	–‖	–‖	–‖
DPA	1.00 (reference)	0.63 (0.23–1.69)	0.58 (0.21–1.59)	0.65 (0.24–1.77)	0.65 (0.24–1.77)	0.46
DHA	1.00 (reference)	1.01 (0.45–2.31)	1.01 (0.45–2.27)	0.60 (0.24–1.49)	0.61 (0.23–1.60)	0.194
Total ω3-PUFAs	1.00 (reference)	0.87 (0.48–1.60)	0.75 (0.40–1.40)	0.93 (0.5–1.72)	0.60 (0.30–1.24)	0.22

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Appendix Table 4—**Continued**

Variable	Hazard Ratio (95% CI), by Quintile of Phospholipid FA Level					*P* Value for Trend
	I	II	III	IV	V	
Hemorrhagic stroke (65 cases)						
EPA	1.00 (reference)	1.76 (0.13–23.4)	1.00 (0.06–15.7)	0.63 (0.04–11.1)	0.21 (0.01–4.87)	0.23
DPA	1.00 (reference)	0.47 (0.05–4.37)	0.22 (0.02–2.90)	0.67 (0.08–5.38)	0.56 (0.07–4.71)	0.68
DHA	1.00 (reference)	0.03 (0.00–0.21)	0.01 (0.00–0.05)	124 (11.3–1352)	0.11 (0.01–0.90)	0.62
Total ω3-PUFAs	1.00 (reference)	1.08 (0.24–4.85)	5.25 (1.35–20.5)	0.43 (0.08–2.34)	1.80 (0.38–8.48)	0.78

CHD = coronary heart disease; DHA = docosahexaenoic acid; DPA = docosapentaenoic acid; EPA = eicosapentaenoic acid; FA = fatty acid; PUFA = polyunsaturated fatty acid.

* Based on multivariate regression dilution correction and measurement error correction, derived from the variance–covariance error structure of repeatedly measured exposures and covariates in subsets of participants, including EPA, DPA, and DHA level (serial measures at baseline, 6 y, and 13 y); physical activity (baseline and 3 y); alcohol use (baseline and 3 y); and dietary habits (baseline and 7 y), including total energy intake and consumption of fish, red meat, fruits, vegetables, and dietary fiber. The multivariate regression dilution ratio was used to correct the β-coefficient and SE for each risk estimate to account for bias due to changes in exposures and covariates. Such methods correct the risk estimate, widen the CI, and alter the statistical significance (*P* value); see the **Appendix** for details. Hazard ratios and 95% CIs were adjusted for the covariates in the multivariate- and diet-adjusted model in **Table 1**: age (years); sex; race (white or nonwhite); education (less than high school, high school, some college, or college graduate); enrollment site (4 sites); FA measurement batch (1994–1996 or 2007–2010); smoking status (never, former, or current); prevalent diabetes (yes or no); prevalent atrial fibrillation (yes or no); prevalent drug-treated hypertension (yes or no); leisure-time physical activity (mcal/wk); body mass index (kg/m^2); waist circumference (cm); alcohol use (6 categories); and consumption of tuna or other broiled or baked fish (servings/wk), fried fish (servings/wk), red meat (servings/wk), fruits (servings/d), vegetables (servings/d), and dietary fiber (g/d).

† Subsets of CHD mortality, with adjudication on whether the underlying event was arrhythmic or nonarrhythmic.

‡ Includes 371 nonfatal myocardial infarctions and 259 CHD deaths. Analyses of incident CHD deaths included an additional 100 deaths that occurred with additional follow-up after an incident nonfatal myocardial infarction.

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

‖ The model did not converge (i.e., the multivariate regression dilution–corrected risk was not estimable).

Appendix Table 5. **Prospective Association of Plasma Phospholipid EPA, DPA, and DHA Levels With Total Mortality, CHD Mortality, and Nonfatal Myocardial Infarction, With and Without Simultaneous Adjustment for EPA, DPA, and DHA Levels**

Variable	Hazard Ratio (95% CI), by Quintile of Phospholipid FA Level*					*P* Value for Trend
	I	II	III	IV	V	
Total mortality						
EPA						
Full model	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
Full model plus DPA	1.00 (reference)	1.04 (0.89–1.21)	0.92 (0.78–1.09)	0.85 (0.71–1.01)	0.89 (0.73–1.07)	0.085
Full model plus DHA	1.00 (reference)	1.01 (0.87–1.17)	0.89 (0.76–1.04)	0.82 (0.70–0.96)	0.90 (0.75–1.08)	0.066
Full model plus DPA plus DHA	1.00 (reference)	1.05 (0.90–1.22)	0.94 (0.79–1.11)	0.87 (0.73–1.05)	0.96 (0.78–1.19)	0.44
DPA						
Full model	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
Full model plus EPA	1.00 (reference)	0.79 (0.67–0.93)	0.86 (0.73–1.02)	0.89 (0.75–1.05)	0.84 (0.70–1.01)	0.145
Full model plus DHA	1.00 (reference)	0.78 (0.67–0.92)	0.83 (0.72–0.97)	0.85 (0.73–0.99)	0.79 (0.67–0.92)	0.018
Full model plus EPA plus DHA	1.00 (reference)	0.80 (0.68–0.93)	0.86 (0.73–1.01)	0.88 (0.75–1.05)	0.83 (0.69–1.00)	0.095
DHA						
Full model	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
Full model plus EPA	1.00 (reference)	0.99 (0.85–1.15)	0.96 (0.83–1.12)	0.94 (0.80–1.11)	0.83 (0.69–1.00)	0.077
Full model plus DPA	1.00 (reference)	0.99 (0.85–1.15)	0.96 (0.82–1.12)	0.93 (0.79–1.09)	0.82 (0.69–0.98)	0.013
Full model plus EPA plus DPA	1.00 (reference)	0.99 (0.85–1.15)	0.96 (0.83–1.12)	0.94 (0.80–1.11)	0.83 (0.69–1.01)	0.052
CHD mortality						
EPA						
Full model	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
Full model plus DPA	1.00 (reference)	1.00 (0.71–1.40)	0.97 (0.68–1.39)	0.90 (0.62–1.32)	0.78 (0.51–1.18)	0.21
Full model plus DHA	1.00 (reference)	1.00 (0.72–1.39)	0.98 (0.70–1.36)	0.97 (0.69–1.36)	0.96 (0.65–1.42)	0.71
Full model plus DPA plus DHA	1.00 (reference)	1.02 (0.72–1.43)	1.02 (0.71–1.47)	0.99 (0.68–1.46)	0.98 (0.62–1.54)	0.94
DPA						
Full model	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
Full model plus EPA	1.00 (reference)	0.70 (0.49–1.00)	1.05 (0.74–1.49)	0.89 (0.61–1.29)	0.88 (0.59–1.31)	0.86
Full model plus DHA	1.00 (reference)	0.71 (0.50–1.01)	1.03 (0.75–1.43)	0.86 (0.61–1.20)	0.84 (0.59–1.19)	0.54
Full model plus EPA plus DHA	1.00 (reference)	0.71 (0.50–1.01)	1.03 (0.73–1.47)	0.86 (0.59–1.25)	0.84 (0.57–1.26)	0.62
DHA						
Full model	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
Full model plus EPA	1.00 (reference)	0.98 (0.71–1.36)	0.96 (0.70–1.32)	0.77 (0.55–1.09)	0.61 (0.40–0.92)	0.009
Full model plus DPA	1.00 (reference)	0.98 (0.71–1.36)	0.98 (0.71–1.35)	0.77 (0.55–1.09)	0.62 (0.42–0.90)	0.003
Full model plus EPA plus DPA	1.00 (reference)	0.98 (0.71–1.36)	0.98 (0.71–1.35)	0.78 (0.55–1.10)	0.63 (0.41–0.94)	0.008
Nonfatal myocardial infarction						
EPA						
Full model	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
Full model plus DPA	1.00 (reference)	1.19 (0.85–1.65)	0.88 (0.61–1.27)	1.05 (0.73–1.52)	0.74 (0.49–1.12)	0.051
Full model plus DHA	1.00 (reference)	1.15 (0.84–1.58)	0.84 (0.60–1.19)	1.03 (0.74–1.43)	0.74 (0.50–1.09)	0.071
Full model plus DPA plus DHA	1.00 (reference)	1.19 (0.86–1.66)	0.88 (0.61–1.27)	1.06 (0.73–1.54)	0.75 (0.48–1.17)	0.088
DPA						
Full model	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
Full model plus EPA	1.00 (reference)	0.79 (0.56–1.10)	0.85 (0.60–1.20)	0.82 (0.57–1.18)	0.97 (0.67–1.40)	0.78
Full model plus DHA	1.00 (reference)	0.78 (0.56–1.09)	0.83 (0.60–1.15)	0.76 (0.55–1.07)	0.89 (0.64–1.23)	0.51
Full model plus EPA plus DHA	1.00 (reference)	0.79 (0.56–1.10)	0.85 (0.60–1.21)	0.82 (0.57–1.18)	0.97 (0.67–1.41)	0.80
DHA						
Full model	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
Full model plus EPA	1.00 (reference)	0.85 (0.61–1.18)	1.09 (0.79–1.51)	0.97 (0.70–1.36)	0.92 (0.63–1.35)	0.80
Full model plus DPA	1.00 (reference)	0.84 (0.60–1.17)	1.05 (0.77–1.45)	0.93 (0.67–1.29)	0.81 (0.57–1.16)	0.32
Full model plus EPA plus DPA	1.00 (reference)	0.85 (0.61–1.18)	1.10 (0.80–1.51)	0.98 (0.70–1.38)	0.93 (0.63–1.36)	0.82

CHD = Coronary heart disease; DHA = docosahexaenoic acid; DPA = docosapentaenoic acid; EPA = eicosapentaenoic acid; FA = fatty acid.

* Hazard ratios and 95% CIs were adjusted for the covariates in the multivariate- and diet-adjusted model in **Table 1**: age (years); sex; race (white or nonwhite); education (less than high school, high school, some college, or college graduate); enrollment site (4 sites); FA measurement batch (1994–1996 or 2007–2010); smoking status (never, former, or current); prevalent diabetes (yes or no); prevalent atrial fibrillation (yes or no); prevalent drug-treated hypertension (yes or no); leisure-time physical activity (mcal/wk); body mass index (kg/m^2); waist circumference (cm); alcohol use (6 categories); and consumption of tuna or other broiled or baked fish (servings/wk), fried fish (servings/wk), red meat (servings/wk), fruits (servings/d), vegetables (servings/d), and dietary fiber (g/d).