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Circulating Long-Chain Omega-3 Fatty Acids and Incidence of Congestive Heart Failure in Older Adults: the Cardiovascular Health Study

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Abstract

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Availability to Readers:
Study Protocol: Available to interested readers by contacting Dr. Mozaffarian at dmozaffa@hsph.harvard.edu
Statistical Code: Available to interested readers by contacting Dr. Mozaffarian at dmozaffa@hsph.harvard.edu
Data: Available to interested readers through established Cardiovascular Health Study procedures for obtaining and analyzing data; see www.chs-nhlbi.org/CHS_DistribPolicy.htm

Background—Few prior studies have evaluated long-chain omega-3 fatty acids and incidence of congestive heart failure (CHF), typically based on diet questionnaires and with conflicting results. Circulating fatty acid levels provide objective biomarkers of exposure.

Objective—We investigated whether plasma phospholipid levels of long-chain omega-3 fatty acids, including eicosapentaenoic acid (EPA, 20:5n-3), docosapentaenoic acid (DPA, 22:5n-3), and docosahexaenoic acid (DHA, 22:6n-3) were associated with incident CHF.

Design—Prospective cohort study, 1992–2006.

Setting—Four U.S. communities.

Patients—2,735 U.S. adults free of prevalent heart disease in the Cardiovascular Health Study.

Measurements—Plasma phospholipid fatty acids and other cardiovascular risk factors were measured in 1992 using standardized methods. Relationships with incident CHF (555 cases during 26,490 person-years, adjudicated using medical records) were assessed using Cox proportional-hazards.

Results—After multivariable-adjustment, plasma phospholipid EPA was inversely associated with incident CHF, with approximately 50% lower risk in the highest vs. lowest quartile [hazard ratio (95%CI)=0.52 (0.38–0.72), P-trend=0.001]. In similar analyses, trends toward lower risk were seen for DPA [0.76 (0.56–1.04), P-trend=0.057] and total long-chain n-3 fatty acids [0.70 (0.49–0.99); P-trend= 0.062], but not DHA [0.84 (0.58–1.21); P-trend=0.38]. In analyses censored to mid-follow-up (7 years) to minimize exposure misclassification over time, multivariable-adjusted hazard ratios (95%CI) were 0.48 for EPA (0.32–0.71; P-trend=0.005); 0.61 for DPA (0.39–0.95; P-trend=0.033); 0.64 for DHA (0.40–1.04; P-trend=0.057); and 0.51 for total n-3 fatty acids (0.32–0.80; P-trend=0.003).

Limitations—Temporal changes in fatty acid levels over time may have caused underestimation of associations. Unmeasured or imperfectly measured covariates may have caused residual confounding.

Conclusions—Circulating individual and total n-3 fatty acids are associated with lower incidence of CHF in older adults.

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INTRODUCTION

Evidence from observational studies and several, although not all, randomized controlled trials suggests that seafood-derived long-chain omega-3 polyunsaturated fatty acids may reduce risk of coronary heart disease, in particular coronary death (1). However, effects of n-3 fatty acids on other cardiovascular outcomes, such as congestive heart failure (CHF), are much less well-established. Although mortality from coronary heart disease is declining in many nations, the incidence and costs of CHF are steadily rising (2). CHF is a condition quite distinct from coronary heart disease. Although a subset of some patients can have both coronary heart disease and CHF, many patients with coronary heart disease (either with or without left ventricular systolic dysfunction) do not develop CHF, and many patients with CHF do not have clinically significant coronary heart disease. This is especially true among older adults, the population with the highest burdens of incident CHF, in whom diastolic dysfunction predominates, often related to aging- or hypertension-related microstructural abnormalities and reduced left ventricular compliance (2–4). Even with optimal medical treatment, congestive heart failure (CHF) causes large public health burdens of morbidity, mortality, and health care utilization (2, 4). Among U.S. adults aged 65 or over, the fastest growing segment of the population, CHF is the leading cause of all hospitalizations (5).

Identification of novel targets for preventing CHF is clearly a priority, particular among older adults.

Several mechanistic effects of n-3 fatty acids have been demonstrated which could, in sum, reduce risk of CHF, including effects on left ventricular diastolic and systolic function, myocardial efficiency, blood pressure, heart rate (HR), arteriolar resistance, endothelial function, blood lipids, inflammation, and autonomic function (1, 6–23). However, in contrast to extensive prior research on dietary factors and risk of coronary heart disease, little is known regarding the role of most nutritional factors for prevention of CHF, and the National Institutes of Health has identified this a critical area of uncertainty requiring investigation (24). Few prior studies have evaluated how n-3 fatty acid consumption relates to incident CHF, typically based on estimates from dietary questionnaires, and with conflicting results (25–28). In contrast to questionnaire estimates, circulating concentrations of n-3 fatty acids provide objective biomarkers of exposure that reflect both dietary consumption and relevant biologic processes such as absorption, incorporation, and metabolism. Additionally, biomarker levels allow direct evaluation of specific individual n-3 fatty acids, such as eicosapentaenoic acid (EPA, 20:5n-3), docosapentaenoic acid (DPA, 22:5n-3), and docosahexaenoic acid (DHA, 22:6n-3), that may each have differing biologic effects. Measurement of circulating fatty acids is laborious, time-consuming, and expensive, and only one prior analysis has evaluated biomarkers of n-3 fatty acids and incident CHF (29). In that report, only hospitalized events were captured, and multivariable-adjusted associations were not statistically significant overall (29), perhaps limited by relatively few (n=197) cases.

We tested the hypothesis that long-chain n-3 fatty acids may reduce the onset of CHF in older adults by prospectively evaluating the associations of EPA, DPA, and DHA, evaluated as objective circulating biomarkers, with incident CHF in the Cardiovascular Health Study (CHS). We hypothesized that both total and individual long-chain n-3 fatty acids would be associated with lower risk of CHF. 5

METHODS

Design and Population

CHS is an NHLBI-sponsored, community-based, multicenter prospective cohort of older U.S. adults (30). Briefly, 5,201 ambulatory, non-institutionalized adults≥age 65 were randomly selected and enrolled in 1989–90 from Medicare eligibility lists in 4 U.S. communities (Forsyth County, North Caroline; Sacramento County, California; Washington County, Maryland; Allegheny County, Pennsylvania); an additional 687 black participants were similarly recruited and enrolled from these communities in 1992–93. Among all eligible adults contacted, 57% agreed to enroll. Annual study-clinic evaluations were performed by trained personnel using standardized methods and included physical examination, diagnostic testing, and questionnaires on health status, medical history, and cardiovascular and lifestyle risk factors. Blood was drawn after 12-hours fasting, stored (−70°C), and shipped on dry ice for long-term storage (−80°C). Each center's institutional review committee approved the study; all participants provided informed written consent.

Study Measures

Fatty acids were measured in 3,630 participants from stored blood samples taken in 1992–93, the baseline for the present analysis, including 3,130 randomly selected from among those with available blood samples and 500 in whom we had previously measured fatty acids as part of a nested case-control study of incident myocardial infarction in CHS (31). All analyses accounted for this sampling within the cohort by using inverse-probability-of-

sampling weights. We excluded participants with prevalent CHF (n=214) or coronary heart disease (n=681) at the time of blood-sampling, resulting in 2,735 participants for this analysis. All fatty acids, other risk factors, and metabolic outcomes were assessed similarly in all participants using the 1992–93 visit and blood sampling, except for dietary habits that were assessed at enrollment 3 years earlier (see below).

Fatty Acid Measurements

Plasma phospholipid fatty acids were measured at the Fred Hutchinson Cancer Research Center, providing quantitative measurement of 45 fatty acids as percentage of total fatty acids. Phospholipids represent a biomarker of longer-term (4–8 week) circulating fatty acids, with similar responses as levels in erythrocyte membranes (32). Under blood storage conditions in CHS, we have observed no degradation, lipolysis, or oxidation after 10 years in prior studies (33). Total lipids were extracted from plasma (34), and phospholipids separated from neutral lipids by one-dimensional thin-layer-chromatography. Fatty-acid-methyl-ester samples were prepared by direct transesterification (35) and separated using gas-chromatography (5890 gas-chromatograph/flame-ionization-detector, Agilent Technologies, Palo Alto, California; SP-2560 fused-silica 100m capillary column, Supelco, Belefonte, Pennsylvania; initial 160°Cx16min, ramp 3.0°C/min to 240°C, hold 15min). Identification, precision, and accuracy were continuously evaluated using model mixtures of known fatty-acid-methylesters and established in-house controls. CVs were <3% for major fatty acids and for EPA, DPA, and DHA.

We assessed long-term reproducibility of n-3 fatty acid levels using serial blood draws (1992–93, 1998–99, 2005–06) in a subset of 100 participants, that would capture laboratory error, biologic variability, and dietary changes over time. For EPA, DPA, and DHA, 6-year and 13-year correlations with baseline levels were 0.55 and 0.50, 0.67 and 0.52, and 0.82 and 0.60, respectively, comparable to within-individual correlations over time for other common risk factors such as blood pressure (36). In prospective studies assessing relationships of risk factors to subsequent disease events, such normal biologic fluctuation in levels of physiologic risk factors over time leads to underestimation of strength of true associations. We used the reproducibility measurements to correct for this regression dilution bias to determine the association of “usual” fatty acid levels with disease risk, using methods established in analyses of blood pressure and cholesterol levels and cardiovascular risk (37, 38). Such methods correct the risk estimate, widen the confidence intervals, and leave the statistical significance (P-value) unchanged.

Other Risk Factors

Standardized methods were used to assess body mass index, waist circumference, HR, and blood pressure; fasting blood lipids, glucose, insulin, and Homeostasis-Assessment-Model-of-Insulin-Resistance (HOMA-IR); and fibrinogen and C-reactive protein; at the same 1992–93 visit used for blood sampling (30). Physical activity was assessed at this same visit using a modified Minnesota Leisure-Time Activities questionnaire, evaluating frequency and duration of 15 different common activities. Dietary habits were assessed 3 years earlier, in 1989–90, using a picture-sort food frequency questionnaire validated against six detailed 24-h diet recall interviews spaced approximately 1 month apart (39).

Ascertainment of CHF

Participants were followed by means of annual study-clinic examinations with interim phone contacts for 10 years, with telephone contacts every 6 months thereafter. Follow-up for vital status was 100% complete; <1% of all person-time was otherwise missing and censored early. For each suspected case of incident CHF, information from outpatient and inpatient medical records, diagnostic tests and consultations, and interviews, as appropriate, were

obtained and reviewed by a centralized CHS committee who were unaware of participants' fatty acid status. Confirmation of definite CHF required *each* of three criteria: (a) CHF diagnosis by a treating physician; (b) either CHF symptoms (shortness of breath, fatigue, orthopnea, paroxysmal nocturnal dyspnea) plus signs (edema, rales, tachycardia, gallop rhythm, displaced apical impulse); or supportive clinical findings on echocardiography, contrast ventriculography, or chest radiography; and (c) medical therapy for CHF, defined as diuretics plus either digitalis or a vasodilator (angiotensin-converting-enzyme inhibitors, hydralazine, long-acting nitrates) (3). Medical records were also reviewed to obtain additional data (e.g., catheterization results, ejection fraction, wall-motion abnormalities, presence of valvular disease) for subclassification of CHF etiology. Because subclassification could be imperfect due to overlapping etiologies in many patients and also because sufficient test results for subclassification were not available in all cases, we only used these data in secondary analyses to explore relations between n-3 fatty acids and CHF subtypes.

Statistical Analysis

Fatty acid levels were evaluated in quartiles as indicator variables, with significance of trends across categories evaluated by entering the categorical variable as an ordinal variable. Dose-response relationships were evaluated nonparametrically using restricted cubic splines, a method that tests for nonlinearity in such relationships (40). Covariates were selected based on biologic interest, well-established relations to metabolic risk, or present associations with exposures/outcomes. Missing covariates (most factors=0.1–1.9%; alcohol=0.04%; dietary factors=4–10%) were imputed by best-subset-regression using 11 demographic/risk variables; results excluding missing values were similar. Cox proportional-hazards were used to estimate the hazard ratio (hereafter relative risk=RR) of incident CHF, with time-at-risk until first diagnosis, death, or latest adjudicated date of follow-up in 2006. The proportional-hazards assumption for each fatty acid and total n-3 fatty acids was not rejected based on Schoenfeld residuals. In addition to evaluating results after regression dilution bias correction (38), we also performed analyses limited to midpoint of follow-up (7 years) to minimize exposure misclassification with increasing duration of follow-up. Multivariable-adjusted associations of fatty acids with physiologic risk factors were evaluated using linear regression with robust variance estimation; regression dilution corrections were not performed for these cross-sectional analyses. Effect modification was evaluated in stratified analyses for age, gender, and drug-treated hypertension at baseline, with statistical significance assessed using the Wald test for the multiplicative interaction term. Analyses utilized Stata10.1, two-tailed-α=0.05.

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RESULTS

Mean (5^{th}, 95^{th} percentile) plasma phospholipid levels of EPA were 0.59 (0.25, 1.18); DPA, 0.83 (0.58, 1.12); and DHA, 3.03 (1.77, 4.91) percent of total fatty acids (Table 1). EPA correlated modestly with both DPA and DHA; DPA and DHA were not highly correlated. Each person's reported usual consumption of tuna or other broiled or baked fish (assessed 3 years earlier) correlated most with DHA levels, less so with EPA levels, and not at all with DPA levels, similar to recent findings from another cohort (41).

In bivariate (unadjusted) analyses, n-3 fatty acid levels were only modestly related to most demographic characteristics and CHF risk factors (Table 2). These findings help guide

consideration of potential important confounders, i.e. factors that may be related to both n-3 fatty acid levels and incident CHF. Interestingly, the three different n-3 fatty acids (EPA, DPA, and DHA) had varying relationships with several potentially relevant confounders. For example, only EPA was associated with differences in age, sex, and alcohol use, whereas only DHA was associated with race. Only EPA and DHA, but not DPA, were associated with educational status and fish consumption. All three n-3 fatty acids were associated with modestly lower smoking; this was not statistically significant for EPA.

During 26,490 person-years of follow-up, there were 555 cases of incident CHF. The incidence rate was 2.1 per 100 person-years, and the cumulative incidence 20.3% over 14 years, highlighting the high risk of new-onset CHF after age 65. After adjustment for demographic, cardiovascular, and lifestyle risk factors, plasma phospholipid EPA was inversely associated with incident CHF, with approximately 50% lower risk in the highest vs. lowest quartile (P-trend=0.001) (Table 3). After correction for regression dilution bias in EPA levels, approximately 70% lower risk was observed in the highest vs. lowest quartile. DPA and total long-chain n-3 fatty acids were each associated with trends toward lower CHF risk (P-trend=0.057, 0.062, respectively); DHA was not associated with risk (P-trend=0.38).

We evaluated the association between EPA levels and risk of CHF using restricted cubic splines, a method that tests for potential nonlinearity in the relationship (Figure). Higher EPA levels were associated with lower incidence of CHF in a monotonic fashion (P=0.001), without statistical evidence for nonlinearity (P-for-nonlinearity=0.33).

We performed prespecified analyses censored at the midpoint of follow-up to minimize effects of exposure misclassification over time (Table 4). After multivariable-adjustment, EPA, DPA, DHA, and total n-3 fatty acid levels were each associated with lower incidence of CHF. Compared with the lowest quartile, individuals in the highest quartile of EPA had approximately 50% lower risk (P-trend=0.005); of DPA, approximately 40% lower risk (P-trend=0.033); of DHA, approximately 35% lower risk (P-trend= 0.057); and of total n-3 fatty acids, approximately 50% lower risk (P-trend=0.003). After correction for regression dilution bias in levels of these fatty acids, risk differences were modestly greater.

Further adjustment for other demographic factors such as income or other dietary factors including consumption of total fat, red meat, fruits, vegetables, carbohydrates, and dietary fiber had little effect on results (not shown). We also assessed the mutual independence of relationships between fish consumption, phospholipid n-3 fatty acid levels, and CHF risk. Fish consumption was associated with a trend toward lower CHF risk when evaluated without adjustment for n-3 fatty acid levels (compared with consumption <1/month, multivariable-adjusted RRs were 0.85, 0.79, and 0.69 for consumption 1–3/month, 1–2/week, and 3+/week, respectively; P-trend=0.118). After adjustment for EPA, these associations were attenuated, with corresponding RRs of 0.91, 0.91, and 0.80 (P-trend=0.39). In contrast, with further adjustment for fish consumption, the multivariable-adjusted RR's for phospholipid n-3 fatty acid levels were virtually unchanged; for example, the extreme-quartile multivariable-adjusted RR for EPA was 0.53 (95%CI=0.38–0.74); P-trend=0.001.

There was also little evidence that relationships of these n-3 fatty acids with CHF varied according to age, gender, or drug-treated hypertension (12 comparisons; P-interaction>0.150 for each), except for a potential interaction between age and DHA, in which the inverse association between DHA levels and CHF appeared to be more pronounced with increasing age (P-interaction=0.009). Only 3.3% of participants reported use of fish oil supplements,

and their exclusion did not appreciably alter results (not shown). Findings were also similar when restricted to nondrinkers (not shown).

In multivariable-adjusted analyses, EPA, DPA, and DHA were each inversely associated with physiologic risk factors for CHF, but specific relationships varied (Table 5). For example, EPA was associated with higher high-density-lipoprotein-cholesterol (P<0.001), lower C-reactive protein (P=0.027), and lower fibrinogen (P=0.017), but not significantly with other risk factors. DPA was associated with lower high-density-lipoprotein-cholesterol (P<0.001) but also significantly lower Creactive protein (P<0.001) and fibrinogen (P=0.008). DHA was associated with lower HR (P<0.001) and trend toward lower systolic blood pressure (P=0.053), but not significantly with other risk factors.

In a multivariable model adjusting for all three n-3 fatty acids simultaneously, the inverse association between EPA and incidence of CHF was not appreciably altered (extreme quartile multivariable-RR=0.49, 95%CI=0.34–0.73), whereas associations for both DPA (multivariable-RR=1.08, 95%CI=0.76–1.52) and DHA (multivariable-RR=1.05, 95%CI=0.71–1.56) were no longer significant.

For more than 90% of cases, sufficient clinical information was available to classify CHF as ischemic (n=257) or nonischemic (n=288), and valvular (n=155) or nonvalvular (n=393). For 60% of cases, ejection fraction data were available to classify CHF as systolic (n=191) or diastolic (n=142). In these subtype analyses, we focused on relationships of EPA with incident CHF. Comparing quartile 4 vs. 1, the multivariable-adjusted RR was 0.51for nonischemic (95%CI=0.34–0.78) and 0.59 for ischemic (95%CI=0.36–0.96), 0.48 for nonvalvular (95%CI=0.33–0.69) and 0.67 for valvular (95%CI=0.36–1.25), and 0.53 for diastolic (95%CI=0.26–1.05) and 0.61 for systolic (95%CI=0.35–1.06) etiologies.

DISCUSSION

In this prospective cohort study of older U.S. adults, circulating levels of individual and total long-chain n-3 fatty acids were associated with lower incidence of CHF. Associations were strongest for EPA, with approximately 50% lower risk among individuals in the highest quartile. In analyses limited to the first half of follow-up, that would limit misclassification due to changes in fatty acid levels over time, all three n-3 fatty acids as well as total n-3 levels were inversely associated with CHF. Compared with self-reported estimates of fish consumption, these biomarkers provide a more objective measure of dietary n-3 consumption, allow evaluation of specific individual fatty acids, and also account for potential nondietary processes (e.g., endogenous elongation of EPA to DPA) that might influence CHF risk.

The biologic plausibility of our findings is supported by animal-experiments (20–23) and several placebo-controlled trials (10–19) evaluating physiologic risk factors related to CHF. Several studies suggest that n-3 fatty acid consumption improves left ventricular diastolic filling (7, 13, 18, 20–22). Such beneficial effects would be particularly relevant to CHF risk in older adults, in whom diastolic dysfunction is common. n-3 fatty acid consumption also improves cardiac efficiency, reducing myocardial oxygen consumption at any given workload (17), and increases stroke volume (7, 18, 20, 21). Increased stroke volume could result from slower resting HR (16) and enhanced diastolic filling, rather than improved contractile function (7, 13, 20–22), although in two recent controlled trials, n-3 fatty acids improved ejection fraction, functional class, and peak VO_2 among patients with nonischemic dilated cardiomyopathy (18) and improved ejection fraction among patients with symptomatic CHF (19).

n-3 fatty acid consumption also lowers resting blood pressure (15), with favorable effects on arteriolar resistance (7, 23), large artery compliance (12), and vasodilatory responses (14). Molecular mechanisms may include effects on nitric oxide production (42, 43), endothelial activation (44–46), or vasoconstrictive responses (10, 11, 14), perhaps partly mediated by effects on cell membrane caveolae proteins including eNOS (47, 48). Over the long-term, lower blood pressure and particularly systemic vascular resistance would be important for reducing risk of both diastolic and systolic dysfunction, especially with advancing age. n-3 fatty acid consumption also modestly increases high-density-lipoprotein-cholesterol, a protective risk factor for CHF (49), and may inhibit proinflammatory responses related to CHF risk, such as production of interleukin-1β and tumor necrosis factor-α (50).

Adjusting for each n-3 fatty acids simultaneously, EPA was most robustly associated with risk. Given their similar dietary sources, as well as direct synthesis of DPA from EPA, the circulating levels of these fatty acids are causally related to one another, and thus such findings should be interpreted cautiously. In other words, effects of residual variation in DHA levels for a given level of EPA, or in DPA levels for a given level of EPA, may be biologically interesting but have less relevance to real world exposures. Interestingly, DPA was weakly associated with dietary fish or n-3 fatty acid consumption, suggesting that circulating levels of DPA may derive more from endogenous synthesis than from diet.

Available experimental and clinical evidence does not allow strong inferences about potentially different cardiovascular effects of each long-chain n-3 fatty acid. In myocardial membranes, DHA is present at 5–10 fold higher concentrations than EPA and 2–3 fold higher concentrations than DPA (51), which has led to particular focus on DHA for protection against cardiac arrhythmias. Conversely, myocardial EPA concentrations are most responsive to dietary changes; consumption of 300 mg/d induces nearly 3-fold increases in myocardial levels (51). Little human evidence is available to establish potential differences in levels or effects of EPA, DPA, and DHA in many other relevant tissues, such as endothelial or vascular smooth muscle cells. In one randomized trial, supplementation with purified EPA reduced risk of nonfatal coronary events, especially unstable angina (52); while in a recent nested case-control study, circulating EPA and DPA levels appeared to have stronger inverse relationships with risk of nonfatal myocardial infarction than did DHA (41). These findings, when combined with our results and those of prior experimental studies, suggest the possibility that EPA, and its metabolite DPA that has received comparably little attention, may be especially relevant for protection against non-arrhythmia-related cardiovascular events, potentially due to long-term modest improvements in multiple risk factors. Our and prior investigations of risk factors suggests that each of these three long-chain n-3 fatty acids may have distinct and potentially complementary physiologic benefits.

Results of prior observational studies of n-3 fatty acids and incident CHF have been conflicting. In the only prior biomarker analysis, circulating DHA levels were associated with lower CHF risk in women but not in men, EPA was not associated with risk, and DPA was not evaluated (29). Compared with our results, this prior study evaluated a younger population (aged 45–64 at baseline), included relatively few events (110 in men, 87 in women), and only captured hospitalized (and thus presumably more severe) CHF cases. Future appropriately powered biomarker studies in middle-aged populations, including both hospitalized and nonhospitalized cases, are needed before strong conclusions can be made about effects of n-3 fatty acids on incident CHF in middle-age. In a randomized controlled trial among 6,975 patients with established CHF who were receiving optimal pharmacologic therapy, supplementation with approximately 1 g/d of EPA+DHA reduced total mortality – the hardest of endpoints – by 8% (p=0.009) (53). Such results cannot be generalized to

effects of n-3 fatty acids on new-onset CHF, but support the importance of these fatty acids in CHF-related pathways.

Our analysis has several strengths. Information on demographics, cardiovascular risk factors, and lifestyle habits were prospectively collected in a well-established multicenter cohort with little loss to follow-up. The cohort focused on older adults, in whom CHF is most problematic. Circulating biomarkers provided objective measures of individual fatty acids. Incidence of CHF was prospectively adjudicated using medical records, and large numbers of events provided statistical power. Population-based enrollment from several U.S. communities increased generalizability. We adjusted for multiple relevant covariates, minimizing confounding. We were able to demonstrate independent relationships between n-3 fatty acid levels and several plausible CHF risk factors, and exploratory analyses suggested potentially stronger associations with nonischemic, nonvalvular, and diastolic etiologies, arguing for causal mechanisms that may influence these pathways of CHF.

Potential limitations should be considered. Fatty acid levels were measured at baseline, and normal dietary and metabolic fluctuations over time would result in increasing exposure misclassification during follow-up, causing underestimation of true relationships with incident CHF. We partly corrected for such regression dilution bias by using information from serial fatty acid measures in a subset of participants. Nevertheless, without repeated measures in all subjects, exposure misclassification over time is still present, and our results restricted to the first half of follow-up may represent the best estimates of associations. This cohort was comprised of older U.S. men and women, and results may not be generalizable to causes of CHF in younger adults or in developing nations.

This is an observational analysis, and residual confounding by unknown or unmeasured factors may be present. However, results were robust to adjustment for multiple CHF risk factors. Also, the relationships with several relevant confounders varied for each of the n-3 fatty acids. For example, DPA was unassociated with education or fish consumption, limiting potential confounding from these factors or their correlates. Similarly, although individuals with greater fish consumption may often have healthier lifestyles in general, DHA was the best correlate of fish consumption, but EPA was most strongly associated with CHF, suggesting specificity for EPA rather than for fish consumption per se.

Many nutritional supplements have not reduced cardiovascular events in controlled trials (54). Several large randomized trials of fish or fish oil have demonstrated reductions in coronary events, although others have not (1). No controlled trials have evaluated effects of n-3 fatty acids on onset of CHF, which is a distinct outcome from myocardial infarction or cardiac death. Our observational findings complement prior controlled trials among patients with established CHF (18, 19, 53) by evaluating the relationship of n-3 fatty acids with new onset of CHF. The results support an expansion of scientific inquiry into potential benefits of n-3 fatty acids for preventing CHF. In the present study, we investigated biomarkers of n-3 fatty acids that were generally derived from seafood intake and perhaps partly from endogenous metabolism (e.g., DPA), rather than from fish oil supplements. Ranges of dietary n-3 fatty acid exposure were also generally much lower than would be seen for supplements.

The large and growing personal and public health burdens and costs of CHF in older adults make identification of novel preventive measures especially timely and important. Our findings demonstrate that long-chain n-3 fatty acids, and in particular EPA, are associated with lower risk of CHF in older adults. These observational results support existing recommendations that adults consume fish, especially oily fish, at least 2 servings per week (55), including later in life. Our findings also support the need for additional well-designed

and powered experimental and interventional studies to elucidate the discrete and potentially complementary health effects and related biologic pathways of EPA, DPA, and DHA that may prevent CHF.

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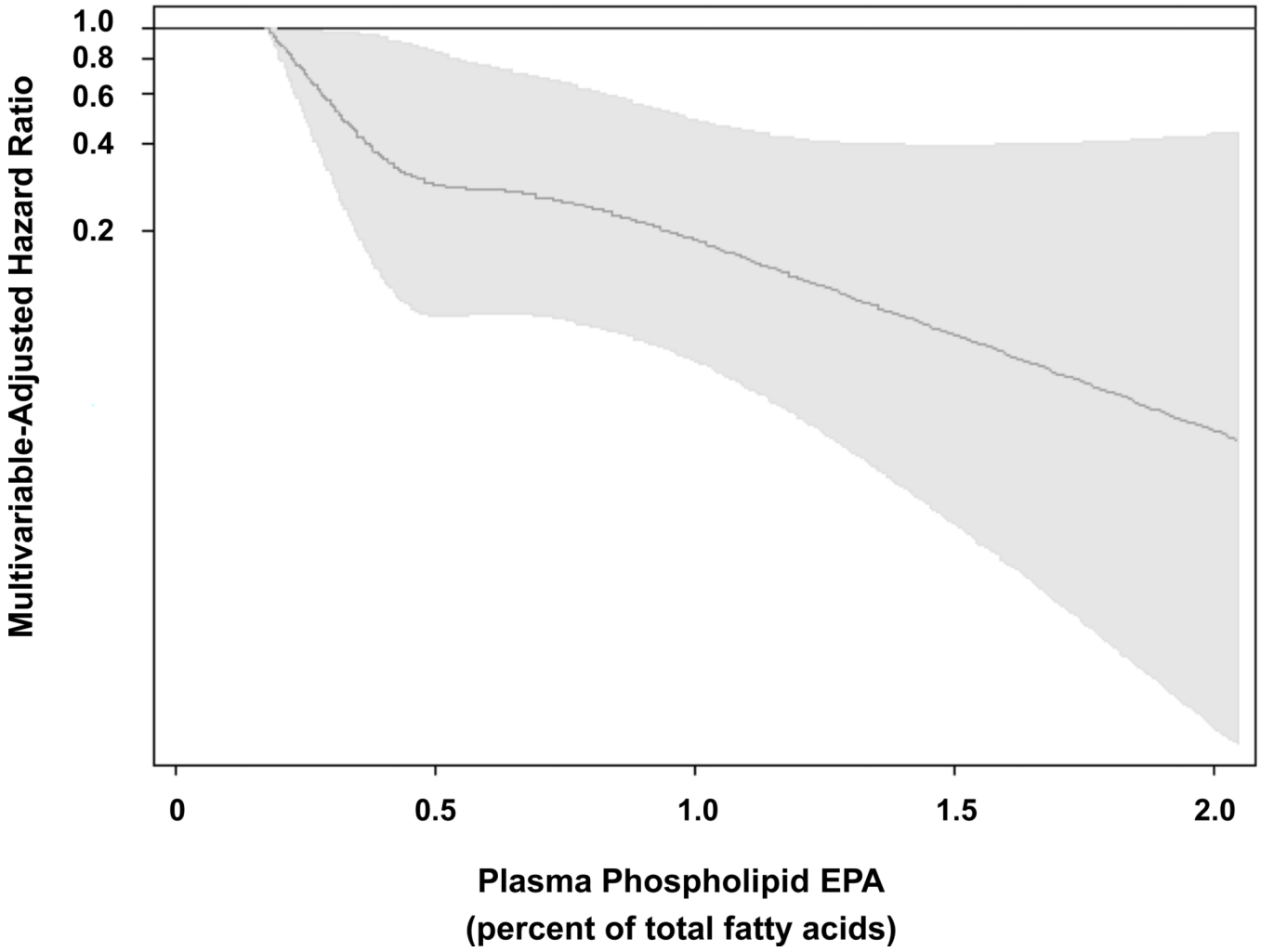

Figure.

Nonparametric multivariable-adjusted relationship between plasma phospholipid EPA levels and incidence of CHF, evaluated using restricted cubic splines and with covariates and regression dilution correction as in Table 3 footnote. The solid line and shaded area represent the central risk estimate and 95% CIs, respectively. Higher EPA levels were associated with lower incidence of CHF (P=0.001), without statistical evidence for a nonlinear dose-response (P-for-nonlinearity=0.33).

Table 1

Mean Levels, Range, and Spearman Correlations Between Plasma Phospholipid EPA, DPA, and DHA as well as Estimated Dietary Intake of Fish and n-3 Polyunsaturated Fatty Acids (n=2,735).

	Plasma Phospholipid Fatty Acids			
	EPA	**DPA**	**DHA**	**Total n-3 Fatty Acids**
Mean±SD, % total fatty acids	0.59 ± 0.38	0.83 ± 0.17	3.03 ± 0.98	4.46 ± 1.29
5th, 95th percentile	0.25, 1.18	0.58, 1.12	1.77, 4.91	2.91, 6.95
Spearman correlations with:				
Plasma phospholipid DPA	0.52			
Plasma phospholipid DHA	0.43	0.14		
Fish consumption *	0.31	0.11	0.44	0.44
n-3 fatty acid consumption *	0.29	0.04	0.46	0.45

* Based on self-reported dietary habits assessed in 1989–90, which were compared with plasma phospholipid fatty acid measurements assessed in a subset of 164 participants using stored blood samples from the same 1989–90 visit. Correlations of the 1989–90 self-reported dietary habits with plasma phospholipid fatty acid measurements using stored blood samples from 1992–93 among all 2,735 participants were modestly (approximately 10%) lower, as would be expected.

Table 2

Baseline Characteristics According to Plasma Phospholipid EPA, DPA, and DHA Among 2,735 U.S. Adults.

	Quartiles of EPA				Quartiles of DPA				Quartiles of DHA			
	I	II	III	IV	I	II	III	IV	I	II	III	IV
% total fatty acids	0.31±0.06	0.45±0.04	0.59±0.05	1.04±0.50	0.62±0.09	0.77±0.03	0.88±0.03	1.06±0.13	1.98±0.26	2.60±0.15	3.17+0.19	4.39±0.75
range	0.11–0.38	0.39–0.50	0.51–0.68	0.69–8.52	0.11–0.71	0.72–0.81	0.82–0.93	0.94–1.63	1.07–2.33	2.34–2.86	2.87–3.54	3.55–8.17
n	(n=691)	(n=680)	(n=681)	(n=683)	(n=687)	(n=682)	(n=687)	(n=679)	(n=684)	(n=685)	(n=683)	(n=683)
Age, years	76±6	75±5	75±5	75±5 *	75±5	75±5	75±5	75±5	75±5	75±5	75±5	76±5
Sex, % male	46	43	37	42 *	39	44	41	45	41	44	42	41
Race, % white	89	89	86	87	89	86	87	88	96	92	82	82 *
Education > high school, %	41	42	48	55 *	44	51	47	46	41	41	48	57 *
Current smoking, %	10	11	7	6	10	10	9	5 *	13	9	7	5 *
Diabetes mellitus, %	13	14	15	14	15	15	13	13	12	12	17	15
Atrial fibrillation, %	7	5	5	6	6	6	6	5	5	7	4	7
Treated hypertension, %	40	42	41	38	43	40	39	39	36	39	46	38
Body-mass-index, kg/m^2	26.0±4.3	26.9±4.6	27.0±4.4	26.5±4.1 *	26.7±4.8	27.0±4.5	26.6±4.2	26.2±3.9	26.1±4.2	26.9±4.5	27.0±4.4	26.5±4.3
Waist circumference, cm	96±12	98±13	98±13	97±12	97±14	98±13	97±12	96±11	96±12	98±13	98±13	97±13
Physical activity, mcal/wk	1.0±1.4	1.1±1.4	1.0±1.3	1.2±1.5	1.0±1.3	1.1±1.5	1.1±1.4	1.1±1.2	1.1±1.4	1.1±1.4	1.1±1.4	1.0±1.4
Alcohol, drinks/week	0.7±2.4	1.9±4.8	2.5±9.1	3.5±6.7 *	1.9±4.7	2.4±5.3	2.1±4.5	2.4±9.3	2.4±5.3	2.0±9.0	2.5±6.0	1.9±4.5
Tuna/dark fish, servings/wk	1.2±1.1	1.4±1.3	1.6±1.4	1.9±1.3 *	1.5±1.3	1.6±1.5	1.5±1.3	1.6±1.3	1.0±1.1	1.4±1.2	1.7±1.3	2.1±1.4 *
Total fat, % energy	32.7±5.7	32.3±5.9	32.2±5.5	31.6±5.5 *	32.8±5.4	32.1±5.8	32.4±5.8	31.6±5.6 *	33.4±6.1	32.7±5.4	31.8±5.5	30.9±5.3 *
Carbohydrate, % energy	52.3±7.6	52.5±7.7	52.2±7.2	52.1±7.1	51.7±6.9	52.4±7.7	51.9±7.8	52.9±7.0 *	51.9±8.1	51.7±7.4	52.7±7.1	52.7±6.9 *
Total energy, mcal/d	2.1±0.7	2.1±0.7	2.1±0.7	2.0±0.6	2.1±0.7	2.1±0.7	2.0±0.6	2.1±0.7	2.1±0.7	2.1±0.7	2.1±0.7	2.0±0.6

Values are mean±SD (continuous variables) or percent (categorical variables).

* P-trend<0.05 across quartiles.

Table 3

Multivariable-Adjusted Incidence of Congestive Heart Failure According to Plasma Phospholipid EPA, DPA, and DHA Levels Among 2,735 U.S. Adults.

	Fatty Acid Quartiles				P for Trend
	I	II	III	IV	
EPA					
Person-years	6,198	6,503	6,993	6,797	
No. of incident cases	174	131	126	124	
Hazard ratio (95%CI)					
Age- and sex-adjusted	1.0 (reference)	0.65 (0.48–0.88)	0.68 (0.50–0.94)	0.52 (0.38–0.70)	<0.001
Multivariable *	1.0 (reference)	0.61 (0.45–0.83)	0.65 (0.47–0.90)	0.52 (0.38–0.72)	0.001
Multivariable corrected †	1.0 (reference)	0.37 (0.20–0.68)	0.42 (0.22–0.81)	0.27 (0.14–0.51)	0.001
DPA					
Person-years	6,540	6,583	6,732	6,635	
No. of incident cases	147	145	131	132	
Hazard ratio (95%CI)					
Age- and sex-adjusted	1.0 (reference)	0.88 (0.65–1.19)	0.69 (0.51–0.95)	0.73 (0.53–1.00)	0.032
Multivariable *	1.0 (reference)	0.89 (0.66–1.22)	0.73 (0.53–1.00)	0.76 (0.56–1.04)	0.057
Multivariable corrected †	1.0 (reference)	0.80 (0.45–1.42)	0.55 (0.29–1.01)	0.59 (0.33–1.06)	0.057
DHA					
Person-years	6,533	6,415	6,794	6,750	
No. of incident cases	141	144	143	127	
Hazard ratio (95%CI)					
Age- and sex-adjusted	1.0 (reference)	0.95 (0.70–1.28)	0.96 (0.71–1.32)	0.79 (0.57–1.11)	0.175
Multivariable *	1.0 (reference)	0.90 (0.66–1.21)	0.91 (0.67–1.25)	0.84 (0.58–1.21)	0.38
Multivariable corrected †	1.0 (reference)	0.84 (0.50–1.41)	0.85 (0.51–1.42)	0.75 (0.40–1.39)	0.38
Total Long-Chain Omega-3's					
Person-years	6,379	6,605	6,595	6,912	
No. of incident cases	143	153	135	124	
Hazard ratio (95%CI)					
Age- and sex-adjusted	1.0 (reference)	0.81 (0.59–1.10)	0.84 (0.61–1.14)	0.63 (0.46–0.87)	0.007

	Fatty Acid Quartiles				P for Trend
	I	II	III	IV	
Multivariable *	1.0 (reference)	0.82 (0.61–1.11)	0.80 (0.58–1.10)	0.70 (0.49–0.99)	0.062
Multivariable corrected †	1.0 (reference)	0.72 (0.44–1.18)	0.69 (0.40–1.18)	0.55 (0.30–1.00)	0.062

*Adjusted for age (years), sex (male/female), race (white/nonwhite), education (<high school, high school, some college, college graduate), enrollment site (4 sites), smoking (never, former, current), prevalent diabetes (yes/no), prevalent atrial fibrillation (yes/no), leisure activity (kcal/wk), body mass index (kg/m2), waist circumference (cm), and alcohol use (6 categories).

†Further corrected for regression dilution bias over time by means of repeated measurements over 13-years follow-up in a subset of participants to assess the association of “usual” fatty acid levels over this period with disease risk.(37, 38)

Table 4

Multivariable-Adjusted Incidence of Congestive Heart Failure During the First Seven Years of Follow-up According to Plasma Phospholipid EPA, DPA, and DHA Levels Among 2,735 U.S. Adults.

	Fatty Acid Quartiles				P for Trend
	I	II	III	IV	
EPA					
Person-years	4,054	4,083	4,279	4,181	
No. of incident cases	103	65	69	69	
Hazard ratio (95%CI)					
Age- and sex-adjusted	1.0 (reference)	0.48 (0.32–0.71)	0.64 (0.42–0.99)	0.46 (0.31–0.69)	0.002
Multivariable *	1.0 (reference)	0.45 (0.30–0.67)	0.62 (0.39–0.97)	0.48 (0.32–0.71)	0.005
Multivariable corrected †	1.0 (reference)	0.23 (0.11–0.49)	0.42 (0.18–0.97)	0.26 (0.13–0.55)	0.005
DPA					
Person-years	4,150	4,123	4,197	4,128	
No. of incident cases	82	79	73	72	
Hazard ratio (95%CI)					
Age- and sex-adjusted	1.0 (reference)	0.73 (0.48–1.12)	0.68 (0.45–1.04)	0.58 (0.38–0.89)	0.015
Multivariable *	1.0 (reference)	0.78 (0.51–1.18)	0.75 (0.49–1.14)	0.61 (0.39–0.95)	0.033
Multivariable corrected †	1.0 (reference)	0.69 (0.37–1.30)	0.65 (0.34–1.23)	0.48 (0.25–0.93)	0.033
DHA					
Person-years	4,132	4,105	4,210	4,151	
No. of incident cases	80	88	70	68	
Hazard ratio (95%CI)					
Age- and sex-adjusted	1.0 (reference)	0.90 (0.61–1.34)	0.72 (0.47–1.12)	0.64 (0.41–0.99)	0.032
Multivariable *	1.0 (reference)	0.83 (0.56–1.23)	0.67 (0.43–1.06)	0.64 (0.40–1.04)	0.057
Multivariable corrected †	1.0 (reference)	0.80 (0.49–1.29)	0.61 (0.36–1.05)	0.58 (0.33–1.03)	0.057
Total Long-Chain Omega-3's					
Person-years	4,084	4,174	4,125	4,215	
No. of incident cases	84	88	70	64	
Hazard ratio (95%CI)					
Age- and sex-adjusted	1.0 (reference)	0.72 (0.48–1.07)	0.61 (0.40–0.94)	0.46 (0.30–0.71)	0.001

	Fatty Acid Quartiles				P for Trend
	I	II	III	IV	
Multivariable *	1.0 (reference)	0.74 (0.50–1.10)	0.57 (0.36–0.90)	0.51 (0.32–0.80)	0.003
Multivariable corrected†	1.0 (reference)	0.69 (0.42–1.12)	0.50 (0.28–0.88)	0.44 (0.24–0.77)	0.003

*Adjusted for age (years), sex (male/female), race (white/nonwhite), education (<high school, high school, some college, college graduate), enrollment site (4 sites), smoking (never, former, current), prevalent diabetes (yes/no), prevalent atrial fibrillation (yes/no), leisure activity (kcal/wk), body mass index (kg/m2), waist circumference (cm), and alcohol use (6 categories)

†Further corrected for regression dilution bias over time by means of repeated measurements over 13-years follow-up in a subset of participants to assess the association of “usual” fatty acid levels over this period with disease risk.(37, 38)

Table 5

Multivariable-Adjusted Differences in Physiologic Risk Factors for CHF According to Plasma Phospholipid EPA, DPA, and DHA (n=2,735).

	EPA	DPA	DHA
For Each 0.5% Higher Fatty Acid Levels, Difference in:			
Systolic blood pressure, mm Hg	−0.2 (−4.0 to +3.6)	+1.0 (−4.6 to +6.7)	−0.9 (−1.8 to 0.02)
Heart rate, bpm	−0.6 (−2.7 to +1.4)	−2.5 (−5.4 to +0.4)	−0.7 (−1.2 to −0.3) §
HDL-cholesterol, mmol/L [mg/dl]	+0.11 [+4.2] (+0.05 to +0.17) § [+1.8 to +6.5]	−0.17 [−6.5] (−0.26 to −0.07) § [−10.0 to −2.9]	0.0 [−0.3] (−0.02 to +0.01) [−0.9 to +0.3]
C-reactive protein, mg/L	−1.6 (−3.0 to −0.2) †	−4.8 (−7.1 to −2.6) §	−0.0 (−0.3 to +0.3)
Fibrinogen, mg/dl	−12.0 (−22.5 to −1.6) †	−22.1 (−38.3 to −5.9) ‡	+2.1 (−0.4 to +4.7)
HOMA-IR, units	−0.3 (−1.3 to +0.8)	−1.0 (−2.6 to +0.6)	−0.1 (−0.2 to +0.1)

Values are multivariable-adjusted differences (95%CI) in levels of each physiologic risk factor for each higher 0.5% levels of each n-3 fatty acid as percent of total fatty acids. Adjusted for age (years), sex (male/female), race (white/nonwhite), education (<high school, high school, some college, college graduate), enrollment site (4 sites), smoking (never, former, current), prevalent diabetes (yes/no), prevalent atrial fibrillation (yes/no), leisure activity (kcal/wk), body mass index (kg/m2), waist circumference (cm), and alcohol use (6 categories).

† P<0.05,

‡ P<0.01,

§ P<0.001

HDL=High-density-lipoprotein