ORIGINAL RESEARCH

Circulating and Dietary Omega-3 and Omega-6 Polyunsaturated Fatty Acids and Incidence of CVD in the Multi-Ethnic Study of Atherosclerosis

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***Background*—**Dietary guidelines support intake of polyunsaturated fatty acids (PUFAs) in fish and vegetable oils. However, some controversy remains about benefits of PUFAs, and most prior studies have relied on self-reported dietary assessment in relatively homogeneous populations.

***Methods and Results*—**In a multiethnic cohort of 2837 US adults (whites, Hispanics, African Americans, Chinese Americans), plasma phospholipid PUFAs were measured at baseline (2000–2002) using gas chromatography and dietary PUFAs estimated using a food frequency questionnaire. Incident cardiovascular disease (CVD) events (including coronary heart disease and stroke; n=189) were prospectively identified through 2010 during 19 778 person-years of follow-up. In multivariable-adjusted Cox models, circulating n-3 eicosapentaenoic acid and docosahexaenoic acid were inversely associated with incident CVD, with extreme-quartile hazard ratios (95% CIs) of 0.49 for eicosapentaenoic acid (0.30 to 0.79; P_{trend}=0.01) and 0.39 for docosahexaenoic acid (0.22 to 0.67; P_{trend}<0.001). n-3 Docosapentaenoic acid (DPA) was inversely associated with CVD in whites and Chinese, but not in other race/ethnicities (*P*-interaction=0.01). No significant associations with CVD were observed for circulating n-3 alpha-linolenic acid or n-6 PUFA (linoleic acid, arachidonic acid). Associations with CVD of self-reported dietary PUFA were consistent with those of the PUFA biomarkers. All associations were similar across racial-ethnic groups, except those of docosapentaenoic acid.

***Conclusions*—**Both dietary and circulating eicosapentaenoic acid and docosahexaenoic acid, but not alpha-linolenic acid or n-6 PUFA, were inversely associated with CVD incidence. These findings suggest that increased consumption of n-3 PUFA from seafood may prevent CVD development in a multiethnic population. **(*J Am Heart Assoc*. 2013;2:e000506 doi: 10.1161/JAHA.113.000506)**

Key Words: cardiovascular disease prevention • cardiovascular risk factors • diet • fatty acids

Current dietary guidelines emphasize increasing consumption of omega-3 (n-3) and omega-6 (n-6) long-chain polyunsaturated fatty acids (PUFAs), found in fish and vegetable oils, as a healthy substitute for saturated fatty acids.[1,2] These recommendations are based on the beneficial effects of PUFAs on blood cholesterol and blood pressure, as well as epidemiologic and clinical trial evidence suggesting cardiovascular benefits of PUFA consumption.[3–6] However, a number of important questions remain unanswered. First, most prior observational studies have evaluated associations with self-reported PUFA intake, which may be influenced by reporting bias and limited by nutrient database information. The use of objective biomarkers of these fatty acids minimizes reporting bias and also allows direct investigation of individual circulating fatty acids. Yet few prior studies have evaluated both dietary and circulating PUFA biomarkers. Second, recent randomized, controlled trials of fish oil supplements in high-risk patients have generally shown null results,[3] raising concerns for true cardiovascular disease (CVD) benefits of n-3 PUFAs. In addition, whereas meta-analyses of prospective cohorts and of older clinical trials have seen lower CVD risk

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with higher consumption of n-6 PUFAs, largely linoleic acid (LA),[4,6] concerns remain regarding the health risks of this PUFA, for example, because of concern that LA and its metabolite arachidonic acid (AA) may increase inflammation and thrombosis.[7] Yet very few studies have assessed how objective biomarkers of LA or AA relate to CVD risk. Finally, evidence for cardiovascular effects of alpha-linolenic acid (ALA), the plant-derived n-3 PUFA, and docosapentaenoic acid (DPA), a long-chain n-3 PUFA of metabolic origin, remains limited and inconclusive.[8,9] Notably, most prior evidence on PUFAs and CVD was also based on studies in racially homogeneous populations, which could have limited generalizability to more diverse groups. To address these key gaps in knowledge, we evaluated associations between both circulating biomarker and dietary n-3 and n-6 PUFAs and incident CVD events in the Multi-Ethnic Study of Atherosclerosis (MESA).

Methods

Study Design

MESA is a prospective cohort study designed to investigate risk factors associated with subclinical cardiovascular disease across race/ethnicities. MESA recruited 6814 participants in 6 US study centers (38% white, 28% African American, 22% Hispanic, and 12% Chinese American) aged 45 to 84 years at baseline (2000–2002) who were free of clinical CVD at that time.[10] Health information was assessed during cohort examinations in 2002–2003, 2004–2005, 2005–2007, and 2010–2011 and follow-up calls. Protocols were approved by local institutional review boards, and all participants gave written informed consent. Plasma phospholipid fatty acids were measured at baseline in a subset of 2880 participants, randomly selected from within each race/ethnic stratum to provide similar proportions of participants from each group. In this study, we included participants whose plasma phospholipid fatty acids measurements and follow-up information were available (n=2837). For dietary PUFA analysis, we further excluded participants whose dietary data did not meet quality control checks[11] (n=390) and those taking fish oil supplements (n=118), which resulted in a subset with 2372 participants.

Measurement of Biochemical Variables

Fasting blood samples were collected at baseline examination and subsequently processed and stored according to the study protocol.[10,12] Plasma phospholipid fatty acids were measured in MESA because of their correlation with key dietary fatty acids, including n-3 and n-6 PUFAs, and with fatty acid content in tissue membranes.[13] Individual phospholipid fatty acids were extracted from EDTA plasma using a chloroform/methanol extraction method,[13] and subsequently separated from cholesterol esters, triglycerides, and free fatty acids by thin-layer chromatography. Individual phospholipid fatty acids were derivatized to methyl esters and detected by gas chromatography flame ionization. Phospholipid fatty acid measurements were expressed as a percentage of total fatty acids. Our primary focus of interest was n-3 and n-6 PUFAs (ALA, eicosapentaenoic acid [EPA], DPA, docosahexaenoic acid [DHA], EPA+DPA+DHA, LA, AA, LA+AA). For each fatty acid, the automated (computer software) limit of detection was 0.03%. Interassay coefficents of variation were 13.5% (ALA), 7.6% (EPA), 8.3% (DPA), 8.5% (DHA), 6.8% (LA), and 7.4% (AA).

Dietary Assessment and Other Covariates

Usual dietary intake over the previous year was assessed at baseline using a Block-type,[14] 120-item food frequency questionnaire, modified to include Chinese foods.[15] Nutrient intake was estimated for each food frequency questionnaire item using the Nutrition Data System for Research (NDS-R database; Nutrition Coordinating Center, Minneapolis, MN). Criterion validity of macronutrient intake estimates has been evaluated using baseline plasma lipid measurements.[16]

Information on medical history, medication use, demographics, and smoking status was collected at baseline using interviewer-administered and self-completed questionnaires. Physical activity was assessed using a validated questionnaire designed to assess time and frequency of various physical activities.[17]

CVD Risk Factors and Biomarkers of Inflammation

Resting seated blood pressure was measured 3 times using a Dinamap model Pro 100 automated oscillometric sphygmomanometer (Critikon). The mean of the last 2 measurements was used in the analysis. Triglyceride was measured in EDTA plasma using Triglyceride GB reagent (Roche Diagnostics). Total cholesterol was measured in EDTA plasma using a cholesterol oxidase method (Roche Diagnostics). After precipitation of non-high-density lipoprotein cholesterol (non-HDL-C) with Mg/dextran, HDL cholesterol was measured using the same method. Low-density lipoprotein cholesterol (LDL-C) was calculated in plasma specimens having a triglyceride value <4.52 mmol/L using the formula of Friedewald et al.[18] Anthropometric measurements were performed using standard procedures. Plasma interleukin-6 (IL-6) was measured by ultrasensitive ELISA (Quantikine HS Human IL-6 Immunoassay; R&D Systems). Plasma C-reactive protein (CRP) was measured with a particle-enhanced immunonephelometric

assay by a BNII nephelometer (High Sensitivity CRP; Dade Behring). Tumor necrosis factor-α soluble receptor 1 (sTNF-R1) was measured using an ultrasensitive ELISA assay (Quantikine Human sTNF RI Immunoassay; R&D Systems, Minneapolis, MN).

Diagnosis of CVD Incidence

Information on new CVD events was collected during each cohort examination and every 9 to 12 months during follow-up calls to each participant. Self-reported events were confirmed by medical record abstractions, death certificates, autopsy reports, and/or obituaries. Medical records were obtained for 98% of reported hospitalized events and 95% of outpatient procedures.[19] Selected cardiovascular diagnoses and procedures were reviewed by a medical end-points committee. Total CVD events comprised myocardial infarction, resuscitated cardiac arrest, coronary heart disease (CHD) death, other atherosclerotic death, angina, stroke, stroke death, or other CVD death. CHD events comprised myocardial infarction, resuscitated cardiac arrest, CHD death, and angina. We also assessed "hard" total CVD, that is, excluding angina. A standard protocol was used to classify events and assign incident dates based on available records.[20,21]

Statistical Analysis

We estimated partial Spearman correlation between plasma phospholipid fatty acids and plasma phospholipid fatty acid and plant- and seafood sources adjusting for age (years), sex, race/ethnicity, and energy intake (kcal/day). We used linear regression with robust variance estimators to assess associations of each circulating PUFA with CVD risk factors. For prospective analyses, we used Cox proportional hazards to estimate the hazard ratio (HR) of incident CVD, with time at risk until the first CVD event, death, or last follow-up in 2009–2010. Associations with each dietary and circulating fatty acid were evaluated in quartiles and continuously per interquartile median range. We found no evidence of violation of the assumptions underlying the proportional hazard models on the basis of the Schoenfeld residuals and of significance test of time-dependent covariates included in Cox models (ie, interactions of the predictors and a function of follow-up time). In addition, we found no evidence of nonlinear associations between each PUFA and the outcomes of interest based on restricted cubic spline analysis.[22] We used serial models adjusting for potential confounders selected on the basis of biologic interest and/or well-established relationships with CVD risk. Linear trend was tested by assigning the median value in each quartile to participants and assessing this variable continuously. We imputed missing covariate data (<2% for most lifestyle factors; 8% to 12% for dietary factors) using single imputation (SAS proc MI) based on age, sex, race/ethnicity, education, physical activity, body mass index, smoking status, LDL-cholesterol, HDL-cholesterol, cholesterol-lowering medication use, and diabetes mellitus; results were similar when we used multiple imputation or excluded missing values. We evaluated potential effect modification by age, sex, and race/ethnicity. We found no evidence of evidence of a threshold or nonlinear associations between each circulating PUFA biomarker and CVD risk. All P values were 2 sided, with $P<0.05$ indicating statistical significance. All analyses were conducted using SAS version 9.3.

Results

At baseline, the average (SD) age was 61.5 (10.2) years, with similar proportion of whites (n=724), African Americans (n=697), Hispanics (n=705), and Chinese Americans (n=711), and 46.8% were male (Table 1). The mean (SD) plasma phospholipid concentrations of n-3 and n-6 fatty acids ranged from 0.18% (0.08%) for ALA to 21.4% (3.4%) for LA. Multivariate adjusted correlations amongst circulating seafood–n-3 PUFAs were moderate and positive, with correlation coefficients (r) ranging from 0.36 to 0.62 (Table 2), whereas circulating n-6 PUFA showed overall moderate inverse correlations amongst themselves and with n-3 PUFA. For example, circulating AA was inversely correlated with circulating ALA ($r=-0.34$; Table 2). Dietary estimates of seafood-derived fatty acids showed moderate positive correlations with corresponding circulating PUFA, with r ranging from 0.41 to 0.46, whereas dietary plant-derived PUFAs were weakly correlated with circulating fatty acids (r between 0.05 and 0.13).

When comparing participants in extreme quartiles of total circulating seafood n-3 PUFAs (Table 3), those with higher phospholipid seafood n-3 PUFAs were older, were more likely to be Chinese American, had lower body mass index, consumed more fruits and vegetables and less red meat, and were more likely to take lipid-lowering medications. On the other hand, participants with higher circulating n-6 PUFAs were younger, were less physically active, had lower consumption of whole grains, fish, and fruits and vegetables, and were less likely to take lipid-lowering medications when compared with those in the lowest quartile of circulating n-6 PUFAs.

Cross-Sectional Associations of Circulating Seafood-Derived n-3 PUFAs With CVD Risk Factors

In multivariate-adjusted analyses, circulating EPA was positively associated with LDL-C (Table 4) and inversely associated with IL-6, CRP, and s-TNF-R1. Circulating EPA was not

Table 1. Baseline Characteristics of 2837 US Adults in MESA

	Whites (n=724)	African Americans (n=697)	Hispanics (n=705)	Chinese (n=711)	All (n=2837)
Age, y	61.4 (10.5)	61.5 (9.8)	61 (10.1)	62.3 (10.3)	61.5 (10.2)
Sex, % male	47.1	45.5	46.2	48.3	46.8
Education, % some college degree	78.0	69.7	33.9	59.3	60.3
Current smoking, %	15.6	18.9	15.0	5.3	13.7
Alcohol intake, g/day	8.5 (14.3)	3.2 (6.8)	3 (9)	1.4 (6.6)	4.1 (10.1)
Body mass index, kg/m^2	27.8 (5.3)	30.1 (5.8)	29.6 (5.1)	24 (3.3)	27.9 (5.5)
Physical activity, MET-min/week	2617.2 (3004.1)	2823.2 (3344.3)	2065.1 (2539.5)	1736.7 (1950.8)	2309.8 (2790)
Prevalent diabetes, %	5.9	16.8	17.3	12.5	13.1
Antihypertensive medication use, %	32.0	48.8	32.9	28.5	35.5
Lipid-lowering medication use, %	15.9	17.5	14.8	14.0	15.5
Dietary supplement use, %	36.7	48.2	41.1	36.6	40.6
Dietary factors, servings/day					
Whole grains	0.7 (0.6)	0.6 (0.5)	0.5 (0.5)	0.3 (0.5)	0.5 (0.5)
Meat	0.4 (0.3)	0.4 (0.4)	0.4 (0.5)	0.4 (0.4)	0.4 (0.4)
Seafood	0.3 (0.3)	0.4 (0.4)	0.2 (0.3)	0.4 (0.4)	0.3 (0.3)
Total fruits and vegetables	3.4 (2.1)	3.5 (2.3)	3.3 (2.3)	4.4 (2.5)	3.6 (2.3)
Dietary ALA, g/day	1.1 (0.6)	1.1 (0.7)	1.0 (0.6)	0.6 (0.4)	1.0 (0.6)
Dietary LA, g/day	9.8 (5.9)	10.6 (8.0)	8.7 (6.3)	7.3 (4.3)	9.0 (6.3)
Dietary AA, g/day	0.11 (0.08)	0.14 (0.10)	0.11 (0.08)	0.11 (0.07)	0.12 (0.08)
Dietary EPA, mg/day	40 (44)	57 (66)	32 (39)	51 (46)	45 (50)
Dietary DPA, mg/day	20 (21)	25 (25)	15 (16)	19 (17)	20 (20)
Dietary DHA, mg/day	78 (69)	104 (93)	64 (59)	82 (62)	82 (73)
Circulating PUFAs, % of total phospholipid fatty acids					
ALA	0.19 (0.11)	0.16 (0.06)	0.17 (0.08)	0.18 (0.07)	0.18 (0.08)
LA	20.9 (3)	19.8 (2.8)	21.7 (3.2)	23.3 (3.6)	21.4 (3.4)
AA	12.1 (2.2)	13.8 (2.3)	11.7 (2.6)	10.5 (2.2)	12.0 (2.6)
EPA	1.0 (0.7)	1.0 (0.8)	0.7 (0.5)	1.3 (1.3)	1.0 (0.9)
DPA	1.0 (0.2)	1.0 (0.2)	0.9 (0.2)	1.0 (0.2)	1.0 (0.2)
DHA	3.7 (1.4)	4.6 (1.4)	3.4 (1.2)	5.1 (1.5)	4.2 (1.6)

Values are mean (SD) for continuous variables and percent for categorical variables. AA indicates arachidonic acid; ALA, alpha-linolenic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; LA, linoleic acid; MESA, Multi-Ethnic Study of Atherosclerosis; MET, metabolic equivalent; PUFA, polyunsaturated fatty acid; SD, standard deviation.

associated with blood pressure, plasma triglycerides, HDL-C, or total:HDL-C. Similarly, circulating DPA was not associated with blood pressure; however, it was inversely associated with inflammatory markers IL-6 and CRP, but not sTNF-R1. In contrast, circulating DPA showed overall harmful associations with plasma lipids. For example, phospholipid DPA was positively associated with triglycerides, LDL-C, and total:HDL-C and inversely associated with HDL-C. Associations with circulating DHA were similar to those of circulating EPA, except for an inverse trend toward lower plasma triglycerides and an inverse association with HDL-C. Overall, circulating seafood n-3 PUFAs showed beneficial associations with markers of inflammation, mixed associations with plasma lipids, and no association with blood pressure (Table 4).

Cross-Sectional Associations of Circulating n-3 ALA and n-6 PUFA With CVD Risk Factors

Circulating ALA was positively associated with plasma triglycerides, inversely associated with LDL-C, and CRP, and showed a trend toward higher plasma HDL-C (P_{trend}=0.08; Table 4) and higher sTNF-R1 (P_{trend}=0.08). Circulating ALA

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Table 2. Correlations* Between Plasma Circulating PUFAs and Self-Reported PUFA Intake in MESA (n=2837)

	Phospholipid ALA, % Total FA	Phospholipid LA, % Total FA	Phospholipid AA, % Total FA	Phospholipid LA+AA, % Total FA	Phospholipid EPA, % Total FA	Phospholipid DPA, % Total FA	Phospholipid DHA, % Total FA	Phospholipid EPA+DPA+DHA, % Total FA	Plant Foods,† servings/day	Seafood,‡ servings/day
Dietary ALA, g/day	0.05		0.08	0.06					0.37	0.09
Dietary LA, g/day		0.13		0.17		−0.06			0.14	0.05
Dietary AA, g/day	−0.05		0.05	0.05	0.10		0.19	0.17	−0.04	0.35
Sum of dietary LA+AA, g/day		0.13		0.17		−0.06			0.14	0.05
Dietary EPA, g/day		−0.06	−0.04	−0.11	0.34	0.12	0.46	0.45	0.12	0.89
Dietary DPA, g/day		−0.09		−0.12	0.33	0.12	0.41	0.41	0.14	0.80
Dietary DHA, g/day		−0.07		−0.09	0.31	0.09	0.43	0.42	0.10	0.81
Sum of dietary EPA+DPA+DHA, g/day	−0.02	−0.07		−0.11	0.33	0.11	0.45	0.44	0.12	0.85
Phospholipid ALA, % total FA		0.28	−0.34		0.16	0.05	−0.05		0.08	−0.02
Phospholipid LA, % total FA			−0.53	0.63	−0.35	−0.38	−0.24	−0.30		−0.13
Phospholipid AA, % total FA				0.25	0.06	0.14		0.06		0.03
Sum of phospholipid LA+AA, % total FA					−0.35	−0.32	−0.23	−0.28		−0.13
Phospholipid EPA, % total FA						0.58	0.62	0.77	0.14	0.30
Phospholipid DPA, % total FA							0.36	0.51	0.05	0.11
Phospholipid DHA, % total FA								0.97	0.17	0.39
Sum of phospholipid EPA+DPA+DHA, % total FA									0.17	0.39
Plant foods,† servings/day										0.18

AA indicates arachidonic acid; ALA, alpha-linolenic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; LA, linoleic acid; MESA, Multi-Ethnic Study of Atherosclerosis; PUFA, polyunsaturated fatty acid.

*Partial Spearman correlations adjusting for age, sex, race/ethnicity, and energy intake. Only statistically significant correlations at the 0.05 significance level are shown.

†Plant food sources[23]: almonds, walnuts, pecans, other nuts; sunflower, pinyon, other seeds; peanuts, peanut butter; miso soup or sauce with soybean paste; soy milk; pinto, black, baked, butter, or red beans, pork and beans, black-eyed peas; broccoli, cabbage, cauliflower, brussels sprouts, sauerkraut, kimchi; tossed salad with spinach, romaine, or dark greens, cooked spinach, turnip greens, collards; tossed salad with iceberg or light green lettuce; green beans, peas, snow peas; pea, lentil, black bean, *potaje* soups; corn, hominy; oatmeal, other hot cereal (grits, cream of wheat, mush, congee); brown or wild rice; white, Mexican, or sticky rice; avocado, guacamole; strawberries, blueberries, other berries; butter, margarine, or oil on vegetables, rice, or potatoes, margarine or mayonnaise on bread or rolls; stir-fried tofu or tempeh with vegetables; dessert made with tofu.

‡Seafood: fried fish or fish sandwich, fried shrimp, calamari; shrimp, lobster, crab, oysters, mussels (not fried); tuna, salmon, sardines (including sashimi or sushi); other broiled, steamed, baked, or raw fish (trout, sole, halibut, poke, grouper).

Table 3. Baseline Characteristics of 2837 US Adults With PUFA Biomarker Measurements in the Multi-Ethnic Study of Atherosclerosis by Quartiles of Total Phospholipid Seafood-Derived PUFAs

	Quartiles of Phospholipid EPA+DPA+DHA			
	1	2	3	4
Age, y	60.4 (10.4)	61.9 (10.2)	61.8 (9.9)	62.1 (10.2)
Sex, % male	48.8	46.1	48.4	48.7
Race/ethnicity				
Whites, %	35.2	31.0	19.0	19.2
African Americans, %	12.4	24.7	34.6	27.4
Hispanics, %	45.3	27.0	15.8	9.6
Chinese, %	7.1	17.3	30.6	43.7
Education, % some college degree	54.0	60.1	63.4	67.0
Current smoking, %	20.9	14.7	12.4	7.3
Alcohol intake, g/day	4.6 (10.1)	3.8 (8.9)	4.6 (12.1)	3.2 (8.8)
Body mass index, kg/m^2	29 (5.5)	28.6 (5.9)	27.3 (5.1)	26.5 (5)
Physical activity, MET-min/week	2217.1 (2883.9)	2262.4 (2724.5)	2367.8 (2636.7)	2394.6 (2904.5)
Prevalent diabetes, %	14.6	10.9	13.0	12.3
Antihypertensive medication use, %	30.8	35.7	41.0	33.8
Lipid-lowering medication use, %	10.2	15.3	17.8	18.1
Dietary factors				
Whole grains, servings/day	0.5 (0.5)	0.5 (0.5)	0.5 (0.6)	0.5 (0.5)
Meat, servings/day	0.5 (0.5)	0.4 (0.4)	0.4 (0.3)	0.4 (0.4)
Total fruits and vegetables, servings/day	3.1 (2.1)	3.5 (2.4)	3.8 (2.3)	4.2 (2.5)
PUFA plant sources, servings/day	2.6 (1.7)	2.7 (1.7)	2.7 (1.5)	2.8 (1.5)
PUFA seafood sources, servings/day	0.1 (0.2)	0.2 (0.3)	0.2 (0.2)	0.3 (0.3)
Transfat, % energy	0.9 (0.3)	0.8 (0.3)	0.7 (0.4)	0.6 (0.3)
Saturated fat, % energy	11.2 (3.2)	10.3 (3.2)	9.4 (3.1)	8.7 (2.9)
Monounsaturated fat, % energy	12.6 (2.7)	12 (2.9)	11.6 (2.9)	11 (3)
Polyunsaturated fat, % energy	6.2 (1.8)	6.2 (1.9)	6.3 (1.8)	6.2 (1.7)
Carbohydrate, % energy	51.9 (8.2)	53.2 (9.2)	53.8 (9.1)	54.4 (9.5)
Protein, % energy	15.3 (3)	15.9 (3.3)	16.4 (3.3)	17.5 (3.6)
Dietary ALA, g/day	1.1 (0.6)	1 (0.6)	0.9 (0.6)	0.8 (0.5)
Dietary LA, g/day	10.5 (6.8)	9.4 (7.3)	8.6 (5.4)	7.9 (5.2)
Dietary AA, g/day	0.12 (0.09)	0.12 (0.08)	0.12 (0.08)	0.12 (0.08)
Dietary EPA, mg/day	28 (37)	41 (44)	47 (42)	67 (63)
Dietary DPA, mg/day	13 (15)	18 (18)	20 (17)	28 (25)
Dietary DHA, mg/day	62 (61)	76 (67)	84 (61)	108 (88)
Circulating PUFAs				
ALA, % of total phospholipid fatty acids	0.18 (0.07)	0.17 (0.07)	0.18 (0.07)	0.18 (0.11)
LA, % of total phospholipid fatty acids	22.7 (3.1)	21.5 (3.2)	21.2 (3.2)	20.3 (3.6)
AA, % of total phospholipid fatty acids	11.4 (2.3)	12.6 (2.6)	12.5 (2.7)	11.5 (2.5)
EPA, % of total phospholipid fatty acids	0.48 (0.17)	0.62 (0.22)	0.84 (0.31)	1.92 (1.35)
DPA, % of total phospholipid fatty acids	0.83 (0.17)	0.9 (0.18)	0.96 (0.17)	1.14 (0.25)
DHA, % of total phospholipid fatty acids	2.5 (0.5)	3.5 (0.4)	4.6 (0.5)	6.3 (1.1)

AA indicates arachidonic acid; ALA, alpha-linolenic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; LA, linoleic acid; MET, metabolic equivalent; PUFA, polyunsaturated fatty acid.

Table 4. Multivariable-Adjusted Cross-Sectional Associations of Circulating PUFAs With CVD Risk Factors in MESA

	n	Quartiles of Plasma Phospholipid Fatty Acids				P_{trend}
		1	2	3	4	
EPA, % total FA (median)		0.39	0.6	0.86	1.62	
Systolic BP, mm Hg	2834	125.7 (0.8)	125.8 (0.7)	125.8 (0.7)	126.7 (0.8)	0.34
Diastolic BP, mm Hg	2834	71.7 (0.4)	71.5 (0.4)	72.1 (0.4)	72.3 (0.4)	0.22
Triglycerides, mg/dL	2833	130.3 (3.7)	139.4 (3.5)	142.3 (3.1)	130.7 (3)	0.44
HDL-C, mg/dL	2833	49.6 (0.5)	50.8 (0.4)	51.2 (0.5)	50.7 (0.5)	0.33
LDL-C, mg/dL	2788	115.3 (1.2)	116.8 (1.1)	116.3 (1.2)	119.8 (1.2)	0.01
Total:HDL-C	2833	4.1 (0.05)	4.1 (0.04)	4.1 (0.04)	4.1 (0.04)	0.99
IL-6, pg/mL	2774	1.6 (0.05)	1.6 (0.05)	1.5 (0.04)	1.4 (0.04)	0.01
CRP, mg/L	2826	4.2 (0.3)	3.6 (0.2)	3.3 (0.2)	3.3 (0.2)	0.06
sTNF-R1, pg/mL	2834	1418 (16)	1363 (16)	1359 (15)	1320 (13)	<0.0001
DPA, % total FA (median)		0.72	0.88	1.01	1.21	
Systolic BP, mm Hg	2834	126.3 (0.7)	126.8 (0.8)	124.4 (0.7)	126.6 (0.7)	0.75
Diastolic BP, mm Hg	2834	72 (0.3)	72 (0.4)	71.6 (0.4)	72 (0.4)	0.92
Triglycerides, mg/dL	2833	131.2 (3.4)	131.7 (3.6)	135.6 (3.1)	145.2 (3.1)	0.003
HDL-C, mg/dL	2833	51.4 (0.5)	50.3 (0.4)	51 (0.4)	49.4 (0.5)	0.01
LDL-C, mg/dL	2788	112.8 (1.2)	115 (1.1)	120.6 (1.1)	120 (1.2)	<0.0001
Total:HDL-C	2833	4 (0.05)	4 (0.04)	4.1 (0.04)	4.3 (0.04)	<0.0001
IL-6, pg/mL	2774	1.6 (0.05)	1.5 (0.04)	1.5 (0.04)	1.4 (0.04)	<0.0001
CRP, mg/L	2826	4.2 (0.2)	3.7 (0.2)	3.5 (0.2)	2.8 (0.1)	<0.0001
sTNF-R1, pg/mL	2834	1344 (15)	1380 (17)	1354 (14)	1389 (17)	0.11
DHA, % total FA (median)		2.5	3.5	4.5	6.0	
Systolic BP, mm Hg	2834	126.8 (0.8)	125.2 (0.7)	126 (0.8)	126.1 (0.8)	0.80
Diastolic BP, mm Hg	2834	71.9 (0.4)	71.5 (0.3)	72.2 (0.4)	72 (0.4)	0.68
Triglycerides, mg/dL	2833	142.1 (5.1)	135.4 (2.6)	133.6 (2.7)	131.6 (3.1)	0.08
HDL-C, mg/dL	2833	51.7 (0.5)	50.2 (0.5)	50.5 (0.5)	50 (0.5)	0.07
LDL-C, mg/dL	2788	116 (1.4)	117.2 (1.1)	115.8 (1.2)	119.1 (1.3)	0.13
Total:HDL-C	2833	4.1 (0.05)	4.1 (0.04)	4.1 (0.04)	4.1 (0.04)	0.34
IL-6, pg/mL	2774	1.5 (0.05)	1.6 (0.05)	1.5 (0.05)	1.4 (0.04)	0.01
CRP, mg/L	2826	3.2 (0.2)	4.1 (0.2)	3.8 (0.3)	3.3 (0.2)	0.47
sTNF-R1, pg/mL	2834	1409 (17)	1358 (15)	1374 (17)	1323 (15)	0.003
EPA+DPA+DHA, % total FA (median)		3.9	5.0	6.3	8.7	
Systolic BP, mmHg	2834	126.3 (0.8)	125.6 (0.7)	126.1 (0.8)	125.9 (0.8)	0.84
Diastolic BP, mmHg	2834	71.8 (0.4)	71.7 (0.3)	72.1 (0.4)	72.1 (0.4)	0.59
Triglycerides, mg/dL	2833	137 (4.5)	137.1 (2.9)	138.6 (2.8)	129.9 (3)	0.13
HDL-C, mg/dL	2833	51.9 (0.5)	49.9 (0.5)	50.3 (0.5)	50.2 (0.5)	0.15
LDL-C, mg/dL	2788	114.9 (1.3)	117.9 (1.2)	115.2 (1.2)	120.2 (1.3)	0.013
Total:HDL-C	2833	4 (0..05)	4.2 (0.04)	4.1 (0.04)	4.1 (0.04)	0.28
IL-6, pg/mL	2774	1.6 (0.05)	1.6 (0.05)	1.5 (0.05)	1.3 (0.04)	<0.0001
CRP, mg/L	2826	3.6 (0.2)	3.9 (0.2)	3.5 (0.2)	3.4 (0.2)	0.42
sTNF-R1, pg/mL	2834	1389.2 (16.3)	1385 (16.6)	1370.5 (16.8)	1317 (14)	0.001
Plant n-3 PUFA						
ALA, % total FA (median)		0.11	0.15	0.19	0.25	
Systolic BP, mm Hg	2834	125.9 (0.6)	125.7 (0.8)	125.6 (0.7)	126.8 (0.8)	0.25

Continued

Table 4. Continued

	n	Quartiles of Plasma Phospholipid Fatty Acids				P_{trend}
		1	2	3	4	
Diastolic BP, mm Hg	2834	71.5 (0.3)	72.5 (0.4)	71.6 (0.3)	72.2 (0.4)	0.36
Triglycerides, mg/dL	2833	126.6 (2.3)	132.6 (3.4)	139.1 (3.4)	146.9 (4.4)	<0.0001
HDL-C, mg/dL	2833	50 (0.4)	50.7 (0.5)	50.6 (0.4)	51.2 (0.5)	0.08
LDL-C, mg/dL	2788	118.6 (1.1)	119.3 (1.3)	115.9 (1.1)	114 (1.2)	0.002
Total:HDL-C	2833	4.1 (0.04)	4.1 (0.05)	4.1 (0.04)	4.1 (0.05)	0.22
IL-6, pg/mL	2774	1.5 (0.04)	1.5 (0.05)	1.5 (0.04)	1.5 (0.05)	0.92
CRP, mg/L	2826	4.0 (0.2)	3.5 (0.2)	3.5 (0.2)	3.2 (0.2)	0.02
sTNF-R1, pg/mL	2834	1350 (13)	1355 (17)	1375 (15)	1385 (17)	0.08
n-6 PUFA						
LA, % total FA (median)		17.6	20.2	22.4	25.2	
Systolic BP, mm Hg	2834	126.7 (0.7)	126.7 (0.7)	125.4 (0.7)	125.2 (0.8)	0.09
Diastolic BP, mm Hg	2834	72.2 (0.4)	72.1 (0.4)	71.8 (0.3)	71.5 (0.4)	0.17
Triglycerides, mg/dL	2833	146.2 (3)	136.3 (2.7)	129.7 (2.8)	130.3 (4.7)	0.001
HDL-C, mg/dL	2833	50.8 (0.5)	50.2 (0.4)	50.1 (0.4)	51.3 (0.5)	0.53
LDL-C, mg/dL	2788	115.4 (1.3)	118 (1.2)	118.3 (1.1)	116.4 (1.2)	0.55
Total:HDL-C	2833	4.1 (0.04)	4.1 (0.04)	4.1 (0.04)	4.1 (0.05)	0.69
IL-6, pg/mL	2774	1.4 (0.04)	1.5 (0.04)	1.6 (0.04)	1.5 (0.05)	0.07
CRP, mg/L	2826	4.2 (0.3)	3.4 (0.2)	3.5 (0.2)	3.2 (0.2)	0.01
sTNF-R1, pg/mL	2834	1346.7 (14.9)	1349.5 (13)	1376.1 (17.5)	1390 (16)	0.04
AA, % total FA (median)		9.0	11.0	12.8	15.1	
Systolic BP, mm Hg	2834	126 (0.8)	125.7 (0.7)	126.1 (0.7)	126.1 (0.8)	0.79
Diastolic BP, mm Hg	2834	72 (0.4)	71.6 (0.4)	72.1 (0.4)	71.9 (0.4)	0.89
Triglycerides, mg/dL	2833	145.2 (4.7)	132.1 (2.9)	134.8 (3)	130.5 (2.7)	0.012
HDL-C, mg/dL	2833	48.9 (0.5)	50.8 (0.5)	50 (0.5)	52.6 (0.5)	<0.0001
LDL-C, mg/dL	2788	117 (1.2)	118.6 (1.2)	117.1 (1.2)	115.4 (1.3)	0.23
Total:HDL-C	2833	4.3 (0.05)	4.1 (0.04)	4.1 (0.04)	3.9 (0.04)	<0.0001
IL-6, pg/mL	2774	1.6 (0.05)	1.5 (0.04)	1.5 (0.04)	1.5 (0.05)	0.060
CRP, mg/L	2826	3.5 (0.2)	3.1 (0.2)	3.5 (0.2)	4.2 (0.3)	0.03
sTNF-R1, pg/mL	2834	1435.8 (17.2)	1349.3 (14.6)	1343 (15.6)	1335 (16)	<0.0001
LA+AA, % total FA (median)		30.1	32.6	34.4	36.7	
Systolic BP, mmHg	2834	127.3 (0.7)	126.6 (0.7)	125.8 (0.7)	124.3 (0.7)	0.004
Diastolic BP, mmHg	2834	72.6 (0.4)	71.9 (0.4)	71.8 (0.3)	71.3 (0.4)	0.01
Triglycerides, mg/dL	2833	150.6 (3.1)	143.2 (3.6)	128.6 (2.7)	119.9 (3.6)	<0.0001
HDL-C, mg/dL	2833	49.2 (0.5)	50.2 (0.5)	50.9 (0.4)	52 (0.5)	<0.0001
LDL-C, mg/dL	2788	116.8 (1.2)	118 (1.1)	117.4 (1.2)	115.9 (1.2)	0.54
Total:HDL-C	2833	4.2 (0.04)	4.2 (0.04)	4.1 (0.04)	4 (0.04)	<0.0001
IL-6, pg/mL	2774	1.5 (0.04)	1.5 (0.05)	1.5 (0.04)	1.5 (0.04)	0.54
CRP, mg/L	2826	3.7 (0.2)	3.6 (0.2)	3.7 (0.2)	3.3 (0.2)	0.25
sTNF-R1, pg/mL	2834	1389.3 (14.7)	1349.5 (15.4)	1369.7 (15)	1354 (16)	0.20

Values are mean (SE). Means are adjusted for age (years), sex, race/ethnicity (white, African American, Hispanic, Chinese), education (<high school, high school, some college, college graduate), field center, smoking status (never, former, current) and pack-years of cigarette smoking, prevalent diabetes (yes, no), alcohol use (g/day), physical activity (active and inactive leisure, MET-min/week), and body mass index. (kg/m^2). AA indicates arachidonic acid; ALA, alpha-linolenic acid; BP, blood pressure; CRP, C-reactive protein; CVD, cardiovascular disease; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; HDL-C, high-density lipoprotein cholesterol; IL, interleukin; LA, linoleic acid; LDL-C, low-density lipoprotein cholesterol; MESA, Multi-Ethnic Study of Atherosclerosis; MET, metabolic equivalent; PUFA, polyunsaturated fatty acids SE, standard error of the mean; sTNF-R1, tumor necrosis factor-α soluble receptor 1.

was not associated with systolic blood pressure, diastolic blood pressure, total:HDL-C, or IL-6. Similarly, circulating n-6 PUFAs showed no associations with blood pressure, except for a trend toward lower systolic blood pressure for higher circulating LA levels. LA and AA were inversely associated with triglycerides and showed no associations with LDL-C; however, phospholipid AA, but not LA, showed beneficial associations with HDL-C and total:HDL-C (Table 4).

All statistically significant associations remained unchanged after additional adjustment for other dietary factors, including intakes of fruits, vegetables, and whole grains; or for use of lipid-lowering and anti-hypertensive medication (not shown).

Prospective Associations of Seafood n-3 PUFA With Incident CVD

A total of 189 new CVD cases were identified during 19 778 person-years of follow-up from 2000 to 2010. After adjustment for potential confounders, circulating EPA and DHA, but not DPA, were inversely associated with lower risk of CVD incidence, with a 51% lower risk in the highest quartile of EPA (HR [95% CI], 0.49 [0.30 to 0.82]), and 61% lower risk in the highest quartile of DHA (HR [95% CI], 0.39 [0.22 to 0.67]) (Table 5). Findings were similar when evaluating fatty acids continuously (HR [95% CI] for each interquartile median range difference increase of circulating PUFAs was 0.59 [0.40 to 0.86]) for EPA, 0.71 [0.49 to 1.02] for DPA, and 0.48 [0.30 to 0.75] for DHA). Findings of dietary estimated seafood n-3 PUFA intake with incident CVD were similar; however, associations were smaller and CIs wider, consistent with high within-person variation in the dietary measures.

Prospective Associations of ALA and n-6 PUFA With Incident CVD

In contrast to favorable associations with seafood n-3 PUFA, we found no statistically significant associations between biomarkers or self-reported measures of ALA or AA and incident CVD in MESA (Table 6). Multivariate-adjusted HR (95% CI) in the highest quartiles was 1.19 (0.79 to 1.78; P_{trend}=0.51) for phospholipid ALA, and 0.88 (0.55 to 1.41; P_{trend}=0.67) for circulating AA. On the other hand, we found evidence of a trend toward increased CVD risk associated with higher LA concentrations (HR [95% CI] in the highest quartile, 1.75 [1.11 to 2.75; P_{trend}=0.08]). This association was not significant for dietary LA or when circulating LA was evaluated continuously. Overall, findings were similar for CHD outcomes, although associations of circulating seafood-derived PUFA with CHD outcomes were slightly stronger than those of total CVD (Tables 7 and 8).

Sensitivity Analyses

Results did not change substantially when we restricted the analysis to participants who were not taking fish oil supplements and in similar analysis excluding angina. For example, the multivariate-adjusted HR (95% CI) after excluding angina (n=132 cases) was 0.40 (0.23 to 0.72; P_{trend}=0.004) and 0.48 (0.25 to 0.93; P_{trend}=0.02) for the highest quartiles of EPA and DHA, respectively.

Influence of Potential Mediators

Further adjustment for factors that could be potential mediators or confounders, including plasma triglycerides, HDL-cholesterol, and LDL-cholesterol, use of lipid-lowering medication, and baseline inflammation (plasma IL-6, CRP, and sTNF-R1) did not appreciably alter our findings. For example, HR (95% CI) of CVD in the highest quartiles was 0.46 (0.29 to 0.75) for circulating EPA and 0.38 (0.22 to 0.67) for DHA.

Heterogeneity by Sex and Race/Ethnicity

Associations of circulating n-3 and n-6 PUFAs with CVD were similar across sex. For example, HR (95% CI) in the highest quartiles of EPA was 0.39 (0.17 to 0.88) in women and 0.58 (0.30 to 1.11) in men, whereas HR (95% CI) for high DHA levels was 0.32 (0.12 to 0.87) in women and 0.41 (0.20 to 0.86) in men. Associations for each n-3 and n-6 PUFA were also similar in each race/ethnic group, except for circulating DPA. The multivariate-adjusted HR (95% CI) for each interquartile range difference in circulating DPA levels was 0.46 (0.24 to 0.92) in whites, 0.27 (0.10 to 0.70) in Chinese, 1.51 (0.75 to 3.03) in African Americans, and 1.33 (0.62 to 2.85) in Hispanics (*P* for interaction=0.01; Table 9).

Discussion

In this large prospective cohort of multiethnic Americans, higher circulating EPA and DHA were each inversely associated with markers of inflammation and prospectively associated with lower CVD incidence. Circulating DPA, a fatty acid largely derived from endogenous metabolism, was inversely associated with CVD events in whites and Chinese, but not in African Americans and Hispanics. Conversely, circulating plant-derived n-3 PUFA (ALA) was not associated with most CVD risk factors or with CVD incidence. In addition, although higher total circulating n-6 PUFAs were inversely associated with systolic and diastolic blood pressure, plasma triglycerides, and total:HDL-C ratio, there were no significant associations between total or individual n-6 PUFAs and CVD risk. When we evaluated n-3 and n-6 dietary PUFAs, nearly all findings were generally

Table 5. Hazard Ratios (95% CIs) of Total CVD* by Sex-Specific Quartiles and Interquartile Median Range Units of Seafood n-3 PUFA in US Adults

	Sex-Specific Quartiles of Plasma Phospholipid Fatty Acids (n=2837)					
	1	2	3	4	P_{trend}	HR (95% CI) for 1-IQR†—Unit Difference in FA Concentrations
Phospholipid EPA, % total FA (median)	0.40	0.60	0.86	1.62		
n	732	711	695	699		
Cases	66	48	47	28		
Multivariate model‡	REF	0.75 (0.51, 1.09)	0.84 (0.56, 1.25)	0.49 (0.30, 0.79)	0.01	0.59 (0.40, 0.86)
Phospholipid DPA, % total FA (median)	0.72	0.88	1.01	1.21		
n	752	701	747	637		
Cases	56	58	43	32		
Multivariate model‡	REF	1.05 (0.72, 1.53)	0.77 (0.51, 1.16)	0.75 (0.48, 1.18)	0.11	0.71 (0.49, 1.02)
Phospholipid DHA, % total FA (median)	2.5	3.5	4.5	6.0		
n	694	738	693	712		
Cases	59	61	46	23		
Multivariate model‡	REF	0.95 (0.65, 1.39)	0.70 (0.45, 1.08)	0.39 (0.22, 0.67)	<0.001	0.50 (0.32, 0.78)
Phospholipid EPA+DPA +DHA, % total FA (median)	3.9	5.0	6.3	8.7		
n	736	688	713	700		
Cases	63	59	40	27		
Multivariate model‡	REF	0.97 (0.67, 1.40)	0.64 (0.41, 1.00)	0.47 (0.28, 0.79)	0.002	0.46 (0.29, 0.72)
	Sex-specific quartiles of dietary fatty acids (n=2372)					
	1	2	3	4		
Dietary EPA, mg/day (median)	10	20	40	90		
n	599	547	585	641		
Cases	56	40	33	32		
Multivariate model‡	REF	0.94 (0.62, 1.42)	0.65 (0.42, 1.03)	0.60 (0.37, 0.98)	0.03	0.67 (0.46, 0.97)
Dietary DPA, mg/day (median)	4.0	10	19	39		
n	622	618	559	573		
Cases	53	50	30	28		
Multivariate model‡	REF	1.10 (0.74, 1.63)	0.67 (0.42, 1.07)	0.59 (0.35, 0.97)	0.02	0.70 (0.48, 1.02)
Dietary DHA, mg/day (median)	20	50	80	150		
n	606	572	600	594		
Cases	46	45	42	28		
Multivariate model‡	REF	0.98 (0.64, 1.49)	0.92 (0.59, 1.44)	0.60 (0.35, 1.02)	0.048	0.66 (0.44, 1.00)
Dietary EPA+DPA +DHA, mg/day (median)	40	80	140	280		
n	600	546	651	575		
Cases	47	47	39	28		
Multivariate model‡	REF	1.16 (0.77, 1.75)	0.81 (0.52, 1.28)	0.64 (0.38, 1.09)	0.05	0.66 (0.44, 0.98)

BMI indicates body mass index; CHD, coronary heart disease; CI, confidence interval; CVD, cardiovascular disease; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; MI, myocardial infarction; PUFA, polyunsaturated fatty acid.

*CHD events comprise MI, resuscitated cardiac arrest, definite and probable angina (if followed by revascularization), and CHD death.

†1-interquartile median ranges (IQR)=1.2 (phospholipid EPA), 0.49 (phospholipid DPA), 3.5 (phospholipid DHA), 4.8 (phospholipid EPA+DPA+DHA), 80 (dietary EPA), 35 (dietary DPA), 130 (dietary DHA) and 240 (dietary EPA+DPA+DHA).

‡Multivariate model: field center, age (years), sex, race/ethnicity (Whites, African American, Hispanic, and Chinese), education (<high school, high school, >high school), cigarette smoking (never, current or former smokers, and pack-years of cigarette smoking), alcohol (g/day), physical activity (active and inactive leisure in metabolic equivalents [min/week]), BMI (kg/m^2), prevalent diabetes (yes/no), total energy intake (kcal/day), weekly dietary supplement use (yes/no), and hypertensive medication use (yes/no), fruits and vegetables (servings/day), fiber (g/day), processed and unprocessed meat (servings/day), vitamin E (IU/day), saturated fat (% energy), and transfat intake (g/day).

Table 6. Hazard Ratios (95% CIs) of Total CVD* by Sex-Specific Quartiles and Interquartile Median Range Units of n-3 and n-6 PUFA in US Adults

	Sex-Specific Quartiles of Plasma Phospholipid Fatty Acids (n=2837)					
	1	2	3	4	P_{trend}	HR (95% CI) for 1-IQR†—Unit Difference in FA Concentrations
Phospholipid ALA, % total FA (median)	0.11	0.15	0.19	0.25		
n	883	569	757	628		
Cases	63	37	46	43		
Multivariate model‡	REF	0.95 (0.63, 1.43)	0.92 (0.62, 1.35)	1.19 (0.79, 1.78)	0.51	1.03 (0.83, 1.28)
Phospholipid LA, % total FA (median)	17.6	20.2	22.4	25.2		
n	714	697	718	708		
Cases	45	56	38	50		
Multivariate model‡	REF	1.39 (0.93, 2.08)	0.95 (0.61, 1.48)	1.75 (1.11, 2.75)	0.08	1.35 (0.92, 1.97)
Phospholipid AA, % total FA (median)	9.0	11.0	12.8	15.1		
n	705	724	702	706		
Cases	41	47	52	49		
Multivariate model‡	REF	0.97 (0.63, 1.50)	1.06 (0.69, 1.63)	0.88 (0.55, 1.41)	0.67	0.90 (0.61, 1.32)
Phospholipid LA+AA, % total FA (median)	30.1	32.6	34.4	36.7		
n	710	712	714	701		
Cases	41	58	43	47		
Multivariate model‡	REF	1.36 (0.90, 2.04)	1.03 (0.66, 1.60)	1.31 (0.84, 2.02)	0.44	1.22 (0.86, 1.74)
	Sex-specific quartiles of dietary fatty acids (n=2372)					
	1	2	3	4		
Dietary ALA, mg/day (median)	390	690	1020	1610		
n	700	592	555	525		
Cases	44	42	43	32		
Multivariate model§	REF	0.88 (0.56, 1.34)	0.94 (0.55, 1.59)	0.61 (0.29, 1.28)	0.20	0.69 (0.39, 1.22)
Dietary LA, mg/day (median)	3870	6230	9220	14 980		
n	638	581	605	548		
Cases	53	24	46	38		
Multivariate model§	REF	0.47 (0.28, 0.78)	0.87 (0.54, 1.41)	0.74 (0.38, 1.43)	0.81	0.83 (0.55, 1.25)
Dietary AA, mg/day (median)	40	80	120	200		
n	605	530	667	570		
Cases	40	36	42	43		
Multivariate model§		0.96 (0.60, 1.53)	1.06 (0.66, 1.67)	1.26 (0.72, 2.20)	0.34	1.08 (0.71, 1.63)
Dietary LA+AA, mg/day (median)	4000	6300	9400	15 200		
n	630	583	607	552		
Cases	52	25	46	38		
Multivariate model§	REF	0.49 (0.30, 0.81)	0.87 (0.54, 1.41)	0.74 (0.38, 1.44)	0.77	1.14 (0.63, 2.05)

AA indicates arachidonic acid; ALA, alpha-linolenic acid; BMI, body mass index; CI, confidence interval; CVD, cardiovascular disease; LA, linoleic acid; PUFA, polyunsaturated fatty acid.
*CHD events comprise MI, resuscitated cardiac arrest, definite and probable angina (if followed by revascularization), and CHD death.
†Interquartile median ranges (IQR): 0.14 (phospholipid ALA), 7.6 (phospholipid LA), 6.1 (phospholipid AA), 6.6 (phospholipid LA+AA), 1200 (dietary ALA), 11 100 (dietary LA), 160 (dietary AA), 11 200 (dietary LA+AA).
‡Multivariate model: field center, age (years), sex, race/ethnicity (Caucasian, African American, Hispanic and Chinese), education (<high school, high school, >high school), cigarette smoking (never, current or former smokers, and pack-years of cigarette smoking), alcohol (g/day), physical activity (active and inactive leisure in metabolic equivalents per min/week), BMI (kg/m^2), prevalent diabetes (yes/no), total energy intake (kcal/day),weekly dietary supplement use (yes/no) and hypertensive medication use (yes/no), fruits and vegetables (serving/day), fiber (g/day), processed and unprocessed meat (serving/day), vitamin E (IU/day), saturated fat (% energy) and trans fat intake (g/day).
§This model included all variables listed in ‡, except dietary vitamin E.

Table 7. Hazard Ratios (and 95% Confidence Intervals) of Total CHD* by Sex-Specific Quartiles and Interquartile Median Range Units of Seafood-Derived PUFA in 2837 Adults

	Sex-Specific Quartiles of Plasma Phospholipid Fatty Acids (n=2837)					
	1	2	3	4	P_{trend}	HR (95% CI) for 1-IQR†–Unit Difference in FA Concentrations
Phospholipid EPA, % total FA (median)	0.40	0.60	0.86	1.62		
n	732	711	695	699		
Cases	49	38	37	17		
Multivariate plus dietary factors‡	REF	0.82 (0.53, 1.26)	0.93 (0.59, 1.46)	0.42 (0.23, 0.75)	0.004	0.51 (0.31, 0.82)
Phospholipid DPA, % total FA (median)	0.72	0.88	1.01	1.21		
n	752	701	747	637		
Cases	41	43	33	24		
Multivariate plus dietary factors‡	REF	1.10 (0.71, 1.70)	0.84 (0.53, 1.35)	0.80 (0.48, 1.35)	0.29	0.71 (0.47, 1.09)
Phospholipid DHA, % total FA (median)	2.5	3.5	4.5	6.0		
n	694	738	693	712		
Cases	48	45	34	14		
Multivariate plus dietary factors‡		0.87 (0.57, 1.34)	0.66 (0.4, 1.09)	0.29 (0.15, 0.58)	0.0002	0.43 (0.25, 0.82)
Phospholipid EPA+DPA +DHA, % total FA (median)	3.9	5.0	6.3	8.7		
n	736	688	713	700		
Cases	50	42	30	19		
Multivariate plus dietary factors‡	REF	0.92 (0.60, 1.41)	0.66 (0.4, 1.10)	0.45 (0.25, 0.82)	0.006	0.40 (0.23, 0.69)
	Sex-specific quartiles of dietary fatty acids (n=2392)					
	1	2	3	4		
Dietary EPA, mg/day (median)	7.3	21	40	85		
n	599	547	585	641		
Cases	40	33	28	21		
Multivariate plus dietary factors‡	REF	1.12 (0.70, 1.80)	0.82 (0.50, 1.37)	0.61 (0.34, 1.10)	0.06	0.66 (0.43, 1.02)
Dietary DPA, Mg/day (median)	4.3	11	19	39		
n	622	618	559	573		
Cases	40	40	24	18		
Multivariate plus dietary factors‡	REF	1.19 (0.76, 1.86)	0.74 (0.50, 1.26)	0.54 (0.29, 0.99)	0.02	0.65 (0.41, 1.03)
Dietary DHA, mg/day (median)	24	49	80	150		
n	606	572	600	594		
Cases	34	34	36	18		
Multivariate plus dietary factors‡	REF	1.02 (0.63, 1.67)	1.12 (0.68, 1.86)	0.57 (0.30, 1.09)	0.09	0.64 (0.39, 1.05)
Dietary EPA+DPA +DHA, mg/day (median)	38	82	140	280		
n	600	546	651	575		
Cases	33	39	32	18		
Multivariate plus dietary factors‡	REF	1.39 (0.86, 2.23)	1.00 (0.60, 1.69)	0.64 (0.34, 1.22)	0.08	

BMI indicates body mass index; CHD, coronary heart disease; CRP, C-reactive protein; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; MI, myocardial infarction; PUFA, polyunsaturated fatty acid; sTNF-R1, tumor necrosis factor-a soluble receptor 1.

*CHD events comprise MI, resuscitated cardiac arrest, definite and probable angina (if followed by revascularization), and CHD death.

†One interquartile median range is 1.2 for phospholipid EPA, 0.49 for phospholipid DPA, 3.5 for phospholipid DHA, 4.8 for phospholipid EPA+DPA+DHA, 80 for dietary EPA, 35 for dietary DPA, 130 for dietary DHA, and 240 for dietary EPA+DPA+DHA.

‡Multivariate model: field center, age (years), sex, race/ethnicity (Caucasian, African American, Hispanic, and Chinese), education (<high school, high school, >high school), cigarette smoking (never, current, or former smokers and pack-years of cigarette smoking), alcohol (g/day), physical activity (active and inactive leisure in metabolic equivalents [min/week]), BMI (kg/m^2), prevalent diabetes (yes/no), total energy intake (kcal/day), weekly dietary supplement use (yes/no), hypertensive medication use (yes/no), fruits and vegetables (servings/day), processed and unprocessed meat (servings/day), fiber (g/day), vitamin E (IU/day), saturated fat (% energy), and trans-fat intake (g/day).

Table 8. Hazard Ratios (and 95% Confidence Intervals) of Total CHD* by Sex-Specific Quartiles and Interquartile Median Range Units of n-3 and n-6 PUFAs in 2837 Adults

	Sex-Specific Quartiles of Plasma Phospholipid Fatty Acids (n=2837)					
	1	2	3	4	P_{trend}	HR (95% CI) for 1-IQR†—Unit Difference in FA Concentrations
Phospholipid ALA, % total FA (median)	0.11	0.15	0.19	0.25		
n	883	569	757	628		
Cases	46	27	37	31		
Multivariate plus dietary factors‡	REF	0.93 (0.58, 1.51)	1.02 (0.65, 1.58)	1.18 (0.74, 1.91)	0.48	1.04 (0.83, 1.32)
Phospholipid LA, % total FA (median)	17.6	20.2	22.4	25.2		
n	714	697	718	708		
Cases	31	42	30	38		
Multivariate plus dietary factors‡	REF	1.47 (0.92, 2.37)	1.07 (0.64, 1.81)	1.88 (1.11, 3.19)	0.07	1.46 (0.94, 2.26)
Phospholipid AA, % total FA (median)	9.0	11.0	12.8	15.1		
n	705	724	702	706		
Cases	30	36	37	38		
Multivariate plus dietary factors‡	REF	1.01 (0.61, 1.65)	1.02 (0.62, 1.69)	0.99 (0.58, 1.69)	0.98	1.00 (0.64, 1.55)
Phospholipid LA+AA, % total FA (median)	30.1	32.6	34.4	36.7		
n	710	712	714	701		
Cases	30	40	30	41		
Multivariate plus dietary factors‡	REF	1.29 (0.80, 2.09)	1.01 (0.60, 1.69)	1.63 (1.00, 2.67)	0.10	1.47 (0.96, 2.17)
	Sex-specific quartiles of dietary fatty acids (n=2372)					
	1	2	3	4		
Dietary ALA, mg/day (median)	450	760	1080	1690		
n	700	592	555	525		
Cases	34	33	33	22		
Multivariate plus dietary factors‡§	REF	0.93 (0.55, 1.56)	0.99 (0.53, 1.83)	0.60 (0.25, 1.41)	0.24	0.67 (0.30, 1.31)
Dietary LA, mg/day (median)	3870	6230	9220	14 980		
n	638	581	605	548		
Cases	43	18	33	28		
Multivariate plus dietary factors‡	REF	0.45 (0.25, 0.80)	0.79 (0.45, 1.37)	0.71 (0.33, 1.51)	0.73	1.20 (0.60, 2.40)
Dietary AA, mg/day (median)	40	80	120	200		
n	605	530	667	570		
Cases	30	30	30	32		
Multivariate plus dietary factors‡	REF	1.08 (0.64, 1.84)	1.00 (0.58, 1.74)	1.30 (0.68, 2.48)	0.45	1.06 (0.67, 1.69)
Dietary LA+AA, mg/day (median)	4000	6300	9400	15 200		
n	630	583	607	552		
Cases	42	19	33	28		
Multivariate plus dietary factors‡	REF	0.48 (0.27, 0.85)	0.79 (0.45, 1.38)	0.72 (0.33, 1.53)	0.71	1.20 (0.60, 2.41)

AA indicates arachidonic acid; ALA, alpha-linolenic acid; BMI, body mass index; CHD, coronary heart disease; CVD, cardiovascular disease; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; LA, linoleic acid; MI, myocardial infarction; PUFA, polyunsaturated fatty acid.

*CHD events comprise MI, resuscitated cardiac arrest, definite and probable angina (if followed by revascularization), and CVD death.

†One interquartile median range is 0.14 for phospholipid ALA, 1.2 for phospholipid EPA, 0.49 for phospholipid DPA, 3.5 for phospholipid DHA, 4.8 for phospholipid EPA+DPA+DHA, 80 for dietary EPA, 35 for dietary DPA, 130 for dietary DHA, and 240 for dietary EPA+DPA+DHA. ‡Interquartile median ranges: 0.14 for phospholipid ALA, 7.6 for phospholipid LA, 6.1 for phospholipid AA, 6.6 for phospholipid LA+AA, 1200 for dietary ALA, 11 100 for dietary LA, 160 for dietary AA, and 11 200 for dietary LA+AA.

‡Multivariate model: field center, age (years), sex, race/ethnicity (Caucasian, African American, Hispanic, and Chinese), education (<high school, high school, >high school), cigarette smoking (never, current, or former smoker and pack-years of cigarette smoking), alcohol (g/day), physical activity (active and inactive leisure in metabolic equivalents [min/week]), BMI (kg/m2), prevalent diabetes (yes/no), total energy intake (kcal/day), weekly dietary supplement use (yes/no), hypertensive medication use (yes/no), processed and unprocessed meat (servings/day), fiber (g/day), fruits and vegetables (servings/day), vitamin E (IU/day), saturated fat (% energy), and trans-fat intake (g/day).

§Included all variables listed in ‡, except dietary vitamin E.

similar, although with broader confidence intervals. These findings build and expand on prior studies investigating the relationship between n-3 and n-6 PUFAs and CVD risk by providing evidence from both biomarker and dietary fatty acids in a large, prospective multiethnic cohort.

Several mechanisms may explain the favorable associations between EPA and DHA and CVD risk. In experimental studies, higher n-3 PUFA levels alter cell membrane fluidity and receptor responses, regulate gene transcription, and serve as metabolic precursors to potent anti-inflammatory molecules.[9] These molecular effects may underlie their demonstrated systemic and cardiac benefits, such as on inflammatory responses, autonomic control, vascular and cardiac hemodynamics, endothelial function, blood lipids, and possibly thrombosis.[9,24] The observed inverse associations with inflammatory markers in the present study support both previous observations[25,26] and recent experimental studies suggesting anti-inflammatory effects of n-3 PUFA and their downstream metabolites.[27] Our cross-sectional findings for blood lipids are also broadly consistent with results from clinical trials that demonstrated that EPA+DHA supplementation lowers triglyceride levels and slightly increases LDL-cholesterol levels,[28,29] the latter being largely related to increases in LDL particle size rather than particle number (apolipoprotein B levels). In contrast, DPA was associated with higher triglyceride levels; that DPA is not strongly correlated with dietary intake may reflect endogenous metabolism, and its lipid and metabolic effects are understudied. Taken together, these results build on prior studies and add new evidence of potential pathways for CVD development.

Prospective associations of EPA and DHA are consistent with previous studies. In a meta-analysis including >300 000 participants, the pooled relative risk (95% CI) of total CHD for extreme quartiles of dietary seafood n-3 PUFA intake was 0.86 (0.75 to 0.97)[30]; associations with CHD mortality were stronger.[5] Fewer observational studies have evaluated circulating biomarkers of seafood-PUFA and incident CVD, mostly in white participants; these studies have generally seen inverse associations with risk.[31–36] Our results in a multiethnic cohort demonstrate, for the first time to our knowledge, the generalizability of these favorable associations across race/ethnicities. In contrast to these generally consistent results from observational studies of dietary EPA and DHA, the effects of fish oil supplements on CVD in randomized, controlled trials have been mixed,[3] with older trials showing benefits and more recent studies showing no significant effects.[3] Heterogeneity in population characteristics, background dietary fish consumption, and length of follow-up may each contribute to these inconsistent findings from different study designs. For example, prior evidence suggests that risk reduction for CHD mortality is maximal

Table 9. Hazard Ratios (and 95% Confidence Intervals) of Total CVD for Circulating Seafood-Derived PUFAs in 2837 US Adults According to Race/Ethnicity

	HR (95% CI) per IQR*, MV1†	HR (95% CI) per IQR*, MV2‡
Phospholipid EPA		
All	0.60 (0.41 to 0.87)	0.59 (0.40 to 0.86)
White	0.45 (0.22 to 0.92)	0.38 (0.18 to 0.79)
Chinese	0.49 (0.22 to 1.10)	0.57 (0.25 to 1.28)
African American	0.76 (0.40 to 1.47)	0.77 (0.40 to 1.50)
Hispanic	0.74 (0.28 to 1.92)	0.87 (0.34 to 2.22)
P-interaction	0.90	0.90
Phospholipid DPA		
All	0.72 (0.50 to 1.03)	0.71 (0.49 to 1.02)
White	0.46 (0.24 to 0.92)	0.41 (0.21 to 0.82)
Chinese	0.27 (0.10 to 0.70)	0.30 (0.11 to 0.81)
African American	1.51 (0.75 to 3.03)	1.51 (0.74 to 3.09)
Hispanic	1.33 (0.62 to 2.85)	1.33 (0.61 to 2.90)
P-interaction	0.01	0.01
Phospholipid DHA		
All	0.50 (0.32 to 0.78)	0.48 (0.30 to 0.75)
White	0.45 (0.20 to 1.01)	0.34 (0.15 to 0.81)
Chinese	0.30 (0.11 to 0.84)	0.37 (0.12 to 1.08)
African American	0.42 (0.17 to 1.04)	0.42 (0.17 to 1.05)
Hispanic	0.68 (0.25 to 1.84)	0.73 (0.25 to 2.13)
P-interaction	0.80	0.85
Phospholipid EPA+DPA+DHA		
All	0.48 (0.30 to 0.75)	0.46 (0.29 to 0.72)
White	0.37 (0.16 to 0.86)	0.28 (0.12 to 0.68)
Chinese	0.31 (0.11 to 0.82)	0.37 (0.13 to 1.03)
African American	0.51 (0.21 to 1.26)	0.51 (0.20 to 1.27)
Hispanic	0.71 (0.25 to 2.00)	0.79 (0.26 to 2.38)
P-interaction	0.84	0.88

Total number of participants (cases) per racial/ethnic stratum: 724 (62) whites, 712 (28) Chinese, 696 (48) African Americans, 705 (51) Hispanics. AA indicates arachidonic acid; CI, confidence interval; CVD, cardiovascular disease; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; HR, hazard ratio; IQR, interquartile median range; MV, multivariate model; PUFA, polyunsaturated fatty acid.

*One IQR is 1.2 for phospholipid EPA, 0.49 for phospholipid DPA, 3.5 for phospholipid DHA, 4.8 for phospholipid EPA+DPA+DHA.

†Multivariate model: field center, age (years), sex, race/ethnicity (Whites, African Americans, Hispanics, and Chinese Americans), education (<high school, high school, >high school), cigarette smoking (never, current, or former smokers and pack-years of cigarette smoking), alcohol (g/day), physical activity (active and inactive leisure in metabolic equivalents [min/week]), BMI (kg/m^2), prevalent diabetes (yes/no), total energy intake (kcal/day), weekly dietary supplement use (yes/no), and hypertensive medication use (yes/no).

‡Multivariate model 1+fruits and vegetables (servings/day), fiber (g/day), processed and unprocessed meat (servings/day), vitamin E (IU/day), saturated fat (% energy), and transfat intake (g/day).

when going from zero intake to modest intake (≈250 mg/day EPA+DHA), with little to no additional benefit at higher levels of intake.[5] Thus, in more recent trials that included populations with relatively high background fish consumption, the addition of fish oil supplementation may have only had a small impact on CVD, at least over only a few years. Because few participants in MESA were taking fish oil supplements, our results support the benefits of higher blood levels of n-3 PUFA across dietary levels of consumption for primary prevention of CVD in multiethnic populations.

In contrast to EPA and DHA, associations between circulating DPA and CVD risk were heterogeneous across race/ethnicities. Consistent with earlier studies,[31,35] we found weak associations between fish consumption and phospholipid DPA, suggesting that endogenous metabolism influences circulating DPA concentrations, for example, by chain elongation and desaturation of EPA. Genetic variants of enzymes involved in this metabolic conversion are associated with DPA levels.[37] Furthermore, these genetic variants are associated with altered inflammatory responses and risk of CHD.[38] Whether race-specific genetic differences in n-3 PUFA metabolism could explain the observed heterogeneity by race in associations of circulating DPA with CVD deserves further investigation. These results could also be due to chance. Our findings support the need to understand the dietary and metabolic determinants and cardiovascular effects of circulating DPA in different ethnic groups.

Although ALA improved several CVD risk factors in experimental studies,[39–41] we did not find significant associations between this plant-derived n-3 PUFA and CVD events. Our findings are in line with a recent systematic review and meta-analysis that identified substantial inconsistencies and overall weak associations of dietary and biomarker ALA with CVD risk in observational studies.[42] We also found no evidence of inverse associations with CVD of circulating or dietary LA or AA; these findings contrast with evidence from meta-analyses of observational studies and clinical trials that estimated a 10% to 13% reduction in incident CHD with replacement of 5% energy from saturated fat with PUFAs.[4,6] Indeed, we found a nonsignificant trend toward higher CVD risk with higher circulating LA, as also seen in older US adults.[32] Differences in food sources of ALA and LA may at least partly explain inconsistencies across prior studies. For example, some of the largest sources of ALA and LA in the US diet are refined-grain breads, desserts, pizza, popcorn, french fries, potato chips, burgers, and processed meats,[43,44] together accounting for ≈30% and 40% of ALA and LA intake, respectively.[43,44] In contrast, most randomized trials used vegetable oils as the source of PUFAs, whereas observational studies showing favorable associations evaluated replacement of saturated fat or carbohydrate with PUFA intake. These diverse potential sources suggest that other components present in less healthy food sources might be counterbalancing potential beneficial effects of ALA and LA.

Our study has important strengths. The use of both circulating PUFA biomarkers and dietary estimates provided a comprehensive evaluation of the relationships of interest, including exposures with different and complementary strengths, limitations, and sources of bias. The prospective cohort design minimized recall and selection bias and allowed inference on temporality of associations. Adjustment for a variety of demographic, lifestyle, and other risk factors minimized the potential for confounding. Finally, the multiethnic nature of the cohort allowed separate evaluations of different race/ethnic groups and increased the generalizability of our findings.

Potential limitations should be considered. Cross-sectional associations with cardiovascular risk factors could be limited by reverse causation, yet several of these associations were consistent with the demonstrated effects of n-3 and n-6 PUFA in prior metabolic studies, as well as with prospective analysis of CVD events in our study. Imprecision in measurement of both circulating and dietary PUFAs may result in exposure misclassification, attenuating measures of association toward the null. Thus, our findings could have underestimated the true associations. Finally, although we carefully adjusted for several potential confounders, we cannot exclude the possibility of residual confounding. Nevertheless, our observed associations were robust to adjustment for a range of major potential risk factors.

In conclusion, we found an inverse associations of seafood-derived long-chain n-3 PUFAs, but not plant-derived n-3 or n-6 PUFAs, with CVD incidence in a multiethnic cohort. These findings suggest that increased consumption of n-3 PUFAs from seafood may prevent CVD development in multiethnic populations.

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