ORIGINAL RESEARCH

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Circulating Omega-3 Polyunsaturated Fatty Acids and Subclinical Brain Abnormalities on MRI in Older Adults: The Cardiovascular Health Study

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***Background*—**Consumption of tuna or other broiled or baked fish, but not fried fish, is associated with fewer subclinical brain abnormalities on magnetic resonance imaging (MRI). We investigated the association between plasma phospholipid omega-3 polyunsaturated fatty acids (PUFAs), objective biomarkers of exposure, and subclinical brain abnormalities on MRI.

***Methods and Results*—**In the community-based Cardiovascular Health Study, 3660 participants aged ≥65 underwent brain MRI in 1992–1994, and 2313 were rescanned 5 years later. MRIs were centrally read by neuroradiologists in a standardized, blinded manner. Participants with recognized transient ischemic attacks or stroke were excluded. Phospholipid PUFAs were measured in stored plasma collected in 1992–1993 and related to cross-sectional and longitudinal MRI findings. After multivariable adjustment, the odds ratio for having a prevalent subclinical infarct was 0.60 (95% CI, 0.44 to 0.82; *P* for trend=0.001) in the highest versus lowest long-chain omega-3 PUFA quartile. Higher long-chain omega-3 PUFA content was also associated with better white matter grade, but not with sulcal or ventricular grades, markers of brain atrophy, or with incident subclinical infarcts. The phospholipid intermediate-chain omega-3 PUFA alpha-linolenic acid was associated only with modestly better sulcal and ventricular grades. However, this finding was not supported in the analyses with alpha-linolenic acid intake.

Conclusions*—**Among older adults, higher phospholipid long-chain omega-3 PUFA content was associated with lower prevalence of subclinical infarcts and better white matter grade on MRI. Our results support the beneficial effects of fish consumption, the major source of long-chain omega-3 PUFAs, on brain health in later life. The role of plant-derived alpha-linolenic acid in brain health requires further investigation. (J Am Heart Assoc.*** **2013;2:e000305 doi: 10.1161/JAHA.113.000305)**

Key Words: fatty acids • fish • magnetic resonance imaging • lacunar infarct • white matter disease

Findings on magnetic resonance imaging (MRI), including subclinical (lacunar) infarcts, white matter abnormalities (leukoaraiosis), and ventricular and sulcal enlargement, that is, brain atrophy, are common in the elderly. Even in the absence of overt clinical events, such as transient ischemic attacks (TIAs) or stroke, these subclinical MRI findings are associated with impairments in health. Subclinical infarcts and white matter abnormalities are likely related to vascular disease of small cerebral vessels[1,2] and are associated with impairments in cognition, gait, and mood with increased risk of subsequent dementia, stroke, and death.[3–8] White matter changes over time have also been found to accelerate cognitive decline and increase risk of cardiovascular disease and death.[8–10] Brain atrophy has been associated with increased risk of physical functional decline, cognitive decline, and dementia.[11–13] Therefore, identification of modifiable risk factors for these MRI findings would open the possibility of preventing these devastating outcomes in the elderly.

Fish consumption is associated with lower risk of stroke[14] and also of dementia and cognitive decline,[15] for which subclinical brain abnormalities are a risk factor.[6] We have shown that consumption of tuna or other broiled or baked fish, but not fried fish, was associated with lower risk of

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stroke[16] and subclinical brain abnormalities.[17] Tuna/other fish, but not fried fish, also correlated with plasma phospholipid long-chain omega-3 polyunsaturated fatty acids (PUFAs),[16] suggesting that these fatty acids could be at least partly responsible for the benefits of fish consumption.

Circulating biomarkers of omega-3 PUFAs provide objective measures that reflect both dietary consumption (eg, fish and fish oil) and relevant biologic processes (eg, elongation of the intermediate-chain omega-3 PUFA alpha-linolenic acid [ALA] to longer-chain omega-3 PUFAs). Biomarker levels also permit direct assessment of specific individual omega-3 PUFAs, including the long-chain eicosapentaenoic acid (EPA, 20:5n-3), docosapentaenoic acid (DPA, 22:5n-3), and docosahexaenoic acid (DHA, 22:6n-3), and the intermediate-chain ALA (18:3n-3), for which prior data on brain health is limited and conflicting.[18–20] Yet few studies have investigated associations between circulating omega-3 PUFAs and MRI findings.[21–23] We investigated the association of plasma phospholipid omega-3 PUFAs, a biomarker for longer-term circulating concentrations, with subclinical brain abnormalities in older adults, including both cross-sectional and prospective analyses based on serial MRIs.

Methods

Study Population

The Cardiovascular Health Study (CHS) is a prospective cohort study of 5888 older adults. The design and recruitment experience have been described.[24,25] Briefly, 5201 men and women aged ≥65 at baseline were randomly selected and enrolled in 1989–1990 from Medicare eligibility lists in 4 US communities: Forsyth County, North Carolina; Sacramento County, California; Washington County, Maryland; and Allegheny County, Pennsylvania. An additional 687 black participants were similarly recruited and enrolled in 1992–1993. Each center's institutional review committee approved the study. All subjects gave informed written consent.

Blood Sample Collection and Fatty Acid Measurements

Plasma phospholipid omega-3 PUFAs were measured in stored plasma specimens taken at the 1992–1993 examination in 3941 participants, as described.[26] Blood was drawn after 12 hours of fasting, stored at −70°C, and shipped on dry ice for long-term storage at −80°C until analyzed.[26] As previously described,[26] lipid fractions were separated by thin-layer chromatography, and 45 individual fatty acids were measured as a percentage of total concentrations using gas chromatography. Phospholipid fatty acids represent a biomarker of longer-term (4- to 8-week) circulating concentrations, with similar responses as fatty acids in erythrocyte membranes.[27] No degradation, lipolysis, or oxidation has been observed after 10 years in the blood storage conditions in CHS.[28] Interassay CVs were 2.1% for EPA, 1.5% for DPA, 1.6% for DHA, and 3.1% for ALA. Long-term reliability of these measures has also been described[26] and is similar to other major risk factors such as blood pressure.[29]

Brain Imaging

All CHS participants were invited to undergo MRI scanning in 1991–1994. The average time between blood collection and MRI scanning was 112 days (range, −517 to 650 days). A total of 3660 (62%) underwent scanning and were slightly younger and healthier than those who did not.[1] All participants were again invited to undergo MRI scanning 5 years later, in 1997–1999, and 2313 were scanned. A total of 2116 participants underwent both scans and were healthier than the 1544 who underwent only the initial scan, including a lower prevalence of cardiovascular disease, hypertension, diabetes, current smoking, and higher income and education.[5]

The cranial MRI scanning protocol included sagittal T1-weighted localizer images and axial T1-, spin-density-, and T2-weighted images.[30] Without knowledge of participants' clinical information, neuroradiologists at the centralized CHS reading center identified infarcts and estimated white matter, ventricular, and sulcal grades, as detailed previously.[1,31,32] Brain infarct was defined as an area of abnormal signal intensity ≥3 mm in size in a vascular distribution that lacked mass effect.[32] Grades were defined using a semiquantitative 10-point scale from 0 to 9 (most abnormal) based on comparison with templates.[1] Ventricular grades ranged from slit-like ventricles (grade 0) to markedly enlarged ventricles (grade 9). Sulcal grades ranged in a similar fashion. White matter grades were estimated by the total extent of periventricular and subcortical white matter signal abnormality on spin-density-weighted axial images graded by successive increase from no changes or barely detectable changes (grades 0 and 1, respectively) to almost all white matter involved (grade 9).[31] As described,[33] ventricular and sulcal grades were grouped for analysis as ≤2, 3, 4, and ≥5 and white matter grade as ≤1, 2, 3, and ≥4.

To evaluate changes in white matter between the serial MRI scans, all scans were reread side by side without knowledge of their order or their previous readings to minimize any potential reader bias.[9] Changes in ventricular and sulcal grades were not estimated. Because of technical limitations, 197 of the 2116 paired scans could not be reread. Demographics, cardiovascular risk factors, and prevalent cardiovascular disease were similar in the 1919 included and 197 excluded participants (data not shown).

Consistent with prior analyses,[17] for cross-sectional analyses of subclinical infarcts, we excluded participants with prevalent clinical TIAs or stroke or with possible (<3-mm) infarcts or prior hemorrhage at the first MRI (Figure 1). For prospective analyses we also excluded participants with subclinical infarcts at the first MRI or with clinically diagnosed TIAs or stroke between the 2 MRIs (Figure 2). For cross-sectional analyses of white matter, ventricles, and sulci, we excluded participants with prevalent clinical TIAs or stroke. For serial changes in white matter grade we also excluded participants with TIAs or stroke before the second MRI.

Diet and Other Risk Factors

All participants underwent extensive baseline evaluations including standard questionnaires, physical examination, performance measures, and laboratory testing.[24,25,32] Prevalent coronary heart disease, stroke, TIA, hypertension, and diabetes were defined using patients' reports and confirmed by centralized review of hospital and clinic records.[24,25] Dietary habits were assessed by reproducible and validated semiquantitative food-frequency questionnaires (FFQ) in 1989–1990 and in 1995–1996.

For the analyses with incident subclinical infarcts in the secondary analyses with dietary ALA, we calculated the cumulative average intake of the 2 FFQs or used data from only 1 if the other was not available. In the analyses with prevalent subclinical findings, we used data from the first FFQ. We also examined whether our previously observed inverse association of consumption of tuna/other fish with risk of MRI findings in this cohort[17] would be affected by adjustment for phospholipid long-chain omega-3 PUFA. Fish intake was assessed in 1989–1990 as previously described.[17]

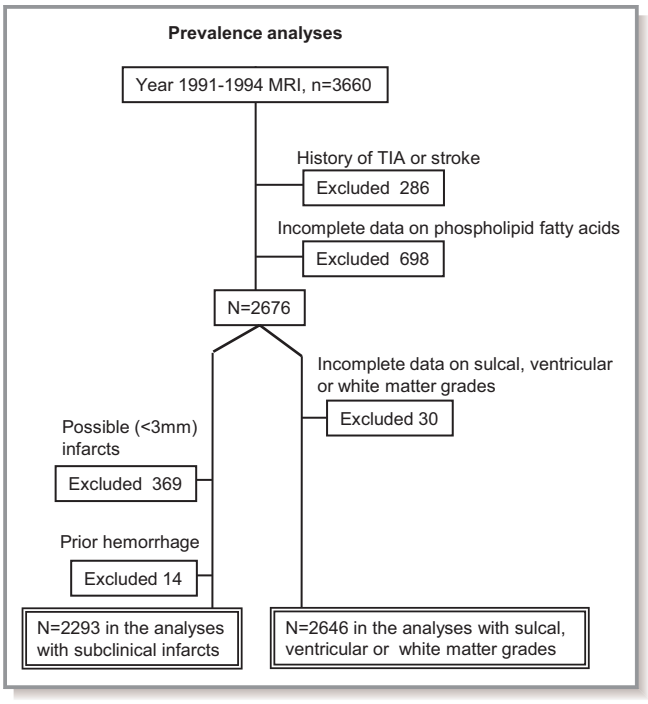

Figure 1. Participants included in the cross-sectional analyses of circulating omega-3 polyunsaturated fatty acids and subclinical magnetic resonance imaging (MRI) abnormalities. TIA indicates transient ischemic attack.

Statistical Methods

We explored the univariate relationships between phospholipid EPA+DPA+DHA and ALA and baseline characteristics by means and linear regression (for continuous variables) or χ^2 tests (for dichotomous variables). We related the phospholipid PUFA to the risk of subclinical infarcts and worsening white matter grade by logistic regression. Only 61 subjects experienced white matter worsening by ≥2 grades between scans, so they were grouped with those experiencing worsening by 1 grade. Risk of number of subclinical infarcts (range, 0 to 4) was evaluated using ordinal logistic regression. Sulcal, ventricular, and white matter grades in quartiles of phospholipid PUFAs were evaluated using analysis of covariance (see list of covariates below). Absolute risk reduction was calculated by multiplying the absolute risk in the reference group by the multivariable-adjusted risk reduction in the comparison group.

Multivariate models included confounders that were selected on the basis of the previously observed associations between fish consumption and MRI findings.[17] The final models included age (years), sex, race (white/other), enrollment center (4 sites), diabetes (yes/no), education (<high school, high school, >high school), smoking (never/former/current), pack-years of smoking, body mass index (kg/m^2), prevalent coronary heart disease (yes/no), alcohol use (beverages/week), physical activity (kcal/week), energy intake (kcal/day), and meat consumption and vegetable consumption (servings/week in quartiles). Further adjustment for proportions of other phospholipid fatty acid biomarkers or the time between blood collection and the MRI scanning did not appreciably alter (<5%) the risk estimates (data not shown). The cohort mean was used to replace missing values in covariates (<12.5% in dietary variables, <3.0% in others). Results were similar if missing values were excluded. We also conducted a sensitivity analysis by evaluating the impact of adjusting for phospholipid long-chain omega-3 PUFAs on the previously observed association between tuna/other fish intake and MRI findings[10] in participants with data on both exposures (n=2079). Likelihood ratio tests using multiplicative interaction terms were used to explore potential effect modification by age, sex, race, education, diabetes, coronary heart disease,

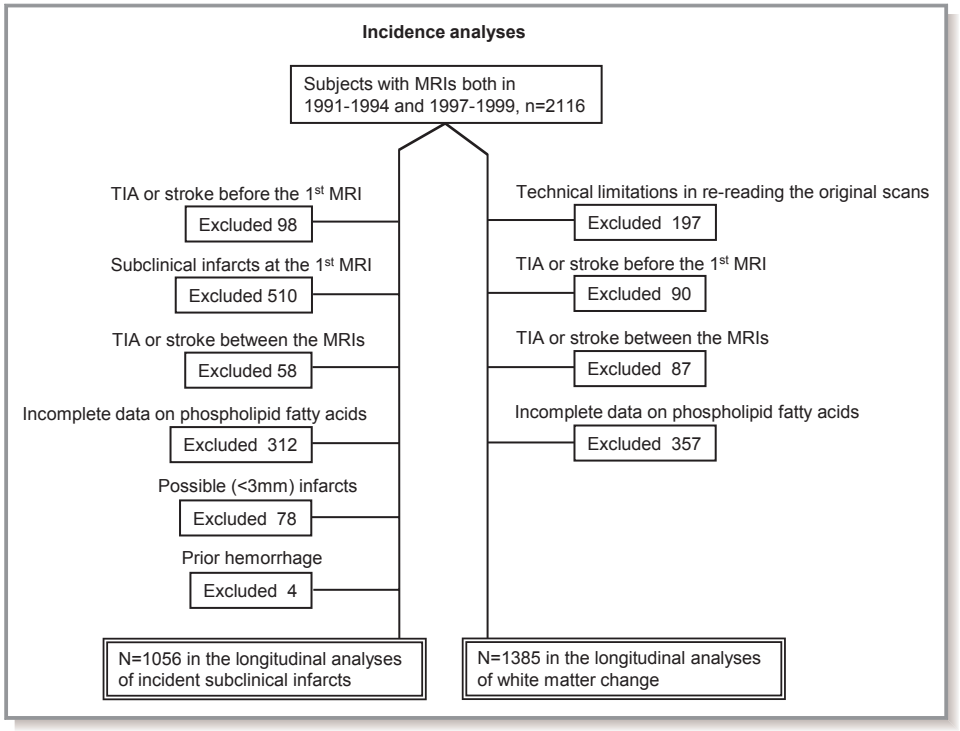

Figure 2. Participants included in the longitudinal analyses of circulating omega-3 polyunsaturated fatty acids and subclinical magnetic resonance imaging (MRI) abnormalities. TIA indicates transient ischemic attack.

hypertension, alcohol intake, smoking, or use of aspirin, lipid-lowering, or hypertension medications. Correlations were estimated by Spearman correlation coefficients. All *P* values were 2-tailed (α=0.05). Analyses were performed using SPSS 19.0 for Windows (SPSS Inc, Chicago, IL).

Results

Descriptive Data

The mean (±SD) phospholipid proportions were 0.60% (0.39%) for EPA, 0.84% (0.17%) for DPA, 3.06% (0.98%) for DHA and 0.15% (0.05%) for ALA of total fatty acids. At baseline, higher EPA+DPA+DHA proportion was related to nonwhite race, higher education, and lower triglyceride concentrations (Table 1). Higher EPA+DPA+DHA proportion was also positively associated with consumption of tuna/other fish and estimated EPA+DHA consumption, but not with fried fish consumption, as well as with lower intake of red meat and higher intake of fruits and vegetables. Baseline characteristics according to EPA, DPA, and DHA have been published previously.[26]

Higher phospholipid ALA proportion was related to female sex, white race, current smoking, and higher education (Table 1). Higher ALA proportion was also related to lower BMI, serum LDL cholesterol, and intake of red meat; higher intakes of tuna/other fish, fruits, and vegetables; and higher estimated intakes of EPA+DHA and ALA.

Phospholipid Long-Chain Omega-3 PUFAs and Subclinical Infarcts

A total of 534 of 2293 participants (23.3%) had 1 or more prevalent subclinical infarcts on the first MRI. In multivariable-adjusted analyses, the odds for having a subclinical infarct were 40% lower (OR, 0.60; 95% CI, 0.44 to 0.82; *P* for trend=0.001) in the highest versus the lowest phospholipid EPA+DPA+DHA quartile (Table 2). On the basis of an absolute risk of 27.1% in the first quartile, the absolute risk reduction for low EPA+DHA+DHA consumption was 10.8%. Evaluating the number of subclinical infarcts, the odds of each additional multiple infarct was 41% lower in the highest versus the lowest quartile (OR, 0.59; 95% CI, 0.44 to 0.80; *P* for trend<0.001). When each long-chain omega-3 PUFA was evaluated individually, only DHA was significantly associated with lower risk (Table 2) (reference group absolute risk=25.1%; absolute risk reduction in the highest DHA quartile=8.0%). The OR of each additional multiple infarct in the highest versus the lowest DHA quartile was 0.67 (95% CI, 0.49 to 0.90; *P* for trend=0.001). Simultaneous adjustment for EPA and DPA had no effect on this association (data not shown).

A total of 170 participants (16.1%) experienced an incident first subclinical infarct between the 2 MRI scans. The associations between total or individual long-chain omega-3 PUFAs and incident subclinical infarct did not achieve statistical significance (Table 3), but directions of association were similar to those seen for prevalent subclinical infarcts.

Table 1. Baseline Characteristics According to Plasma Phospholipid Omega-3 Polyunsaturated Fatty Acids

Characteristic	EPA+DPA+DHA, %				ALA, %			
	<3.64 (n=573)	3.64 to 4.22 (n=573)	4.23 to 5.04 (n=574)	>5.04 (n=573)	<0.11 (n=580)	0.11 to 0.14 (n=566)	0.15 to 0.18 (n=576)	>0.18 (n=571)
Age, y	75.1 (4.9)	75.3 (5.0)	75.1 (4.9)	75.0 (5.3)	74.9 (4.9)	74.9 (5.0)	75.5 (5.3)	75.2 (4.9)
Male sex, %	44	39	38	39	48	38	39	34*
White race, %	96	92	87	80*	86	88	90	90*
Current smoker, %	11	8	7	8	11	9	7	7*
Education ≥high school diploma, %	69	70	78	80*	68	74	75	79*
Coronary heart disease, %	20	21	21	19	21	21	22	18
BMI, kg/m^2	26.2 (4.4)	27.0 (4.5)	26.9 (4.7)	26.3 (4.4)	27.5 (4.5)	26.9 (4.6)	26.4 (4.5)	25.6 (4.2)*
Systolic blood pressure, mm Hg	134 (20)	135 (21)	135 (20)	133 (20)	133 (20)	135 (21)	135 (20)	134 (21)
LDL-C, mg/dL	127 (35)	125 (36)	129 (32)	126 (32)	129 (34)	127 (34)	127 (32)	123 (34)*
Triglycerides, mg/dL	145 (95)	145 (76)	143 (74)	131 (76)*	141 (68)	141 (76)	144 (91)	139 (87)
Leisure-time activity, kcal/week	1856 (4544)	2042 (7828)	1454 (1608)	1734 (4492)	1889 (6026)	1521 (1840)	1633 (4406)	2039 (6136)
Alcohol, drinks/week	2.0 (4.8)	1.8 (5.0)	2.3 (5.0)	2.2 (4.6)	2.0 (5.0)	1.8 (4.7)	2.2 (4.9)	2.3 (4.9)
Tuna/other fish, servings/week	1.2 (1.3)	1.3 (1.1)	1.8 (1.4)	2.3 (1.6)*	1.5 (1.4)	1.6 (1.3)	1.6 (1.4)	1.8 (1.5)*
Fried fish, servings/week	0.3 (0.5)	0.3 (0.5)	0.3 (0.5)	0.3 (0.6)	0.3 (0.5)	0.3 (0.5)	0.3 (0.6)	0.3 (0.4)
Beef or pork, servings/day	1.0 (0.8)	0.8 (0.6)	0.8 (0.6)	0.6 (0.6)*	0.9 (0.7)	0.8 (0.7)	0.8 (0.7)	0.7 (0.6)*
Fruits, servings/day	2.0 (1.1)	2.1 (1.1)	2.2 (1.1)	2.4 (1.1)*	2.0 (1.1)	2.1 (1.1)	2.2 (1.1)	2.3 (1.1)*
Vegetables, servings/day	2.3 (1.3)	2.4 (1.3)	2.6 (1.4)	2.8 (1.4)*	2.3 (1.3)	2.5 (1.3)	2.5 (1.4)	2.7 (1.4)*
EPA+DHA, g/day	0.20 (0.21)	0.26 (0.22)	0.32 (0.20)	0.42 (0.25)*	0.28 (0.21)	0.30 (0.23)	0.29 (0.26)	0.31 (0.22)*
ALA, g/day	1.60 (0.53)	1.60 (0.53)	1.60 (0.51)	1.61 (0.54)	1.53 (0.51)	1.59 (0.51)	1.63 (0.55)	1.67 (0.53)*

Values are means (SDs) for continuous variables or percentages for categorical variables. ALA indicates alpha-linolenic acid; BMI, body mass index; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; LDL-C, low-density lipoprotein cholesterol.
*P<0.05 across categories of plasma phospholipid omega-3 polyunsaturated fatty acids.

Phospholipid Long-Chain Omega-3 PUFAs and White Matter, Sulcal, and Ventricular Grades

In cross-sectional analyses, higher phospholipid EPA+DPA+DHA was associated with better white matter grade (Table 4). After multivariable adjustment, those in the highest quartile had 9.2% better white matter grade, compared with those in the lowest quartile (P for trend=0.001). The associations were similar for EPA (difference between the extreme quartiles 9.3%, P for trend=0.002) and DHA (difference, 8.5%; P for trend=0.001), but appeared weaker with DPA (difference, 3.6%; P for trend=0.08). When EPA, DPA, and DHA were included in the model simultaneously, only the association with DHA remained statistically significant (difference, 7.2%; P for trend=0.01). EPA+DPA+DHA or individual fatty acids were not significantly associated with markers of brain atrophy, that is, sulcal or ventricular grades (P>0.05 for each, Table 4).

In prospective analyses, 383 of 1385 participants (27.7%) experienced a worsening of white matter grade (a decrement of ≥1 grade) between the MRI scans. In multivariable-adjusted analyses, participants in the highest EPA+DPA+DHA quartile had 42% lower odds of developing worsening white matter (OR, 0.58; 95% CI, 0.40 to 0.84; P for trend=0.002) compared with the lowest quartile (Table 5). When individual long-chain omega-3 PUFAs were evaluated separately, only DHA was significantly associated with lower risk (Table 5).

Phospholipid and Dietary ALA and Subclinical MRI Findings

Phospholipid ALA content was not associated with prevalent or incident subclinical infarcts or white matter grade (Tables 2 through 5). However, higher phospholipid ALA was associated with modestly better sulcal grade (in the highest quartile, 3.8% better; *P* for trend=0.02) and ventricular grade (3.0% better, *P* for trend=0.03) compared with the lowest quartile. Adjustment for phospholipid long-chain n-3 PUFAs had no effect on these associations (data not shown).

In secondary analyses, dietary ALA was not significantly associated with any of the MRI findings, except for a cross-sectional association with white matter grade, for which those in the highest quartile of consumption had a 4.4% better grade (*P* for trend=0.04) compared with the lowest quartile (other data not shown).

Sensitivity Analyses

Before adjustment for phospholipid long-chain omega-3 PUFAs, the multivariable-adjusted ORs of prevalent subclinical infarct across categories of fish intake were 1, 0.95, 0.84, and 0.70 (*P* for trend=0.09). Adjustment for phospholipid DHA substantially attenuated the association; the ORs were 1, 0.98, 0.96, and 0.87 (*P* for trend=0.54). In contrast, adjustment for fish intake had no effect on the association between phospholipid long-chain omega-3 PUFAs and MRI findings. For example, the extreme-quartile OR for prevalent subclinical infarct in the quartiles of phospholipid DHA was 0.70 (95% CI, 0.51 to 0.96; *P* for trend=0.003) after adjustment. Similar results were also observed with the other MRI findings (data not shown).

We did not find evidence for significant effect modification by age, sex, race, education, diabetes, coronary heart disease, hypertension, alcohol intake, smoking, or

Table 2. Plasma Phospholipid Omega-3 Polyunsaturated Fatty Acids and Risk of Prevalent Subclinical Infarcts

	Fatty Acid Quartile				
	1 (n=573)	2 (n=573)	3 (n=574)	4 (n=573)	*P* Trend
EPA+DPA+DHA, %	<3.64	3.64 to 4.22	4.23 to 5.04	>5.04	
Number of cases	155	145	126	108	
Model 1	1	0.89 (0.68 to 1.16)	0.74 (0.56 to 0.97)	0.60 (0.45 to 0.79)	<0.001
Model 2	1	0.91 (0.69 to 1.19)	0.75 (0.57 to 0.99)	0.60 (0.44 to 0.82)	0.001
EPA, %	<0.39	0.39 to 0.51	0.52 to 0.68	>0.68	
Number of cases	142	147	121	124	
Model 1	1	1.05 (0.80 to 1.37)	0.84 (0.63 to 1.11)	0.84 (0.64 to 1.11)	0.12
Model 2	1	1.05 (0.80 to 1.38)	0.87 (0.65 to 1.16)	0.88 (0.66 to 1.18)	0.27
DPA, %	<0.72	0.72 to 0.82	0.83 to 0.94	>0.94	
Number of cases	141	136	128	129	
Model 1	1	0.93 (0.71 to 1.23)	0.84 (0.64 to 1.10)	0.87 (0.66 to 1.15)	0.25
Model 2	1	0.98 (0.74 to 1.30)	0.87 (0.65 to 1.15)	0.92 (0.69 to 1.22)	0.41
DHA, %	<2.36	2.36 to 2.88	2.89 to 3.58	>3.58	
Number of cases	144	165	112	113	
Model 1	1	1.21 (0.93 to 1.58)	0.70 (0.53 to 0.93)	0.69 (0.52 to 0.92)	0.001
Model 2	1	1.22 (0.94 to 1.60)	0.72 (0.54 to 0.96)	0.68 (0.50 to 0.92)	0.001
ALA, %	<0.11	0.11 to 0.14	0.15 to 0.18	>0.18	
Number of cases	138	126	132	138	
Model 1	1	0.91 (0.69 to 1.21)	0.92 (0.70 to 1.22)	1.01 (0.77 to 1.33)	0.85
Model 2	1	0.93 (0.70 to 1.24)	0.95 (0.72 to 1.26)	1.07 (0.80 to 1.42)	0.55

Values are odds ratios (95% CIs). Model 1 adjusted for age (years), sex, and race (white, nonwhite). Model 2 adjusted for model 1 and enrollment center (4 sites), diabetes (yes/no), education (<high school, high school, >high school), smoking status (never, former, current), smoking history (pack-years), body mass index (kg/m^2), CHD at the time of MRI (yes/no), alcohol use (beverages/week), physical activity (kcal/week), total energy intake (kcal/day), and meat and vegetable consumption (quartiles). ALA indicates alpha-linolenic acid; CHD, coronary heart disease; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; MRI, magnetic resonance imaging.

Table 3. Plasma Phospholipid Omega-3 Polyunsaturated Fatty Acids and Risk of Incident Subclinical Infarcts

	Fatty Acid Quartile				
	1 (n=264)	2 (n=264)	3 (n=264)	4 (n=264)	*P* Trend
EPA+DPA+DHA, %	<3.71	3.71 to 4.31	4.32 to 5.16	>5.16	
Number of cases	42	51	43	34	
Model 1	1	1.27 (0.81 to 2.00)	1.04 (0.65 to 1.67)	0.78 (0.47 to 1.28)	0.17
Model 2	1	1.23 (0.78 to 1.96)	1.00 (0.62 to 1.61)	0.77 (0.46 to 1.31)	0.20
EPA, %	<0.40	0.40 to 0.53	0.54 to 0.70	>0.70	
Number of cases	42	51	43	34	
Model 1	1	1.28 (0.82 to 2.02)	1.04 (0.65 to 1.67)	0.81 (0.49 to 1.32)	0.21
Model 2	1	1.26 (0.79 to 1.99)	0.99 (0.61 to 1.60)	0.80 (0.47 to 1.34)	0.23
DPA, %	<0.72	0.72 to 0.82	0.83 to 0.94	>0.94	
Number of cases	42	45	46	37	
Model 1	1	1.06 (0.67 to 1.68)	1.08 (0.68 to 1.71)	0.84 (0.52 to 1.37)	0.50
Model 2	1	1.09 (0.68 to 1.74)	1.09 (0.68 to 1.74)	0.84 (0.51 to 1.39)	0.49
DHA, %	<2.37	2.37 to 2.96	2.97 to 3.64	>3.64	
Number of cases	38	58	41	33	
Model 1	1	1.69 (1.07 to 2.66)	1.09 (0.67 to 1.77)	0.84 (0.50 to 1.40)	0.15
Model 2	1	1.62 (1.01 to 2.57)	1.05 (0.64 to 1.71)	0.81 (0.47 to 1.39)	0.17
ALA, %	<0.11	0.11 to 0.14	0.15 to 0.18	>0.18	
Number of cases	39	40	49	42	
Model 1	1	1.06 (0.65 to 1.71)	1.30 (0.82 to 2.07)	1.07 (0.66 to 1.72)	0.71
Model 2	1	1.11 (0.68 to 1.81)	1.38 (0.85 to 2.23)	1.19 (0.72 to 1.97)	0.44

Values are odds ratios (95% CIs). Model 1 adjusted for age (years), sex, and race (white, nonwhite). Model 2 adjusted for model 1 and enrollment center (4 sites), diabetes (yes/no), education (<high school, high school, >high school), smoking status (never, former, current), smoking history (pack-years), body mass index (kg/m^2), CHD at the time of MRI (yes/no), alcohol use (beverages/week), physical activity (kcal/week), total energy intake (kcal/day), and meat and vegetable consumption (quartiles). ALA indicates alpha-linolenic acid; CHD, coronary heart disease; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; MRI, magnetic resonance imaging.

use of aspirin, lipid-lowering, or hypertension medications (*P* for interactions>0.05). We also did not find evidence for interaction between phospholipid long-chain n-3 PUFAs and ALA proportions (*P*>0.05). Exclusion of participants who used fish oil supplements (3.8%) did not change the associations (data not shown).

Discussion

In this study among older men and women, plasma phospholipid long-chain omega-3 PUFAs, and in particular DHA, were associated with specific findings consistent with better brain health, including lower risk of prevalent subclinical (lacunar) infarcts, better white matter grade, and lower risk of worsening white matter. Although the direction of the associations with incident subclinical infarct was similar to the observed lower risk of prevalent subclinical infarcts in the present analysis and also incident ischemic stroke,[34] the associations were not statistically significant, possibly because of lower power in these analyses. The phospholipid long-chain omega-3 PUFAs were not associated with other MRI metrics, such as markers of brain atrophy, that is, sulcal and ventricular grades. Phospholipid ALA, an intermediate-chain omega-3 PUFA from plants, only had borderline associations with better sulcal and ventricular grades.

The results from the current study extend our previous findings that intake of tuna/other fish, but not fried fish, was associated with lower risk of subclinical infarcts and better white matter grade, but not with sulcal and ventricular grades.[17] In contrast to tuna/other fish meals, the types of fish used in fried fish meals, such as fish burgers or fish sticks, are typically low in EPA+DHA. Tuna/other fish, but not fried fish, correlated with plasma phospholipid long-chain omega-3 PUFAs, which suggests that these fatty acids at least partly mediate the beneficial effects of fish consumption. This is also supported by the attenuation of the associations with tuna/other fish consumption after adjustment for phospholipid DHA.

Fish is the major dietary source of the long-chain omega-3 PUFAs, and DHA is the most abundant in diet and in

Table 4. Plasma Phospholipid Omega-3 Polyunsaturated Fatty Acids and White Matter, Sulcal, and Ventricular Grades

	Fatty Acid Quartile				
	1 (n=662)	2 (n=661)	3 (n=662)	4 (n=661)	*P* Trend
EPA+DPA+DHA, %	<3.64	3.64 to 4.24	4.25 to 5.06	>5.06	
White matter grade	2.29 (2.18 to 2.39)	2.23 (2.13 to 2.34)	2.23 (2.13 to 2.33)	2.08 (1.98 to 2.19)	0.001
Sulcal grade	3.40 (3.31 to 3.48)	3.33 (3.24 to 3.41)	3.35 (3.27 to 3.43)	3.36 (3.28 to 3.45)	0.93
Ventricular grade	3.53 (3.44 to 3.62)	3.60 (3.51 to 3.70)	3.62 (3.53 to 3.71)	3.53 (3.43 to 3.62)	0.46
EPA, %	<0.39	0.39 to 0.51	0.52 to 0.69	>0.69	
White matter grade	2.23 (2.13 to 2.34)	2.30 (2.19 to 2.40)	2.23 (2.13 to 2.34)	2.07 (1.97 to 2.18)	0.002
Sulcal grade	3.41 (3.32 to 3.49)	3.39 (3.31 to 3.47)	3.34 (3.26 to 3.42)	3.30 (3.21 to 3.38)	0.10
Ventricular grade	3.60 (3.51 to 3.70)	3.63 (3.54 to 3.72)	3.50 (3.41 to 3.60)	3.54 (3.45 to 3.63)	0.13
DPA, %	<0.73	0.73 to 0.82	0.83 to 0.94	>0.94	
White matter grade	2.25 (2.15 to 2.35)	2.26 (2.16 to 2.37)	2.15 (2.05 to 2.25)	2.17 (2.07 to 2.27)	0.08
Sulcal grade	3.36 (3.27 to 3.44)	3.43 (3.35 to 3.52)	3.35 (3.27 to 3.43)	3.30 (3.21 to 3.38)	0.07
Ventricular grade	3.69 (3.60 to 3.78)	3.51 (3.42 to 3.60)	3.50 (3.41 to 3.59)	3.59 (3.49 to 3.68)	0.11
DHA, %	<2.36	2.36 to 2.90	2.91 to 3.59	>3.59	
White matter grade	2.24 (2.14 to 2.35)	2.27 (2.17 to 2.38)	2.26 (2.16 to 2.37)	2.05 (1.95 to 2.16)	0.001
Sulcal grade	3.38 (3.29 to 3.46)	3.35 (3.27 to 3.44)	3.33 (3.25 to 3.41)	3.38 (3.29 to 3.47)	0.75
Ventricular grade	3.55 (3.46 to 3.65)	3.55 (3.46 to 3.64)	3.63 (3.54 to 3.72)	3.54 (3.45 to 3.64)	0.84
ALA, %	<0.11	0.11 to 0.14	0.15 to 0.18	>0.18	
White matter grade	2.17 (2.07 to 2.28)	2.32 (2.21 to 2.42)	2.21 (2.10 to 2.31)	2.15 (2.04 to 2.25)	0.29
Sulcal grade	3.44 (3.36 to 3.53)	3.36 (3.28 to 3.44)	3.32 (3.24 to 3.40)	3.31 (3.23 to 3.40)	0.02
Ventricular grade	3.62 (3.52 to 3.71)	3.65 (3.56 to 3.74)	3.51 (3.42 to 3.60)	3.51 (3.42 to 3.60)	0.03

Values are adjusted for age (years), sex, race (white, nonwhite), enrollment center (4 sites), diabetes (yes/no), education (<high school, high school, >high school), smoking status (never, former, current), smoking history (pack-years), body mass index (kg/m^2), CHD at the time of MRI (yes/no), alcohol use (beverages/week), physical activity (kcal/week), total energy intake (kcal/day), and meat and vegetable consumption (quartiles). ALA indicates alpha-linolenic acid; CHD, coronary heart disease; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; MRI, magnetic resonance imaging.

circulation. The endogenous synthesis of DHA from DPA is very limited,[35] so circulating DHA is a good biomarker for fish consumption. On the other hand, although fish contains small amounts of DPA, circulating DPA does not correlate with fish intake,[26] because DPA is mainly synthesized endogenously from EPA.[35] Although EPA and DHA are intercorrelated,[26] EPA can also be synthesized endogenously from ALA in limited amounts, so it correlates less strongly with fish consumption.[26,35] On the other hand, DHA is the most abundant long-chain omega-3 PUFA also in the brain, representing >95% of omega-3 PUFAs.[36] Thus, DHA may truly be most relevant for brain health. Although EPA and DHA both have physiological effects that could result in improved brain health, including reducing inflammation, oxidative stress, and platelet aggregation and improving arterial compliance, DHA also has favorable effects on blood pressure.[35] Hypertension is a major risk factor for subclinical infarcts and white matter abnormalities.[37] However, in our analyses the associations of DHA with MRI findings were independent of the baseline measure of blood pressure. Whether benefits could partly relate to long-term changes in blood pressure deserves further investigation.

Few studies have investigated the relations of circulating omega-3 PUFAs with MRI findings, but the cross-sectional analyses in the Framingham Study[21] and the Oregon Brain Aging Study[22] and the prospective analyses in the Three-City Study[23] support the beneficial associations of long-chain omega-3 PUFAs with subclinical brain abnormalities.

Phospholipid ALA was associated with better ventricular and sulcal grades but not with other MRI findings, suggesting potentially specific associations with brain atrophy. Brain atrophy is associated with increased risk of cognitive decline and dementia,[11,12] and dietary or circulating ALA has been associated with lower risk of cognitive decline in some[38–40] although not all studies.[41–43] The associations we observed for phospholipid ALA were of borderline statistical significance, and our secondary analyses with dietary ALA did not support the findings observed with phospholipid ALA. Thus, these findings should be interpreted cautiously, and confirmation in additional studies is needed, especially in populations with

Table 5. Plasma Phospholipid Omega-3 Polyunsaturated Fatty Acids and Risk of Worsening of White Matter by ≥1 Grade Between the 2 MRI Scans

	Fatty Acid Quartile				
	1 (n=345)	2 (n=346)	3 (n=346)	4 (n=345)	*P* Trend
EPA+DPA+DHA, %	<3.70	3.70 to 4.29	4.30 to 5.12	>5.12	
Number of cases	116	99	80	88	
Model 1	1	0.80 (0.58 to 1.10)	0.59 (0.42 to 0.83)	0.68 (0.48 to 0.96)	0.02
Model 2	1	0.81 (0.58 to 1.14)	0.60 (0.42 to 0.85)	0.58 (0.40 to 0.84)	0.002
EPA, %	<0.41	0.41 to 0.52	0.53 to 0.70	>0.70	
Number of cases	100	100	96	87	
Model 1	1	0.97 (0.70 to 1.36)	0.94 (0.67 to 1.31)	0.85 (0.61 to 1.19)	0.32
Model 2	1	1.02 (0.72 to 1.44)	0.94 (0.66 to 1.34)	0.75 (0.52 to 1.08)	0.09
DPA, %	<0.73	0.73 to 0.82	0.83 to 0.94	>0.94	
Number of cases	109	96	82	96	
Model 1	1	0.83 (0.60 to 1.16)	0.68 (0.49 to 0.96)	0.83 (0.60 to 1.16)	0.21
Model 2	1	0.83 (0.59 to 1.16)	0.72 (0.51 to 1.02)	0.85 (0.60 to 1.19)	0.31
DHA, %	<2.38	2.38 to 2.93	2.94 to 3.63	>3.63	
Number of cases	105	109	83	86	
Model 1	1	1.04 (0.75 to 1.43)	0.73 (0.52 to 1.02)	0.76 (0.54 to 1.07)	0.05
Model 2	1	1.06 (0.76 to 1.49)	0.75 (0.53 to 1.07)	0.64 (0.44 to 0.93)	0.01
ALA, %	<0.11	0.11 to 0.14	0.15 to 0.18	>0.18	
Number of cases	91	94	101	97	
Model 1	1	0.97 (0.69 to 1.36)	1.14 (0.81 to 1.59)	1.05 (0.75 to 1.47)	0.63
Model 2	1	0.97 (0.69 to 1.38)	1.14 (0.80 to 1.61)	1.04 (0.73 to 1.49)	0.69

Values are odds ratios (95% CIs). Model 1 adjusted for age (years), sex, race (white, nonwhite), and white matter grade at initial MRI scan. Model 2 adjusted for model 1 and enrollment center (4 sites), diabetes (yes/no), education (<high school, high school, >high school), smoking status (never, former, current), smoking history (pack-years), body mass index (kg/m^2), CHD at the time of MRI (yes/no), alcohol use (beverages/week), physical activity (kcal/week), total energy intake (kcal/day), and meat and vegetable consumption (quartiles). ALA indicates alpha-linolenic acid; CHD, coronary heart disease; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; MRI, magnetic resonance imaging.

low fish intake. On the other hand, because the majority of dietary ALA is oxidized and used for energy,[44] dietary ALA does not correlate well with circulating ALA concentrations. Thus, circulating ALA concentrations have other metabolic determinants that could at least partly explain the different results for phospholipid versus dietary ALA. Few studies have investigated the associations between ALA and MRI findings. Recently, plasma ALA was not associated with any of the studied MRI findings, including brain volume, in elderly subjects.[22] Our findings for ALA build on this preliminary research and indicate a need for further investigation to elucidate the potential role of ALA in brain health.

An important strength of our investigation is the use of circulating biomarkers of omega-3 PUFAs, which provide objective measures of exposures. Other strengths were the community-based recruitment, which increased generalizability; the large numbers of men and women enrolled, which increased power; the availability of 2 MRI scans for many participants for evaluation of longitudinal changes; the exclusion of participants with clinical TIA or stroke before MRI scan, which minimizes reverse causation and allowed us to focus on subclinical findings; and the standardized measurements of multiple covariates, which allowed us to adjust for a range of potential factors to minimize confounding.

A potential limitation was the availability of circulating PUFA measurements at only 1 time, which may cause bias by misclassification because of changes in PUFA concentrations over time and thus attenuate the associations toward the null in longitudinal analyses. The participants who underwent MRI scans were somewhat healthier than those who did not, so results may not be fully applicable to the sickest individuals in a general elderly population. We have adjusted for all measured health-related potential determinants of the second MRI in multivariate models. This will remove bias from selection related to these factors. In addition, by examining interactions of these health-related potential determinants of the second MRI with PUFAs, we have assessed evidence for

differential effects of PUFA among those with the second MRI. No such interactions were evident, strengthening the evidence that the observed findings are generalizable beyond the subgroup with the second MRI. Because serial MRI scans were not available for the whole study population, we may have had insufficient power to find statistically significant associations with incident subclinical infarcts. Although interreader reliabilities of white matter and ventricular grades were good, estimates of sulcal grade have greater interreader variability,[31] which would make it more difficult to detect associations with this latter end point.

In conclusion, our findings in these older men and women suggest that circulating long-chain omega-3 PUFA concentrations, a biomarker of regular fish consumption, are associated with lower risk and could be beneficial for the prevention of certain subclinical brain abnormalities that are commonly observed in the elderly. The potential role of the plant-based, intermediate-chain omega-3 PUFA ALA is less evident. Our results support the need for additional prospective observational studies using fatty acid biomarkers, as well as randomized intervention studies to evaluate the role of omega-3 PUFAs in subclinical brain health and disease later in life.

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