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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Background—Experimental studies suggest that long-chain n-3 polyunsaturated fatty acids (n-3 PUFAs) may reduce the 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 PUFAs provide an objective measurement of exposure.

Methods and Results—Among 3326 US men and women ≥65 years of age and free of AF or heart failure at baseline, plasma phospholipid levels of eicosapentaenoic acid, docosapentaenoic acid, and docosahexaenoic acid were measured at baseline by use of standardized methods. Incident AF (789 cases) was identified prospectively from hospital discharge records and study visit ECGs during 31 169 person-years of follow-up (1992–2006). In multivariable Cox models adjusted for other risk factors, the relative risk in the top versus lowest quartile of total n-3 PUFAs (eicosapentaenoic acid+docosapentaenoic acid+docosahexaenoic acid) levels was 0.71 (95% confidence interval, 0.57–0.89; *P* for trend=0.004) and of DHA levels was 0.77 (95% confidence interval, 0.62–0.96; *P* for trend=0.01). Eicosapentaenoic acid and docosapentaenoic acid levels were not significantly associated with incident AF. Evaluated nonparametrically, both total n-3 PUFAs and docosahexaenoic acid 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 docosahexaenoic acid 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 the primary prevention of AF. (***Circulation*. 2012;125:1084-1093.**)

Key Words: atrial fibrillation ■ biological markers ■ epidemiology ■ fatty acids

Atrial fibrillation (AF) is the most common chronic arrhythmia in adults, and risk increases markedly with age.[1] Age-adjusted incidence of AF has increased in the United States, perhaps related to the increasing prevalence of risk factors such as obesity and diabetes mellitus.[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.[3] AF is associated with fatigue, reduced exercise tolerance, and higher risk for stroke, dementia, heart failure, and total mortality.[4] Once AF has developed, treatment options are limited, with rate control and anticoagulation being mainstay therapies. The 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.

Clinical Perspective on p 1093

Long-chain n-3 polyunsaturated fatty acids (n-3 PUFAs), obtained mainly in the diet from seafood, have several important biological effects on a range of cellular functions that may reduce the onset of AF. In animal studies and

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short-term clinical trials, increased intake of n-3 PUFAs improves multiple indexes of hemodynamic and cardiac function, including blood pressure, systemic vascular resistance, and myocardial efficiency,[5–8] and provides antiinflammatory and antifibrotic effects that may reduce long-term atrial remodeling and limit substrate for AF development.[9–11] Cellular studies suggest that n-3 PUFAs may also directly affect cardiac electrophysiology through modulation of ion channels, potentially increasing myocardial electric stability.[12–14] In several animal models of AF, treatment with n-3 PUFAs suppressed atrial structural remodeling and reduced susceptibility to AF.[15–17]

These experimental studies are promising, but few studies have assessed whether n-3 PUFAs are linked to lower onset of AF in general populations. Although some prior prospective studies have assessed the association of n-3 PUFAs with incident AF, nearly all of these studies assessed estimated dietary n-3 PUFA intake through the use of questionnaires with conflicting results.[18–22] Such dietary estimates also limit separate assessment of individual n-3 PUFAs, which may have differing effects.[23] In addition, most prior studies have 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 PUFAs 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 PUFAs, eg, in circulating phospholipids, provide objective measures of exposure, incorporating influences of dietary intake and other potential physiologically relevant processes such as absorption, membrane incorporation, or metabolism.[24] Measurement of biomarkers has the additional advantage of allowing direct quantification of individual n-3 PUFAs, 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.[25,26] Medicare eligibility lists from 4 US communities (Sacramento County, California; Washington County, Maryland; Forsyth County, North Carolina; and Allegheny County, PA) were used to randomly select and enroll 5201 men and women ≥65 years of age in 1989 to 1990; an additional black subcohort of 687 individuals were similarly recruited and enrolled in 1992 to 1993. Participants were included if they were ≥65 years old, noninstitutionalized, expected to remain in their current community for >3 years, and not under active hospice or cancer treatment. Fifty-seven percent 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.[25–29] Assessments included validated questionnaires on health status, medical history, and cardiovascular and lifestyle risk factors; physical examination; diagnostic testing, including 12-lead ECGs; and laboratory evaluations. We measured plasma phospholipid fatty acids in 3941 participants using stored samples from 1992 to 1993, the baseline for all present analyses. This included 3448 participants with available blood samples and 493 participants for whom samples were no longer available but who had prior fatty acid measures from a nested case-control study of incident myocardial infarction (MI).[30] All participants were free of cardiovascular disease at the time that the blood samples 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 AF, 183 with prevalent congestive heart failure, and 120 using fish oil supplements at the time of blood sampling, resulting in 3326 participants included in the present analyses.

Fatty Acid Analysis

Individual plasma phospholipid fatty acids were measured as percent of total fatty acids by the Fred Hutchinson Cancer Research Center Biomarker Laboratory. Concentrations of n-3 PUFAs in the atrium and plasma phospholipids are highly correlated (r=0.87, r=0.54, and r=0.72 for EPA, DPA, and DHA, respectively),[31] indicating that phospholipid levels provide a reasonable estimate of myocardial tissue exposure where they could influence cardiac metabolism and electrophysiology. In CHS, blood was sampled after a 12-hour fast and stored at −70°C before being shipped 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 to 1993 samples in 163 subjects. Sample means were very similar, and intraclass correlation coefficients of these repeated measures were high (r=0.91, r=0.92, and r=0.92 for EPA, DPA, and DHA, respectively), confirming both storage and laboratory measurement stability of fatty acids, consistent with prior reports.[32] Total lipids were extracted from plasma according to Folch et al,[33] and phospholipid was separated from neutral lipid by the use of 1-dimensional thin-layer chromatography. We followed the method of Lepage and Roy[34] to prepare transesterified fatty acid methyl esters, which were analyzed with gas chromatography (Agilent 5890 gas chromatograph flame ionization detector, Agilent Technologies, Palo Alto, CA; fused silica capillary column SP-2560 [100 m×0.25 mm; 0.2 μm]), Supelco, Belefonte, PA; initial, 160°C for 16 minutes; ramp, 3°–240°C/min; hold, 15 minutes). Identification, precision, and accuracy were evaluated continuously with model mixtures of known fatty acid methyl esters and an established in-house control pool, with identification confirmed by gas chromatography–mass spectrometry at the US Department of Agriculture (Peoria, IL). Interassay coefficients of variation were 2.1%, 1.5%, and 1.6% for EPA, DPA, and DHA, respectively.

In longitudinal studies assessing associations of baseline physiological risk factors with future disease outcomes, fluctuations in levels of these risk factors as a result of measurement error and true biological variability over time will lead to an underestimation of the strength of the true associations (regression dilution bias).[35,36] For example, in the case of n-3 PUFAs, baseline phospholipid levels represent objective exposure over the preceding few weeks[24] but do not capture potential dietary changes over time. To evaluate and correct for changes in exposure over time, we used methods established in prior analyses evaluating the relation of blood pressure and cholesterol levels with cardiovascular risk.[36,37] Serial phospholipid fatty acid measures were obtained in a subset of 100 participants using blood samples drawn in 2005 to 2006, 13 years after baseline. Within-individual fatty acid correlations comparing 1992 to 1993 and 2005 to 2006 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 PUFAs, comparable to other within-individual correlations over time for major risk factors such as blood pressure.[38] Both the β coefficient and the SE 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 (CIs), and leave the statistical significance (P value) unchanged.[36,37]

Assessment of Other Covariates

Other risk factors were assessed by use of standardized procedures at the same baseline visit (1992–193) as the blood sampling.[26–29] 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, US), and C-reactive protein (in-house, validated, high-sensitivity ELISA). Usual frequency of consumption and types of alcoholic beverages consumed (wine, beer, or liquor) were assessed with a standardized questionnaire.[39] A modified Minnesota Leisure-Time Activities questionnaire was used to evaluate frequency and duration of leisure-time activity.[40] Echocardiography was completed in the main cohort in 1989 to 1990 (3 years earlier than the present 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 was ≥0.55, borderline if ejection fraction was 0.45–0.54, or impaired if ejection fraction was <0.45).[41,42] A picture-sort food frequency questionnaire was used to assess dietary habits in 1989 to 1990 and was validated against 6 detailed 24-hour diet recall interviews and against plasma phospholipid fatty acids from stored blood in 1989 to 1990.[28,43]

Identification of Incident AF

CHS participants were followed up 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,[44] or taken from hospital discharge diagnoses (*International Classification of Disease,* ninth revision, code 427.3, 427.31, or 427.32). Compared with direct review of medical records, the positive predictive value of the hospital discharge codes for diagnosing AF was 98.6%.[45] In a substudy 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 and was not identified by either the annual ECG or hospital discharge codes as having AF.[21] We also evaluated incident congestive heart failure and MI as potential mediating conditions that might partly account for any observed relation between n-3 PUFAs and incident AF. Suspected cases of incident congestive heart failure and MI were reviewed and confirmed by centralized CHS committees through the use of medical records, diagnostic tests, and consultations using standardized algorithms.[46,47]

Statistical Analysis

We evaluated the sum of long-chain n-3 PUFAs (EPA+DPA+DHA) and each fatty acid individually. Fatty acid levels were assessed in quartiles as indicator variables and 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 nonlinear associations were evaluated semiparametrically by use of restricted cubic splines.

Risk of incident AF was evaluated with 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 n-3 PUFAs and each individual n-3 PUFA was tested and not rejected on the basis of the Schoenfeld 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.[36,37] To minimize potential confounding, covariates were selected on the basis of biological interest, because they were well-established risk factors for AF risk, or because of associations with exposures and outcomes in the present cohort. From these considerations and the goal of parsimony in covariate selection, 3 final multivariate models were fitted: (1) adjusted for age, sex, race education, enrollment site, smoking status, prevalent diabetes mellitus, treated hypertension, history of MI, 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 that 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 that could be potential mediators, including incident nonfatal MI and congestive heart failure as time-varying covariates. Potential effect modification was investigated for age, sex, and ethnicity by assessing the significance of multiplicative interaction terms through the use of 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[21] could be mediated by phospholipid n-3 PUFA levels. Fish intake was assessed 3 years before the present study baseline in 1989 to 1990 as previously described.[21] The multivariable-adjusted association of tuna/other broiled or baked fish consumption with incident AF was assessed with and without adjustment for plasma phospholipid n-3 PUFAs.

Missing covariates (<2% for most factors; 7% to 10% for dietary factors) were imputed by single imputation (impute command in Stata) through the use of baseline data on age, sex, race, smoking status, alcohol use, education, physical activity, body mass index, coronary heart disease, diabetes mellitus, and stroke. Single imputation methods perform similarly to multiple imputation methods when the percentage of missing data is not high.[48,49] For missing echocardiography values (valvular disease, left ventricular function; <10% missing), we used a missing indicator category. Results were similar when participants with missing values were excluded. All *P* values were 2 tailed (α=0.05). Analyses were performed with Stata 10.1 (Stata Corp, College Station, TX).

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% 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 PUFAs 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 PUFAs had varying patterns of associations with most other demographic, medical, and dietary variables, suggesting that there may not be 1 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 adjustment for age and sex, total n-3 PUFA levels were inversely associated with incident AF, with 36% lower risk (relative risk [RR], 0.64; 95% CI, 0.52–0.79; *P* for 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* for trend=0.004) in the highest quartile. When evaluated continuously as percent of total fatty acids, each 1% higher total n-3 PUFA was associated with 9% (RR, 0.91; 95% CI, 0.85–0.97) lower risk of AF. Further adjustment for potential intermediate risk factors, including systolic and diastolic blood pressures, left

Table 1. Baseline Characteristics According to Plasma Phospholipid Eicosapentaenoic Acid, Docosapentaenoic Acid, and Docosahexaenoic Acid Among 3326 US Adults

	Quartiles of EPA				Quartiles of DPA				Quartiles of DHA			
	1	2	3	4	1	2	3	4	1	2	3	4
Phospholipid fatty acid concentration, %	0.30±0.06	0.45±0.04	0.58±0.05	0.99±0.49	0.63±0.07	0.77±0.03	0.87±0.03	1.05±0.11	1.98±0.26	2.61±0.16	3.19±0.19	4.37±0.76
Range	0.11–0.39	0.40–0.51	0.52–0.67	0.68–8.52	0.11–0.72	0.73–0.82	0.83–0.93	0.94–1.63	0.78–2.35	2.36–2.88	2.89–3.54	3.55–8.17
n	834	834	827	831	832	831	843	820	834	829	832	831
Age, y	74.9±5.6	74.1±5.2	73.7±5.0	73.8±4.8*	73.8±5.0	74.2±5.4	74.3±5.2	74.2±5.1	74.1±5.1	74.0±5.1	74.1±5.2	74.3±5.2
Male sex, %	46	41	33	39*	37	42	38	42	43	38	39	39
White, %	88	88	85	87	88	87	87	87	96	91	84	78*
Education more than high school, %	38	44	46	55*	43	46	46	47	38	42	46	56*
Current smoking, %	11	11	8	8*	12	9	8	9*	13	10	7	8*
Diabetes mellitus, %	16	18	16	14	18	16	15	15	15	16	18	15
Coronary heart disease, %	20	21	18	16*	22	19	18	17*	18	19	19	19
Treated hypertension, %	46	47	49	44	48	47	47	44	42	47	51	46
Borderline/low EF (<0.55), %	6.8	6.9	5.0	4.7*	6.2	6.2	6.3	4.7	5.7	6.2	5.7	5.7
Valvular heart disease, %	7.9	8.7	7.4	10	7.9	11	6.1	9.6	8.5	7.9	8.5	9.4
Aspirin >2 d in 2 wk, %	37	36	35	36	36	37	37	34	34	35	36	39*
Lipid-lowering medication, %	7.7	7.1	8.1	6.4	10.2	7.3	4.9	6.4*	6.6	7.6	7.6	7.0
Body mass index, kg/m^2	26.1±4.6	26.9±4.7	27.1±4.7	26.6±4.3	26.7±4.9	27.2±4.7	26.7±4.5	26.1±4.1*	26.3±4.5	26.9±4.5	27.1±4.7	26.5±4.7
Waist circumference, cm	96±13	98±13	98±13	96±13	97±14	98±13	97±13	96±12*	97±13	98±13	98±13	96±13
Alcohol, drinks/wk	1.0±3.5	1.7±4.4	2.4±9.2	3.3±6.2*	1.9±4.5	2.3±5.3	2.1±5.0	2.2±9.2	2.3±5.4	2.1±9.0	2.1±5.1	1.9±4.5
Tuna/other fish, servings/wk	1.2±1.1	1.5±1.4	1.7±1.3	2.0±1.4*	1.5±1.3	1.7±1.4	1.6±1.3	1.7±1.3*	1.1±1.2	1.4±1.1	1.7±1.3	2.2±1.5*
Total fat, % energy	32.8±5.9	32.3±5.9	31.7±5.7	31.1±5.7*	32.5±5.7	32.1±5.9	32.0±5.9	31.4±5.7*	33.2±6.0	32.5±5.9	31.7±5.4	30.6±5.6*
Carbohydrates, % energy	52.1±7.8	52.3±7.7	52.6±7.5	52.6±7.4	51.8±7.4	52.3±7.7	52.3±7.8	53.2±7.4*	52.0±7.9	52.0±8.1	52.6±7.1	53.0±7.1*
Total energy, kcal/d	2097±669	2049±619	2016±602	2015±576*	2024±648	2077±604	2014±609	2063±608	2069±668	2043±637	2043±612	2022±548

EPA indicates eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; and EF, ejection fraction. Values are mean±SD for continuous variables and percent for categorical variables. Echocardiography (ejection fraction and valvular heart disease) and dietary variables (tuna or other broiled or baked fish intake, total fat, carbohydrate, and total energy) were assessed in 1989 to 1990, 3 years before the baseline of the present analysis.

*$P<0.05$ for trend across quartiles.

ventricular systolic function, plasma C-reactive protein, and fibrinogen, did not greatly alter the observed associations (not shown). Because the risk estimates were based on a single baseline fatty acid measurement and therefore subject to regression dilution,[35,36] 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 PUFAs was 0.57 (95% CI, 0.39–0.82) compared with the lowest quartile.

When each individual n-3 PUFA was examined separately, DHA was associated with lower risk of AF, with 23% lower risk in the highest quartile compared with the lowest (RR, 0.77; 95% CI, 0.62–0.96; *P* for trend=0.01; Table 2). When evaluated continuously as percent of total fatty acids, each 0.5% higher DHA was associated with 6% lower risk (RR, 0.94; 95% CI, 0.90–0.98) of AF. In sensitivity analysis adjusted 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 after further adjustment for potential intermediate risk factors (not shown).

Semiparametric analyses using restricted cubic splines suggested relatively linear inverse associations of both total n-3 PUFAs and DHA with the incidence of AF (Figure). EPA

Table 2. Relative Risk of Incident Atrial Fibrillation According to Plasma Phospholipid Long-Chain n-3 Polyunsaturated Fatty Acids in 3326 US Adults

	Quartiles of Fatty Acid Levels				*P* for Trend*
	1	2	3	4	
Total long-chain n-3 PUFAs					
Person-years	7510	7788	7676	8195	
Cases, n	220	210	204	155	
Hazard ratio (95% CI)					
Age- and sex-adjusted	1.0 (Reference)	0.92 (0.76–1.11)	0.94 (0.77–1.13)	0.64 (0.52–0.79)	<0.001
Multivariable†	1.0 (Reference)	0.93 (0.77–1.12)	0.97 (0.80–1.18)	0.71 (0.57–0.89)	0.004
DHA					
Person-years	7771	7476	7852	8070	
Cases, n	214	219	201	155	
Hazard ratio (95% CI)					
Age- and sex-adjusted	1.0 (Reference)	1.09 (0.90–1.31)	0.96 (0.79–1.16)	0.70 (0.57–0.86)	<0.001
Multivariable†	1.0 (Reference)	1.08 (0.89–1.30)	0.98 (0.80–1.19)	0.77 (0.62–0.96)	0.01
DPA					
Person-years	7616	7828	7842	7882	
Cases, n	204	200	212	173	
Hazard ratio (95% CI)					
Age- and sex-adjusted	1.0 (Reference)	0.93 (0.76–1.13)	0.98 (0.81–1.19)	0.78 (0.64–0.96)	0.03
Multivariable†	1.0 (Reference)	0.97 (0.79–1.18)	1.06 (0.87–1.29)	0.86 (0.70–1.06)	0.24
EPA					
Person-years	7227	7778	8004	8160	
Cases, n	209	188	210	182	
Hazard ratio (95% CI)					
Age- and sex-adjusted	1.0 (Reference)	0.86 (0.71–1.05)	0.99 (0.82–1.20)	0.81 (0.66–0.99)	0.08
Multivariable†	1.0 (Reference)	0.88 (0.72–1.07)	1.01 (0.83–1.23)	0.86 (0.69–1.06)	0.30

n-3 PUFA indicates n-3 polyunsaturated fatty acid; CI, confidence interval; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; and EPA, eicosapentaenoic acid.

*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.

†Adjusted for age (years), sex (male/female), race (white/nonwhite), education (less than high school, high school, more than high school), enrollment site (4 sites), body mass index (kg/m^2), prevalent treated hypertension (yes/no), prevalent diabetes mellitus (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).

and DPA were not associated with the risk of incident AF across the range of their plasma phospholipid levels in this study (Figure). Visual inspection of the splines also suggested a trend toward 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, nonsteroidal antiinflammatory agents, antihypertensive medications, or lipid-lowering medications; fasting plasma low-density lipoprotein, high-density lipoprotein, triglycerides, glucose, insulin, or resting heart rate; or timing of fatty acid measurements (recent measures versus prior measures from the nested study of MI; 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 PUFAs and DHA with incident AF were mediated by the effects on MI or CHF. After additional adjustment for MI or CHF as a time-varying covariate, those in the highest versus lowest quartile of total n-3 PUFAs 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, sex, 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 PUFAs were significantly associated with lower AF risk in both whites (n=2897; extreme-quartile RR, 0.72; 95% CI, 0.57–0.91) and blacks (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

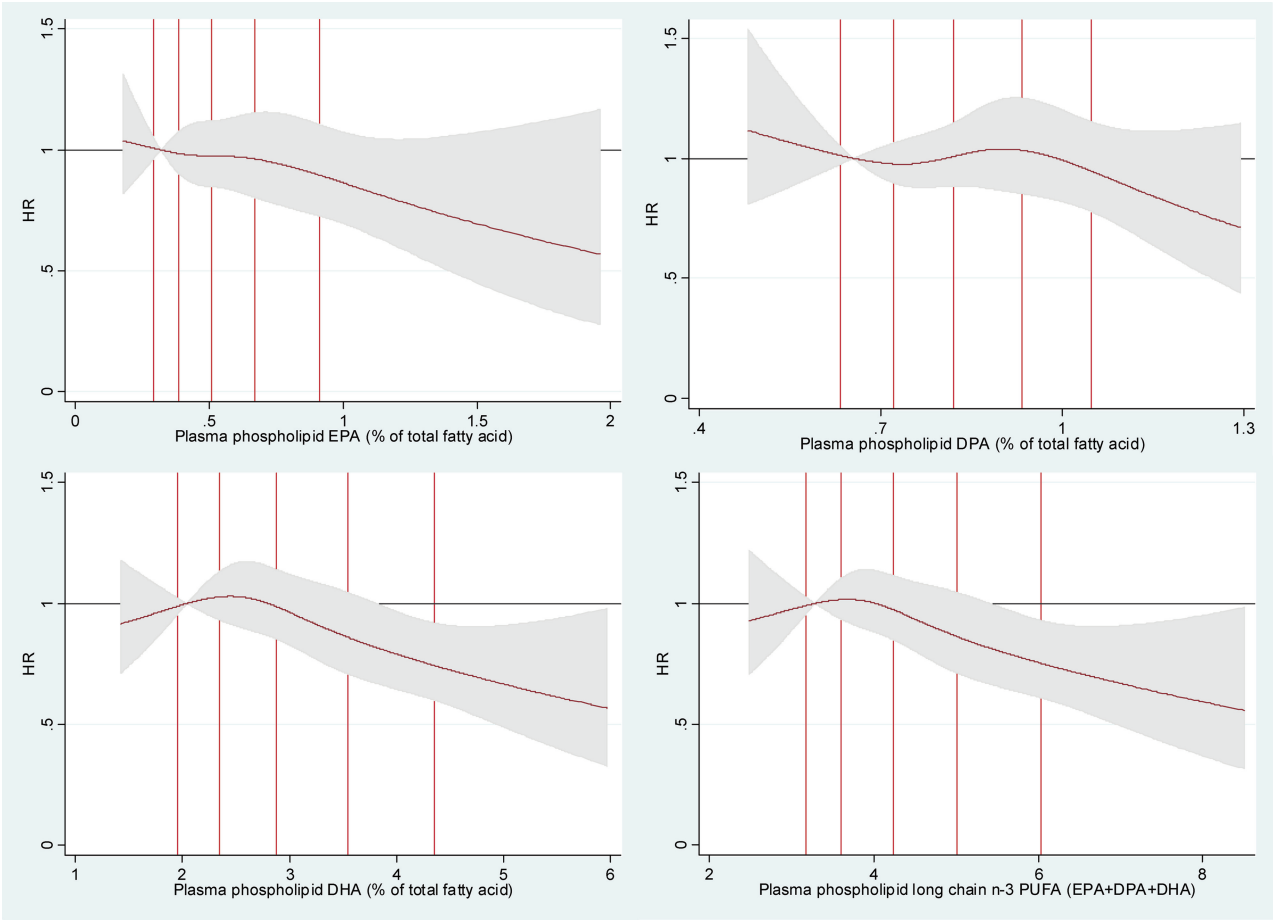

Figure. Semiparametric multivariable-adjusted relationship of plasma phospholipid eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA) and total long-chain n-3 polyunsaturated fatty acids with incident atrial fibrillation evaluated by use of restricted cubic splines after the exclusion participants with values below the first or above the 99th percentile to remove the effects of outliers. The multivariable model was adjusted for age (years), sex (male/female), race (white/nonwhite), education (less than high school, high school, more than high school), enrollment site (4 sites), body mass index (kg/m^2), prevalent treated hypertension (yes/no), prevalent diabetes mellitus (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 hazard ratio (HR) and 95% confidence intervals, respectively. The red vertical lines correspond to the 10th, 25th, 50th, 75th, and 90th percentiles for each fatty acid.

tuna or other broiled or baked fish (Table I in the online-only Data Supplement).[21] In a multivariable model, additional adjustment for plasma phospholipid total n-3 PUFAs led to appreciable attenuation of the relationship between fish consumption and incident AF, including 82% attenuation among those consuming 1 to 4 fish servings a week and 43% attenuation among those consuming ≥5 fish servings a week (Table I in the online-only Data Supplement). When n-3 PUFAs 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 PUFAs 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 PUFAs 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 PUFAs or DHA had ≈25% lower risk. Additional analyses suggested that plasma phospholipid n-3 PUFAs may at least partly mediate the previously observed inverse association between fish consumption and incidence of AF.[21] To the best of 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 biological plausibility of these findings. n-3 PUFAs improve hemodynamic parameters, including lowering blood pressure and systemic vascular resistance[5,8]; lower heart rate and augment vagal activity[50,51]; enhance myocardial metabolic efficiency and left ventricular diastolic filling[6,7,52,53]; and may have antiinflammatory effects.[10,11] 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.[15–17,54] Experi-

Table 3. Prior Prospective Cohort Studies of Fish or n-3 Polyunsaturated Fatty Acids and Incidence of Atrial Fibrillation

Authors (Year)	Country	Mean Age, y	Men, %	Mean Follow-Up, y	Ascertainment of Atrial Fibrillation	Events, n	Exposure Comparison	RR (95% CI) for Atrial Fibrillation
Fish or n-3 PUFA consumption assessed by dietary questionnaire								
Mozaffarian et al,[21] (2004)	United States	73	43	8.8	Annual study clinic 12-lead ECGs or hospital discharge diagnoses	980	Tuna or other broiled or baked fish consumption, ≥5/wk vs <1/mo	0.70 (0.53–0.93)
Frost et al,[20] (2005)	Denmark	56	47	5.7	Hospital discharge diagnoses	556	EPA+DHA consumption, top vs bottom quintile	1.34 (1.02–1.76)
Brouwer et al,[19] (2006)	Netherlands	67	41	6.4	Study clinic 12-lead ECGs, diagnosis obtained from general practitioners, or hospital discharge diagnoses	312	Fish consumption, top vs bottom tertile	1.17 (0.87–1.57)
							EPA+DHA consumption, top vs bottom tertile	1.18 (0.88–1.57)
Berry et al,[18] (2010)	United States	63	0	6	Study clinic 12-lead ECGs	378	Nonfried fish consumption, top vs bottom quartile	1.02 (0.73–1.42)
							EPA+DHA consumption, top vs bottom quartile	1.02 (0.73–1.44)
Shen et al,[22] (2010)	United States	62	44	4	Study center and external clinic ECGs	296	Fish consumption, ≥5/wk vs <1/wk	1.25 (0.84–1.86)
							EPA+DHA consumption, top vs bottom quartile	1.18 (0.85–1.64)
n-3 PUFA levels assessed as circulating biomarkers								
Virtanen et al,[56] (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	0.61 (0.41–0.90)
							DHA concentrations, top vs bottom quartile*	0.58 (0.39–0.87)

RR indicates relative risk; CI, confidence interval; n-3 PUFA, n-3 polyunsaturated fatty acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; and DPA, docosapentaenoic acid.

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

mental studies also suggest that EPA and DHA modulate myocyte ion channels,[12–14] although whether these potential effects might influence the induction of AF requires investigation.

Fish are the major source of dietary n-3 PUFAs in the US population.[55] Several national and international dietary guidelines recommend 1 to 2 servings of fish per week (preferably oily fish) to obtain ≈250 mg or more n-3 PUFAs per day, based on the consistency of evidence supporting the efficacy of n-3 PUFAs to reduce the risk of coronary heart disease mortality.[23] Our findings provide evidence that dietary n-3 PUFAs 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 after adjustment for phospholipid n-3 PUFA levels further supports the hypothesis that n-3 PUFAs are a major bioactive component in fatty fish that could lower risk of AF.

In our analyses, lower risk was most robust for total n-3 PUFAs and for DHA. Similarly, in a prior Finnish study of n-3 PUFA biomarkers, only total n-3 PUFAs and DHA were significantly associated with lower risk of AF.[56] DHA is present in higher (3- to 9-fold higher) levels in myocardial membranes than EPA or DPA.[31] In experimental studies, the preferential accumulation of DHA in the myocardium has been suggested to contribute to better protection against ventricular fibrillation compared with EPA.[14] 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 either EPA or DHA alone. 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,[57] therefore suggesting that DHA may be most important for certain cardiac risk factors. On the other hand, although only findings for DHA

were statistically significant in the present analysis, the semiparametric analyses of EPA and DPA suggested possible trends toward protective associations at higher levels of these fatty acids, although the CIs could not exclude no effect. Our results emphasize the need to further elucidate potential differences between the physiological effects of individual n-3 PUFA and how they 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).[18–22] In the only prior biomarker study of incident AF, middle-aged (age, 42–60 years at baseline) Finnish men in the top versus bottom quartile of serum n-3 PUFAs had 39% lower risk of incident AF diagnosed by hospitalization records.[56] Our results confirm and extend these prior findings by evaluating an older population (age ≈75 years 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 versus 240 cases); and documenting both hospitalized and outpatient (routine ECG diagnosed) AF cases. The consistency of the findings across 2 distinct populations with different background diets, lifestyle habits, and comorbidities lends support to a potential protective role of n-3 PUFAs and DHA for new-onset AF.

Several small randomized controlled trials have assessed the effect of n-3 PUFA supplementation to prevent postoperative AF after cardiac surgery or on recurrent AF in patients with established paroxysmal or persistent AF.[58,59] 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 PUFAs on these end points but also noted that the small sample sizes and significant heterogeneity in the methods limited strong conclusions.[58] The generalizability of these trials of relatively short-term fish oil supplementation and risk of postoperative or recurrent AF to effects of habitual dietary n-3 PUFA consumption on risk of new onset of AF may also be limited. Overall, the present results and prior studies highlight the need for additional investigation into the potential of n-3 PUFAs to protect against AF, particularly in the setting of primary prevention among older adults, in appropriately designed and powered studies.

Our analysis has several strengths. Dietary questionnaires can effectively estimate intake of total n-3 PUFAs, but sources of imprecision could lead to misclassification or bias, especially for individual fatty acids. We used phospholipid n-3 PUFAs 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 minimized selection and recall bias; the 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 an aim of this investigation and can be considered in future studies. Residual confounding resulting from unmeasured or imprecisely measured factors cannot be excluded. On the other hand, total n-3 PUFAs 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 levels 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 by 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 owing 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 may differ compared with older adults.

Our findings suggest that n-3 PUFAs could be beneficial for the prevention of onset of AF in older individuals, a group 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 PUFAs and to test the efficacy of n-3 PUFAs for preventing new onset of AF among older adults in a randomized intervention.

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Disclosures

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CLINICAL PERSPECTIVE

Atrial fibrillation (AF) is the most common chronic arrhythmia in adults, and risk increases markedly with age. Experimental studies suggest that long-chain n-3 polyunsaturated fatty acids (n-3 PUFA) from seafood may reduce onset of AF, but most prior studies of n-3 PUFA and new-onset AF in ambulatory populations have used estimates based on dietary questionnaires and were not focused on older adults, the general population at highest risk. We prospectively investigated the associations of circulating blood levels of the n-3 PUFA eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA) with incidence of AF among 3,326 older men and women (≥65y) from 4 US communities over a 14-year period. We excluded study subjects taking fish oil supplements. After adjustment for other risk factors, participants in the top quartile of total n-3 PUFA (EPA+DPA+DHA) levels had 29% lower risk of AF, compared with the lowest quartile (P trend <0.004). Among the individual n-3 fatty acids, only DHA was significantly associated with AF, with a 23% lower risk among participants in the highest versus lowest quartile ($P=0.01$). These associations were consistent among different subgroups, including men and women as well as whites and blacks. Although the observational nature of this study does not prove a causal relationship, these findings suggest that increased dietary intake of total n-3 PUFA or DHA from seafood may reduce incidence of AF in older adults.