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Higher RBC EPA + DHA corresponds with larger total brain and hippocampal volumes

WHIMS-MRI Study

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ABSTRACT

Objective: To test whether red blood cell (RBC) levels of marine omega-3 fatty acids measured in the Women's Health Initiative Memory Study were related to MRI brain volumes measured 8 years later.

Methods: RBC eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and MRI brain volumes were assessed in 1,111 postmenopausal women from the Women's Health Initiative Memory Study. The endpoints were total brain volume and anatomical regions. Linear mixed models included multiple imputations of fatty acids and were adjusted for hormone therapy, time since randomization, demographics, intracranial volume, and cardiovascular disease risk factors.

Results: In fully adjusted models, a 1 SD greater RBC EPA + DHA (omega-3 index) level was correlated with 2.1 cm³ larger brain volume ($p = 0.048$). DHA was marginally correlated ($p = 0.063$) with total brain volume while EPA was less so ($p = 0.11$). There were no correlations between ischemic lesion volumes and EPA, DHA, or EPA + DHA. A 1 SD greater omega-3 index was correlated with greater hippocampal volume (50 mm³, $p = 0.036$) in fully adjusted models. Comparing the fourth quartile vs the first quartile of the omega-3 index confirmed greater hippocampal volume (159 mm³, $p = 0.034$).

Conclusion: A higher omega-3 index was correlated with larger total normal brain volume and hippocampal volume in postmenopausal women measured 8 years later. While normal aging results in overall brain atrophy, lower omega-3 index may signal increased risk of hippocampal atrophy. Future studies should examine whether maintaining higher RBC EPA + DHA levels slows the rate of hippocampal or overall brain atrophy. *Neurology*® 2014;82:435-442

GLOSSARY

AD = Alzheimer disease; **CRP** = C-reactive protein; **DHA** = docosahexaenoic acid; **EPA** = eicosapentaenoic acid; **FA** = fatty acid; **HDL** = high-density lipoprotein; **HT** = hormone therapy; **Q1** = first quartile; **Q4** = fourth quartile; **RBC** = red blood cell; **WHI** = Women's Health Initiative; **WHIMS** = Women's Health Initiative Memory Study.

Aging is associated with increased risk of dementia and cognitive impairment, with exponentially increasing prevalence expected in the next several decades.¹ Based on data from the Framingham study, a middle-aged woman in North America of European descent has approximately 20% risk of developing dementia of some type in her lifetime, which usually involves Alzheimer disease (AD).² Reduced brain volume is an important part of the pathology of AD, and hippocampal atrophy is frequently observed before symptomatic impairment.³

Omega-3 fatty acids (FAs) from marine sources could have an important role in maintaining brain structure and function with advancing age. Approximately 30% to 40% of FAs in gray matter of the cortex are docosahexaenoic acid (DHA),⁴ and this FA is particularly concentrated in synaptic membranes.⁵ However, in white matter, DHA constitutes only 4% of total FAs.⁶ Patients with AD have been reported to have decreased serum, brain, and neuronal DHA and/or eicosapentaenoic acid (EPA) compared with controls without dementia.⁷⁻¹¹ DHA levels within plasma phosphatidylcholine, in the highest quartile, were associated with a 40% to 50%

Supplemental data at
www.neurology.org

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reduction in risk of developing all-cause dementia or AD in the original Framingham cohort.¹² Most relevant to the present study, total brain volumes were directly related to RBC omega-3 levels in the Framingham Offspring cohort.¹³ In our study, we hypothesized that marine omega-3 FAs would be directly related to total brain volumes determined by MRI 8 years later, and then explored the relations in ischemic tissue and 13 gray and white matter anatomical regions.

METHODS MRI data. The MRI standardized protocol has been described previously,^{14–16} and is briefly reported here: T1-weighted, T2-weighted, proton density-weighted, and fluid-attenuated inversion recovery scans were acquired from each individual. The multimodality images were coregistered by standard protocol, extracranial material was removed, and the T1-weighted brain tissue mask was used for segmentation into gray and white matter and CSF. Anatomical region (i.e., 4 lobes, limbic, basal ganglia, corpus callosum, hippocampus) volumetric measurements were obtained using an automated computer-based template warping method that summed the number of respective voxels.¹⁷ Intracranial volume was estimated as total cerebral hemispheric volumes plus the ventricular CSF. Ischemic lesion volume segmentation used a support vector machine classifier trained on expert-defined lesions that has been validated in other studies.^{16,18,19}

RBC FA analysis. In this study, RBC FA composition was analyzed using gas chromatography with flame ionization detection, and expressed as a weight percent of total identified FAs.²⁰ The omega-3 index was defined as EPA + DHA, which is a documented marker of omega-3 biostatus.²¹ The intra-assay coefficient of variation for EPA + DHA was 1.6% and 0.8% for the low and high controls, respectively; the interassay coefficient of variation was 3.8% and 1.7%. During the aliquoting phase, the RBC samples were stored improperly at -20°C for a period of approximately 2 weeks, causing oxidative degradation of the polyunsaturated FA before measurement. The original FA levels were estimated with multiple imputations using independent data on FA degradation rates and the length of time the samples were exposed to -20°C .²⁰ After correcting the bias in the entire Women's Health Initiative Memory Study (WHIMS) cohort, 3.1% of samples remained severely degraded and were recommended for exclusion in subsequent analyses.²⁰ In our MRI cohort, 63 subjects were thus excluded.

Subjects. All subjects were participants in the Women's Health Initiative (WHI) randomized, placebo-controlled clinical trial to test the effects of hormone therapy (HT) (conjugated equine estrogen therapy [0.625 mg/d] with or without medroxyprogesterone acetate [2.5 mg/d]) on primary outcomes of heart disease and breast cancer. A subset was included in the WHIMS, which examined the effects of HT on loss of cognitive function.^{22,23} At the time of enrollment in WHIMS, the subjects were 65 to 80 years of age and free of dementia.²⁴ A further subset was recruited from 14 of the 39 US research centers to participate in the WHIMS Brain MRI Study.¹⁴ These women had an average age (range) of 78 years (71–88) at the time of MRI.

There were 1,380 women with RBC FA and MRI data. The WHI screening visits, when blood samples were collected, occurred from 1995 to 1998. The MRI brain scans were conducted a median of 8.0 years after blood was drawn. Sixty-three

subjects were excluded because their RBC samples were degraded as explained above.²⁰ There were 38 subjects who experienced a stroke or TIA between the screening visit and the MRI brain scans; because these events could have altered MRI outcomes independent of FA exposure, they were also excluded. An additional 168 participants were excluded because of poor quality scans (27) or missing variables central to the analysis (141). Thus, the total number of women included in this analysis was 1,111. All analyses were performed for each FA imputation, and then the inferences were combined using Rubin's technique.²⁵

Standard protocol approvals, registrations, and patient consents. All participants gave written informed consent, and institutional review boards approved the study protocols.

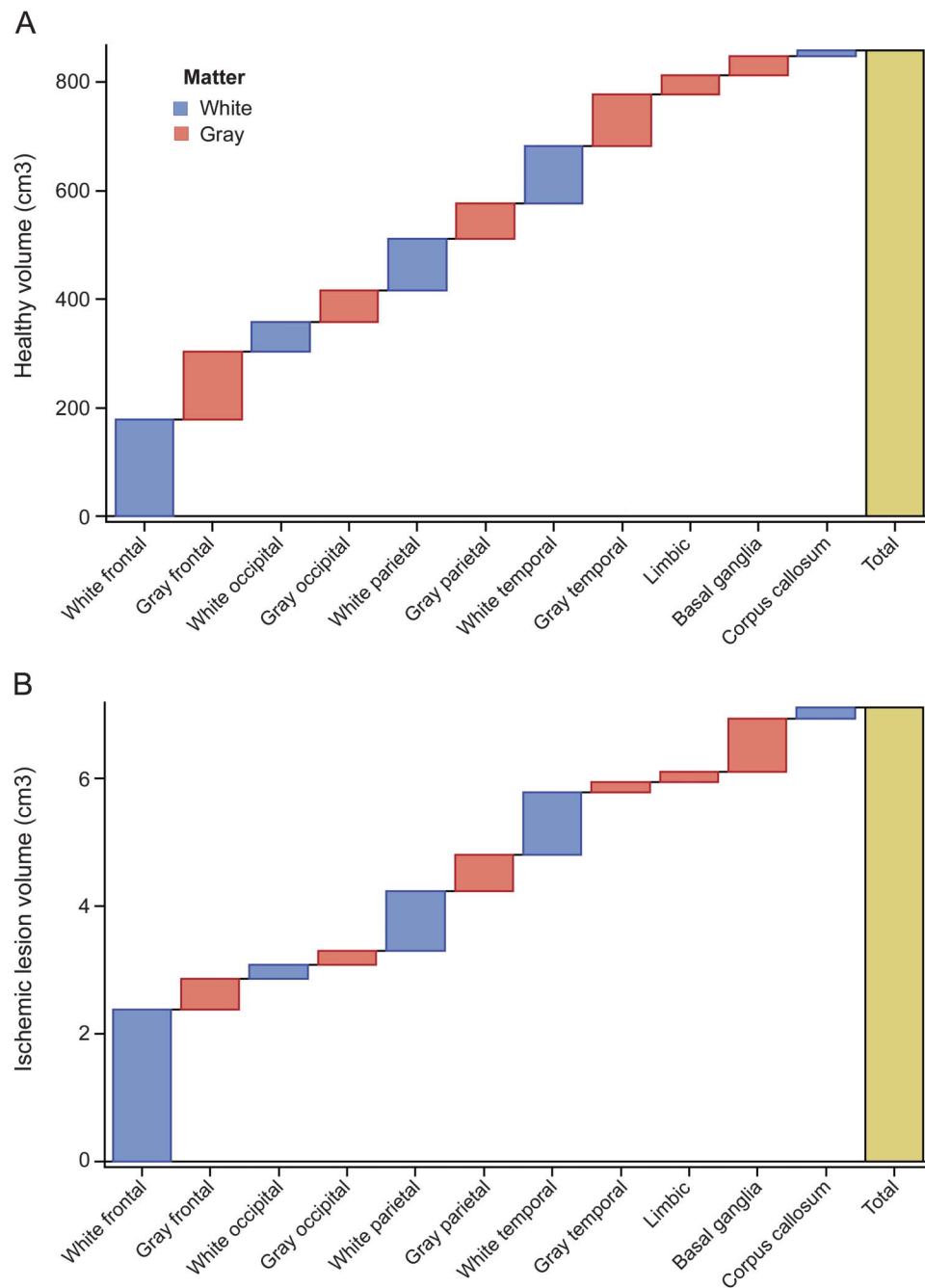
Statistical methods. Bivariate relations that quartiles of normal and ischemic brain volumes had with clinical factors were tested using 1-way analysis of variance and χ^2 tests for continuous and categorical data, respectively. Racial differences were controlled for by forming the quartiles independently for white and nonwhite subjects, and then combining the groups.

Volumes in cubic centimeters of total, normal, and natural logarithm of ischemic lesions were the dependent variables in linear mixed models. The primary hypothesis was that total brain volume would be associated with the omega-3 index; the component FAs DHA and EPA were also assessed individually. Three levels of covariate adjustment were determined a priori. The first model included study design variables (i.e., HT treatment assignment, time from randomization to MRI scan, and a covariance component was included to model the potential correlation from clusters of subjects within clinical sites) and fixed covariates (i.e., age, intracranial volume, race, and highest level of education obtained). Education is an indicator of socioeconomic status, which can be considered a fixed factor for this cohort of postmenopausal women. A second model additionally adjusted for modifiable, environmental factors (i.e., current smoking, physical activity, alcohol intake, and body mass index), and disease comorbidities (i.e., prior cardiovascular disease, diabetes, and treated and untreated hypertension). The third model included all blood biomarkers obtained at the WHI screening visit, i.e., high-density lipoprotein (HDL) cholesterol, non-HDL cholesterol, triglycerides, C-reactive protein (CRP), creatinine, insulin, and glucose. Triglycerides, CRP, insulin, and glucose had right-skewed distributions so they were transformed using a natural logarithm to reduce leverage of extreme values. Lastly, an interaction was tested between the omega-3 index and HT treatment assignment using the Wald test.

In an exploratory analysis, the relations between the omega-3 index and 13 anatomical regions (5 white matter and 8 gray matter) were analyzed in fully adjusted models. As a sensitivity analysis, the omega-3 index was included as a continuous and ordinal (i.e., quartiles) variable in separate models. A critical level ≤ 0.05 was used for testing the primary hypothesis and for the regional exploratory analyses; for the univariate analysis, the critical level was controlled at 0.05 using Bonferroni correction. Analyses were performed using SAS software (version 9.3; SAS Institute Inc., Cary, NC).

RESULTS On average, normal and ischemic tissue constituted 99% and 1% of the total brain volume, respectively. Overall, the tissue was 56% white matter and 44% gray matter; figure 1 shows the distribution of white and gray matter by anatomical region. Table 1 shows the demographic and clinical factors associated with quartiles of normal brain volumes, and table e-1, on the *Neurology*[®] Web site at www.neurology.org, shows the ischemic brain volumes by

Figure 1 Mean normal (A) and ischemic (B) lesion brain volumes in cubic centimeters by anatomical region



quartiles. Older age was directly related to the extent of ischemic lesion volume and inversely to amount of normal brain tissue. Intracranial volume was directly related to both normal and ischemic tissue volumes. In the bivariate analyses, there were no significant relations between the omega-3 index, DHA or EPA, and normal or ischemic brain volumes. However, factors that differ between omega-3 index quartiles may confound the relation between the index and brain volumes; these include education, physical activity, body mass index, CRP, HDL cholesterol, and triglycerides (table e-2).

DHA, EPA, and their sum (i.e., the omega-3 index) were tested in separate models as predictor variables of total brain volume, and the constituents of normal and ischemic tissue with 3 models of covariate adjustment. In the fully adjusted model, total brain volume was directly related to the omega-3 index ($p = 0.048$); a 1-SD increase was associated with 2.1 cm³ (95% confidence interval: 0.0–4.3 cm³) larger brain volume (table 2). Nearly identical results were obtained in normal brain volume. The association between a 1-SD increase in DHA was marginally significant (2.0 cm³ for both, $p = 0.063$ and 0.059)

Table 1 Patient characteristics by quartiles of normal (i.e., nonischemic) brain volume (stratified by race)

Variable (N = 1,111)	1st Q, 763 cm ³	2nd Q, 831 cm ³	3rd Q, 881 cm ³	4th Q, 958 cm ³	p Value ^a
Age at MRI, y	79.5 (3.7)	78.7 (3.8)	78.4 (3.6)	77.3 (3.2)	<0.0001 ^b
Intracranial volume, cm ³	984 (56)	1,059 (43)	1,116 (53)	1,206 (76)	<0.0001 ^b
White race	262 (95)	262 (94)	262 (94)	262 (94)	1.00
Hormone therapy active treatment	136 (49)	129 (46)	146 (53)	138 (50)	0.55
Estrogen + progestin trial	178 (64)	177 (64)	183 (66)	171 (62)	0.76
Time from randomization to MRI, y	8.1 (0.6)	8.0 (0.6)	7.9 (0.6)	8.0 (0.6)	0.045
Highest education					0.60
High school/votech	120 (43)	118 (42)	119 (43)	105 (38)	
Associate/bachelor	100 (36)	109 (39)	96 (35)	107 (38)	
Graduate or professional	57 (21)	51 (18)	63 (23)	66 (24)	
Physical activity, MET h/wk	11.3 (13.3)	11.1 (12.0)	12.3 (12.3)	10.7 (10.8)	0.46
Alcohol intake, servings/wk	2.4 (6.3)	2.3 (4.7)	2.2 (4.1)	3.0 (5.5)	0.26
BMI, kg/m ²	28.0 (5.2)	27.8 (5.2)	28.6 (6.0)	28.0 (5.2)	0.33
Current smoking	7 (3)	12 (4)	12 (4)	15 (5)	0.40
Prior CVD ^c	44 (16)	38 (14)	41 (15)	28 (10)	0.21
Hypertension					0.028
Never	158 (57)	189 (68)	171 (62)	193 (69)	
Untreated	32 (12)	19 (7)	29 (10)	17 (6)	
Treated	87 (31)	70 (25)	78 (28)	68 (24)	
Diabetes	11 (4)	6 (2)	10 (4)	6 (2)	0.46
Omega-3 index, % of total	5.1 (1.6)	5.2 (1.6)	5.4 (1.6)	5.2 (1.6)	0.24
DHA, % of total	4.4 (1.4)	4.6 (1.4)	4.7 (1.4)	4.5 (1.4)	0.28
EPA, % of total	0.7 (0.3)	0.7 (0.4)	0.7 (0.4)	0.7 (0.4)	0.48
Glucose, mg/dL	100 (20)	97 (19)	97 (21)	97 (18)	0.38
Insulin, mg/dL	56 (41)	54 (37)	56 (39)	50 (35)	0.17
Creatinine, mg/dL	0.78 (0.15)	0.76 (0.14)	0.75 (0.13)	0.74 (0.13)	0.029
C-reactive protein, mg/dL	4.09 (6.60)	3.30 (4.03)	3.70 (5.14)	3.51 (4.65)	0.33
HDL cholesterol, mg/dL	53 (12)	53 (13)	54 (12)	55 (13)	0.16
Non-HDL cholesterol, mg/dL	183 (43)	181 (37)	179 (35)	182 (42)	0.66
Triglycerides, mg/dL	146 (76)	143 (74)	138 (71)	137 (73)	0.41

Abbreviations: BMI = body mass index; CVD = cardiovascular disease; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; HDL = high-density lipoprotein; MET = metabolic equivalent; Q = quartile.

Data are presented as mean (SD) or n (%) for continuous and categorical variables, respectively. Mean volumes are given below each quartile.

^aThe critical level was set to 0.05/23 factors = 0.0022 for statistical significance using Bonferroni correction.

^bSignificant values.

^cPrior CVD includes myocardial infarction, congestive heart failure, coronary artery disease, peripheral artery disease, angina, and coronary revascularization.

with total and normal brain volume, respectively; however, EPA was not significantly related in fully adjusted models (1.7 and 1.6 cm³, $p = 0.11$ and 0.15, respectively). There were no associations between the natural logarithm of ischemic lesion volumes and any of the omega-3 FA metrics. There were also no significant interactions between the omega-3 index and HT on total or normal brain volumes ($p = 0.41$ and 0.32, respectively).

Next, the association of the omega-3 index with normal brain volume was explored in 13 anatomical regions, including 5 white matter (i.e., the 4 lobes and corpus callosum) and 8 gray matter (i.e., the 4 lobes, basal ganglia, limbic system, hippocampus, and parahippocampal gyrus). In fully adjusted models, the omega-3 index was included as either a continuous variable (per 1 SD = 1.6%) or by comparing the fourth quartile (Q4) vs the first quartile (Q1), which had mean levels

Table 2 Associations between brain volumes and a 1-SD increase in RBC omega-3 fatty acids (N = 1,111)

Brain tissue	Model ^a	DHA (1 SD = 1.4%)			EPA (1 SD = 0.4%)			EPA + DHA (1 SD = 1.6%)					
		Est.	95% CI	p Value	Est.	95% CI	p Value	Est.	95% CI	p Value			
Total, cm ³	1	1.8	-0.3	3.9	0.097	1.5	-0.6	3.5	0.16	1.9	-0.2	4.0	0.077
	2	1.9	-0.2	4.0	0.070	1.8	-0.3	3.9	0.085	2.1	0.0	4.3	0.048 ^b
	3	2.0	-0.1	4.1	0.063	1.7	-0.4	3.8	0.11	2.1	0.0	4.3	0.048 ^b
Normal, cm ³	1	1.8	-0.3	3.9	0.095	1.5	-0.6	3.7	0.17	1.9	-0.2	4.0	0.074
	2	2.0	-0.1	4.1	0.068	1.8	-0.4	4.0	0.12	2.1	0.0	4.3	0.050 ^b
	3	2.0	-0.1	4.1	0.059	1.6	-0.6	3.9	0.15	2.2	0.0	4.3	0.047 ^b
Ln (ischemic), ^c % change	1	2.8	-5.3	10	0.49	1.3	-6.1	8.3	0.72	2.7	-5.0	9.9	0.48
	2	3.0	-5.1	11	0.45	0.5	-7.2	7.7	0.90	2.8	-5.0	10	0.48
	3	2.9	-5.2	11	0.47	1.0	-6.8	8.3	0.79	2.8	-5.0	10	0.47

Abbreviations: CI = confidence interval; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; Est. = estimated; Ln = natural logarithm; RBC = red blood cell.

^aModel 1 = study design variables (i.e., active vs placebo hormone therapy [HT], type of HT, time from HT randomization to MRI, and clinical site), fixed covariates (i.e., age, intracranial volume, white race, and highest level of education). Model 2 = model 1 + environmental variables (i.e., current smoking, body mass index, physical activity metabolic equivalent h/wk, and alcohol servings/wk), disease comorbidities (i.e., prior cardiovascular disease, treated and untreated hypertension, and diabetes). Model 3 = model 2 + blood biomarkers, i.e., high-density lipoprotein cholesterol, non-high-density lipoprotein cholesterol, Ln (triglycerides), Ln (C-reactive protein), creatinine, Ln (glucose), and Ln (insulin).

^bSignificant values.

^cMissing n = 3.

of 7.5% vs 3.4%. In white matter, a 1-SD increase in the index was associated with a 66 mm³ (95% confidence interval: 1–130 mm³) larger corpus callosum (table 3). However, the corpus callosum volume was not different between Q4 and Q1 ($p = 0.16$). In gray matter, the hippocampus was directly related to the omega-3 index

on both a per-SD basis (50 mm³, $p = 0.036$; figure 2) and comparing Q4 and Q1 (159 mm³, $p = 0.034$).

DISCUSSION We found that a 2 SD (3.2% absolute) higher omega-3 index was associated with a 0.48% (4.2 cm³) larger total brain volume measured 8 years

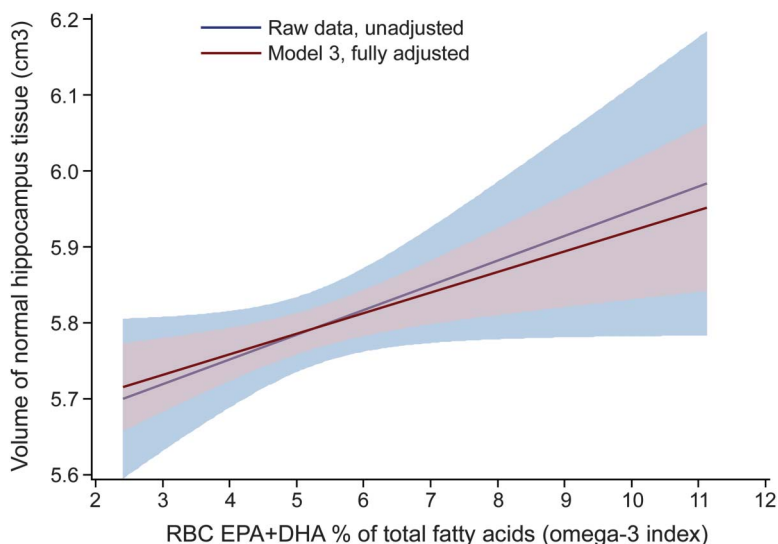
Table 3 Associations between normal (i.e., nonischemic) brain volumes (cm³) and the omega-3 index, in fully adjusted models (N = 1,111)

Brain region	Per 1 SD (1.6%)			Q4 (7.5%) vs Q1 (3.4%)				
	Est.	95% CI	p Value	Est.	95% CI	p Value		
White matter								
Frontal lobe	0.413	-0.542	1.369	0.40	1.022	-1.806	3.851	0.48
Occipital lobe	-0.044	-0.437	0.349	0.83	-0.058	-1.252	1.135	0.92
Parietal lobe	0.356	-0.174	0.886	0.19	0.623	-1.152	2.398	0.48
Temporal lobe	0.438	-0.104	0.979	0.11	0.909	-0.568	2.386	0.23
Corpus callosum	0.066	0.001	0.130	0.046 ^a	0.141	-0.054	0.336	0.16
Gray matter								
Frontal lobe	0.297	-0.377	0.972	0.39	1.017	-1.018	3.052	0.33
Occipital lobe	0.067	-0.303	0.438	0.72	0.114	-0.987	1.214	0.84
Parietal lobe	0.205	-0.212	0.621	0.33	0.684	-0.560	1.927	0.28
Temporal lobe	0.237	-0.298	0.772	0.38	0.868	-0.771	2.507	0.30
Basal ganglia	0.000	-0.171	0.170	1.00	0.109	-0.462	0.680	0.70
Limbic	0.066	-0.147	0.279	0.54	0.339	-0.362	1.039	0.34
Parahippocampal gyrus	0.018	-0.006	0.042	0.14	0.052	-0.016	0.120	0.13
Hippocampus	0.050	0.003	0.097	0.036 ^a	0.159	0.013	0.305	0.034 ^a

Abbreviations: CI = confidence interval; Est. = estimated; Q1 = first quartile; Q4 = fourth quartile.

^aSignificant values.

Figure 2 Mean normal hippocampal brain volume in cubic centimeters by omega-3 index using linear regression with 95% confidence bands



The fully adjusted model has a slightly attenuated slope compared with the unadjusted model, but reduced variability; a 3.2% greater omega-3 index is correlated with 100 mm³ greater hippocampus volume ($p = 0.036$). DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; RBC = red blood cell.

later. The 4.1% absolute mean difference between Q1 and Q4 in the omega-3 index level was associated with a 0.67% (5.8 cm³) larger brain volume. Similarly, in the Framingham Offspring cohort ($n = 1,575$, 54% women, average age 67 years), participants with an omega-3 index in the first quartile had a smaller average brain volume (−0.49%) compared with those in the upper 3 quartiles, which was described as being equivalent to approximately 2 years of normal brain aging.¹³

In our multicenter, women-only study, we found no relations between ischemic lesion volumes (which represented both diffuse small-vessel disease and the hyperintensities that surround focal lesions) and marine omega-3 FAs. This is in contrast to the Framingham study, which reported greater white matter hyperintensity volumes as a percentage of intracranial volume with lower DHA levels.¹³ A plasma nutrient biomarker pattern that included EPA, DHA, and 12 other nutrients was also inversely related to white matter hyperintensity volumes.^{e1} The Cardiovascular Health Study ($n = 2,465$, 59% women, average age 75 years) reported less likelihood of subclinical infarcts, defined as ischemic lesions ≥ 3 mm in diameter, for participants who ate fatty, nonfried fish more frequently.²⁶

In a previous study with 192 men and women (average age 78 years), the mean decrease in total brain volume was −0.45% and −0.98% per year in subjects without and with dementia, respectively, over an average of 2 years follow-up.²⁷ Interestingly, they also found that the slope of the cross-sectional relationship between age and brain volumes in

subjects without dementia was similar to the longitudinal rate of change. Our cohort included more than 1,100 women 65 years or older and free of dementia at WHIMS screening, and only one woman who developed dementia participated in the MRI study.

We also found that higher levels of RBC EPA + DHA were associated with larger hippocampal volumes, but significant differences were not detected in other specific regions between the upper and lower quartiles of the index. Because our study was cross-sectional, we cannot determine whether the volumetric differences found in the hippocampus would be suggestive of early neurodegenerative pathology. In a study of 55 men and women with a mean age of 45 years, the highest tertile of EPA + DHA dietary intake was associated with larger hippocampal volume in models minimally adjusted for age and sex.²⁸ Because the intake of EPA + DHA is highly correlated with RBC levels,^{29,30} this finding would be consistent with ours. More recently, a randomized controlled trial (2.2 g/d of EPA + DHA vs placebo for 6 months) in 65 men and women (average age 64 years) detected increased volume in the hippocampus, and the total gray matter declined only in the placebo group.^{e2}

A recent dose-response study found that 12 months of 0.93 and 1.86 g/d of EPA + DHA increased RBC levels by 3.6% and 4.5%, respectively.³¹ Therefore, changes in the omega-3 index that can be achieved through diet modification and/or supplementation are similar to those associated with 1 to 2 years of normal, age-related brain atrophy.

The primary findings of this study were that total normal (nonischemic) brain and hippocampal volumes were directly associated with RBC EPA + DHA levels, the omega-3 index. Together with the findings of others, our study suggests that a higher tissue reserve of omega-3 FAs may slow the loss of cognitive function or disease that can accompany brain atrophy. Although the omega-3 index was not related to cognitive domains in WHISCA (Women's Health Initiative Study of Cognitive Aging),³² a study is currently under way to examine the relations between it and clinically adjudicated probable dementia in WHIMS. Animal and human studies have demonstrated that DHA deficiency during the prenatal period results in cognitive or behavioral deficits, and that these changes reverse with omega-3 supplementation.³³ It has been shown that DHA supplementation promotes neuron growth in hippocampus cells in vitro and in rat models.³⁴ Normal brain functions such as synaptic plasticity and neurotransmission remain dependent on adequate DHA intake throughout the lifespan.³⁵ Even though DHA has a role in brain development, the role of dietary DHA in maintaining normal brain function remains controversial. DHA brain metabolism was studied in 14 healthy adult men and

women between 19 and 64 years of age using radiolabeled DHA and PET.³⁶ The net rate of human brain DHA uptake was 3.8 ± 1.7 mg/d, which implied a 2.5-year half-life assuming 5 g of total DHA in the brain. To the extent that incorporation of DHA into neuronal membranes is important in maintaining cognitive function, future supplementation studies may need to be several years long.

EPA + DHA are very inefficiently (i.e., <5%) produced from the plant essential omega-3 FA α -linolenic acid and are thus best obtained preformed in the diet. In approximately 3,200 Framingham Offspring subjects, a combination of EPA + DHA intake and the use of fish oil supplements explained 40% of the total variability in the omega-3 index; an additional 25% was due to heritability.³⁰ The heritability aspect reflects the metabolic efficiency with which dietary omega-3 FAs are absorbed and incorporated into RBC membranes, and as such the omega-3 index is a postmetabolism biomarker used in place of dietary intake.

In past studies, omega-3 FA amounts have been reported in a variety of ways: as weight %, mol %, or concentration (by volume or cell count), and in many types of blood/tissue. The main debate is the reporting of relative vs absolute amounts, while the different tissue types can represent different durations of exposure.³⁷ Absolute concentrations rise and fall with total lipoprotein levels because the majority of lipids they carry contain FAs, whereas using relative %, particularly for membranes such as RBCs, more fully reflects the composition (and thus function) of other cell membranes. In a population, plasma % and RBC % are strongly correlated³¹; however, omega-3 RBC % has the advantage of lower within-person variability than plasma %, and resistance to a single large dose of fish oil.^{38,39}

Although the omega-3 index was measured only once 8 years before the MRI scan, it should be stable in subjects who did not alter/initiate fish oil supplementation. This was shown in 250 Framingham Offspring participants with levels measured 7 years apart.⁴⁰ Also in that study, the percentage of participants who started taking fish oil supplements increased from 3% around 1999 to 13% in 2006. These dates overlap nicely with the current study whereby the WHI screening visit blood draw and MRI scan dates were around 1998 and 2006, respectively. This suggests that approximately 10% of the WHIMS subjects may have begun taking fish oil supplements after the screening visit, although this variable was not captured in the trial. If so, this would have potentially preserved some brain volume, which would cause the reported associations to be underestimated.

In this cohort of postmenopausal women, lower RBC EPA + DHA levels correlated with smaller total and hippocampal brain volumes, the former being an indication of cognitive aging and the latter being

centrally involved with AD pathology. This study thus adds to the growing literature suggesting that higher omega-3 FA tissue levels, which can be achieved by dietary changes, may hold promise for delaying cognitive aging and/or dementia.

AUTHOR CONTRIBUTIONS

Dr. Pottala drafted and edited the manuscript, analyzed the data, interpreted the findings, and designed the study. Dr. Yaffe reviewed and edited the manuscript, and interpreted the findings. Dr. Robinson and Dr. Espeland reviewed and edited the manuscript, interpreted the findings, and designed the study. Dr. Wallace reviewed and edited the manuscript, and interpreted the findings. Dr. Harris reviewed and edited the manuscript, collected the RBC fatty acid data, interpreted the findings, and designed the study.

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DISCLOSURE

J. Pottala reports no disclosures. K. Yaffe serves on the WHI scientific advisory board, the Brain Center Medical Advisory Board, and the DSMB for NIA and Takeda; she is also a consultant for Lilly and Novartis. J. Robinson has served as principal investigator for grants received by the University of Iowa from Amarin, Amgen, Daiichi-Sankyo, Genentech/Hoffmann La Roche, and GlaxoSmithKline. M. Espeland serves on Data and Safety Monitoring Boards for the KOWA Research Institute and the Terumo Medical Corporation, and a Steering Committee for Boehringer-Ingelheim Pharmaceuticals. R. Wallace reports no disclosures. W. Harris owns OmegaQuant Analytics, LLC, and is a Senior Research Scientist at Health Diagnostic Laboratory, Inc., both of which offer red blood cell fatty acid tests. He is also a scientific advisor to Omthera Pharmaceuticals and Aker BioMarine Antarctic. Go to Neurology.org for full disclosures.

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