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Association of Plasma Phospholipid Long-Chain Omega-3 Fatty Acids with Incident Atrial Fibrillation in Older Adults: The Cardiovascular Health Study

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Abstract

Background—Experimental studies suggest long-chain n-3 polyunsaturated fatty acids (n-3 PUFA) may reduce risk of atrial fibrillation (AF). Prior studies evaluating fish or n-3 PUFA consumption from dietary questionnaires and incident AF have been conflicting. Circulating levels of n-3 PUFA provide an objective measurement of exposure.

Methods and Results—Among 3,326 US men and women age≥65y and free of AF or heart failure at baseline, plasma phospholipid levels of eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA) were measured at baseline using standardized methods. Incident AF (789 cases) was prospectively identified from hospital discharge records and study visit electrocardiograms during 31,169 person-years of follow-up (1992-2006). In
multivariable Cox models adjusted for other risk factors, the RR in the top versus lowest quartile of total n-3 PUFA (EPA+DPA+DHA) levels was 0.71 (95%CI=0.57-0.89, P-trend=0.004); and of DHA levels, 0.77 (95%CI=0.62-0.96, P-trend=0.01). EPA and DPA levels were not significantly associated with incident AF. Evaluated non-parametrically, both total n-3 PUFA and DHA showed graded and linear inverse associations with incidence of AF. Adjustment for intervening events such as heart failure or myocardial infarction during follow-up did not appreciably alter results.

Conclusions—In older adults, higher circulating total long-chain n-3 PUFA and DHA levels were associated with lower risk of incident AF. These results highlight the need to evaluate whether increased dietary intake of these fatty acids could be effective for primary prevention of AF.

Keywords
atrial fibrillation; biomarkers; epidemiology; fatty acids

Introduction
Atrial fibrillation (AF) is the most common chronic arrhythmia in adults, and risk increases markedly with age.\textsuperscript{1} Age-adjusted incidence of AF has increased in the US, perhaps related to increasing prevalence of risk factors such as obesity and diabetes.\textsuperscript{2} Together with the aging of the population, these factors will contribute to dramatic increases in prevalence of AF, which is projected to more than double and afflict 7.5 million Americans by 2050.\textsuperscript{3} AF is associated with fatigue, reduced exercise tolerance, and higher risk for stroke, dementia, heart failure, and total mortality.\textsuperscript{4} Once AF has developed, treatment options are limited, with rate control and anticoagulation being mainstay therapies. These tremendous societal burdens prioritize the identification of novel strategies to prevent the initial onset of AF, especially among older adults who are at highest risk.

Long-chain n-3 polyunsaturated fatty acids (n-3 PUFA), mainly obtained in the diet from seafood, have several important biologic effects on a range of cellular functions that may reduce onset of AF. In animal studies and short-term clinical trials, increased intake of n-3 PUFA improves multiple indices of hemodynamic and cardiac function, including blood pressure, systemic vascular resistance, and myocardial efficiency;\textsuperscript{5-8} and also provides anti-inflammatory and anti-fibrotic effects that may reduce long term atrial remodeling and limit substrate for AF development.\textsuperscript{9-11} Cellular studies suggest n-3 PUFA may also directly affect cardiac electrophysiology through modulation of ion channels, potentially increasing myocardial electrical stability.\textsuperscript{12-14} In several animal models of AF, treatment with n-3 PUFA suppressed atrial structural remodeling and reduced susceptibility to AF.\textsuperscript{15-17}

These experimental studies are promising, but few studies have assessed whether n-3 PUFA are linked to lower onset of AF in general populations. While some prior prospective studies have assessed the association of n-3 PUFA with the incident AF, nearly all these studies assessed estimated dietary n-3 PUFA intake using questionnaires, with conflicting results.\textsuperscript{18-22} Such dietary estimates also limit separate assessment of individual n-3 PUFA, which may have differing effects.\textsuperscript{23} Most prior studies have also focused on predominantly middle-aged populations, rather than older adults who represent the general population at highest risk.

To address these issues, we investigated how circulating biomarker levels of n-3 PUFA related to incident AF in the Cardiovascular Health Study (CHS), a community-based longitudinal cohort of older US men and women. Biomarker concentrations of n-3 PUFA, for example in circulating phospholipids, provide objective measures of exposure,
incorporating influences of dietary intake as well as other potential physiologically relevant processes such as absorption, membrane incorporation, or metabolism. Measurement of biomarkers has the additional advantage of allowing direct quantification of individual n-3 PUFA, including eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA), to determine their individual associations with AF risk.

Methods

Design and Population

CHS is a community-based cohort established by the National Heart, Lung, and Blood Institute to study risk factors for cardiovascular disease in older adults. Medicare eligibility lists from 4 US communities (Sacramento County, CA; Washington County, MD; Forsyth County, NC; and Allegheny County, PA) were used to randomly select and enroll 5,201 men and women ≥65 years of age in 1989-90; an additional African-American sub-cohort of 687 individuals was similarly recruited and enrolled in 1992-93. Participants were included if they were ≥65 years old, non-institutionalized, expected to remain in their current community for >3 years, and not under active hospice or cancer treatment. 57% of eligible adults agreed to enroll. The institutional review committee from each center approved the study, and all participants gave written informed consent. At baseline and annually for the first 10 years, participants attended in-clinic evaluations carried out by trained personnel using standardized protocols. Assessments included validated questionnaires on health status, medical history, and cardiovascular and lifestyle risk factors; physical examination; diagnostic testing including 12-lead electrocardiograms (ECG); and laboratory evaluations. We measured plasma phospholipid fatty acids in 3,941 participants using stored samples from 1992-1993, the baseline for all present analyses. This included 3,448 participants with available blood samples and 493 participants in whom samples were no longer available but who had prior fatty acid measures from a nested case-control study of incident myocardial infarction (MI). All participants were free of cardiovascular disease at the time the bloods were obtained for latter fatty acid measurements, including those from the nested case-control study. All fatty acid measurements were performed by the same laboratory using standard methods (see below). For this analysis, we excluded 312 individuals with prevalent atrial fibrillation, 183 with prevalent congestive heart failure, and 120 using fish oil supplements at the time of blood sampling, resulting in 3,326 participants included in the current analyses.

Fatty acid analysis

Individual plasma phospholipid fatty acids were measured as percent of total fatty acids by the Fred Hutchinson Cancer Research Center Biomarkers Laboratory. Concentrations of n-3 PUFA in the atrium and plasma phospholipids are highly correlated (r=0.87, 0.54, and 0.72 for EPA, DPA, and DHA, respectively), indicating that phospholipid levels provide a reasonable estimate of myocardial tissue exposure where they could affect cardiac metabolism and electrophysiology. In CHS, blood was sampled after a 12-hour fast and stored at -70°C before shipping on dry ice for centralized long-term storage at -80°C. To assess storage stability and laboratory drift, we assessed repeat measurements performed 10 years apart on the same stored 1992-93 samples in 163 subjects. Sample means were very similar and intra-class correlation coefficients of these repeated measures were high (r=0.91, 0.92, and 0.92 for EPA, DPA, and DHA, respectively), confirming both storage and laboratory measurement stability of fatty acids, consistent with prior reports. Total lipids were extracted from plasma according to Folch, and phospholipid separated from neutral lipid using one dimensional thin layer chromatography. We followed Lepage's method to prepare transesterified fatty acid methyl esters (FAMEs), which were analyzed using gas chromatography (Agilent 5890 Gas Chromatograph flame ionization detector, Agilent...
Technologies, Palo Alto, California; fused silica capillary column SP-2560 [100m × 0.25mm, 0.2μm], Supelco, Belefonte, Pennsylvania; initial 160°C for 16min, ramp 3°C/min to 240°C, hold for 15min). Identification, precision, and accuracy were continuously evaluated using model mixtures of known FAMEs and an established in-house control pool, with identification confirmed by gas chromatography-mass spectrometry at the US Department of Agriculture (Peoria, IL). Inter-assay CVs were 2.1%, 1.5%, and 1.6% for EPA, DPA, and DHA, respectively.

In longitudinal studies assessing associations of baseline physiological risk factors to future disease outcomes, fluctuations in levels of these risk factors due to measurement error and true biologic variability over time will lead to underestimation of the strength of the true associations (regression dilution bias). For example, in the case of n-3 PUFA, baseline phospholipid levels represent objective exposure over the preceding few weeks, but do not capture potential dietary changes over time. To evaluate and correct for changes in exposure over time, we utilized methods established in prior analyses evaluating the relation of blood pressure and cholesterol levels with cardiovascular risk. Serial phospholipid fatty acid measures were obtained in a subset of 100 participants using blood samples drawn in 2005-06, 13 years after baseline. Within-individual fatty acid correlations comparing 1992-93 and 2005-06 measures were used to define regression dilution ratios, which were 0.50 for EPA, 0.52 for DPA, 0.60 for DHA, and 0.60 for total n-3 PUFA, comparable to other within-individual correlations over time for major risk factors such as blood pressure. Both the beta coefficient and standard error for the relation of each baseline fatty acid measurement to incident AF were divided by the corresponding regression dilution ratio to obtain the adjusted estimates for how usual n-3 PUFA levels relate to AF. Such methods correct the risk estimate, widen the confidence intervals, and leave the statistical significance (P-value) unchanged.

Assessment of other covariates

Other risk factors were assessed using standardized procedures at the same baseline visit (1992-93) as the blood sampling. These included anthropometric parameters (weight, height and waist circumference); seated resting blood pressure (model 7076 random-zero sphygmomanometer, Hawksley and Sons, UK); fasting fibrinogen (BBL fibrometer, Becton Dickinson, USA) and C-reactive protein (in-house, validated, high-sensitivity enzyme-linked immunosorbent assay). Usual frequency and types of alcoholic beverages consumed (wine, beer or liquor) were assessed using a standardized questionnaire. A modified Minnesota Leisure-Time Activities questionnaire was used to evaluate frequency and duration of leisure-time activity. Echocardiography was completed in the main cohort in 1989-90 (3 years earlier than the current study baseline) and was used to assess prevalent valvular disease (categorized as present if a participant had at least moderate aortic or mitral regurgitation and/or stenosis) and left ventricular systolic function (categorized as normal if ejection fraction ≥0.55, borderline if ejection fraction 0.45 to 0.54, or impaired if ejection fraction <0.45). A picture-sort food frequency questionnaire was used to assess dietary habits in 1989-90 and was validated against six detailed 24-h diet recall interviews and against plasma phospholipid fatty acids from stored blood in 1989-90.

Identification of incident AF

CHS participants were followed by means of annual study clinic visits for 10 years with interim 6-month telephone contact, and then telephone contacts every 6 months thereafter. Additionally, information on all hospitalizations was collected. Incident cases of AF including atrial flutter were diagnosed from annual study clinic 12-lead ECGs, read by a centralized ECG reading center, or hospital discharge diagnoses (ICD-9 codes 427.3, 427.31, or 427.32). Compared to direct review of medical records, the positive predictive
value of the hospital discharge codes for diagnosing AF was 98.6%. In a sub-study among 819 CHS participants who underwent 24-hour Holter monitoring in year 5 of CHS, only 1 participant (0.1%) had sustained or intermittent AF on the Holter who was not identified by either the annual ECG or hospital discharge codes as having AF. We also evaluated incident congestive heart failure and myocardial infarction as potential mediating conditions that might partly account for any observed relation between n-3 PUFA and incident AF. Suspected cases of incident congestive heart failure and MI were reviewed and confirmed by centralized CHS committees based on medical records, diagnostic tests and consultations using standardized algorithms.

Statistical analysis

We evaluated the sum of long-chain n-3 PUFA (EPA+DPA+DHA) and also each fatty acid individually. Fatty acid levels were assessed in quartiles as indicator variables and also continuously as percent of total fatty acids. Linear trend was tested by assigning to participants the median value in each quartile and assessing this as a continuous variable. Possible non-linear associations were evaluated semi-parametrically using restricted cubic splines.

Risk of incident AF was evaluated using multivariable-adjusted Cox proportional hazards, with time-at-risk until first diagnosis, death, or the latest adjudicated date of follow-up in 2006. The Cox proportional hazards assumption for total and each individual n-3 PUFA was tested and not rejected based on Schoenfeld’s residuals. Thirteen-year within-individual fatty acid correlation coefficients were used as regression dilution ratios to correct the Cox regression estimates for regression dilution bias. To minimize potential confounding, covariates were selected based on biologic interest, being well-established risk factors for AF risk, or associations with exposures and outcomes in the current cohort. Based on these considerations and the goal of parsimony in covariate selection, 3 final multivariate models were fitted: (1) adjusted for age, gender, race education, enrollment site, smoking status, prevalent diabetes, treated hypertension, history of myocardial infarction, history of valvular disease, body mass index, leisure-time physical activity, alcohol use, saturated fat intake, fruit and vegetable intake and total energy intake; (2) further adjusted for factors which could be potential confounders or mediators including systolic blood pressure, diastolic blood pressure, left ventricular systolic function, plasma C-reactive protein, and fibrinogen; (3) and further adjusted for disease conditions which could be potential mediators including incident nonfatal MI and congestive heart failure as time-varying covariates. Potential effect modification was investigated for age, gender and ethnicity by assessing the significance of multiplicative interaction terms using Wald tests.

In secondary analysis, we examined the extent to which the previously observed inverse association of consumption of tuna/other broiled or baked fish with risk of incident AF in this cohort could be mediated by phospholipid n-3 PUFA levels. Fish intake was assessed 3 years prior to the current study baseline in 1989-90 as previously described. The multivariable-adjusted association of tuna/other broiled or baked fish consumption with incident AF was assessed with and without adjusting for plasma phospholipid n-3 PUFA.

Missing covariates (<2% for most factors; 7-10% for dietary factors) were imputed by single imputation (impute command in Stata) using baseline data on age, gender, race, smoking status, alcohol use, education, physical activity, body mass index, coronary heart disease, diabetes and stroke. Single imputation methods perform similarly to multiple imputation methods when the percentage of missing data is not high. For missing echocardiography values (valvular disease, left ventricular function; <10% missing), we used a missing indicator category. Results were similar excluding participants with missing
Values. All p-values were two-tailed (\( \alpha = 0.05 \)). Analyses were performed using Stata 10.1 (Stata Corp, College Station, Texas).

**Results**

At baseline, the mean (±SD) age was 74.1 (±5.2) years, and 60% of participants were women. The mean total n-3 PUFA concentration was 4.5 (±1.3) percent of plasma phospholipid fatty acids, including DHA (3.0±1.0%), DPA (0.83±0.17%), and EPA (0.58±0.36%). Characteristics at baseline according to quartiles of n-3 PUFA are shown in Table 1. Consumption of tuna or other broiled or baked fish was positively associated with EPA and DHA, but not DPA levels. Each individual n-3 PUFA was inversely associated with smoking and total fat consumption. Interestingly, the individual n-3 PUFA had varying patterns of associations with most other demographic, medical, and dietary variables, suggesting that there may not be one single or set of major confounders of their associations with incident AF.

During 31,169 person-years of follow-up, 789 incident cases of AF occurred, an incidence rate of 25.3 per 1000 person-years. After adjusting for age and sex, total n-3 PUFA levels were inversely associated with incident AF, with 36% lower risk (RR=0.64, 95% CI=0.52-0.79, P-trend=0.001) among participants in the highest compared with the lowest quartile (Table 2). After further multivariate adjustment for demographic, cardiovascular, and lifestyle risk factors, total n-3 PUFA levels remained inversely associated with incident AF, with 29% lower risk (RR=0.71, 95% CI=0.57-0.89, P-trend=0.004) in the highest quartile. When evaluated continuously as percent of total fatty acids, each 1 percent higher total n-3 PUFA was associated with 9% lower risk of AF. Further adjustment for potential intermediate risk factors including systolic and diastolic blood pressure, left ventricular systolic function, plasma C-reactive protein and fibrinogen did not greatly alter the observed associations (not shown). As the risk estimates were based on a single baseline fatty acid measurement and therefore subject to regression dilution, we carried out sensitivity analysis to correct for this potential bias. After correction for regression dilution bias, the RR associated with the highest quartile of total n-3 PUFA was 0.57 (95% CI=0.39-0.82), compared to the lowest quartile.

When each individual n-3 PUFA was examined separately, DHA was associated with lower risk of AF, with 23% lower risk associated with the highest quartile compared to the lowest (RR=0.77, 95% CI=0.62-0.96, P-trend=0.01) (Table 2). When evaluated continuously as percent of total fatty acids, each 0.5 percent higher DHA was associated with 6% lower risk of AF. In sensitivity analysis adjusting for regression dilution, higher DHA levels were associated with 35% lower risk (RR=0.65, 95% CI=0.45-0.93) among participants in the highest versus lowest quartile. EPA and DPA were not significantly associated with AF risk in multivariate-adjusted analyses. Results were not appreciably altered following adjustment for potential intermediate risk factors (not shown).

Semi-parametric analyses using restricted cubic splines suggested relatively linear inverse associations of both total n-3 PUFA and DHA with incidence of AF (Figure 1). EPA and DPA were not associated with risk of incident AF across the range of their plasma phospholipid levels in this study (Figure 1). Visual inspection of the splines also suggested a trend towards lower RR in participants with higher EPA or DPA, but these findings were not statistically significant.

Results were not appreciably altered in several sensitivity analyses, including analyses with additional adjustment for other covariates including income; consumption of processed meat or dietary fiber; presence of asthma or emphysema; use of aspirin, estrogen, non-steroidal
anti-inflammatory agents, antihypertensive medications, or lipid-lowering medications; fasting plasma LDL, HDL, triglycerides, glucose, insulin, or resting heart rate; or timing of fatty acid measurements (recent measures vs. prior measures from the nested study of myocardial infarction) (data not shown). Results were also similar after exclusion of current smokers (n=317) (data not shown). We did not find evidence that the observed inverse associations of total n-3 PUFA and DHA with incident AF were mediated by effects on MI or CHF. After additional adjustment for MI or CHF as time-varying covariates, those in the highest vs. lowest quartile of total n-3 PUFA and DHA had 27% (RR=0.73, 95% CI=0.59-0.91) and 23% (RR=0.77, 95% CI=0.62-0.96) lower risk of AF, respectively.

There was also little evidence that age, gender or ethnicity modified the associations between n-3 PUFA levels and incident AF (8 comparisons; P for interaction>0.15 for each). For example, total n-3 PUFA was significantly associated with lower AF risk in both whites (n=2,897, extreme quartile RR=0.72, 95% CI=0.57-0.91), and African-Americans (n=429, extreme quartile RR=0.41, 95% CI=0.17-0.98).

In secondary analysis, we assessed the extent to which phospholipid n-3 PUFA levels might explain the previously observed lower risk of AF seen with higher consumption of tuna/other broiled or baked fish (Supplemental Table 1). In a multivariable model, additional adjustment for plasma phospholipid total n-3 PUFA led to appreciable attenuation of the relationship between fish consumption and incident AF, including 82% attenuation among those consuming 1-4 fish servings/week and 43% attenuation among those consuming 5+ fish servings/week (Supplemental Table 1). When n-3 PUFA were examined individually, attenuation of the association between fish consumption and incident AF was observed after adjustment for EPA and especially DHA. In contrast, the inverse association between higher plasma phospholipid n-3 PUFA and incident AF was minimally affected by additional adjustment for fish consumption, with extreme-quartile multivariable-adjusted RRs of 0.71 (95% CI=0.56-0.89) and 0.77 (95% CI=0.61-0.97) for total n-3 PUFA and DHA, respectively.

Discussion

In this large prospective study among older US adults not taking fish oil supplements, higher plasma phospholipid total n-3 PUFA and DHA levels were associated with lower risk of incident AF. The inverse association appeared to be relatively linear, and people in the top quartile of plasma n-3 PUFA or DHA had ~25% lower risk. Additional analyses suggested that plasma phospholipid n-3 PUFA may at least partly mediate the previously observed inverse association between fish consumption and incidence of AF. To our knowledge, this is the first prospective study to evaluate the association of objective fatty acid biomarkers with incident AF among older US adults.

Several experimental and interventional studies support the biologic plausibility of these findings. n-3 PUFA improve hemodynamic parameters, including lowering blood pressure and systemic vascular resistance, lower heart rate and augment vagal activity, enhance myocardial metabolic efficiency and left ventricular diastolic filling, and may have anti-inflammatory effects. In animal models of AF, fish oil consumption modulates atrial gene expression and protein signaling pathways, contributing to reduced atrial structural remodeling and AF susceptibility. Experimental studies also suggest that EPA and DHA modulate myocyte ion channels, although whether these potential effects might affect induction of AF requires investigation.

Fish are the major source of dietary n-3 PUFA in the US population. Several national and international dietary guidelines recommend 1-2 servings of fish per week (preferably oily...
(fish) to obtain ~250mg or more n-3 PUFA/day, based on consistency of evidence supporting the efficacy of n-3 PUFA to reduce the risk of coronary heart disease mortality. Our findings provide evidence that dietary n-3 PUFA could also provide protection against onset of AF later in life. The observed attenuation of the inverse association between fish consumption and incidence of AF following adjustment for phospholipid n-3 PUFA levels further supports the hypothesis that n-3 PUFA are a major bioactive component in fatty fish which could lower risk of AF.

In our analyses, lower risk was most robust for total n-3 PUFA and for DHA. Similarly, in a prior Finnish study of n-3 PUFA biomarkers, only total n-3 PUFA and DHA were significantly associated with lower risk of AF. DHA is present in higher (3- to 9-fold higher) levels in myocardial membranes than EPA or DPA. In experimental studies, the preferential accumulation of DHA in the myocardium have been suggested to contribute to better protection against ventricular fibrillation, in comparison to EPA. Most prior observational or interventional studies have evaluated fish or fish oil supplements containing both EPA and DHA, making it difficult to attribute observed effects to EPA or DHA. However, a limited number of human studies that evaluated EPA and DHA separately found that purified DHA, but not EPA, lowered BP and heart rate, therefore suggesting DHA may be most important for selected cardiac risk factors. On the other hand, while only findings for DHA were statistically significant in the present analysis, the semi-parametric analyses of EPA and DPA suggested possible trends toward protective associations at higher levels of these fatty acids, although the confidence intervals could not exclude no effect. Our results emphasize the need to further elucidate potential differences between physiologic effects of individual n-3 PUFA and how this may relate to AF development.

A few prior observational studies have found conflicting results for fish or n-3 PUFA consumption estimated by dietary questionnaires and incidence of AF (Table 3). In the only prior biomarker study of incident AF, middle-aged (age 42-60 y at baseline) Finnish men in the top versus bottom quartile of serum n-3 PUFA had 39% lower risk of incident AF as determined by hospitalization records. Our results confirm and extend these prior findings by evaluating an older population (age ~75 y at baseline), who are at highest risk for AF, and including a large proportion of women; assessing n-3 PUFA biomarkers in a larger population with greater statistical power (789 vs. 240 cases); and documenting both hospitalized and outpatient (routine ECG diagnosed) AF cases. The consistency of the findings across two distinct populations with different background diets, lifestyle habits, and co-morbidities lends support to a potential protective role of n-3 PUFA for new-onset AF.

Several small randomized controlled trials have assessed the effect of n-3 PUFA supplementation to prevent post-operative AF following cardiac surgery, as well as on recurrent AF in patients with established paroxysmal or persistent AF. Findings were mixed in these studies, with some trials but not others showing benefits. A recent meta-analysis found no significant overall effect of n-3 PUFA on these end-points, but also noted that the small sample sizes and significant heterogeneity in the methods limited strong conclusions. Generalizability of effects of relatively short-term n-3 PUFA supplementation on post-operative or recurrent AF to effects of habitual n-3 PUFA consumption on new onset of AF may also be limited. Overall, the current results and prior studies highlight the need for additional investigation into the potential of n-3 PUFA to protect against AF, in particular in the setting of primary prevention in appropriately designed and powered studies.

Our analysis has several strengths. Dietary questionnaires can effectively estimate intake of total n-3 PUFA, but sources of imprecision could lead to misclassification or bias, especially for individual fatty acids. We utilized phospholipid n-3 PUFA as objective biomarkers of...
both total and individual n-3 fatty acid exposures. The community-based recruitment in the
CHS enhances generalizability, including particular focus on older adults, the age group at
highest risk for AF, and including both men and women. The prospective cohort design
minimizes selection and recall bias; and thorough follow-up and multiple methods to
diagnose incident AF reduced the potential for missed or misclassified outcomes. The
detailed and standardized collection of demographic, lifestyle and other covariates allowed
adjustment for several relevant potential confounders. A large number of events provided
appropriate statistical power.

Potential limitations should be considered. Whether n-3 PUFA biomarker measurement
might add to clinical algorithms for predicting AF risk was not assessed in this investigation,
and can be considered in future studies. Residual confounding due to unmeasured or
imprecisely measured factors cannot be excluded. On the other hand, total n-3 PUFA and
DHA remained associated with AF risk after adjustment for a range of lifestyle, dietary, and
demographic risk factors; and our findings are consistent with prior biomarker findings in
Finnish men, suggesting that residual confounding may not be the sole explanation for our
findings. n-3 PUFA level were assessed at baseline, and changes in exposure over time lead
to long-term misclassification, resulting in underestimation of the true relationships with AF.
We partly corrected for this regression dilution bias using repeated measures in a subset of
participants, and these corrected estimates may represent the best estimates of associations.
Conversely, such sensitivity analyses should be interpreted cautiously due to potential
limitations of such correction methods 60. CHS comprises older US men and women, and
results may not be generalizable to younger populations, in whom pathophysiology of AF
differ in comparison to older adults.

Our findings suggest that n-3 PUFA could be beneficial for the prevention of onset of AF in
older individuals, a group who are at particularly high risk. Given the aging of the
population, the significant and growing public health burden of AF, and the limited
treatment options once AF develops, our results highlight the need to investigate atrial
physiological and arrhythmic mechanisms affected by total and individual n-3 PUFA, and to
test the efficacy of n-3 PUFA for preventing new onset of AF among older adults in a
randomized intervention.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
Semi-parametric multivariable-adjusted relationship of plasma phospholipid EPA, DPA, DHA, and total long-chain n-3 PUFA with incident AF, evaluated using restricted cubic splines after excluding participants with values below the 1st or above the 99th percentile to remove the effects of outliers. The multivariable model was adjusted for age (years), gender (male/female), race (white/nonwhite), education (<high school, high school, >high school), enrollment site (4 sites), body mass index (kg/m²), prevalent treated hypertension (yes/no), prevalent diabetes (yes/no), prevalent myocardial infarction (yes/no), prevalent valvular disease (yes/no), smoking (never, former, current), leisure time activity (kcal/wk), alcohol intake (6 categories), saturated fat intake (% energy), fruit and vegetable intake (servings per day) and total calories (kcal/d). The solid line and shaded area represent the central risk estimate and 95% CIs, respectively. The red vertical lines correspond to the 10th, 25th, 50th, 75th and 90th percentiles for each fatty acid.
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<td>Education &gt; high school, %</td>
<td>38</td>
<td>44</td>
<td>46</td>
</tr>
<tr>
<td>Current smoking, %</td>
<td>11</td>
<td>11</td>
<td>8</td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
<td>16</td>
<td>18</td>
<td>16</td>
</tr>
<tr>
<td>Coronary heart disease, %</td>
<td>20</td>
<td>21</td>
<td>18</td>
</tr>
<tr>
<td>Treated hypertension, %</td>
<td>46</td>
<td>47</td>
<td>49</td>
</tr>
<tr>
<td>Borderline/low EF (&lt;0.55), %</td>
<td>6.8</td>
<td>6.9</td>
<td>5.0</td>
</tr>
<tr>
<td>Valvular heart disease, %</td>
<td>7.9</td>
<td>8.7</td>
<td>7.4</td>
</tr>
<tr>
<td>Aspirin &gt;2 days in 2wks, %</td>
<td>37</td>
<td>36</td>
<td>35</td>
</tr>
<tr>
<td>Lipid-lowering medication, %</td>
<td>7.7</td>
<td>7.1</td>
<td>8.1</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>26.1±4.6</td>
<td>26.9±4.7</td>
<td>27.1±4.7</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>96±13</td>
<td>98±13</td>
<td>98±13</td>
</tr>
<tr>
<td>Alcohol, drinks/week</td>
<td>1.0±3.5</td>
<td>1.7±4.4</td>
<td>2.4±9.2</td>
</tr>
<tr>
<td>Tuna/other fish, servings/wk</td>
<td>1.2±1.1</td>
<td>1.5±1.4</td>
<td>1.7±1.3</td>
</tr>
<tr>
<td>Total fat, % energy</td>
<td>32.8±5.9</td>
<td>32.3±5.9</td>
<td>31.7±5.7</td>
</tr>
<tr>
<td>Carbohydrate, % energy</td>
<td>52.1±7.8</td>
<td>52.3±7.7</td>
<td>52.6±7.5</td>
</tr>
<tr>
<td>Total energy, kcal/d</td>
<td>2097±669</td>
<td>2049±619</td>
<td>2016±602</td>
</tr>
</tbody>
</table>

Values are mean ± SD for continuous variables and percent for categorical variables. EF, ejection fraction.
† Echocardiography (ejection fraction and valvular heart disease) and dietary variables (tuna/other broiled or baked fish intake, total fat, carbohydrate and total energy) were assessed in 1989-90, 3 years prior to baseline of the present analysis.

* $P < 0.05$ for trend across quartiles.
<table>
<thead>
<tr>
<th>Total long-chain n-3 PUFA</th>
<th>Quartiles of Fatty Acid Levels</th>
<th>1&lt;sup&gt;st&lt;/sup&gt;</th>
<th>2&lt;sup&gt;nd&lt;/sup&gt;</th>
<th>3&lt;sup&gt;rd&lt;/sup&gt;</th>
<th>4&lt;sup&gt;th&lt;/sup&gt;</th>
<th>P for trend&lt;sup&gt;†&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Person-years</td>
<td></td>
<td>7510</td>
<td>7788</td>
<td>7676</td>
<td>8195</td>
<td></td>
</tr>
<tr>
<td>No. of cases</td>
<td></td>
<td>220</td>
<td>210</td>
<td>204</td>
<td>155</td>
<td></td>
</tr>
<tr>
<td>Hazard ratio (95% CI)</td>
<td>Age- and gender-adjusted</td>
<td>1.0 (reference)</td>
<td>0.92 (0.76-1.11)</td>
<td>0.94 (0.77-1.13)</td>
<td>0.64 (0.52-0.79)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Multivariable</td>
<td>1.0 (reference)</td>
<td>0.93 (0.77-1.12)</td>
<td>0.97 (0.80-1.18)</td>
<td>0.71 (0.57-0.89)</td>
<td>0.004</td>
</tr>
<tr>
<td>DHA</td>
<td>Person-years</td>
<td>7771</td>
<td>7476</td>
<td>7852</td>
<td>8070</td>
<td></td>
</tr>
<tr>
<td>No. of cases</td>
<td></td>
<td>214</td>
<td>219</td>
<td>201</td>
<td>155</td>
<td></td>
</tr>
<tr>
<td>Hazard ratio (95% CI)</td>
<td>Age- and gender-adjusted</td>
<td>1.0 (reference)</td>
<td>1.09 (0.90-1.31)</td>
<td>0.96 (0.79-1.16)</td>
<td>0.70 (0.57-0.86)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Multivariable</td>
<td>1.0 (reference)</td>
<td>1.08 (0.89-1.30)</td>
<td>0.98 (0.80-1.19)</td>
<td>0.77 (0.62-0.96)</td>
<td>0.01</td>
</tr>
<tr>
<td>DPA</td>
<td>Person-years</td>
<td>7616</td>
<td>7828</td>
<td>7842</td>
<td>7882</td>
<td></td>
</tr>
<tr>
<td>No. of cases</td>
<td></td>
<td>204</td>
<td>200</td>
<td>212</td>
<td>173</td>
<td></td>
</tr>
<tr>
<td>Hazard ratio (95% CI)</td>
<td>Age- and gender-adjusted</td>
<td>1.0 (reference)</td>
<td>0.93 (0.76-1.13)</td>
<td>0.98 (0.81-1.19)</td>
<td>0.78 (0.64-0.96)</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>Multivariable</td>
<td>1.0 (reference)</td>
<td>0.97 (0.79-1.18)</td>
<td>1.06 (0.87-1.29)</td>
<td>0.86 (0.70-1.06)</td>
<td>0.24</td>
</tr>
<tr>
<td>EPA</td>
<td>Person-years</td>
<td>7227</td>
<td>7778</td>
<td>8004</td>
<td>8160</td>
<td></td>
</tr>
<tr>
<td>No. of cases</td>
<td></td>
<td>209</td>
<td>188</td>
<td>210</td>
<td>182</td>
<td></td>
</tr>
<tr>
<td>Hazard ratio (95% CI)</td>
<td>Age- and gender-adjusted</td>
<td>1.0 (reference)</td>
<td>0.86 (0.71-1.05)</td>
<td>0.99 (0.82-1.20)</td>
<td>0.81 (0.66-0.99)</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>Multivariable</td>
<td>1.0 (reference)</td>
<td>0.88 (0.72-1.07)</td>
<td>1.01 (0.83-1.23)</td>
<td>0.86 (0.69-1.06)</td>
<td>0.30</td>
</tr>
</tbody>
</table>

n-3 PUFA, n-3 polyunsaturated fatty acids; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid.
Adjusted for age (years), gender (male/female), race (white/nonwhite), education (< high school, high school, > high school), enrollment site (4 sites), body mass index (kg/m^2), prevalent treated hypertension (yes/no), prevalent diabetes (yes/no), prevalent myocardial infarction (yes/no), prevalent valvular disease (yes/no), smoking (never, former, current), leisure time activity (kcal/wk), alcohol intake (6 categories), saturated fat intake (% energy), fruit and vegetable intake (servings per day) and total calories (kcal/d).

Linear trend was tested by assigning to participants the median value in each quartile and assessing this as a continuous variable. Findings were very similar with fatty acid concentrations evaluated in their natural units as continuous exposures.
### Table 3

Prospective Cohort Studies of Fish or n-3 PUFA and Incidence of Atrial Fibrillation

<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Country</th>
<th>Mean age, y</th>
<th>Men, %</th>
<th>Mean Follow-Up, y</th>
<th>Ascertainment of Atrial Fibrillation</th>
<th>Number of Events</th>
<th>Exposure comparison</th>
<th>RR (95% CI) for Atrial Fibrillation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fish or n-3 PUFA Consumption Assessed by Dietary Questionnaire</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mozaffarian, (2004)</td>
<td>USA</td>
<td>73</td>
<td>43</td>
<td>8.8</td>
<td>Annual study clinic 12-lead ECGs or hospital discharge diagnoses</td>
<td>980</td>
<td>Tuna or other broiled or baked fish consumption, 5+/week vs. &lt;1/month</td>
<td>0.70 (0.53-0.93)</td>
</tr>
<tr>
<td>Frost (2005)</td>
<td>Denmark</td>
<td>56</td>
<td>47</td>
<td>5.7</td>
<td>Hospital discharge diagnoses</td>
<td>556</td>
<td>EPA+DHA consumption, top vs. bottom quintile</td>
<td>1.34 (1.02-1.76)</td>
</tr>
<tr>
<td>Brouwer (2006)</td>
<td>Netherlands</td>
<td>67</td>
<td>41</td>
<td>6.4</td>
<td>Study clinic 12-lead ECGs, diagnosis obtained from General Practitioners, or hospital discharge diagnoses</td>
<td>312</td>
<td>Fish consumption, top vs. bottom tertile EPA+DHA consumption, top vs. bottom tertile</td>
<td>1.17 (0.87-1.57) 1.18 (0.88-1.57)</td>
</tr>
<tr>
<td>Berry (2010)</td>
<td>USA</td>
<td>63</td>
<td>0</td>
<td>6</td>
<td>Study clinic 12-lead ECGs</td>
<td>378</td>
<td>Non-fried fish consumption, top vs. bottom quartile EPA+DHA consumption, top vs. bottom quartile</td>
<td>1.02 (0.73-1.42) 1.02 (0.73-1.44)</td>
</tr>
<tr>
<td>Shen (2011)</td>
<td>USA</td>
<td>62</td>
<td>44</td>
<td>4</td>
<td>Study center and external clinic ECGs</td>
<td>296</td>
<td>Fish consumption, 5+/week vs. &lt;1/week EPA+DHA consumption, top vs. bottom quartile</td>
<td>1.25 (0.84-1.86) 1.18 (0.85-1.64)</td>
</tr>
</tbody>
</table>

| n-3 PUFA Levels Assessed as Circulating Biomarkers | | | | | | | | |
| Virtanen (2009) | Finland | 53 | 100 | 17.7 | Record linkage to the Finland national computerized hospitalization registry diagnosis | 240 | EPA+DPA+DHA concentrations, top vs. bottom quartile DHA concentrations, top vs. bottom quartile | 0.61 (0.41-0.90) 0.58 (0.39-0.87) |

*Significant associations were not seen for EPA concentrations or DPA concentrations alone.*